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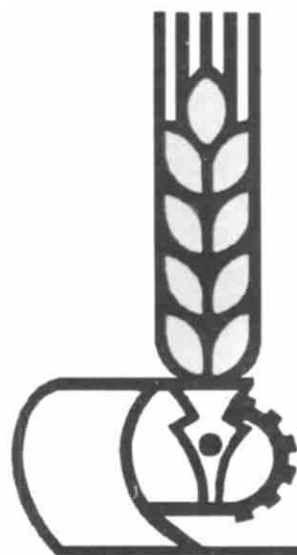
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Analysis of the Factors Affecting Coffee Export in Cameroon: A Gravity Model Approach

Veli Anıl Çakan^{1*}, Amadou Merleau Nsangou Pofoura¹, and Tolga Tipi¹

ABSTRACT

This study investigated the factors affecting coffee exports in Cameroon. For this purpose, we employed the gravity model. Considering the sample characteristics, the model was estimated with the Poisson Pseudo-Maximum Likelihood (PPML) method. The main material of the study was a panel data set covering the years 2001-2021 for ten countries, Cameroon's main coffee export partners. The findings show that the GDP of importing countries, coffee export prices, and Bilateral Investment Treaties (BITs) positively influence exports, whereas distance, exchange rates, and Cameroon's GDP have negative impacts. The results highlight Cameroon's logistics infrastructure deficiencies and the significance of stable, high-quality production. The Cameroonian government should implement policies to improve production quality and efficiency by expanding agricultural extension services and offering farmers input and investment incentives to address these challenges. Additionally, improving port efficiency will necessitate the digitalization of operations, implementation of data-driven planning, and strategic infrastructure investments.

Keywords: BITs, Exchange rate, Export price, FTAs, Gravity Model.

INTRODUCTION

Agricultural sector plays a crucial role in the economy of Sub-Saharan African countries (Senbet and Simbanegavi, 2017). It has a predominant place in the supply of food, employment for rural populations, raw materials, and foreign income, and mainly contributes to the formation of the Gross Domestic Product (GDP). The contribution of agriculture to Cameroon's GDP was approximately 16.97% in 2021. In the same year, it was the leading employer, employing 42.82% of the total workforce, and served as one of the primary sources of foreign currency, contributing 18.63% to merchandise exports. (World Bank, 2024).

Coffee, along with cocoa and cotton, has played a decisive role in Cameroon's national economy (Kufa, 2010; René *et al.*, 2023). The coffee industry in Cameroon is an essential source of income for many

households from various aspects, including production, marketing, and distribution. An historical analysis of coffee production in Cameroon reveals that the past decade marked the lowest levels of production. Between 2011 and 2021, coffee production amounted to 33,527 tons on average, with approximately 1.5% of the arable land allocated for cultivation. In contrast, the peak of coffee production was observed in the 1980s, with production reaching 137,900 tons in 1984. During this peak, 5.7% of the arable land was allocated to coffee, making it the fourth largest agricultural product by land area (FAOSTAT, 2024). Despite the decline in production, coffee continues to play a significant role in the Cameroonian economy due to its substantial contribution to exports. According to TRADEMAP (2024), coffee, tea, maté, and spices (Code: 09) were Cameroon's 8th major export commodity category in 2001-2021. Coffee

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(Code: 0901) constitutes 99.66% of this category. Cameroon directs 93% of its total coffee exports to its top ten coffee importers. Figure 1 illustrates the share of these countries in Cameroon's coffee exports.

Figure 2 presents the data for Cameroon's coffee production, exports, and export-to-production ratio spanning from 2001 to 2021. The figure illustrates that Cameroon's ratio of production to exports was generally above 80% during this period. Nevertheless, there is a noticeable decrease in both the ratio of coffee exports to production and the amount of coffee exports.

The simultaneous decline in production and exports can be attributed to a combination of many structural and cyclical problems. One of the most important is that coffee provides lower returns than substitute crops, such as cocoa, for the farmers. A significant factor contributing to the decline in farmers' revenue is the decrease in coffee export prices. Cameroon's real coffee export price has declined by 24.53% over the past five years (2016-2021) compared with the preceding sixteen-year period. Moreover, Cameroon's real coffee export price was 30% below the world price in 2001-2021 (FAOSTAT, 2024; original calculations). The loss of income leads producers to switch from coffee to cocoa cultivation or to forego

the renewal of their aging coffee trees (Shillie and Egwu, 2020). This change in production patterns may seem suitable, given that opting for a more profitable alternative is likely to result in a favorable impact on the total agricultural production value. Nonetheless, limiting the diversity within a country's production and export portfolio raises risks due to factors such as plant diseases and sector-specific global crises. Moreover, it renders investments made in established industries ineffective for processing abandoned products. The income loss in coffee production stems from export-related issues. Factors such as the limited popularity of Cameroonian origin in the international market, the absence of a strategic marketing approach, fluctuating production, concerns about product quality, high export taxes, and the insufficiency of dynamic promotional initiatives collectively erode the competitiveness in exports (MINADER, 2009; GCP, 2016). The low survival rate of export relationships could also be included among these factors. In Cameroon, the probability of new exporters continuing their activities stands at 30% for the following year, decreasing to approximately 12 % by the third year (World Bank, 2016).

To alleviate the prevailing crisis within the

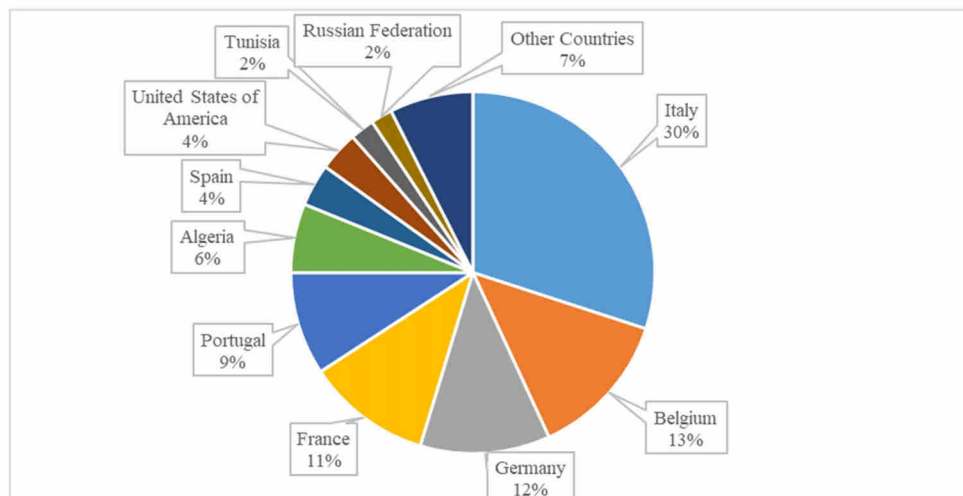


Figure 1. Main coffee export partners of Cameroon in 2001-2021 (TRADEMAP, 2024).

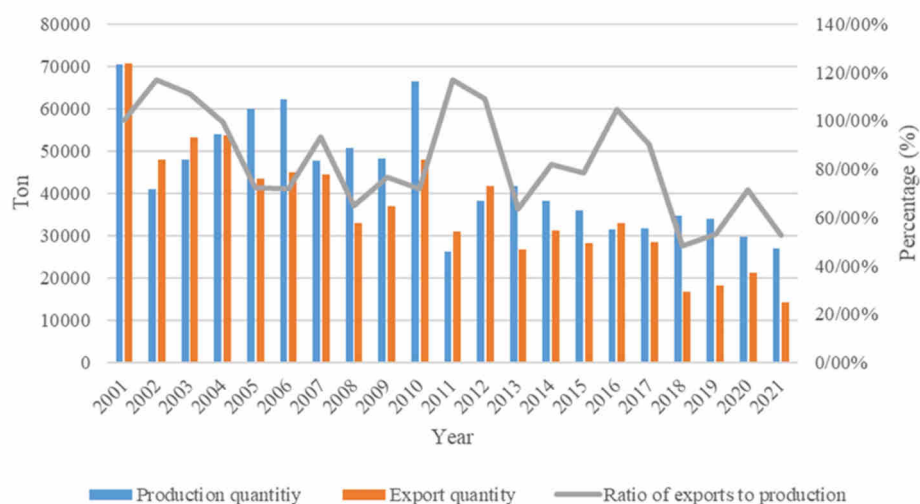


Figure 2. Cameroon's coffee production, export, and export-to-production ratio, between 2001 to 2021 (FAOSTAT, 2024).

coffee sector, the Cameroonian government is concentrating its efforts on increasing domestic consumption (VOA, 2019). Improving local consumption is crucial, not only to ensure access to essential consumer goods within society but also to safeguard producers against potential export crises. Although enhancing local consumption might boost supply through increased demand, the main driver of Cameroon's coffee industry development is the prioritization of exports. This is primarily because the price level incentivizing producers to engage in coffee production can be attained through exports. Besides, international trade is essential for economic growth (Karambakuwa and Nwadi, 2020). It enhances productivity by driving firms to improve efficiency, while supporting economic development through the expansion of exports and imports (Kircicek and Ozparlak, 2023).

Exports play a critical role in propelling the development of developing countries (Sanjuán-López and Dawson, 2010). Therefore, it is crucial to undertake more studies to identify the determinants exports in developing countries and to provide recommendations within this framework. The gravity model has been widely used in

trade economics to elucidate the bilateral trade flows between countries. This model has been refined over the years, and scholars across the globe have extensively explored the various factors affecting the export of commodities. Among these factors, the exchange rate is frequently mentioned. Studies have shown that the impact of exchange rates on agricultural export flows can vary significantly depending on the country and sector in question (Abdullahi *et al.*, 2021; Abdullahi *et al.*, 2022; Yadav and Chattopadhyay, 2024). The effects of free trade agreements on exports have also been discussed in the literature, demonstrating that Free Trade Agreements (FTAs) facilitate increased export volumes by reducing trade barriers and fostering market access (Nsabimana and Tirkaso, 2020; Jagdambe and Kannan, 2020). Price is one of the key factors that shape demand for goods within a country. A decline in export prices typically leads to increased demand in importing countries, stimulating higher export volumes. Multiple works in the literature have reached similar conclusions (Yusiana *et al.*, 2022, Phung and Nguyen, 2022). Recent researches have investigated the effects of Bilateral Investment Agreements (BITs) on exports,



demonstrating that these agreements significantly boost exports (Heid and Vozzo, 2020; Xiong, 2022).

Despite the growing body of literature, research on sub-Saharan Africa remains limited. Further research is needed to understand the factors driving strategic product exports and develop strategies in this area. This study assists in filling this gap by employing the gravity model to analyze factors affecting coffee exports in Cameroon. In its basic form, the gravity model provides ideas about the impact of transportation costs and trade partners' income on exports. However, this research extends the model by incorporating variables related to the impact of international agreements and export price elasticity. These factors have received limited attention in the literature regarding their impact on exports, particularly in developing countries. This versatile framework enables us to conduct a comprehensive foreign trade

analysis for Cameroon, providing actionable findings to support policymakers in crafting and refining strategies for more effective trade planning and development.

MATERIALS AND METHODS

Material

The main material of this study consisted of data obtained from international bodies. The data set covers the period 2001-2021 and includes the ten countries to which Cameroon exports the most in the relevant period. These countries are Italy, Belgium, Germany France, Portugal, Algeria, Spain, the USA, Tunisia and Russian Federation, respectively. Information on the variables included in the model is given in Table 1.

Table 1. Description of the Variables.

Variable ^a	Description ^a	Unit	Source	Expected Sign
EX_{ij}	The value of coffee ^b exports from Cameroon to country j	US\$, in 2015 Prices	TRADEMAP (2024), original calculations	N/A
GDP_i	Gross domestic product of Cameroon	US\$ per capita, in 2015 Prices	FAOSTAT (2024)	+
GDP_j	Gross domestic product of importing country j	US\$ per capita, in 2015 Prices	FAOSTAT (2024)	-/+
$DIST_{ij}$	The geographical distance between Cameroon and the importing country j	km	CEPII (2024)	-
RXP_i	The coffee export price of Cameroon	US\$, in 2015 Prices	TRADEMAP (2024), original calculations	-
ER_{ij}	Exchange rate: This indicates how much of the Central African CFA Franc is required to purchase one unit importing countries' own money.	Central African CFA franc	FXTOP (2024)	-/+
FTA_{ij}	Free Trade Agreements between Cameroon and the importing country j	Dummy	CEPII (2024)	+
BIT_{ij}	Bilateral Investment Treaties between Cameroon and the importing country j	Dummy	UNCTAD (2024)	+

^a Here i represents Cameroon and j represents one of Cameroon's top ten trading partners.

^b "Product: 0901 Coffee, whether or not roasted or decaffeinated; coffee husks and skins; coffee substitutes containing coffee in any proportion" (TRADEMAP, 2024).

Methods

This study analyzes the factors affecting coffee export in Cameroon. The gravity model is used for this purpose. In recent decades, this model has become a widely used tool for elucidating international trade determinants (Sharma *et al.*, 2023). The primary reason for this is the model's ability to offer a comprehensive analysis of the revealed trade data (Jadhav and Ghosh, 2023). The gravity model of international trade elucidates bilateral trade flows by incorporating the economic scale of the trading partners and the geographical distance that separates them (Golovko and Sahin, 2021). Tinbergen's (1962) pioneering work established the framework for this model and, subsequently, early studies further developed and applied its principles.

The gravity model in the logarithmic transformation of the variables, as commonly used in the literature, can be expressed as follows:

$$\ln EX_{ij} = \beta_0 + \beta_1 \ln GDP_i + \beta_2 \ln GDP_j + \beta_3 \ln DIST_{ij} + e_{ij}$$

Where, i represents Cameroon, j represents trading partners, EX_{ij} represents the value of bilateral coffee Exports from Cameroon to its trading partners, GDP_i represents the Gross Domestic Product of the country i , GDP_j represents the Gross Domestic Product of the country j , and $DIST_{ij}$ represents the geographical Distance between Cameroon and its trading partners.

The basic structure of the gravity model has evolved in multiple ways to accommodate the needs of researchers (Nawrot, 2023). In country- and product-specific studies, such modifications yield empirical insights that not only enrich the literature but also assist policymakers in formulating effective strategies. Therefore, we modified our model by integrating variables linked to the primary factors behind the decline in Cameroon's coffee exports, as outlined in the introduction. We tackled the problem of low export prices by including variables such as the real export

price and exchange rates. To address the issue of low survival rates in export relationships, we included dummy variables related to free trade agreements and bilateral investment treaties. The modified model is formulated as follows:

$$\ln EX_{ij} = \beta_0 + \beta_1 \ln GDP_i + \beta_2 \ln GDP_j + \beta_3 \ln DIST_{ij} + \beta_4 \ln RXP_i + \beta_5 \ln ER_{ij} + \beta_6 \ln FTA_{ij} + \beta_7 \ln BIT_{ij} + e_{ij}$$

Where, RXP_i is the coffee Export Price of Cameroon, ER_{ij} is the Exchange Rate between Cameroon and its trading partners, FTA_{ij} is the Free Trade Agreements between Cameroon and its trading partners, and BIT_{ij} is the Bilateral Investment Treaties between Cameroon and its trading partners.

Estimation of the gravity model is a problematic issue in most cases and has long been debated by researchers. The primary cause behind this is the fluctuating nature of the bilateral trade flows. Economic and political circumstances have the potential to either excessively boost trade or entirely impede its occurrence. This phenomenon is especially evident in developing economies or emerging sectors of a country. An example of this is Cameroon, which has not traded with major coffee export partners for some years.

Zero trade flows create substantial econometric challenges, as the loss of observations in log-linear transformations leads to information loss and biased results (Gómez-Herrera, 2013; Borojo *et al.*, 2022). To simply solve the zero-trade problem, removing these observations from the data set (Bikker, 1987) and formulating the dependent variable as $\ln(\text{Export}+1)$ (Eichengreen and Irwin, 1995; Guo, 2004) are commonly used. However, Santos Silva and Tenreiro (2006) showed that these traditional methods lead to inconsistent estimates, especially in cases where the heteroscedasticity problem exists, and suggested the Poisson Pseudo-Maximum Likelihood (PPML) model for estimating gravity models. The use of the PPML estimator in the gravity model has been criticized on the grounds that it may yield biased results in situations where zero trade

flows predominate and there is over-dispersion (Burger *et al.*, 2009; Martínez-Zarzoso, 2013). Nevertheless, a substantial amount of research has shown that PPML maintains its robustness, even in situations of frequent zero trade flows or overdispersion (Santos Silva and Tenreiro, 2011, 2022; Ghazalian, 2019).

Due to its advantages, we used the PPML method for estimation. This model can be estimated only when the dependent variable consists of integer count data. Hence, we rounded the data in our dependent variable, which included fractional observations to integer values. Our gravity model to be estimated with PPML can be formulated as an exponential function as follows:

$$EX_{ij} = \exp[\beta_0 + \beta_1 \ln GDP_i + \beta_2 \ln GDP_j + \beta_3 DIST_{ij} + \beta_4 \ln RXP_i + \beta_5 \ln ER_{ij} + \beta_6 FTA_{ij} + \beta_7 BIT_{ij}] + e_{ij}$$

Given that the model is in exponential form, dummy variables are interpreted similarly to semi-logarithmic models. In this study, the approach proposed by Halvorsen and Palmquist (1980) was employed for interpreting dummy variables. Accordingly, whereas g represents the relative effect, the percentage effect is calculated using the following formula:

$$100 * g = 100 * (e^{\beta} - 1)$$

To test the robustness of the estimation methodology, we employed alternative commonly used estimators. These are the Negative Binomial Maximum Likelihood (NBML) model proposed by Burger *et al.* (2009) and the OLS with $\ln(\text{Export}+1)$ modification. Since the negative binomial model is categorized as an integer count model, we used the same rounding modification as the PPML for the dependent variable. In the findings section, we presented the outcomes derived from these estimators along with PPML.

RESULTS AND DISCUSSION

Table 2 presents descriptive statistics of the variables included in the model. As FTA and BIT are dummy variables, their means reflect their respective frequencies. FTAs, for instance, account for 20% of the observations. Cameroon's sole FTA with the countries in the dataset is the agreement with the EU, which came into effect on August 14, 2014. However, as the CEPII database registers agreements that take effect after July 1st in the following year, FTAs are recorded from 2015 onwards. BITs account for 39% of the observations. Cameroon has BIT agreements only with Italy, the USA, Belgium, and Germany, among the countries included in the dataset for this study. Of these, only the BIT with Italy occurred during the observation period, on April 1, 2004. The agreements with other countries came into force prior to the observation period.

Table 3 shows the estimation results of the gravity model. Here, the PPML model is employed as the base model for interpretation, while NBML and OLS (Ordinary Least Squares) models are added for robustness check. Examination of the estimation results indicates that the estimators produce largely consistent outcomes regarding both the direction and significance levels of the relationships. The only difference is that the OLS method yields statistically significant results for the FTA_{ij} .

The estimation results for our base model show that all variables, with the exception of FTA, are statistically significant. The GDP of importing countries has the highest positive impact of 1.50%, followed by Cameroon's real coffee export price at 0.63%. Additionally, the percentage impact of BITs is measured at 54%. The variable with the most substantial negative effect is Cameroon's GDP, with -5.62%. This is followed by distance and exchange rates with -3.99 and -0.35%, respectively.

Table 2. Descriptive statistics.

Variables	Mean	Std. Dev.	Maximum	Minimum
EX_{ij}	5857.27	7658.47	52590.00	0.00
$\ln GDP_i$	7.18	0.07	7.28	7.05
$\ln GDP_j$	9.85	0.95	11.03	7.91
$\ln DIST_{ij}$	8.51	0.27	9.17	8.20
$\ln RXP_i$	0.52	0.20	0.91	0.17
$\ln ER_{ij}$	5.55	1.69	6.60	1.41
FTA_{ij}	0.20	0.40	1.00	0.00
BIT_{ij}	0.39	0.49	1.00	0.00

Table 3. Estimation results for Gravity Model.

Variables	PPML ^a	NBML ^a	OLS $\ln(EX_{ij} + 1)$
Constant	69.41878*** (11.59736)	58.74402*** (10.81458)	75.23906*** (15.07565)
$\ln GDP_i$	-5.624733*** (1.430220)	-4.627639*** (1.523776)	-10.56881*** (2.059889)
$\ln GDP_j$	1.497604*** (0.260985)	1.191910*** (0.152143)	2.016000*** (0.239863)
$\ln DIST_{ij}$	-3.994570*** (0.542036)	-3.325517*** (0.359847)	-4.170305*** (0.585373)
$\ln RXP_i$	0.629975* (0.337856)	1.087721*** (0.379283)	2.429299*** (0.606308)
$\ln ER_{ij}$	-0.355475*** (0.098317)	-0.241735*** (0.065761)	-0.568053*** (0.104096)
FTA_{ij}	-0.170373 (0.228515)	-0.029155 (0.259557)	0.826028** (0.405091)
BIT_{ij}	0.430819*** (0.145855)	0.416284*** (0.131776)	0.605192** (0.291410)
Adjusted R ²	0.61	0.50	0.43
N	210	210	210

^a Robust standard errors are in parenthesis, *, **, ***: $P < 0.1$, $P < 0.05$, and $P < 0.01$, respectively.

The negative correlation between Cameroon's GDP per capita growth and coffee exports can be attributed to the increase in domestic purchasing power. This growth enables higher internal consumption, potentially reducing the volume of coffee available for export. Concrete signs of this relationship are evident in Cameroon. The per capita GDP increased by 24% during the study period, indicating growing domestic demand. This is further supported by the rise in coffee processing facilities, with 104 active plants reported by the National Cocoa and Coffee Board (NCCB, 2022). Since 99% of Cameroon's coffee exports are unroasted and only 5% of production is processed domestically (AFCA, 2024), these facilities predominantly serve the local market. Moreover, the negative relationship between income growth and agricultural

product exports has also been previously documented (Abdullahi *et al.*, 2022). This shift in demand aligns with the broader mechanism of income elasticity, which suggests that, as incomes grow, domestic consumption can compete with exports. If managed well, this mechanism can produce positive results for coffee exports in the long term. Because sustainable production is a prerequisite for sustainable exports, and sustainable production is possible with alternative sales channels. Establishing a vibrant domestic market is essential, enabling producers to engage in sales even when confronted with conditions detrimental to exports, such as international crises. Therefore, to safeguard against a potential decline in exports due to increased demand, it is essential to support producers with productivity-enhancing policies, such as

facilitating access to agricultural credit, offering incentives for input use, and promoting mechanization.

Another variable that has a negative effect is distance. Each 1% increase in distance reduces export value by 3.99%. Although past studies reached results consistent with ours regarding the direction of the distance-export relationship, they differ in the magnitude of the effect. In the majority of the studies surveyed, the distance elasticity for coffee exports falls below the level estimated for Cameroon (Sadeghi *et al.*, 2019; Abafita and Tadesse, 2021; Nguyen, 2022). Considering that the distance variable reflects the costs and risks in transportation, this finding may indicate Cameroon's logistical inefficiency in coffee. The inefficiency in the port of Douala, Cameroon's largest port, confirms our explanation. Douala's average dwell time for containers (19 days) differs negatively from other African ports such as Dar es Salam (12 days), Mombasa (11 days), and Durban (4 days) (Raballand *et al.*, 2012; Diarra and Tchapa, 2014; World Bank, 2016). Even more concerning, recent studies have demonstrated that Douala's container dwell time has exceeded 21 days (Awah *et al.*, 2021), placing it 340th out of 370 ports in the 2021 Container Port Performance Index (Worldbank, 2022). The World Bank (2016) pointed to the sector's lack of proper regulation as the explanation for this situation. Furthermore, if we accept that this variable also indirectly expresses cultural distances like language differences and varying institutional frameworks between countries (Van Bergeijk and Brakman, 2010; Golovko and Sahin, 2021), the obtained coefficient can also be associated with marketing failure. The Cameroonian government must invest in intercity transportation infrastructure and enhance port efficiency to address logistics shortcomings. This requires digitalizing port operations, data-driven planning, and infrastructure upgrades. Additionally, expanding the network of asphalt roads is essential, not only to increase the efficiency

of coffee transport but also to enhance the movement of goods and improve domestic mobility across the country.

Our estimation results show that every 1% decrease in the value of the Central African CFA franc reduces coffee exports by 0.35%. The effect of exchange rates on exports is quite controversial, both theoretically and empirically. The appreciation of a country's currency can weaken that country's competitiveness in the international market. Generally, an increase in the exchange rate reduces the comparative price of exports and increases foreign demand by reducing the prices of domestic goods (Nugroho and Lakner, 2022). In reality, various factors tied to both the country and the sector play a role in determining the correlation between the exchange rate and export dynamics. Examining the case of Cameroon, we observe that its export price is approximately half of the global price, as previously noted. Given Cameroon's already highly competitive pricing, an appreciation of its currency may still yield a positive impact on the value of its exports. The literature also reflects on the relative nature of this situation. While a substantial body of evidence supports our study's conclusions (Irshad *et al.*, 2018; Yadav and Chattopadhyay, 2024; Eshetu, 2024), there is also a significant volume of evidence with contradictory outcomes (Abdullahi *et al.*, 2021; Abafita and Tadesse, 2021; Nugroho and Lakner, 2022). The estimation results further indicate a positive and significant relationship between coffee export prices and export value. While this finding diverges from the literature (Phung and Nguyen, 2022), it aligns with our results for the exchange rate. Similar to the exchange rate, the general assumption is that competitive pricing boosts total exports. However, this no longer holds for Cameroon, which already offers highly competitive prices compared to the global market. Therefore, Cameroonian policymakers should prioritize quality-enhancing production policies that increase prices rather than focusing on selling more

products at competitive prices to boost income from coffee exports. This can be achieved by expanding agricultural extension and advisory services. In this way, the necessary technical support is provided to help traditional producers improve the quality of their products. Additionally, producers are informed about certified production techniques, such as organic farming, which offers high-price premiums. In this context, it is crucial to financially support and motivate producers who engage in certified production.

Our estimation results for international agreements revealed that FTAs had no statistically significant effect on coffee exports, whereas BITs have shown a positive effect. This finding is consistent with the literature, which provides evidence of positive relationships between exports and BITs (Heid and Vozzo, 2020; Xiong, 2022). However, this study represents the first documented case in the context of a developing country. It is not surprising that, irrespective of the development level of the countries, BITs and exports display similar effects. One of the most significant advantages of BITs is their capacity to facilitate foreign direct investment, which, as studies have demonstrated (Samantha and Haiyun, 2018; Sahoo and Dash, 2022), enhances exports in both developing and developed countries. Furthermore, BITs offer several additional benefits that can boost export volumes, including promoting trade in specialized intermediate inputs and mitigating risks through enhanced legal protections (Heid and Vozzo, 2020). Contrary to the widely held assumption that FTAs boost exports, recent research on coffee by Abafita and Tadesse (2021) has found no statistically significant relationship, as in our study. They explain this by noting that coffee trade primarily flows from the least developed countries to more developed countries, while most regional trade agreements (RTAs) in their study are intra-regional, which may diminish the impact of inter-regional RTAs on coffee trade. In the Cameroonian case, the most

plausible explanation for the lack of a significant relationship is the steady decline in coffee production since the FTAs came into force. Despite the facilitation of bilateral trade, the continuous drop in production has prevented the Cameroonian coffee sector from benefiting from these agreements. These findings imply that the cornerstone of increasing Cameroon's exports through international agreements is ensuring a steady and consistent production flow. Additionally, the Cameroonian government should analyze the existing agreements and suggest strategic adjustments explicitly tailored to the coffee sector to maximize the impact of FTAs.

CONCLUSIONS

Our study uncovered key insights into the underlying dynamics of Cameroon's coffee exports. The most significant challenge is the negative impact of rising domestic demand for coffee, driven by income growth on export levels. However, if adequately managed through policies aimed at increasing productivity, such as better access to credit and mechanization, this demand could support domestic consumption and sustainable exports. Cameroon's logistical inefficiencies, particularly when compared to its competitors, further constrain exports. On the positive side, international agreements and focus on quality rather than price competition can influence exports positively. To fully realize its potential, Cameroonian government must provide technical and financial support to producers, enhance efficiency at the Douala port, and critically assess its international agreements to develop strategies that maximize their benefits.

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تحلیل عوامل مؤثر بر صادرات قهوه در کامرون: رویکرد مدل جاذبه

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چکیده

این مطالعه عوامل مؤثر بر صادرات قهوه در کامرون را بررسی کرد. برای این منظور، از مدل جاذبه استفاده کردیم. با توجه به ویژگی‌های نمونه مورد نظر، مدل با روش پواسون (Poisson) یعنی درست نمایی شبه حداکثر (PPML) تخمین زده شد. ماده اصلی این مطالعه، مجموعه‌ای از داده‌های پانلی بود که سال‌های 2001 تا 2021 را برای ده کشور، شرکای اصلی صادرات قهوه کامرون، پوشش می‌داد. یافته‌ها نشان می‌دهد که تولید ناخالص داخلی کشورهای واردکننده، قیمت صادرات قهوه و معاهدات سرمایه‌گذاری دوجانبه (BIT) تأثیر مثبتی بر صادرات دارند، در حالی که مسافت، نرخ ارز و تولید ناخالص داخلی کامرون تأثیرات منفی دارند. این نتایج، کاستی‌های زیرساخت‌های لجستیکی کامرون و اهمیت تولید پایدار و با کیفیت بالا را برجسته می‌کند. دولت کامرون باید با گسترش خدمات ترویج کشاورزی و ارائه مشوق‌های سرمایه‌گذاری و مشارکت با کشاورزان، سیاست‌هایی را برای بهبود کیفیت و بهره‌وری تولید اجرا کند تا به این چالش‌ها رسیدگی شود. علاوه بر این، بهبود کارایی در بندر مستلزم دیجیتالی شدن عملیات، اجرای برنامه‌ریزی مبتنی بر داده و سرمایه‌گذاری‌های استراتژیک در زیرساخت‌ها خواهد بود.

An Extension Model Compatible with Drought Management in Iran

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ABSTRACT

The main purpose of this research was to design an extension model compatible with drought management in Iran. The research utilized a mixed research approach, combining both qualitative and quantitative methods. In the qualitative section, data was collected through semi-structured interviews, observation, and review of relevant sources. The participants in this section were 15 of extension experts with significant experience in drought management, selected through purposeful and snowball sampling methods. The data was analyzed using the systematic grounded theory approach with MAXQDA10 software, following Strauss and Corbin's (1998) approach. In the quantitative section, the statistical population included experts, trainers, and professors whose field or organizational post was related to water resources, irrigation and drainage, agricultural extension and development, and drought, working full-time in the Ministry of Agricultural Jihad (N= 6018). The sample size was determined using Cochran's formula, with a total of 372 participants. Structural Equation Modeling (SEM) and PLS software were used for data analysis. The results showed that the main components of the model were the detailed requirements of drought management (coefficient of coefficient 0.013), extensional methods of drought management (0.033), contextual conditions (0.1011), supporting conditions (0.166), conditions and causes (0.102), and consequences of drought management (0.065). Finally, an extension model compatible with drought management in Iran was presented.

Keywords: Contextual conditions, Drought adaptation, Supporting conditions.

INTRODUCTION

Drought is an extreme climatic phenomenon that occurs throughout the world with different intensities, especially in arid and semi-arid regions, and leaves harmful effects on the surface and groundwater resources, agriculture, economy, and generally all aspects of life. Given Iran's location in the arid and semi-arid belt of the world, it is crucial to study drought as a widespread natural disaster with long-term effects in different sectors (Mousavi & Niknami (2021). It can be said that drought is a climatic reality in Iran, considering that 27 droughts have occurred

in this country over the last 40 years (Zarafshani *et al.*, 2016). Drought and its undesirable consequences for natural resources, agricultural production, and economic and social development are some of the fundamental challenges of Iran and drought-prone regions. Considering the frequency and significant extent of its occurrence, it is essential to implement directional mechanisms to counteract it (Savari and Skandari Damaneh, 2025; Solh and Van Ginkel, 2014). According to a UN report, 18 countries in the world will face water shortages in the near future, and it is predicted that more than two-thirds of the world's population will be in severe water

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shortage conditions by 2025 (Pozzi *et al.*, 2013; Shabanali Fami *et al.*, 2020). These disasters are partially caused by climate change (Rahman and Alam, 2016), and developing countries are more strongly affected by their risks than other regions due to deficient knowledge and adaptation to this phenomenon (Xenarios *et al.*, 2016). The main climatic problem in dry areas is not the drought itself but the attitude toward it as an ordinary natural phenomenon and the lack of regulation of various water programs and uses based on that attitude (Khorambakht *et al.*, 2013). Due to its biological nature and strong dependence on nature, agriculture is the largest consumer of water resources in most countries. In Iran, approximately 88% of water resources are used in agriculture (Pouralimoghaddam *et al.*, 2022). Rural communities always face many effects of drought, including economic and social problems, which should not be underestimated, and a comprehensive approach is required to mitigate these impacts and achieve successful adaption (Kiem and Austin, 2013).

Various factors contribute to the occurrence of drought and it is beyond human capabilities to make changes or interventions in these factors. Nevertheless, measures can be taken in different dimensions to cope with and reduce the negative consequences of drought. Water-intensive agriculture has suffered significant damage, resulting in the losses and degradation of rangelands and pastures and the decline in livestock numbers and productivity (FAO, 2017). Economically, drought imposes an average annual loss of 6-8 billion USD globally, with adverse effects on farmers' revenue (production quantity and quality) being the most significant risk (Mardy *et al.*, 2018). Consequently, it can be inferred that drought substantially threatens agriculture-based communities (Campbell *et al.*, 2011; Fanni *et al.*, 2016), impacting the productive, economic, social, and environmental sectors (Naderi and Karami Dehkordi, 2019). Various studies have indicated that drought

has numerous social effects, including reduced social welfare, physical and mental health decline, increased social isolation, heightened conflicts, decreased trust and cohesion, lowered social capital, increased suspicion toward governmental institutions, longer working hours, decreased leisure time, increased divorce rates, and destabilized family systems, posing a fundamental challenge to farmers' livelihood stability (Keshavarz and Karami, 2010; Keshavarz *et al.*, 2013). Therefore, communities exposed to drought are vulnerable to a lower standard of living (De Silva and Kawasaki, 2018). In this regard, improving farmers' capacity in areas like adaptation and resilience in climatic conditions is necessary to sustain livelihoods (Alam *et al.*, 2016), along with strategies for adapting to water scarcity and drought conditions (Yazdanpanah *et al.*, 2015). In this context, the overall objective of this research was to design a model of extension compatible with drought management in Iran.

Climate and environmental changes significantly impact the livelihoods of communities, especially in rural areas, and affect agricultural activities in different ways (Shakouri and Merseli, 2018). Therefore, farmers need to adopt behaviors compatible with the impacts of climate change to protect their livelihoods (Savari and Eskandari Damaneh, 2019) and minimize the adverse effects of these changes (Nilsen *et al.*, 2012). Adaptation strategies mainly consist of medium- and long-term measures that farmers employ to improve the resilience of their farming units to drought-induced stresses (Ghambarali *et al.*, 2012). Adaptation strategies are defined in risk management and crisis management (Kheyri *et al.*, 2021; Tavakoli *et al.*, 2015). In general, they encompass individual, economic, social, environmental, and institutional dimensions, which directly and indirectly affect agricultural production in both predictable and unpredictable ways (Smit and Pilifosova, 2003; Deressa, 2010; Ommani, 2011; FAO, 2012; Gomez and

Blanco, 2012; Feola *et al.*, 2015). Therefore, in agricultural and rural areas, it is impossible to rely solely on agriculture to maintain production or improve people's lives. Instead, a wide range of drought adaptation strategies must be chosen (Thieme, 2006).

In order to face the effects of drought, farmers need to be empowered in various economic, social and technical dimensions. In this regard, it seems that educational and extensional activities can be implemented to improve drought management by farmers. Agricultural extension services not only provide information on various aspects of items mentioned, but help secure agricultural related services from banks, organizations and companies. The most important functions of agriculture extension services, however, are transfer of technologies and agricultural education of farmers to equip them with sufficient and suitable alternatives and solutions and place them in a decision making (Al-Zahrani *et al.*, 2016). Optimal management, extension, and appropriate use of water in agriculture is essential. Agricultural education experts should implement extensional programs to combat water scarcity at the national and provincial levels. These programs should cover producers of agricultural and horticultural products, as well as the implementing agents. By increasing knowledge and skills among producers and implementing agents, we can increase the efficiency of water resources and improve the quantity and quality of production in farms and orchards (Rahimian, 2015). One of the main challenges faced by water conservation experts is the lack of improvement in water management, particularly in irrigation, as well as the absence of proper organizations for farmers to promote their use of water. This is due to the farmers' lack of awareness about the water crisis and their disregard for it, as well as the insufficient knowledge of extension experts in providing effective plans. The low efficiency of irrigation in agricultural lands is also a result of the lack of timely and

effective extension, leading to the loss of water. Furthermore, the absence of integrated water resource management plans in the watershed, and the lack of appropriate farming patterns in accordance with the sustainable capacity of water resources in the region, are also attributed to the lack of extension experts' knowledge. Additionally, the lack of awareness among beneficiaries about modern methods and the absence of specialized and knowledgeable experts in this field are also major issues. Therefore, an extensional model for irrigation management and better coping with the drought in Iran leads to improved irrigation management, increased irrigation efficiency, and improved agricultural development, which is of great importance. Table 1 provides a summary of studies regarding extension and drought management.

MATERIALS AND METHODS

This study used a cross-sectional methodology and a survey to gather descriptive data for a practical goal. Employing a mixed approach, incorporating both quantitative and qualitative methods (Johnson and Onwuegbuzie, 2004). In the initial stage of this research, data collection methods included semi-structured interviews, observation, and review of relevant sources. The systematic grounded theory with MAXQUDA10 software was used for data analysis and using Strauss and Corbin (1998) approach. Coding includes three stages: open coding, axial coding and selective coding (Lee, 2001; Creswell and Creswell, 2017). The qualitative section of the study included a sample of 15 senior experts and academic members with practical and scientific experience in drought (Table 2). They were selected using purposeful and snowball sampling methods.

For the second stage, confirmatory factor analysis, structural equation modeling, and Smart-PLS software were employed. For

**Table 1.** A summary of influential variables in a model of extension compatible with drought crisis management.

Research Title	Author	Method	Findings
Investigating the social consequences of drought on rural areas (Case study: Shosef District, Nehbandan City)	Fal Suleiman <i>et al.</i> (2013)	Survey	In the environmental dimension, drought causes the drying up of surface water, the destruction of vegetation, and an increase in dust. In the economic aspect, the income level has decreased, the unemployment rate has increased, and agricultural and livestock production has decreased.
Extension pattern compatible with drought management in Khorasan Razavi Province, Iran	Mousavi <i>et al.</i> (2021)	Qualitative	Extension compatible with drought management requires cooperation and coordination between different institutions and organizations, and educational and extensional programs can significantly improve drought management.
Extension pattern compatible with drought management in Alborz Province, Iran	Firouzjani (2018)	Qualitative	Planning, development, and implementation of water resources and drought management plans for each region should be based on the conditions and resources available in that region. It is also essential to educate and promote concepts related to drought management.
Development of extension pattern for drought management in Iran	Rahimi <i>et al.</i> (2019)	Qualitative	Drought management requires the development of suitable extension models. Improving the awareness and capability of the society and water users in the field of drought management is one of the main success factors in the implementation of extensional models.
extension management using new media	Azari <i>et al.</i> (2017)	Qualitative	Teaching and extension the concepts related to drought management helps improve the awareness of society and farmers, improving drought management in Iran.
The perception of soil erosion and its social and economic factors in different regions of Sri Lanka.	Udayakumara <i>et al.</i> (2010)	Survey	agricultural workforce, household size, literacy rate, property security, conservation costs, promotional education, membership in local organizations, professional skills, financial capital, distance to land, and farm income are all important factors in understanding soil erosion in the studied region.
Drought management planning policy: from Europe to Spain	Hervás-Gámez & Delgado-Ramos (2019)	Qualitative	A key milestone in terms of European drought-risk management was set by the 2007 EC Communication “Addressing the Challenge of Water Scarcity and Droughts in the European Union”. This presented an initial set of seven policy instruments for tackling water scarcity and drought issues at European, national, and regional levels. These included options in relation to ‘putting the right price tag on water’, ‘allocating water more efficiently’, and ‘fostering water efficient technologies and practices’. The Communication also recommended the development of DMPs.
The Impacts of Drought and the Adaptive Strategies of Small-Scale Farmers in uMsinga, KwaZuluNatal, South Africa	Lottering <i>et al.</i> (2021)	Survey	Farmers adopted various adaptive strategies to adapt to drought such as the use of early-maturing crops, mixed cropping systems and drought-tolerant crops. With regard to mitigation, a majority of farmers did not prepare for drought, and those who did utilized indigenous methods of conserving water such as rainwater harvesting, the use of wells, and migrating for alternative employment.
Assessing agricultural drought management strategies in the Northern Murray–Darling Basin	Aitkenhead <i>et al.</i> (2021)	Qualitative	Government Assistance is the most used ADMS for Paroo Shire, the Maranoa Region and Murweh Shire, Whereas the MDB Plan is mainly used in the Goondiwindi Region.

Table 2. Frequency distribution of demographic and professional characteristics of the studied people.

Characteristic	Strata	Abundance
Age Average= 48.36	< 30	1
	40-31	3
	50-41	6
	60-51	5
Gender	Man	12
	Female	3
Educational level	Master's degree	4
	PhD	11
	Water and irrigation	5
Field of study	Agriculture extension	7
	agricultural development	3

this analysis, the statistical population consisted of 6018. They were experts, trainers, and faculty members whose field or organizational post was related to water resources, irrigation and drainage, drought, agricultural extension and development sciences and were employed full-time in the Ministry of Agricultural Jihad in Iran. The statistical sample was determined using Cochran's formula. The number of samples

was determined to be 372 experts (Table 3). Sampling method was stratified.

Validity and Reliability

Guba, and Lincoln (1985) method was used to check reliability and validity. The indexes used were Dependability and Transferability. Based on this, re-coding was done in two different time periods and two other researchers were used. Based on the results, the dependability index was 74% and the transferability index was 71%. Considering that they were more than 60%, it can be said that the indicators had a favorable condition.

Confirmatory factor analysis was used within the SEM framework to assess the proposed model's validity (Hosseinzare 2017). To examine the reliability of the questionnaire, a pilot study was conducted with non-sampled respondents to make necessary revisions. The reliability or confidence level of the variables was estimated by Cronbach's Alpha coefficient (Table 4).

Table 3. The number of samples in each of the three fields.

Category	Statistical population	Sample size
Experts	4390	271
Trainers and researches	930	57
faculty members	698	43
total	6018	372

Table 4. Cronbach's Alpha coefficient for questionnaire factors.

Row	Variables	Number of items	Cronbach's Alpha coefficients
1	Management before drought	15	0/691
2	Management after drought	11	0/701
3	Management during drought	11	0/630
4	Extension system adapted to drought	13	0/941
5	Supportive policies	9	0/832
6	Consequences of drought	17	0/852
7	Disseminational and educational methods	12	0/754
8	Causal conditions	14	0/775
9	Contextual conditions of drought	12	0/811

**Table 5.** Frequency distribution of demographic and professional characteristics of the studied people.

Characteristic	Strata	Abundance	Percent	Cumulative percentage
Age n= 372 Average= 40.46	20-30	79	3.21	3.21
	40-51	93	25	3.46
	50-61	134	36	3.82
	60-71	66	7.17	100
Gender n= 372	Man	284	3.76	Mode= Man
	Female	88	7.23	
Educational level Mode= Master's degree	Bachelor's degree	60	1.16	1.16
	Master's degree	194	2.52	3.68
	Ph.D.	118	7.31	100
Field of study n= 218	Science	55	8.14	Mode= Agriculture
	Agricultural engineering	165	3.44	
	Humanities	73	6.19	
	Other	33	9.8	
	5-1	6	1.6	
Work experience n= 218 Average= 12.99	6-10	75	20.2	21.8
	11-15	136	36.5	58.3
	16-20	123	33.1	91.4
	21-25	14	3.8	95.2
	26-30	15	4.8	100

RESULTS

Examining the age of the responders showed that the highest frequency (36%) was related to the age group of 41-50 years. Also, 284 (76.3%) of the responders were male (the highest frequency), and 88 (23.7%) were female. In terms of the educational level, the highest frequency was related to the MSc degree with a frequency of 194 (52.2%). Among the study fields, agricultural engineering had the highest frequency of 165 people (44.3%). Regarding experience, the highest frequency was related to 11-15 years with a frequency of 136 (36.5%). (Table 5).

In the structural equation model methodology, it is first necessary to study the validity of the structure in order to determine whether the indicators selected to measure the desired structures have the necessary accuracy i.e., the questions is to check whether the variables were chosen correctly or not? For this purpose, Confirmatory Factor Analysis (CFA) was used. In this method, the factor load of each

indicator with its structure must be higher than 0.4. Factor loadings were calculated by measuring the correlation between indicator and connected construct. This suggests acceptable reliability regarding the measurement model (Table 6).

After ensuring the existence or non-existence of a causal relationship between the research variables and checking the appropriateness of the observed data with the conceptual model, the research hypotheses were also tested using SEM (the PLS approach). Tables 7 and 8 depict the results of running the model, and Tables 11 presents the results of testing the hypotheses.

Table 7 shows the values of R^2 that represent the explained variance. Based on this, supporting conditions with a coefficient of 0.16 has the greatest effect, and consequences of drought with a coefficient of 0.06 has the least effect of drought management. The total variables have explained 0.15 of the variance of the dependent variable.

The values listed in Table 8 shows the T values. For each factor to be significant, the

Table 6. Factor loadings under the modified components of the extension drought management model.

Factors	Manifesting variable	Factor loading
Contextual conditions	The presence of weather and climate information centers	0.731
	Information capacity of agricultural service centers	0.525
	The existence of agricultural and irrigation cooperatives	0.498
	The existence of training centers in the field of drought management	0.494
	Agriculture to financial resources	0.493
	Insensitivity of people and social networks	0.802
	Unauthorized exploitation of water resources	0.462
	The government's insensitivity to the issue	0.460
Causal conditions	Low level of education	0.455
	Weak financial base of farmers	0.447
	Weakness of water infrastructure	0.408
Intervening conditions	Crop insurance coverage	0.675
	Guaranteed purchase of agricultural products	0.671
	Investing in the infrastructure of irrigation networks	0.569
	Water pricing and sale	0.536
	Granting loans and free facilities	0.448
	Effective monitoring of the license of agricultural wells	0.447
	Supporting organizations and cooperative companies in the water sector	0.424
Dissemination variables	Considering and measuring the educational- dissemination needs of farmers	0.612
	Using radio and television agricultural programs (mass media)	0.583
	Holding educational workshops	0.569
	Using dissemination personal messengers	0.558
	Using the Internet and virtual networks	0.516
	Visiting new irrigation systems	0.506
Consequences	Increase in fake jobs	0.905
	Reduction of cultivated area	0.903
	Decrease in income	0.808
	Insecurity	0.795
	Increase in input prices	0.790
	Reducing the price of agricultural land	0.730
	Decrease in production	0.713
	Decreased quality of life	0.707
	Decrease in welfare	0.598
	Reduction of local communication among people	0.591
Requirements for extension drought management	Increase in unemployment rate and immigration	0.582
	Assessing the educational- dissemination needs of farmers	0.842
	Providing extension specialist human resources	0.652
	Reforming the organizational structures of extension	0.591
	Reforming the financial structures of extension organizations	0.462
	Increasing the professional qualifications of extension agents	0.411

Table 7. The measurement of the main model and the results of the hypotheses in the standard mode. Please check the numbers in this Table with the above text.

Variable	R ²	Path coefficient
Drought management (the dependent variable)	0.155	-
Detailed requirements of drought management	0.00	0.013
Extensional methods of drought management	0.00	0.033
Contextual conditions	0.00	0.1011
Supporting conditions	0.00	0.166
Conditions and causes	0.00	0.102
Consequences of drought	0.00	0.065



Table 8. The measurement of the original model and the results of the hypotheses in the standard mode.

Variable	T values
Drought crisis management (The dependent variable)	-
Detailed requirements of drought crisis management	4.874
Extensional methods of drought crisis management	2.207
Contextual conditions	2.094
Supporting conditions	4.661
Conditions and causes	4.812
Consequences of drought	2.029

value of T should be significant at the error level of 0.05, that is, if its value is outside the range (1.96 and -1.96), the effect of this component is significant. Based on the listed results, all paths are significant (Table 8).

Table 9 shows the factor loading values to answer the question of whether the questions to measure the variables are chosen correctly or not. To have the appropriate accuracy, the factor loading should be higher than 0.4. Based on the results listed in Table 9, most factor loadings are greater than 0.4.

Table 10 shows the T values of the indicators used for the structures. For each indicator to be significant, the value of T is significant at the error level of 0.05, that is, its value is outside the range (1.96 and -1.96), and thus, this indicator correctly measures the desired component. Based on the results shown in Table 10, all the indicators used are significant.

The effect of the independent variable on the dependent variables is depicted in Table 11. The significance coefficient (t-statistic) of the output model of SEM was used to test the research hypotheses. If the t-statistic was more than 1.96 or less than -1.96 (with a 5% error level), the hypotheses would be confirmed, and the significant effect of the variable would be achieved. It can also be seen in the measurement model that the factor coefficient for each variable is higher than the value of 0.50%. Table 11 presents a summary of the results of hypothesis testing.

Table 12 presents Composite Reliability (CR), coefficient of determination (R^2), Cronbach's Alpha, communality values, and communal reliability (AVE) for the main components of the research.

To check the model's fit in PLS, we used the global quality criterion proposed by Amato *et al.* (2004).

$$GOF = \sqrt{\text{communality} \times R^2}$$

The index of Fit Of the General model (Goodness-of-Fit Index for PLS Structural Equation Modeling (GOF) was 0.568%, so, it can be accepted that the general model of the research has a good fit. The high fit of the model shows that this model is well explained (Table 13).

DISCUSSION

Agricultural sector requires specific adaptation to cope with water scarcity and drought (Yazdanpanah *et al.*, 2015; Delphian, 2016). To address this challenge, an extension model should be designed based on local needs, culture, local language, and appropriate communication methods in each region to mitigate the negative impacts of these changes (Ifeanyiobi *et al.*, 2012; Engle, 2011).

Due to the level of knowledge and low adaptation to the phenomenon of drought, developing countries are more affected by the risks associated with it than other regions (Xenarios *et al.*, 2016). There are many reasons for this, including the lack of access to water and extension specialists, lack of practical solutions for drought management and lack of extensional recommendations in drought management. Also, the results of studies indicate an increase in the number of droughts in Iran (Firoozi *et al.*, 2019). In this

Table 9. The measurement of the final model in the standard mode.

Variable	Sign	Factor loading	Correlation coefficient
Drought management (The dependent variable)	Critical	1.00	--
Extensional methods of drought crisis management	M1	0.498	0.560
	M2	0.569	
	M3	0.612	
	M4	0.320	
	M5	0.506	
	M6	0.516	
	M7	0.622	
	M8	0.715	
	M9	0.573	
	M10	0.396	
	M11	0.583	
	M12	0.658	
Contextual conditions	AR1	0.407	0.170
	AR2	0.525	
	AR3	0.494	
	AR4	0.731	
	AR5	0.498	
	AR6	0.557	
	AR7	0.435	
	AR8	0.677	
	AR9	0.671	
	AR10	0.438	
	AR11	0.508	
	AR12	0.493	
Supporting conditions	MD1	0.571	0.167
	MD2	0.675	
	MD3	0.424	
	MD4	0.447	
	MD5	0.669	
	MD6	0.538	
	MD7	0.448	
	MD8	0.420	
	MD9	0.497	
Causal conditions	F1	0.460	0.440
	F2	0.802	
	F3	0.447	
	F4	0.455	
	F5	0.462	
	F6	0.408	
	F7	0.505	
	F8	0.446	
	F9	0.477	
	F10	0.471	
	F11	0.461	
	F12	0.595	
	F13	0.584	
	F14	0.776	

Table 9 continued...



Continued of Table 9

Variable	Sign	Factor loading	Correlation coefficient
Drought management (The dependent variable)	Critical	1.00	--
Consequences	CH1	0.808	0.001
	CH2	0.906	
	CH3	0.591	
	CH4	0.571	
	CH5	0.795	
	CH6	0.707	
	CH7	0.490	
	CH8	0.682	
	CH9	0.733	
	CH10	0.598	
	CH11	0.713	
	CH12	0.903	
	CH13	0.510	
	CH14	0.833	
	CH15	0.742	
	CH16	0.601	

Table 10. The measurement of the final model and the results of the hypotheses in the significant state.

Variable	Sign	T value	Correlation coefficient
Drought management (Dependent variable)	Critical	1.00	--
Extensional methods of drought management	M1	3.463	2.428
	M2	3.552	
	M3	3.877	
	M4	3.285	
	M5	2.485	
	M6	2.270	
	M7	2.706	
	M8	2.592	
	M9	2.272	
	M10	2.928	
	M11	3.862	
	M12	0.588	
Contextual conditions	AR1	2.681	2.248
	AR2	2.329	
	AR3	4.399	
	AR4	2.270	
	AR5	2.168	
	AR6	3.043	
	AR7	3.359	
	AR8	2.866	
	AR9	2.678	
	AR10	2.176	
	AR11	3.327	
	AR12	2.027	
Supporting conditions	MD1	3.523	2.931
	MD2	2.329	
	MD3	3.983	
	MD4	3.399	
	MD5	2.844	
	MD6	2.206	
	MD7	3.305	
	MD8	4.597	
	MD9	2.346	

Table 10 continued...

Continued of Table 10

Variable	Sign	T value	Correlation coefficient
Drought management (Dependent variable)	Critical	1.00	--
Causal conditions	F1	3.038	3.719
	F2	3.141	
	F3	2.211	
	F4	3.997	
	F5	2.010	
	F6	3.933	
	F7	2.160	
	F8	4.757	
	F9	2.960	
	F10	3.951	
	F11	2.110	
	F12	2.160	
	F13	1.035	
	F14	2.138	
Consequences	CH1	4.044	2.008
	CH2	3.634	
	CH3	3.107	
	CH4	2.760	
	CH5	3.137	
	CH6	2.682	
	CH7	2.161	
	CH8	2.935	
	CH9	2.751	
	CH10	2.557	
	CH11	3.358	
	CH12	3.747	
	CH13	2.213	
	CH14	4.112	
	CH15	3.512	
	CH16	2.811	

Table 11. A summary of hypotheses testing results.

Hypotheses	Path coefficient	Significance coefficient	Result
Main hypothesis: Drought-adapted extension requirements affect agricultural drought management.	0.113	4.874	Confirmed
The first hypothesis: Extension methods affect the management of agricultural drought.	0.550	2.428	Confirmed
The second hypothesis: Contextual conditions affect the management of agricultural drought.	0.170	2.248	Confirmed
The third hypothesis: Causal conditions affect the management of agricultural drought.	0.440	3.719	Confirmed
The fourth hypothesis: The consequences of drought affect the management of agricultural drought.	0.001	2.008	Confirmed
The fifth hypothesis: Management policies affect the management of agricultural drought.	0.167	2.931	Confirmed

case, there is a need for adaptation and drought management by farmers. The decision-making process around adaptation is complex (Bunham and Ma, 2016; Harmer and Rahman, 2014) and includes a wide and

interconnected range of socio-political, social and environmental factors. Weather, its intensity and the level of confidence of farmers about receiving yield due to adaptation are closely related (Tucker *et al.*,

**Table 12.** The general model's quality criteria.

Research components	Composite Reliability (CR)	Coefficient of determination (R^2)	Cronbach's Alpha	Communal values (Communality)	Shared reliability (AVE)
Methods of extension drought management	0.76	0.58	0.84	0.49	0.43
Support policies	0.80	0.54	0.82	0.41	0.46
Contextual conditions	0.59	0.74	0.75	0.45	0.49
Causal conditions	0.71	0.83	0.88	0.31	0.54
Consequences	0.64	0.55	0.90	0.42	0.52
Background conditions	0.75	0.71	0.79	0.27	0.48
Drought management	1	--	1	1	1

Table 13. The final model's fit.

Index name	R^2	Communality
Methods of extension drought management	0.58	0.49
Consequences	0.63	0.52
Drought support policies	0.54	0.41
Background conditions	0.74	0.45
Causal conditions	0.83	0.31

2011; Anik *et al.*, 2012). People's participation in the adaptation of drought management is one of the necessary things in the success of programs in this field (Wani *et al.*, 2013). Publication of magazines, brochures, books, guidelines and extension books about new methods of irrigation with traditional and old methods and comparing them in a demonstration for a group of farmers. Holding extension meetings in the presence of water and extension experts, extension exhibitions (new irrigation tools and methods) and extension films and videos about new irrigation methods, farmers visits to the office of the Agricultural Extension Service, visit of agricultural extension workers to the farmers, interaction with consulting service companies and extension organizations (Al-Zahrani *et al.*, 2016). These activities are aimed at addressing informational and educational needs related to drought management (Harvey *et al.*, 2014; Singh *et al.*, 2017; Tripathi and Mishra, 2017). Such as to create these conditions need to Existence of extension specialists and access

to them, Expansion of social networks and local networks to disseminate information.

Also, formation of agricultural cooperatives and water bodies in order to create irrigation groups, providing facilities in the field of extension services, supportive policies in low water consumption (Cheng and Tao, 2010; Eriksen and Silva, 2009; Keshavarz and Karami, 2013). It can help a lot to establish an extension model that is compatible with the management of drought. In the end, it can be said that the establishment of this extension model can include the followings:

- Increasing the resilience of farmers in dealing with drought,
- Access to meteorological and drought information,
- Access to drought management information,
- Increasing participation of farmers in drought management.

Figure 1 shows extension model compatible with drought management in Iran.

Based on the results, it is recommended to involve knowledgeable agricultural extension experts in providing the necessary training and technical advice to farmers. It will be helpful to establish constructive communication between farmers and extension agents through social networks to

address the existing water-related issues and convey them to the relevant authorities for appropriate solutions. Also, the importance of water and the impact of water scarcity challenges on economic, social, and security sectors should be recognized. Additionally, it is necessary to prioritize this issue as a

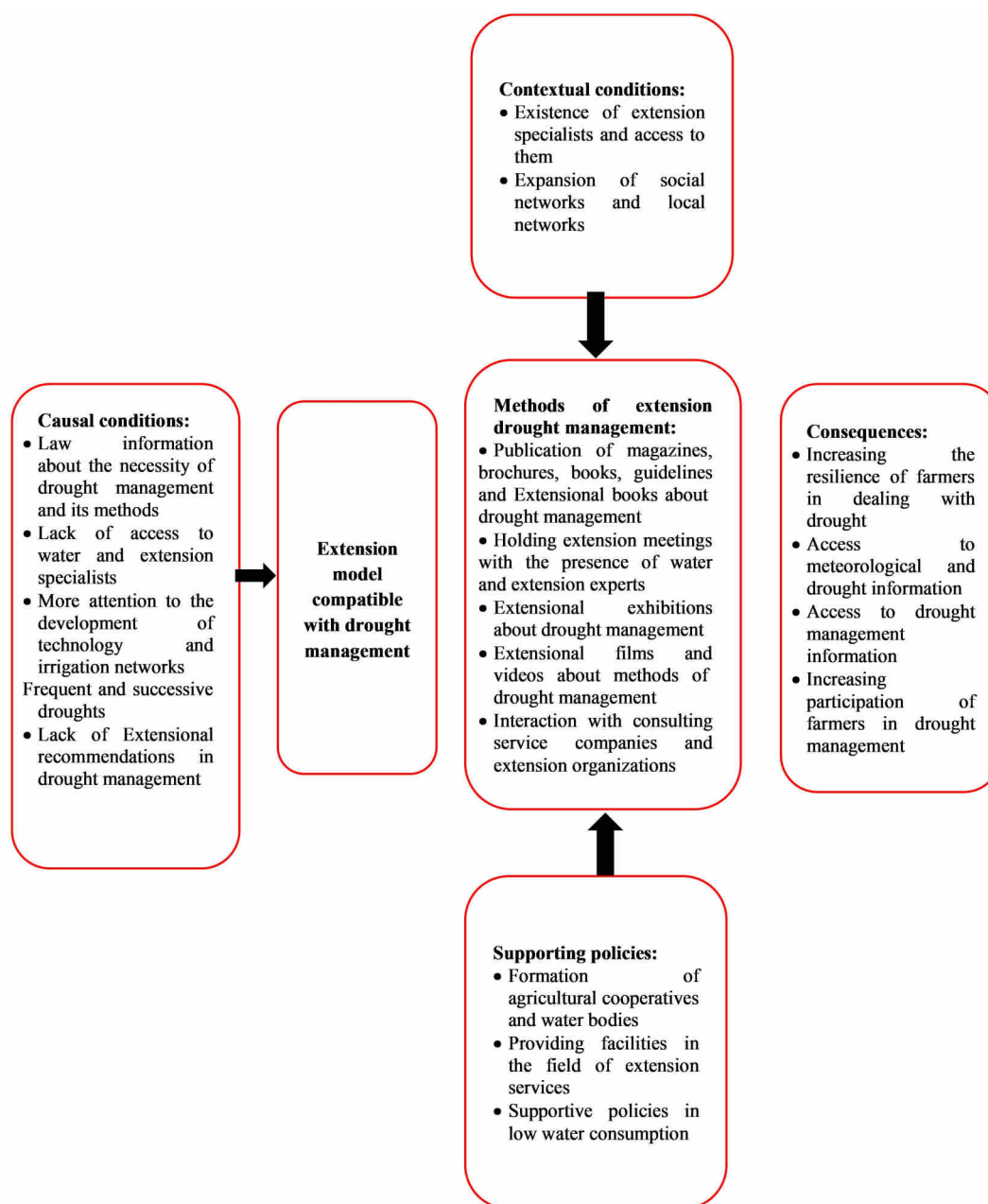


Figure 1. The final extension model compatible with drought crisis management.



fundamental strategy in the annual budget and Iran's Seventh Development Plan. Last but not least, it is recommended that the government supports farmers through facilities such as low-interest loans and subsidies to assist in implementing adaptation strategies and drought management.

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یک مدل ترویجی سازگار با مدیریت خشکسالی در ایران

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چکیده

هدف اصلی این تحقیق، طراحی یک مدل ترویجی سازگار با مدیریت خشکسالی در ایران بود. این تحقیق از رویکرد تحقیق ترکیبی، شامل تلفیق روش‌های کیفی و کمی، استفاده کرد. در بخش کیفی، داده‌ها از طریق مصاحبه‌های نیمه‌ساختاریافته، مشاهده و بررسی منابع مرتبط جمع‌آوری شد. شرکت‌کنندگان در این بخش، ۱۵ نفر از کارشناسان ترویج با تجربه قابل توجه در مدیریت خشکسالی بودند که از طریق روش‌های نمونه‌گیری هدفمند و گلوله برفی انتخاب شدند. داده‌ها با استفاده از رویکرد نظریه زمینه‌ای سیستماتیک با نرم‌افزار MAXQDA10، مطابق با رویکرد (Strauss and Corbin 1998)، تجزیه و تحلیل شدند. در بخش کمی، جامعه آماری شامل کارشناسان، مربیان و اساتیدی بود که رشته یا پست سازمانی آنها مرتبط با منابع آب، آبیاری و زهکشی، ترویج و توسعه کشاورزی و خشکسالی بود و به صورت تمام وقت در وزارت جهاد کشاورزی مشغول به کار بودند. (N= 6018) حجم نمونه با استفاده از فرمول کوکران، در مجموع ۳۷۲ نفر تعیین شد. برای تجزیه و تحلیل داده‌ها از مدل‌سازی معادلات ساختاری (SEM) و نرم‌افزار PLS استفاده شد. نتایج نشان داد که اجزای اصلی مدل شامل الزامات تفصیلی مدیریت خشکسالی (ضریب ۰.۰۱۳)، روش‌های ترویجی مدیریت خشکسالی (۰.۰۳۳)، شرایط زمینه‌ای (۰.۱۰۱۱)، شرایط پشتیبان (۰.۱۶۶)، شرایط و علل (۰.۱۰۲) و پیامدهای مدیریت خشکسالی (۰.۰۶۵) بودند. در نهایت، یک مدل ترویجی سازگار با مدیریت خشکسالی در ایران ارائه شد.

Assessment of Habitat Suitability of *Carissa carandas* L. in India Using Bio-Climatic Variables, GHG Scenarios, Land Use, and Land Cover Predictors

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ABSTRACT

This study was conducted to assess the habitat suitability of *Carissa carandas* in India, which is crucial for its sustainable integration into agriculture under changing climatic conditions. We utilized Maximum Entropy (MaxEnt) modelling to evaluate the species' distribution across current and future scenarios (2050 and 2070) under four Representative Concentration Pathways (RCPs: 2.6, 4.5, 6.0, and 8.5). Results indicated that temperature-related variables, particularly the Minimum Temperature of the Coldest Month (MiTCM, contributing 46.8% in 2070 RCP 2.6) and Isothermality (contributing up to 35.2% in 2070 RCP 8.5), are the dominant climatic drivers. Land Use and Land Cover (LULC) factors such as urbanization (49.8%), total cultivated land (28.1%), and grassland (9.0%) significantly influence habitat suitability. Under the current conditions, optimal habitat spans 4,588 km², decreasing by 38.95% under LULC scenarios. Projected habitat changes indicate 2.04% gain under 2070, but 11.06% decline under 2050 RCP 2.6. Southern and western regions, including Karnataka, Tamil Nadu, Maharashtra, and Gujarat exhibit high suitability. Habitat fragmentation is projected in northern and western India due to climate change and land use modifications. These findings underscore the need for proactive conservation planning and climate-adaptive agricultural strategies to optimize the cultivation of *C. carandas*. Policymakers and stakeholders should focus on preserving suitable regions while mitigating urbanization-induced habitat loss.

Keywords Climate Change, Maximum Entropy modelling, Underutilized crop, Urbanization.

INTRODUCTION

Underutilized plant species are domesticated or wild plant species that have economic, nutritional, medicinal, or ecological value, but are not widely cultivated, traded, or researched (Ghosh *et al.*, 2023). These species are often locally important but remain underdeveloped in terms of agronomic improvements, policy support, and market integration (Padulosi *et al.*, 2013). Such species have some specific traits like limited commercialization, local or indigenous importance, high resilience and adaptability, nutritional and medicinal

benefits and contributing to agrobiodiversity. *Carissa carandas*, *Moringa oleifera*, *Ensete ventricosum* and *Chenopodium quinoa* are the few examples (Knez *et al.*, 2023). Further, such species could lead to innovative crop cultivation. Despite climate change, farmers can increase their income by producing these agricultural commodities sustainably (Akinola *et al.* 2020; Meena *et al.*, 2022; Mugiyo *et al.*, 2022).

Despite their alleged ability to adapt to sub-optimal environments and changes in climate, there is a lack of scholarly studies focused on the consequences of climate

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change on their spatial and temporal distribution. The limited extent of policy and decision-making procedures presents a barrier to the integration of smallholder farmers into adaptation strategies (Olayinka Atoyebi *et al.*, 2017). Given the current circumstances, a cohort of esteemed researchers is advocating for the assimilation of overlooked crop varieties into agricultural and dietary frameworks in response to the ramifications of climate change (Nyathi *et al.*, 2018; Chibarabada *et al.*, 2020). Smallholder farmers integration into adaptation strategies is hindered by policy and decision-making gaps (Olayinka Atoyebi *et al.*, 2017). Due to climate change, esteemed researchers are advocating for the inclusion of overlooked crop varieties in agricultural and dietary frameworks (Chibarabada *et al.*, 2020).

Spatial modelling and analysis can reveal underutilized species distribution patterns (Mathur *et al.*, 2023). Species Distribution Modelling (SDMs) combine species occurrence data with topographical and climatic factors to create cartographic representations of past, present, and future species distributions (Akpoti *et al.*, 2020). The correlation between environmental variables and species occurrence records helps researchers understand ecological or evolutionary mechanisms and predict

macroscopic agro-ecology suitability (Mathur and Mathur, 2023).

Carissa carandas, (Hindi= Karondais) is an Indian Apocynaceae species and commonly called "Christ's Thorn." It's an evergreen shrub (Figure 1-A) that blooms elegant white flowers from December to April (Figure 1-B). Within Indian states like Gujarat, Karnataka, and Uttar Pradesh, gardens, orchards, and small-scale plantations grow this plant for bio-fencing, live-fencing, and aesthetics (Meena *et al.*, 2022). This species can produce 5–8 kilograms of fruit in arid and semi-arid regions with little care (Figure 1-C). According to Krishna *et al.* (2017), the botanical specimen can yield 10–15 kilograms per tree when grown under proper agrarian conditions. It is used as vegetable and immature fruit is usually used in pickling and chutney. However, fully matured fruit is eaten raw or made into confectioneries and natural food colorants (Singh *et al.*, 1998). Iron-rich Karonda fruit treats anemia and scurvy (Kanupriya *et al.*, 2019). Pectin makes pickles and jellies ideal for mature fruit (Figure 1-D). These fruits can also be used to make popular preserves, drinks, and condiments.

The habitat modelling of *Carissa carandas* (karonda) is significant for various



Figure 1. *Carissa carandas* an underutilized evergreen shrub (A), utilize in landscaping for their flower (B), beautiful shiny fruits (C), and cherries are useful for preparation of pickles and vegetable (D). Presence locations of the species, use for habitat suitability modelling (E).

ecological, agricultural, and conservation-related reasons, including the followings:

(a) **Ecological importance:** *C. carandas* is a resilient, drought-tolerant shrub that flourishes in several climatic environments, and comprehending its habitat is essential for evaluating its contribution to biodiversity, particularly its relationships with pollinators and other plant species.

(b) **Agricultural and commercial significance:** The plant yields consumable fruits utilized in traditional medicine, food processing (jams, pickles), and nutraceutical sectors. Habitat modelling facilitates the identification of optimal places for its production, hence enhancing yield and profitability for farmers. It facilitates the advancement of sustainable agroforestry methods through the incorporation of *C. carandas* into agricultural systems.

(c) Conservation and sustainable utilization of its native populations are imperilled by habitat degradation, overharvesting, and alterations in land use. Modelling assists in conservation planning and in pinpointing regions suitable for protection.

(d) **Climate resilience and adaptation:** By examining its habitat preferences, researchers can assess its resilience to drought and fluctuating temperatures, rendering it a valuable species for climate adaptation strategies. Such studies can also be applied to reforestation initiatives aimed at mitigating soil erosion and desertification.

In summary, habitat modelling of *C. carandas* is essential for enhancing its agricultural utilization, preserving its natural populations, and incorporating it into climate-resilient ecosystems.

Additionally, this species' area-yield relationship, market authenticity, cost trends, and ecological studies have knowledge gaps ((Banik *et al.*, 2012; Mahajan *et al.*, 2022; Maanik *et al.*, 2023; Mishra *et al.*, 2024; Sarkar, 2024). These gaps make it difficult to understand how its distribution patterns relate to climate and land use.

Given the scientific knowledge gaps, this study investigated the habitat suitability for

this species. We examined bio-climatic variables over different timescales, GHS scenarios, and land-use predictors. The Maxent model (Mathur *et al.*, 2023) was used to assess how climate change affects the spatial arrangement of arable regions suitable for *Carissa carandas* fruit cultivation in India. Our specific goals were to: (a) Identify the species' habitats, as delineated by current and projected climatic conditions over 2050 and 2070, within four Greenhouse Gas (GHG) scenarios, (b) Quantify the impact of diverse land utilization patterns on the species' habitat appropriateness, and (c) Identify the manifold climatic and land use factors that exert influence on both the fundamental and realized niches of this species.

MATERIAL AND METHODS

Distributional Record

Distributional records for this species were obtained from data repositories such as the Global Biodiversity Information Facility (GBIF, 2023), the Indian Biodiversity Portal (<https://indiabiodiversity.org/species/show/32472>), and published literature ((Singh *et al.*, 2010; CIAH 2014 and 2020; Meghwal *et al.*, 2014; Kanupriya *et al.*, 2019; Meena *et al.*, 2020) and our field work during 2005 to 2014 at various districts of arid and semi-arid areas of Rajasthan, India (Mathur and Mathur, 2023). To reduce spatial autocorrelation and eliminate duplicate records, we followed Sofaer *et al.* (2019) and used the spatial thinning window of "Wallace Software," a user-friendly graphical interface built on the R programming language (Kass *et al.*, 2018), with a thinning distance of 10 kilometres.

Bio-Climatic (Bio) and Non-Bioclimatic Variables (Non-Bio)

Machine learning helps predict species distribution based on their current



range (Praveen *et al.*, 2022). In this study, WorldClim version 2.0 observational bioclimatic data was used to predict species distributions (Fick and Hijmans, 2017). The study used 19 bioclimatic variables from Hijmans *et al.* (2005), extracted at a 30-second spatial resolution ($\sim 1 \text{ km}^2$). DIVA-GIS version 7.5 converted these variables to ASCII or ESRI ASCII (Coban *et al.*, 2020). The 2050- and 2070-time frames, which represent the mean values from 2041 to 2060 and 2061 to 2080, respectively, were used to collect data for the current and two future climatic scenarios, according to Zhang *et al.* (2021). The future datasets are associated with four Representative Concentration Pathways (RCPs): 2.6 W/m^2 (lowest emission), 4.5, 6.0, and 8.5 W/m^2 (highest emission, Chaturvedi *et al.*, 2012). Table 1 summarizes bio-climatic parameters, including units and mathematical expressions.

Land Use and Land Cover (LULC)

Various Land Use and Land Cover

(LULC) predictors, including rain-fed and irrigated land, total cultivated land, forest, Grass/Scrub/Woodland (GRS), barren/very sparsely vegetated land (NVG), urban land, land used for housing and infrastructure, and wet lands, have been employed to forecast the suitability of habitats for this particular species. These variables were downloaded from web at a resolution of $\sim 1 \text{ km}^2$ utilized as recommended by Fischer *et al.* (2008)

Issue of Multicollinearity

The Pearson correlation coefficient (r) was used to examine cross-correlation, and multicollinearity was examined to assess overfitting. We also followed Pradhan *et al.* (2016) to eliminate variables with cross correlation coefficients of 0.85 or higher. This was accomplished through the utilization of the Niche Tool Box, as described by Osorio-Olvera *et al.* (2020a, b). A singular variable, which exhibits substantial cross-correlation and holds biological relevance to the species, was selected from a set of two alternative variables for the purpose of simplifying model

Table 1. Description of predictive bio-climatic variables use in this study.

Code	Environmental variables and their abbreviations	Scaling	Unit
Bio -1	Annual Mean Temperature (AMT)	10	$^{\circ}\text{C}$
Bio -2	Mean Diurnal Range (MeDR)	10	$^{\circ}\text{C}$
Bio -3	Isothermality (Bio 2/Bio 7) ($\times 100$) (Iso)	100	-
Bio -4	Temperature Seasonality (Standard deviation $\times 100$) (TempS)	100	-
Bio -5	Max Temperature of Warmest Month (MaTWaM)	10	$^{\circ}\text{C}$
Bio -6	Min Temperature of Coldest Month (MiTCM)	10	$^{\circ}\text{C}$
Bio -7	Temperature Annual Range (Bio 5- Bio 6) (TAR)	10	$^{\circ}\text{C}$
Bio -8	Mean Temperature of Wettest Quarter (MeTWaQ)	10	$^{\circ}\text{C}$
Bio -9	Mean Temperature of Driest Quarter (MeTDQ)	10	$^{\circ}\text{C}$
Bio -10	Mean Temperature of Warmest Quarter (MeTWaQ)	10	$^{\circ}\text{C}$
Bio -11	Mean Temperature of Coldest Quarter (MeTCQ)	10	$^{\circ}\text{C}$
Bio -12	Annual Precipitation (AnPr)	1	mm
Bio -13	Precipitation of Wettest Month (PrWeM)	1	mm
Bio -14	Precipitation of Driest Month (PrDM)	100	mm
Bio -15	Precipitation Seasonality (Coefficient of Variation) (PrS)	1	Fraction
Bio -16	Precipitation of Wettest Quarter (PrWeQ)	1	mm
Bio -17	Precipitation of Driest Quarter (PrDQ)	1	mm
Bio -18	Precipitation of Warmest Quarter (PrWaQ)	1	mm
Bio -19	Precipitation of Coldest Quarter (PrCQ)	1	mm

interpretation (Mathur and Mathur 2023).

Projection Transformation

The Bio-Climatic (Bio) and Non-Bio variables were obtained from different sources at different resolutions, so, they were standardized before extracting data and generating predictions using machine learning tools. We used ArcMap and ArcToolbox to follow a methodology for analysis. The Data Management Tools interface's "projection and transformation" section explained the projection (Jijon *et al.*, 2021). To quantify area under each habitat suitability class in Arc Map's "calculate geometry" window, we converted the habitat class raster file projections to WGS 1984 web Mercator (auxiliary sphere-3857).

Species Distribution Modelling

The present study used Maxent 3.4.1 (<http://www.cs.princeton.edu/schapiro/Maxent/>) to simulate and predict *C. carandas* plausible geographic distribution likelihood using the current scenario, two future scenarios (2050- and 2070-time frames), and a non-climatic variable. This tool's discrete execution with each predictor in isolation allowed us to accurately measure their impact on the species' distributional pattern. Background points were randomly generated at 10,000 (Zhang *et al.*, 2021). We set the regularization multiplier to 0.1 to avoid test data overfitting. (Phillips *et al.*, 2006), while the rest were left at their software defaults. To calibrate and validate Maxent model evaluation, threshold-independent Receiver-Operating Characteristic (ROC) analyses were used, and an Area Under the receiver operating Curve (AUC) was used to estimate model predictions (Elith *et al.*, 2006). Based on the AUC value, the model was classified using the conservative guide suggested by Thuiller *et al.* (2005) and Kagnev *et al.* (2023) as: failing (0.5-0.6), poor (0.6-0.7), fair (0.7-0.8), good (0.8-0.9), or

excellent (0.9-1). The model performs well with AUC values near one (Mathur *et al.*, 2023).

Variable Importance values and response curves were used to assess how bioclimatic and non-bioclimatic variables affected this species' distribution (Mathur and Mathur, 2023). We then used ArcGIS to convert the Maxent output ASCII file into raster format and classified (Ali *et al.*, 2023) this species' habitat with help of "Raster Calculation Tool" into areas as optimal (1.0 to 0.80), moderate (0.80 to 0.60), marginal (0.60 to 0.40), low (0.40 and 0.20), and absent (< 0.20). Then, the optimum habitat raster file was converted into Keyhole Markup Language (KML) to accurately identify ideal habitat changes across diverse climatic temporal intervals and LULC compared to the current optimal habitats. Percent changes (gain and loss) in areas of optimum habitat suitability under different climatic and non-climatic variables in comparison to current optimum area were calculated using the following formula provided by (Mathur and Mathur, 2023). This exercise allowed us to quantify optimal habitats based on climatic time frames, RCPs, and LULC.

$$\left(\frac{\text{Future Optimum Area} - \text{Current Optimum Area}}{\text{Current Optimum Area}} \right) \times 100$$

Ellipsoid Niche Hypervolume

Machine learning models offer a variety of significant variables to enhance the precision of species localization. The quantification of hypervolumes linked to the niches of this particular species was carried out by employing the top three predictors across all bioclimatic scenarios and RCPs, in addition to LULC variables. In the present study, we utilized NicheToolBox (Osorio-Olvera *et al.*, 2020a) software program coded in the R programming language, necessitates the invocation of the raster output pertaining to BC variables. Ellipsoidal models were constructed through the calculation of the centroid and covariance matrix of the environmental values of the species. The



research region was comprehensively examined, with all potential settings radiating outward from its geographic epicentre. Through the utilization of this particular methodology, we were able to determine the environmental factors that dictate the fundamental and realized niche of the said species.

RESULTS

Multicollinearity and Model Performance

By conducting an extensive examination of a wide array of sources (as mentioned in material and method) originating from the Indian region, we effectively derived a total of 285 locations where this particular species can be found. Using Wallace Software's spatial thin window feature (Kass *et al.*, 2018), we eliminated all instances of a record within a 10-kilometer radius. Integrating 218 *C. carandas* presence points without spatial autocorrelation completed the ENM development process (Figure 1-E). The final bioclimatic variables and their percentage contributions are shown in Table 2, which uses the "x" symbol to exclude variables from their bioclimatic time frame and RCPs. Based on their strong correlations with bioclimatic factors, Bio-1,2, 9,10,11,12,14,15, and 18 were excluded from future analyses. Figures 2-a (current) and -b (LULC) show model quality results in terms of AUC. Additionally, Figures 2-c

to -f show the 2050 climatic time frame results for each Representative Concentration Pathway (RCP). Figures 3-a to -d show the 2070 results and RCPs. Since all AUC curves exceeded 0.80, model performance was good.

Percent Contribution of Bio-climatic and LULC Variables

Table 2 and Figure 4 show bio-climatic and LULC variable percentage contributions. The Minimum Temperature of Coldest Month (Bio-6 MiTCM) is the primary bio-climatic predictor that significantly affects this species' habitat suitability across various climatic time-frames and RCPs, except for 2070 RCPs 6.0 and 8.5. In these situations, isothermality (Bio-3), the ratio between the annual mean temperature and the mean diurnal range, controls the species the most. This climatic variable is the second most influential factor in all future Representative Concentration Pathways. However, given the bioclimatic conditions, temperature seasonality (Bio-4) is the second most important factor affecting this species' habitat suitability. Temperature Annual Range (Bio-7 TAR) and Mean Temperature of the Wettest Quarter (Bio-8 MeTWeQ) are the least effective bioclimatic factors. In Land Use and Land Cover (LULC) variables, urbanization, cultivated land, and grassland are influential.

Table 2. Maxent output showing percent contribution of the different bioclimatic variables to the model with respect to bioclimatic time-frames and their RCPs.

Bio Variables	Current	2050 RCPs				2070 RCPs			
		2.6	4.5	6.0	8.5	2.6	4.5	6.0	8.5
Bio-3	x	28.3	27.4	25.9	33	25	28.7	34.6	35.2
Bio-4	29.2	3.7	3.3	5.2	1.1	2.6	3.1	4.3	4
Bio-5	x	14.7	x	x	9	8.7	9.8	18.5	10.2
Bio-6	36.9	33.3	42.1	38.9	35.6	46.8	36.5	0.7	28.3
Bio-7	0.9	x	2.1	3.5	0.6	3.6	3.9	1.1	0.1
Bio-8	4.7	1.3	7.9	5.1	3	3.8	1	10.4	4.8
Bio-13	12.9	6.7	6.8	10.7	11.5	5.3	3.4	13.2	5.4
Bio-16	5.3	6.4	5.9	6.3	6.2	4.2	7.5	10.8	9
Bio-17	7.3	5.7	x	x	x	x	x	x	x
Bio-19	2.8	x	4.5	4.4	x	x	6.1	6.5	3

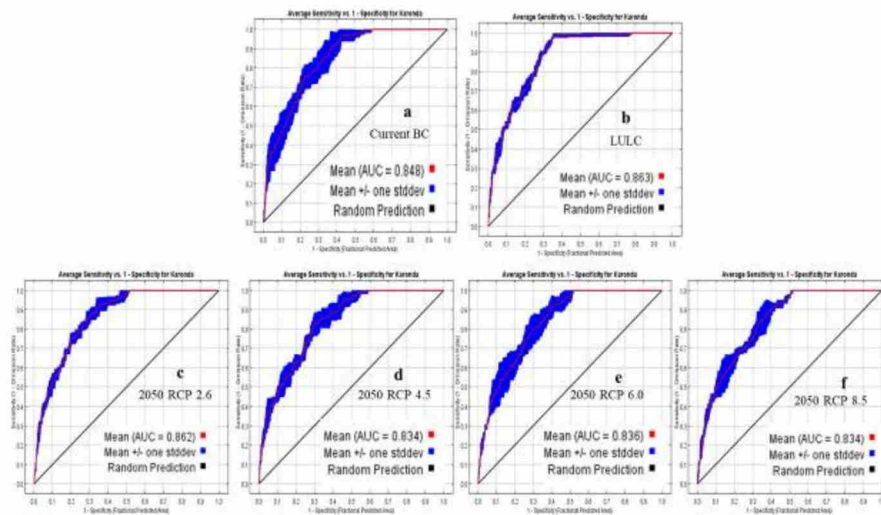


Figure 2. The area under the receiver operating curve with current bio-climatic (a), LULC (b), future climatic time frame (2050) with four RCPs namely 2.6 (c), 4.5 (d), 6.0 (e), and 8.5 (f).

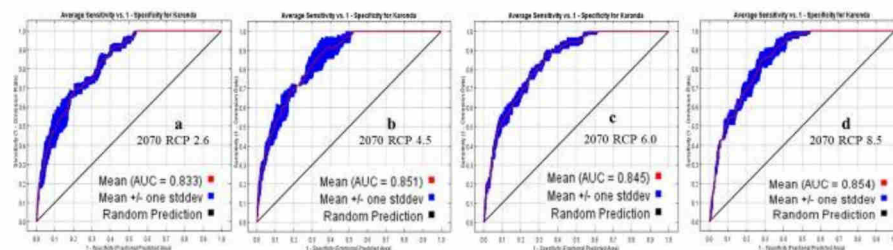


Figure 3. The AUC curve with future climatic time frame (2070) with four RCPs namely 2.6 (a), 4.5 (b), 6.0 (c), and 8.5 (d).

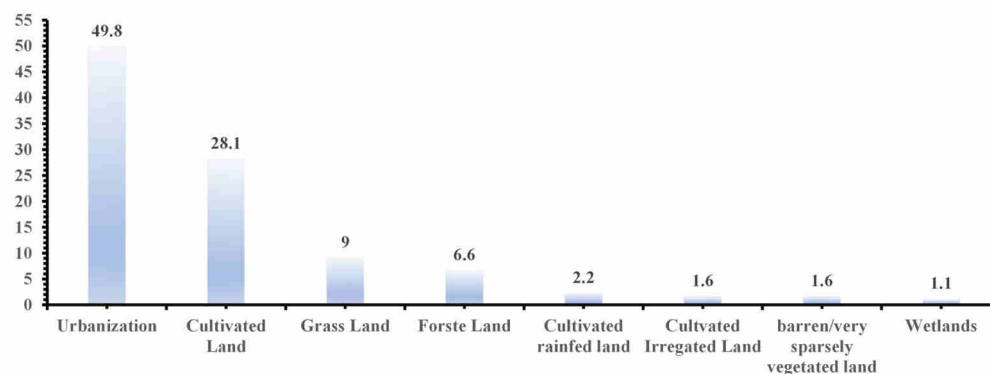


Figure 4. Variable importance values of Maxent output with different variables of LULC.



Their percentage contributions are 49.8, 28.1, and 9. Cultivated rain-fed and irrigated land, barren land, and wetlands have variable importance values below 2.0.

The supplementary material (Figures 1–10) shows response curves for the three most important bio-climatic and land use/land cover variables. The response curves showed that the projected species suitability values with MiTCM variables were highest at 15°C for both present conditions and all future Representative Concentration Pathways (RCPs), except 6.0 and 8.5 in 2070. Isothermality has had the greatest impact on these two RCPs. Additionally, species suitability peaked at 50 and ranged from 40 to 55. Temperature seasonality peaks between 20 and 40°C. Urbanization and grassland have skewed curves in LULC variables. Urbanization peaks between 10 and 20, while grassland peaks between 5 and 10. Cultivated lands have a wider spectrum, peaking at 35 to 60.

Habitat Suitability Areas (km²)

Table 3 shows habitat suitability areas for optimal, moderate, marginal, and low habitat types. The spatial distribution of these areas is shown in Figure 5-a for the current climatic time, and in Figures 5-b to -e for 2050 and its RCPs. Figures 6-a to -d show 2070 habitat suitability and RCPs. Lastly, Figure 7 shows LULC patterns for each habitat type: optimum (Figure 7-a), moderate (Figure 7-b), marginal (Figure 7-c), and low (Figure 7-d). In the optimal class, 2070RCP 2.6 had the largest land area (46.82×10^2 km²). In 2050 RCP 2.6, the bio-climatic variables had the smallest area (40.81×10^2 km²), followed by 2070 RCP 8.5. The optimal habitat type covers 45.88×10^2 km² under current climate conditions. The LULC optimum suitability habitats (km²) had the smallest area, 28.01×10^2 km². The areas with the greatest extent were moderate (70.85×10^2 km²) and low (16.19×10^3 km²), under the current bioclimatic conditions. Conversely, 2070 RCP 4.5 (34.51×10^2) and land use and land

cover change (50.08×10^2) had the smallest areas for above classes. LULC had the highest marginal class area (15.04×10^3).

Based on analysis of habitat suitability classes and their spatial extents, this study proposes the existence of optimal regions in the southern (Karnataka and Tamil Nadu), as well as in the western (covering the western Ghat region of Maharashtra and Goa, and some scattered areas in Gujarat) areas of India, which exhibit similar characteristics across various bio-climatic time frames and RCPs. Nonetheless, LULC predictors have shown fragmented patterns in optimum habitat. Furthermore, this habitat has been observed in both the eastern (Odisha, Jharkhand, West Bengal) and northern (Uttar Pradesh, New Delhi, Uttarakhand) parts of the country. Furthermore, given the current climatic conditions, it is found that specific regions in the west (Gujarat, Rajasthan), north (Uttar Pradesh), and south (Andhra Pradesh) are moderately favourable for this species. Given the steady evolution of climatic conditions projected for 2050, as well as the four RCPs, it is expected that certain portions of western India, particularly Maharashtra, will see the emergence of suitable habitats for this species. However, it is vital to highlight that habitat fragmentation is likely to occur in the country's northern territories, including Uttar Pradesh, as well as western portions (Gujarat and Rajasthan), resulting in the split and isolation of these ecosystems. The moderate portions of Gujarat (western part of the country) will either become a marginal habitat by 2070 (RCP 2.6) or proceed to an optimum habitat under RCP 4.5, 6.0, and 8.5 scenarios. The central areas of India are distinguished by the presence of habitats with marginal or low ecological value. In addition to optimum habitat, we have documented fragmented and patchy habitats classified as moderate, marginal, or low with LULC. This species cannot be grown in the extreme western region (Rajasthan), which has a hot and arid climate and encompasses Barmer and Jaisalmer districts. Similarly, the northern region, such as Jammu and Kashmir, as well as Ladakh, are unsuited for cultivation of this species. Finally, the eastern areas of the country, notably Arunachal Pradesh and Sikkim, do not have ideal circumstances for cultivating this plant.

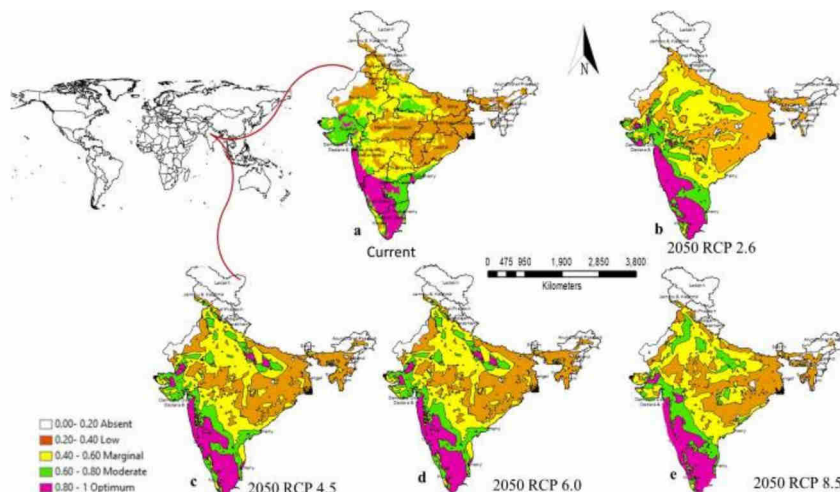


Figure 5. Habitat suitability of *C. carandas* under different classes with current (a) and 2050 bio-climatic time frame with its four RCP 2.6 (b), RCP 4.5 (c), RCP 6.0 (d), and RCP 8.5 (f).

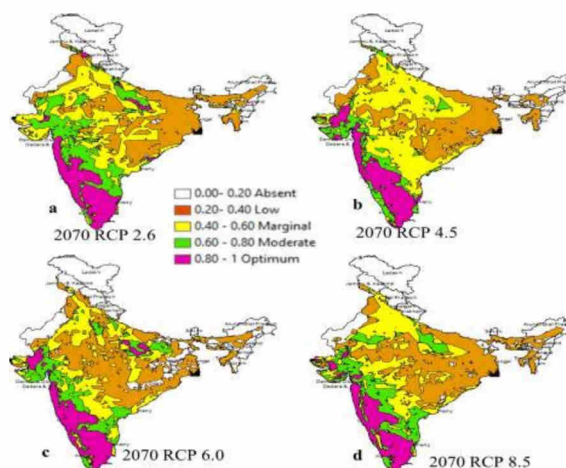


Figure 6. Habitat suitability of *C. carandas* under different classes with 2070 bio-climatic time frame with its four RCP 2.6 (b), RCP 4.5 (c), RCP 6.0 (d), and RCP 8.5 (f).

Spatial Changes in Optimum Habitats

As evaluated using various predictors, Figure 8 shows the percentage changes in the most suitable habitat's extent relative to the optimal area. The spatial distribution of these alterations is shown in Figure 9-a to -e (current+2050, along with their RCPs) and Figures 10-a to -d (current+2070 RCPs) for two future climatic-time frames. Based on LULC parameters, this suitability class is highly fragmented. Comparatively, this fragmentation has decreased by 38.95%. This species has a marginal gain of +2.04 for

2070 under the RCP 2.6 scenario and +1.06 under the RCP 4.5 scenario. With the previous one, hilly regions of norther India (Himachal Pradesh, Uttarakhand, Uttar Pradesh), western parts (covering areas adjoining to Ahmedabad, Morbi, Rapar, Bhabhar, Tharad, Dhanera, Deesa, Raniwara Gir National Park in Gujarat, and Bhinmal Gudamalani in Rajasthan) had the highest gain of 2070RCP4.5. However, optimal habitats decreased by -11.06 and -8.75% under the 2050 and 2070 RCPs 2.6 and 8.5, respectively. RCP 4.5, 6.0, and 8.5 of 2050 showed less than 5% loss in optimum habitats (Figure 8).

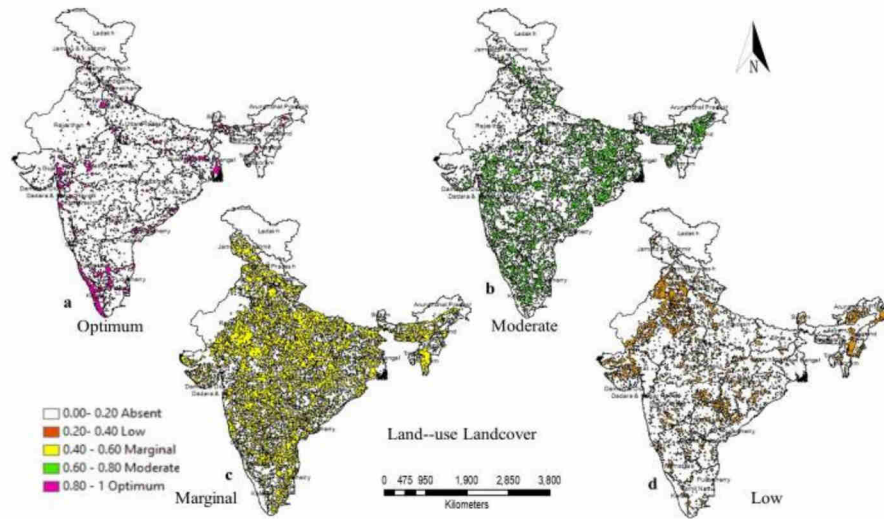


Figure 7. Habitat suitability of *C. carandas* under different classes with LULC: Optimum (a), moderate (b), marginal (c), and low (d).

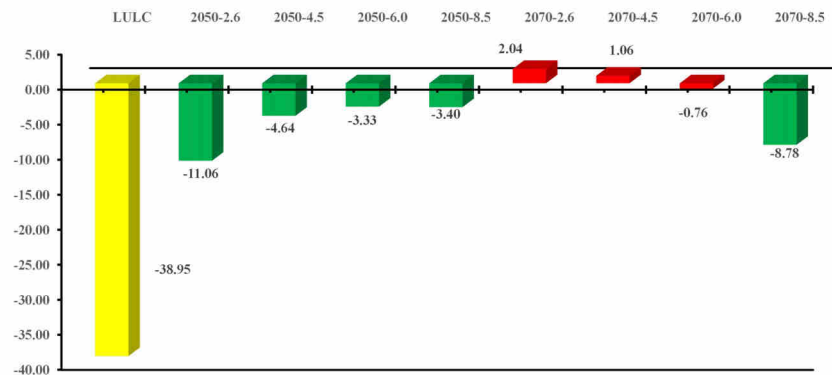


Figure 8. Percent changes (gain and loss) in areas of optimum habitat suitability under different climatic and non-climatic variables in comparison to the current optimum area.

Table 3. Area (km^2) of different habitat suitability classes with studied predictors.

Variables	Optimum	Moderate	Marginal	Low
Current	45.88×10^2	70.85×10^2	12.78×10^3	16.19×10^3
Land use and Landcover	28.01×10^2	53.93×10^2	15.04×10^3	50.08×10^2
2050RCP2.6	40.81×10^2	48.10×10^2	11.49×10^3	10.77×10^3
2050RCP4.5	43.75×10^2	54.09×10^2	11.43×10^3	10.96×10^3
2050RCP6.0	44.35×10^2	50.59×10^2	10.97×10^3	11.75×10^3
2050RCP8.5	44.32×10^2	53.97×10^2	11.42×10^3	10.38×10^3
2070RCP2.6	46.82×10^2	60.92×10^2	10.50×10^3	10.65×10^3
2070RCP4.5	46.37×10^2	34.51×10^2	13.16×10^3	10.21×10^3
2070RCP6.0	45.53×10^2	46.85×10^2	85.74×10^2	11.76×10^3
2070RCP8.5	41.85×10^2	54.29×10^2	92.05×10^2	12.36×10^3

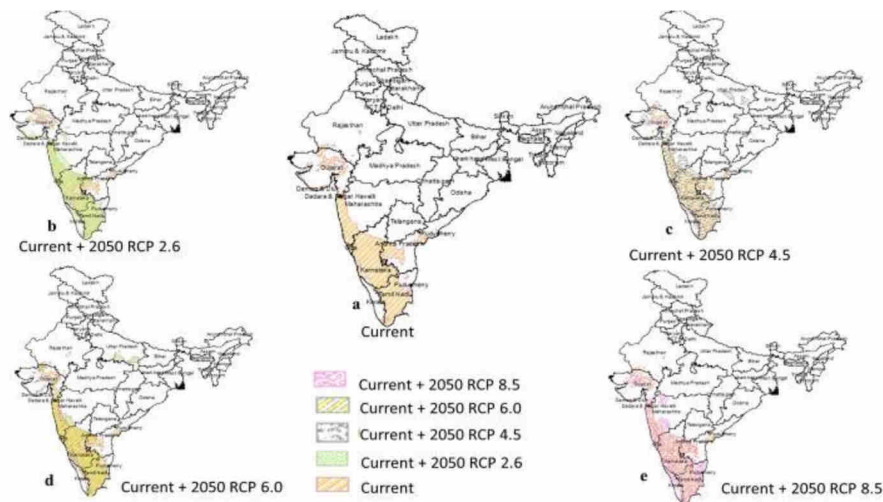


Figure 9. Superimposition of the current optimum suitability sites (a) with different RCPs of 2050 2.6 (b), 4.5 (c), 6.0 (d), and 8.5 (e).

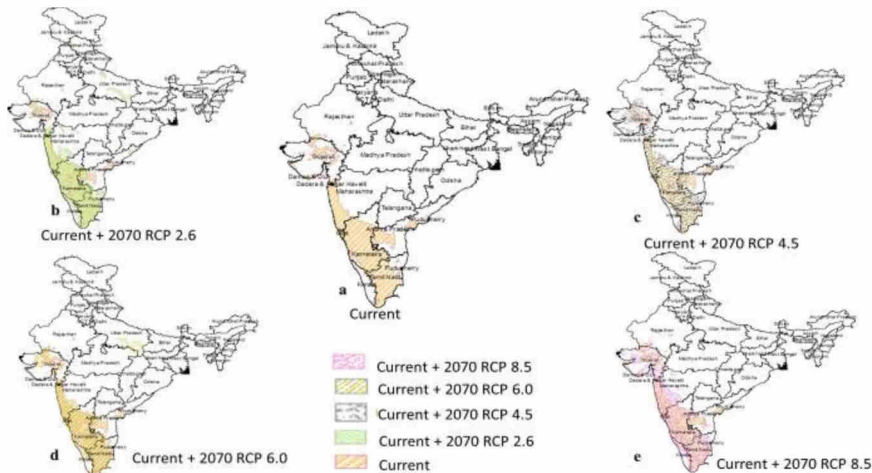


Figure 10. Superimposition of current optimum suitability sites (a) with different RCPs of 2070 2.6 (b), 4.5 (c), 6.0 (d), and 8.5 (e).

Ellipsoid Niche Hypervolume

Using the existing dataset, we constructed an ellipsoid hypervolume, which represents a multidimensional space encompassing the available resources for a given species. This hypervolume was employed to simulate both the fundamental niche, which refers to the species' capacity to persist and reproduce in a wider range of environments in the absence of interspecific competition, and the realized niche, which considers the species' interactions with other coexisting species. To achieve this, we utilized projected

occurrence records of the species *C. carandas*, along with the pertinent environmental variables that were identified as crucial through the Maxent algorithm, presented in the form of raster output. This enables us to discern the variables that dictate both its fundamental and realized niche. The results are display in Figures 11-a (current bio-climatic) and -b (LULC), 12 (a-d) (2050 and its RCPS) and 13 (a-d) (2070 and its RCPS). Within these visual representations, the utilization of the blue hue signifies the concept of niche stability, while the incorporation of the color green



conveys the notion of niche unfilling, denoting the extent to which the native niche remains unoccupied by the exotic niche. Additionally, the inclusion of the red hue serves to symbolize the phenomenon of niche expansion (Mathur and Mathur, 2023). The dimensions of these zones are directly proportional to the magnitude of their respective ecological niche. In terms of bioclimatic space, *C. carandas* ellipsoidal niche had a larger hypervolume ($82.21 \times 10^3 ^\circ\text{C mm}^2$) with the current bioclimatic conditions, followed by 2.6 RCPs of 2050 ($60.24 \times 10^3 ^\circ\text{C mm}^2$) and 2070

($57.16 \times 10^3 ^\circ\text{C mm}^2$), and among the bioclimatic variables it was the smallest ($23.06 \times 10^2 ^\circ\text{C mm}^2$) during the 2050 RCP 4.5. However, with LULC, it was recorded minimum having $19.26 \times 10^2 ^\circ\text{C mm}^2$. The manifestation of environmental factors on the dynamics of ecological niches is denoted by the centroid values associated with these variables. The spatial proximity of these entities to the centroid serves as a reliable indicator of their capacity to exert influence over the suitability of species (Nunez-Penichet *et al.*, 2021). The values pertaining to the centroid of various bio-climatic

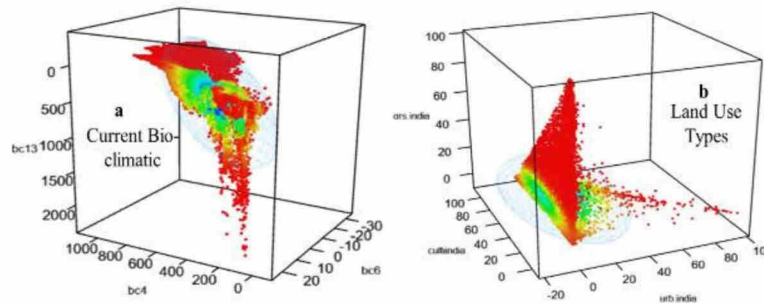


Figure 11. Graphical representation of *C. carandas* niche hypervolume with three most influential variables pertains to the current bioclimatic (a) and LULC (b) predictors.

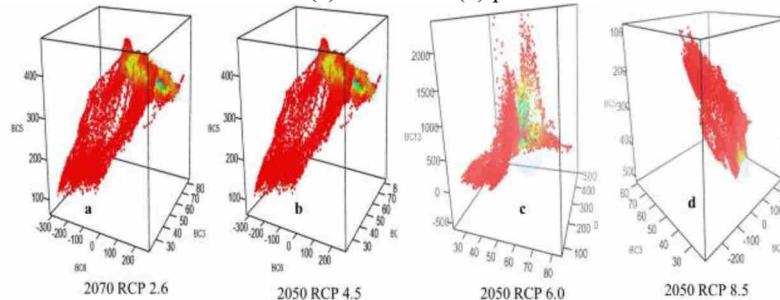


Figure 12. Graphical representation of *C. carandas* niche hypervolume with three most influential variables pertains to 2050 bioclimatic time frame with its four RCPs namely 2.6 (a) 4.5 (b), 6.0 (c) and 8.5 (d).

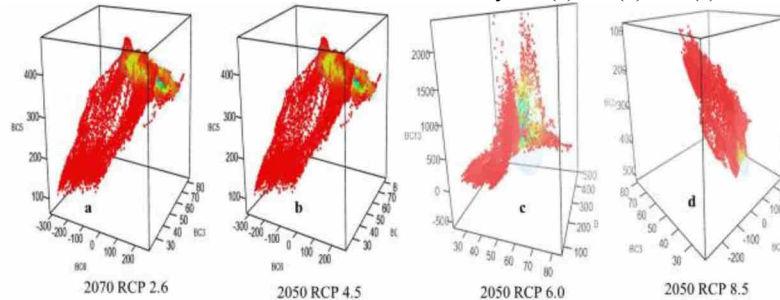


Figure 13. Graphical representation of *C. carandas* niche hypervolume with three most influential variables pertains to 2070 bioclimatic time frame with its four RCPs namely 2.6 (a) 4.5 (b), 6.0 (c) and 8.5 (d).

variables across three distinct time frames are displayed in Table 4.

This species expands its ecological niche mostly beyond its fundamental niche in relation to precipitation levels during the wettest month (Bio-13, the water variable) in the current climate. Table 4 also shows that temperature seasonality (Bio-4) and the minimum temperature during the coldest month (Bio-6) help preserve these niche areas. This analysis of all four RCPs from 2070 shows that the warmest month's maximum temperature (Bio-5) controls the expansion of its fundamental niche. Alternatively, isothermality, the minimum temperature of the coldest month, and the precipitation of the wettest month (2070 RCP 6.0) support these niche areas. Except for RCP 4.5 in 2050, the other RCPs suggest that, like the current situation, its niche expansion is primarily influenced by precipitation levels during the wettest month. In RCP 4.5, the mean temperature of the wettest quarter (Bio-5) dominates this expansion. The centroid value of 52.93 for the LULC variables indicates that cultivated lands control *C. carandas*' fundamental niche expansion. Urbanization (13.29) and barren/sparsely vegetative areas (5.47) help this species maintain its niche.

DISCUSSION

The Asian continent persists in grappling with a significant incidence of malnutrition. The enduring state of malnutrition can be ascribed to a deficiency in dietary variety, coupled with a dearth of diversity in

production. Dietary diversity encompasses the adoption of a nourishing, well-rounded, and heterogeneous dietary pattern, thereby guaranteeing the sufficiency of essential nutrients. The principle of dietary diversity is unequivocally endorsed in all national food-based dietary guidelines. Strategies centred on food that aim to combat malnutrition, particularly deficiencies in essential micronutrients, are intricately intertwined with scientifically substantiated dietary patterns. However, these approaches remain disjointed from the existing agricultural production system. The incorporation of promising, yet underutilized, species characterized by their high nutrient density, climate resilience, profitability, and local availability and adaptability plays a pivotal role in enhancing both dietary and production diversity (Mayes *et al.*, 2012).

By delineating the boundaries of suitable areas, scholar inquiry can significantly strengthen the justification for integrating these crops into a holistic approach to climate adaptation. Furthermore, agronomists have the ability to utilize these maps in order to augment their understanding of the existing and future limitations on resources in each specific region and crop. Upon undergoing scrutiny by an agronomist, it becomes evident that maps possess the inherent capacity to expedite the discernment of the most appropriate agronomic methodology that harmonizes with the particular circumstances of the agriculturalist (Mugiyo *et al.*, 2022; Mathur and Mathur, 2024).

This study used four Representative

Table 4. Values of niche centroid of three most influential bio-climatic variables pertains to various time-frames and RCPs.

Bio variables	Current	2050 RCPs				2070 RCPS			
		2.6	4.5	6.0	8.5	2.6	4.5	6.0	8.5
Bio-3	-	48.52	49.51	48.34	48.9	50.33	49.27	50	49.78
Bio-4	358.72	-	-	-	-	-	-	-	-
Bio-5	-	386.45	-	-	-	384.27	393.86	499.02	402.63
Bio-6	13.69	154.44	164.78	158.81	166.26	163.17	164.4	-	183.03
Bio-8	-	-	279.84	-	-	-	-	-	-
Bio-13	363.06	-	-	465.02	442.04	-	-	391.42	-



Concentration Pathways (RCPs) to assess crop viability at various concentrations. The RCPs included a large trajectory (RCP8.5), a moderate trajectory (RCP4.5 and RCP6.0), and a small trajectory (RCP2.6). We wanted to determine crop sustainability potential across these trajectories. The environmental adaptation and eco-geographic distribution of underutilized species have been widely recognized in scholarly literature (Williams and Haq, 2002; Mugiyo *et al.*, 2022). Many underutilized species have adapted to inhospitable environments, preserving biodiversity and protecting against risks in an ever-changing ecosystem. Thus, understanding their ecological adaptation and ecogeographic dispersion is crucial to selecting crops for future use (Bow and Haq, 2010).

C. carandas is discussed as a climate-resilient, underutilized crop to examine the factors affecting its domesticated areas, the fundamental niche, and its new viable areas, the realized niche, for its introduction. Koch *et al.* (2022) empirically supported our methods. Their research involves developing an ensemble model to characterize the distribution patterns of *Ensete ventricosum*, a perennial banana species grown only in southwestern Ethiopia. Ratnayake *et al.* (2020) advocated for predictive modelling in the management of Neglected and Underutilized Fruit Species (NUFS) in the light of climate change, supporting our methods. The researchers examined *Aegle marmelos*, *Annona muricata*, *Limonia acidissima*, and *Tamarindus indica* species in both present and projected future climates (RCP 4.5 and RCP 8.5) for 2050 and 2070. They used the widely-recognized Maximum entropy (Maxent) Species Distribution Modelling (SDM) approach to predict species distributions. These methods have highlighted the need for climate change adaptation strategies and research to strengthen underutilized fruit crops against climate change.

The current study has furnished a comprehensive nationwide database concerning the geo-tagged spatial

distribution of *C. carandas*. This dataset comprises 218 strategically thinned points, and its implications extend to practical assessments of favourable regions for crop cultivation, accurate productivity forecasting, and facilitation of appropriate markets for these under-utilized crops. Moreover, it represents a crucial step towards the development of a user-friendly mobile application, such as "Kirshi-Kisan" (<https://play.google.com/store/apps/details?id=com.cropdemonstrate&hl=en&gl=US>) by government of India.

The results of our habitat suitability analysis have revealed that the distribution dynamics of this particular species are primarily influenced by temperature-related variables rather than water-related variables such as precipitation. Among temperature variables, isothermality and the minimum temperature during the coldest months have the greatest impact on species distribution. Temperature annual range and the wettest quarter mean temperature affect species distribution less. Moreover, by employing threshold values of the effective temperature variables, such as a minimum temperature of 15°C for the coldest month and an isothermality peak of 50, we can deduce the distribution pattern of this particular species. It becomes apparent that the species is predominantly found in the southern and western regions of the countries, while its presence is notably absent in the northern and eastern regions: In the regions of Gujarat, Karnataka, Tamil Nādu, Andhra Pradesh, and certain areas of Rajasthan. These locations exhibit isothermality, where the diurnal temperature range is half of the annual temperature range. In essence, a numerical value of 100 represents a location where daily temperature fluctuations equal annual temperature variation. However, a numerical value of 50 indicates a location where the 24-hour temperature difference is half of the annual temperature range. According to Kogo *et al.* (2019), environmental factors tend to affect the appropriateness of different regions. Any deviation from these parameters affects crop

suitability, whether positively or negatively. In India, *C. carandas* thrives in hot, humid climates. The main factors limiting *C. carandas* growth and development are temperature and seasonal fluctuations (Meena *et al.*, 2022).

The variables of urbanization, cultivated land, and grassland have been identified as influential factors in Land Use and Land Cover (LULC). Our analysis has shown that as urbanization increases by 10-20% and grassland expands by 5-10%, the likelihood of suitability for this particular species experiences a gradual but limited decrease. Nevertheless, this particular species demonstrates a remarkable adaptability to thrive within cultivated regions, owing to its significantly higher tolerance for land use and land cover changes. As mentioned, these areas are mostly in Karnataka, Tamil Nadu, and the Western Ghats of Maharashtra and Goa. There are also occasional suitable habitats for this species in Gujarat. We included all the relevant bio-climatic temporal variations and RCPs in our analysis. RCPs were used to identify several Rajasthan locations. However, using LULC predictors, we found widely dispersed optimal habitats for this species in Odisha, Jharkhand, West Bengal, Uttar Pradesh, New Delhi, Uttarakhand, and Jharkhand.

By utilizing the LULC variable, we have successfully documented the highest level of fragmentation within the optimal suitability category, resulting in a notable reduction of up to -38.95% compared to its existing climatic extent. The phenomenon of fragmentation has been previously examined and conceptualized by Rathore *et al.* (2022). LULC changes should significantly impact *C. carandas* distribution in the study region. Urban heat islands show that human activity and ecosystem damage can raise local temperatures, so, species composition may differ between urban and rural areas. Urbanization alters soil properties. Heavy metal and organic matter are higher in urban soils (Wang *et al.*, 2016). Bhandari *et al.* (2022) and Padder and Mathavan (2022) quantified how land cover changes adversely

affected rice and maize productivity. Unfortunately, this association for underutilized crops has not been studied. This study helped us understand the causes and effects of underutilized crop productivity and its factors.

Understanding niche dynamics is crucial to creating effective conservation strategies (Atwater *et al.*, 2018; Liu *et al.*, 2020). During habitat colonization, species change their niche space, which can maintain, expand, or contract. Variations in the realized niche—all the biotic and abiotic conditions a species is observed in nature—and the fundamental niche—the abiotic conditions needed for positive population growth without biotic interactions—influence these changes (Guisan and Thuiller, 2005). Jezkova and Wiens (2016) found that changing realized and fundamental niches are distinct processes that do not overlap. We simulate *C. carandas* climatic and non-climatic fundamental and realized niche using a precise predictor. The ecological niche hypervolume analysis has shown that *C. carandas* 'climatic niche is larger than its non-climatic niche, which is supported by Bilton *et al.* (2016). This study found that temperature-related factors are most important in determining the phenomenon's spatial range. Niche analysis has shown that the amount of precipitation received during the wettest month is the main factor affecting its ecological niche expansion during the current and projected 2050 climatic timeframe. Niche expansion is regulated by the warmest month's upper limit in 2070.

CONCLUSIONS

This study provides a comprehensive assessment of the habitat suitability of *Carissa carandas* in India, incorporating bio-climatic variables, Greenhouse Gas (GHG) scenarios, and Land Use/Land Cover (LULC) predictors. Using the MaxEnt model, we identified key environmental factors influencing the species'



distribution, with temperature-related variables, such as the minimum temperature of the coldest month and isothermality, playing a dominant role. Future climate projections for 2050 and 2070 indicate shifts in suitable habitat, with the southern and western regions of India (including Karnataka, Tamil Nadu, Maharashtra, and Gujarat) continuing to be optimal areas, while habitat fragmentation is expected in the northern and western regions due to climate change and land use changes. The findings underscore the importance of integrating *C. carandas* into climate-resilient agricultural and conservation strategies. Given its adaptability and economic potential, promoting its cultivation in suitable regions can enhance biodiversity, support sustainable agriculture, and provide economic benefits to farmers. However, policy interventions are needed to mitigate the effects of urbanization and land-use changes on its habitat. Future research should focus on refining habitat predictions using additional environmental factors and assessing the socio-economic impact of cultivating this underutilized species.

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ارزیابی مناسب بودن زیستگاه گونه *Carissa carandas* L. در هند با استفاده از متغیرهای زیست اقلیمی، سناریوهای گازهای گلخانه‌ای، و پیش‌بینی‌کننده‌های کاربری زمین و پوشش زمین

مانیش ماتور، و پریت ماتور

چکیده

این پژوهش به منظور ارزیابی مناسب بودن زیستگاه کاریسا کارانداس (*Carissa carandas*)، گیاهی که برای ادغام پایدار آن در کشاورزی تحت شرایط آب و هوایی متغیر بسیار مهم است، در هند انجام شد. ما از مدل‌سازی حداکثر آنتروپی (MaxEnt) برای ارزیابی توزیع گونه‌ها در سناریوهای فعلی و آینده (۲۰۵۰ و ۲۰۷۰) در چهار مسیر غلظت نماینده (Representative Concentration Pathways) شامل (RCPs): ۲.۶، ۴.۵، ۶.۰ و ۸.۵ استفاده کردیم. نتایج نشان داد که متغیرهای مرتبط با دما، به ویژه حداقل دمای سردترین ماه (MiTCM)، با سهم ۴۸.۸٪ در RCP ۲.۶ سال ۲۰۷۰ و ایزوترمالیتی (با سهم تا ۳۵/۲٪ در RCP ۸.۵ سال ۲۰۷۰)، محرک‌های اقلیمی غالب هستند. عوامل کاربری و پوشش زمین (LULC) مانند شهرنشینی (۴۹.۸٪)، کل زمین‌های کشت‌شده (۲۸/۱٪) و علفزارها (۹٪) به طور قابل توجهی بر تناسب زیستگاه تأثیر می‌گذارند. در شرایط فعلی، زیستگاه بهینه ۴۵۸۸ کیلومتر مربع را در بر می‌گیرد که تحت سناریوهای LULC ۳۸.۹۵٪ کاهش می‌یابد. تغییرات پیش‌بینی‌شده زیستگاه نشان‌دهنده افزایش ۲.۴۰٪ تا سال ۲۰۷۰ است، اما کاهش ۱۱.۰۶ درصدی تا سال ۲۰۵۰ با RCP ۲.۶ را نشان می‌دهد. مناطق جنوبی و غربی، از جمله کارناتا‌کا، تامیل نادو، مهاراشترا و گجرات، از تناسب بالایی برخوردارند. تکه‌تکه شدن زیستگاه در شمال و غرب هند به دلیل تغییرات اقلیمی و تغییرات کاربری زمین پیش‌بینی می‌شود. تأکید این یافته‌ها بر نیاز به برنامه‌ریزی حفاظتی پیشگیرانه و استراتژی‌های کشاورزی سازگار با آب و هوا برای بهینه‌سازی کشت *C. carandas* می‌باشد. سیاست‌گذاران و ذینفعان باید بر حفظ مناطق مناسب تمرکز کنند و درعین حال از بین رفتن زیستگاه ناشی از شهرنشینی را کاهش دهند.

Explaining and Validating the Green Curriculum's Characteristics Based on the Critical Competencies of Education for the 21st Century

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ABSTRACT

This study aimed to explain and validate the characteristics of a Green Curriculum (GC) based on critical competencies required for the 21st-century education. A mixed-methods approach was employed. In the qualitative phase, experts with experience in sustainability within Iran's higher education system were selected through snowball sampling. Data were collected via semi-structured interviews and analyzed using content analysis. The identified GC characteristics were then quantitatively validated using the Lawshe method. For this purpose, 40 faculty members and doctoral students in educational sciences were randomly selected to evaluate the appropriateness of these characteristics on a three-level scale. The results identified 50 characteristics from interviews and documents, with 48 being validated through the Lawshe method. Additionally, the study found that each of the five competencies for the 21st-century education (learning to know, learning to be, learning to do, learning to live together, and learning to transform oneself and society) aligns with several GC characteristics. Recommendations for operationalizing these competencies within a GC include integrating environmental management into various academic curricula, defining practical projects for students, and encouraging engagement with the environmental organizations and global research communities.

Keywords: Environmental challenges, Green university, Higher Education sustainability, Lawshe method.

INTRODUCTION

Green University

Due to increasing concern about various environmental issues, universities are expected to contribute to solving these problems by generating relevant knowledge and integrating sustainability into their programs. This expectation has led to the introduction of the concept of a green university (Shu *et al.*, 2024; Khoderchah and

Semaan, 2024). Although "green" is a broad and complex concept encompassing environmental concerns, protection of the planet and animals, humanitarian concerns, fair trade, clean water, welfare, equality, and sustainability (Nowak, 2023), in higher education, it represents an approach proposed under the concept of sustainable development, highlighting the serious responsibility of higher education in this regard (Deriu and Gallo, 2024). The first thought that comes to mind when hearing about a green university is often a green campus. However, it refers to all human activities in the 21st century that cause the

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least damage to the environment (Pouramini and Bashokouh, 2024). Following the introduction of the green university concept, several assessment systems have been designed to monitor the activities of green universities worldwide (Figure 1).

As shown in Figure 1, among the six ranking systems defined for green universities, only two systems consider education as a criterion: STARS and Green Metric. The former is specifically designed for American and Canadian universities and is not a global ranking system (Atici *et al.*, 2013). The latter system emphasizes the physical environment, with education presented implicitly alongside research. Chankrajang and Muttarak (2017) studied the contribution of education to pro-

environmental behavior and confirmed that education significantly increased the probability of taking knowledge-based environmentally-friendly actions. Kountouris and Remoundou (2023) also found that education improved some types of environmentally friendly activities, such as waste recycling. Therefore, educational aspects are underestimated in Green University (GU) assessments and need more attention.

Despite the increasing emphasis on sustainability in higher education, the current GU assessment systems inadequately address the educational aspects of sustainability. Most systems focus primarily on the physical environment, neglecting the critical role of education in fostering pro-

The Holcim Awards (Damati, 2013)	• Progress: Innovation, and transferability • People: Ethical Standards and Social Equity • Planet: Environmental Quality and Resource Efficiency	• Prosperity Economic Performance and Compatibility • Proficiency Contextual and Aesthetic Impact
STARS (Atici <i>et al.</i> , 2013)	• Operations: Energy, water, waste, transportation, and other operational aspects. • Academics: Sustainability in curriculum and research • Innovation and Leadership: Innovative and exemplary sustainability practices	• Planning and Administration: Coordination and planning, diversity and affordability, investment and finance, and wellbeing and work • Engagement: Campus engagement and public engagement in sustainability efforts
Green Report System (Shi and Lai, 2013)	• Administration • Climate change and energy • Food and recycling • Green building • Student involvement	• Transportation • Endowment transparency • Investment priorities • Shareholder engagement
Green Building System (Marrone <i>et al.</i> , 2018)	• Energy efficiency • Indoor environmental quality • Sustainable site development • Water efficiency	• Materials and resources • Innovation in design • Transportation
Green Metric (Marrone <i>et al.</i> , 2018)	• Setting and infrastructure • Energy and climate change • Waste and waste management	• Water conservation • Transportation • Education and research

Figure 1. Green Universities Ranking systems.

environmental behaviors and competencies. This oversight limits the effectiveness of GU in promoting comprehensive sustainability education.

Addressing this gap is crucial because education is a powerful tool for instilling sustainable practices and mindsets in future generations. Without a strong educational component, GU cannot fully achieve their potential in driving societal change towards sustainability. This research aims to bridge this gap by validating the characteristics of a green curriculum based on the critical competencies of education for the 21st century.

This study provides a detailed analysis of the educational dimensions of GU, proposing a framework for integrating sustainability into higher education curricula. By focusing on the critical competencies identified by UNESCO, this research offers practical guidelines for developing green curricula that equip students with the knowledge, skills, attitudes, and values necessary for sustainable living.

Green Curriculum

According to the Illinois Central College Curriculum Committee in the United States, a green curriculum is related to sustainability and equips learners with the knowledge and skills to identify, analyze, and solve problems in various social, economic, and environmental domains (Illinois Central College, 2023). The green curriculum aims to integrate sustainability into the teaching process so that the learners obtain sufficient knowledge and skills to become “Sustainability Minded Citizens.” The primary purpose of sustainable thinking is to empower individuals to comprehend the complex issues of sustainability and the challenges facing human society at local and global levels, enabling them to seek logical solutions to these problems (Taqipour *et al.*, 2016; Singer-Brodowski *et al.*, 2018). Previous studies related to the green

curriculum confirm that this type of curriculum focuses on environmental crises and often aims to empower people to solve these crises.

According to UNESCO’s Greening Curriculum Guidance, integrating climate mitigation and adaptation into teaching and learning is essential for fostering a holistic, scientifically accurate, and justice-driven approach to climate change education (UNESCO, 2024). Hays and Reinders (2020) discussed that the concept of Sustainable Learning and Education (SLE) emphasizes creating curricula that instill skills and dispositions necessary for thriving in challenging circumstances. The integration of sustainability in the green curriculum is crucial for developing an environmentally conscious mind-set in students. Gabrys *et al.* (2020) emphasize problem-solving in a coached apprenticeship system, which can be applied to green education through real-world environmental challenges. Avvisati and Borgonovi (2020) show that problem-solving in mathematics enhances analytical skills vital for sustainability. Rebello *et al.* (2017) highlight the need for an interdisciplinary approach to address complex scientific problems, stressing the importance of a green curriculum that incorporates sustainability across various subjects. Furthermore, Karami *et al.* (2020) examined the green curriculum in Iranian universities, emphasizing the water crisis, climate change, and knowledge supply sources. They suggested developing a green curriculum aligned with climate change management in Iran, including creating a department for curriculum development and revision in all universities of the Ministry of Science, Research and Technology according to the country’s water conditions, and dedicating part of the lessons to climate change management at different education levels. Another study considered the green curriculum as a tool for sustainable learning, indicating that it should be used to allow people to solve their problems in communities through radical problem-



solving initiatives and ideas. Transformative solutions, which are holistic, not only are profitable but also save people and the planet (Louw, 2013). Haigh (2005) indicated that universities should equip all their students with “environmental literacy”, and sustainability must be the central point of the curricula and all practices of any university worldwide. Greening the curriculum is considered a plan to ensure students’ ability to face the challenges of the 21st century, such as global warming and climate change, social inequalities, unsustainable lifestyles, and the urgent need to move to an economy based on renewable energy (Greenheart Education, 2019). Accordingly, the green curriculum also covers the critical competencies of education for the 21st century. Competency in this context refers to a combination of knowledge, skills, attitudes, and values that students need to effectively navigate and succeed in various aspects of life, including personal, academic, and professional domains (Koeppen *et al.*, 2008). These competencies, which correspond to basic skills and required characteristics for sustainable activities, are identified by UNESCO and presented in Delors’s report (1996) as follows:

Learning to know: Obtaining the instruments of understanding or learning how to learn, which can be considered a foundation for lifelong learning.

Learning to do: Applying learned knowledge in daily life to improve the ability to be creative and responsible toward the surrounding environment.

Learning to be: Obtaining universally shared values, developing one’s personality, self-identity, and self-knowledge, becoming immersed in one’s culture and wisdom, and being empowered to learn about oneself and become more fully human.

Learning to live together: Education for international and intercultural understanding is essential for fostering the social dimension of human development. It serves as the foundation for cohesion and harmony, conflict avoidance, non-violence, and peaceful coexistence. Recognizing that

difference and diversity are opportunities rather than dangers, this competency emphasizes the value of diversity as a resource for the common good. It promotes the ability to tolerate, respect, welcome, and celebrate differences in people, their histories, traditions, beliefs, values, and cultures, using this diversity to enrich our lives and classrooms.

Learning to transform oneself and society: This competency focuses on working towards a gender-neutral, non-discriminatory society and acting to achieve social solidarity and international understanding. Above all, it emphasizes living sustainably (Shaeffer, 2007).

Given the above explanations, it is clear that the green university movement is a crucial approach to addressing and mitigating environmental concerns in the contemporary era. However, despite the critical role of education in this journey, current ranking systems for green universities have not sufficiently considered educational aspects. Due to the significance of green education, it aligns well with the critical competencies of education for the 21st century, as introduced by UNESCO. Therefore, this study aims to design and validate a green curriculum model based on these critical competencies. To achieve this aim, we have formulated two research questions:

What are the characteristics of a green curriculum?

How can we categorize the characteristics of a green curriculum based on the competencies of education for the 21st century?

MATERIALS AND METHODS

Research Design

This study employed a mixed-methods approach, combining qualitative and quantitative paradigms to explore and validate the characteristics of a green curriculum based on the critical

competencies of education for the 21st century. The exploratory nature of the initial phase justified the use of qualitative methods, allowing for an in-depth understanding of the research topic. The subsequent phase utilized quantitative methods to validate the content of the green curriculum characteristics.

Qualitative Phase

In the first phase, we conducted semi-structured interviews with 12 experts in education and sustainability. These experts were selected using snowball sampling, ensuring a diverse and knowledgeable participant pool. The interview questions focused on identifying the characteristics and components of a green curriculum. Data collection continued until theoretical saturation was reached, indicating comprehensive coverage of the topic. Additionally, we conducted a documentary analysis of 14 published articles and a dissertation related to the green curriculum, using content analysis techniques (Aithal and Rao, 2016; Capdevila *et al.*, 2002; Chakraborty *et al.*, 2018; Cotgrave and Alkhaddar, 2006; Haigh 2005, Karami *et al.*, 2020; Louw, 2013; Okaka, 2016; Okaka, 2007; Şahin, 2008; Şahin *et al.*, 2009; Torre *et al.*, 2017; Wemmenhove and de Groot, 2001; Wu, 2011; Xiong *et al.*, 2013). The accuracy of this procedure was verified through constant comparison (Schilling, 2006). Thus, 10% of all selected documents were examined by two researchers at the same time using research tools (including index cards proposed by Padang *et al.*, 2018) to systematically gather and organize data, ensuring a structured and efficient approach to documentary analysis. Then, the results were compared. Since more than 90% of the results were similar, the accuracy of the research tool was confirmed. To analyze the data, content analysis was used according to Borg and Gall (1984). Accordingly, the texts of both the interviews and documents were carefully studied and, firstly, in the open coding, all statements illustrating characteristics of the

green curriculum were extracted. In the next step, categorization was done thorough the axial coding. Considering that the basis of this research was the key competencies of education for the 21st century, central coding was done on this basis.

Quantitative Phase

In the second phase, we employed the Lawshé method to assess the content validity of the green curriculum characteristics. A questionnaire containing the identified characteristics was distributed to a panel of 40 experts, who evaluated each item as “necessary”, “useful but unnecessary”, or “unnecessary”. The Content Validity Ratio (CVR) was calculated for each item using the following formula:

$$CVR = \frac{ne - N/2}{N/2} \quad (1)$$

Where, (ne) is the number of panelists indicating “necessary” and (N) is the total Number of panelists. The minimum acceptable value of CVR for a panel of 40 members is 0.29 (Paykari *et al.*, 2018). Items with lower values were omitted.

Additionally, the Content Validity Index (CVI) was calculated to assess the simplicity, clarity, and relevance of the characteristics. The CVI was determined using the following formula:

$$CVI = \frac{\text{The number of experts giving rate 3 or 4}}{\text{Total number of experts}} \quad (2)$$

Panel members rated each item on a 4-point scale, and the minimum acceptable CVI value for the remaining items in the questionnaire was 0.79 (Munro, 2005).

RESULTS

Descriptive Findings

To determine the characteristics of the green curriculum, we conducted interviews

**Table 1.** Concepts extracted from interviews regarding the green curriculum characteristics.

Concepts extracted from interviews	Number of citations
Emphasis on creating a positive attitude toward environmental issues among students	6
Increasing students' awareness of the current environmental issues in the world	5
Teaching a critical attitude towards behaviors and activities that affect the environment	5
Benefiting from educators who believe in sustainability	5
Considering social responsibility in the content of the green curriculum	5
Considering the principles of sustainability and environmental protection in the curriculum evaluation stage	5
Providing the opportunity for students to respond to environmental challenges in nature	5
Developing a curriculum based on the ecological needs of the society	5
Using teaching-learning approaches based on interaction in order to promote the spirit of empathy in solving environmental hazards among learners	4
Emphasis on problem-oriented education based on solving environmental problems	4
Teaching the operational principles of paying attention to sustainability in any specialized work related to people's profession	3
Improving people's creativity in solving environmental challenges	3
Emphasizing the necessity of environmental protection in the green curriculum	3
Emphasis on minimizing environmental risks	3
Emphasis on systemic thinking and attention to the relationship between environmental components	3
Paying attention to the education of citizenship or global citizen in the content of the green curriculum	3
Considering the principles of sustainability and environmental protection in the curriculum design phase	3
Introducing knowledge resources related to sustainability to learners as auxiliary resources	3
Introducing knowledge resources related to sustainability to learners as main resources	3
Introducing knowledge resources related to sustainability to learners as non-curriculum resources	3
Enabling the students to consider the consequences of personal behaviors and paying attention to the direct effects of these behaviors	2
Enabling the students to consider the consequences of personal behaviors and paying attention to the indirect effects of these behaviors	2
Integrating different aspects of sustainable development in the content of the green curriculum	2
Teaching the sustainability skills to students (students' ability to combine the principles of sustainability with all aspects of life)	2
Considering the issue of social justice (rights of the next generation) in the content of the green curriculum	2
Considering the issue of environmental culture in the content of the green curriculum	2
Encouraging students to learn lifelong environmental issues	2
Focus on changing students' behavior to achieve sustainability in society	2
Using educational resources related to the environment	1
Informing students about political relations effective on environmental risk management	1
Creating an opportunity for students to exchange their experiences related to environmental management	1
Considering topics related to personal adaptation in the content of the green curriculum	1
Emphasizing public interest repeatedly in the curriculum	1
Encouraging students to pursue environmentally friendly trans-sectoral activity	1
Encouraging students to consider sustainability in economic activities	1
Considering the principles of sustainability and environmental protection in the curriculum implementation phase	1
Using environmental symbols and signs in the learning environment	1
Encouraging students to consider future consequences of various activities	1

with 12 experts in the field of curriculum planning. The total duration of these interviews was 477 minutes, averaging 39.7 minutes per interview. To supplement and enrich the data obtained from the interviews,

we also reviewed a set of related documents. These documents included 15 research studies published in national and international databases, predominantly articles in English, along with a doctoral

Table 2. Concepts extracted from documents regarding the green curriculum characteristics.

Concepts extracted from the reviewed documents	Number of citations
Using environmental symbols and signs in the learning environment*	8
Providing environmental knowledge originated from high-quality research	4
Increasing students' awareness of the current environmental issues in the world*	4
Empowering students for addressing real environmental issues or concerns	4
Emphasis on systemic thinking and attention to the relationship between environmental components*	4
Benefiting from educators who believe in sustainability*	3
Teaching the operational principles of paying attention to sustainability in any specialized work related to people's profession*	2
Developing practical guidelines for environmental education	2
Creating a positive attitude toward environmental protection among students	2
Encouraging students to learn lifelong environmental issues*	2
Integrating green concepts into the curriculum	2
Explaining the philosophy and basic concepts of the green curriculum	2
Considering the principles of sustainability and environmental protection in the curriculum evaluation stage*	2
Considering optional courses for students who are interested in gaining expertise in environmental protection	2
Equipping students with the knowledge and skills to monitor environmental problems and concerns	2
Emphasizing the necessity of environmental protection in the green curriculum*	1
Encouraging students to consider sustainability in economic activities*	1
Defining homework related to the environment to encourage students 'short-term achievements	1
Considering the principles of sustainability and environmental protection in the curriculum implementation phase*	1
Explaining sustainability in various industrial processes	1
Explaining sustainability in various social processes	1
Providing the opportunity for students to respond to environmental challenges in nature*	1
Introducing and teaching the use of green technologies	1

* Common concepts in both interviews and documents. **Note:** More explanations about the characteristics in Tables 1 and 2 is provided in the Appendix (1).

dissertation and a scientific research article in Persian. Detailed bibliographic information on these sources is provided in Appendix 1.

Inferential Findings

The content analysis of the interviews resulted in the identification of 38 characteristics of the green curriculum. Table 1 summarizes the key concepts extracted from the interviews.

In the content analysis of the documents related to the green curriculum, 23 characteristics of the green curriculum were extracted. Among these characteristics, there are 11 similar items to those extracted from the interviews, marked with (*) in Table (2).

In this research, the key competencies of education for the 21st century (Shaeffer, 2007) were used as a theoretical framework. Accordingly, axial coding was performed to check the alignment of all extracted characteristics with these competencies (see Table 3). Subsequently, the values of Content Relevance Value (CRV) and Content Validity Index (CVI) were calculated. In the category of "learning to know," the CRV values for the two items were less than 0.29. However, all items had an acceptable CVI value.

To summarize the key findings, it can be mentioned that this study systematically identified 50 characteristics of a green curriculum through comprehensive expert interviews and an extensive review of

**Table 3.** Adjusting green curriculum characteristics with key competencies of education for the 21st century and the amount of CRV and CVI.

Key competencies of education for the 21st century	Green curriculum characteristics	CRV	CVI
Learning to know	Providing environmental knowledge originated from high-quality research	0.900	1
	Using educational resources related to the environment	0.944	0.875
	Informing students about political relations effective on environmental risk management	0.589	0.923
	Increasing students' awareness of the current environmental issues in the world	0.900	0.897
	Integrating green concepts into the curriculum	0.850	0.925
	Explaining the philosophy and basic concepts of the green curriculum	0.487	0.900
	Benefiting from educators who believe in sustainability	0.589	0.925
	Introducing knowledge resources related to sustainability to learners as auxiliary resources	0.550	0.897
	Introducing knowledge resources related to sustainability to learners as main resources	0.650	0.950
	Introducing knowledge resources related to sustainability to learners as non-curriculum resources	0.250*	0.875
	Considering the principles of sustainability and environmental protection in the curriculum implementation phase	0.350	0.890
	Considering optional courses for students who are interested in gaining expertise in environmental protection	0.800	0.850
	Considering the principles of sustainability and environmental protection in the curriculum design phase	0.250*	0.875
	Equipping students with the knowledge and skills to monitor environmental problems and concerns	0.800	0.925
	Integrating different aspects of sustainable development in the content of the green curriculum	0.846	0.900
	Considering the principles of sustainability and environmental protection in the curriculum evaluation stage	0.500	0.875
Learning to do	Developing practical guidelines for environmental education	0.894	0.950
	Teaching the operational principles of paying attention to sustainability in any specialized work related to people's profession	0.857	0.951
	Improving people's creativity in solving environmental challenges	0.785	0.961
	Using environmental symbols and signs in the learning environment	0.700	0.950
	Encouraging students to learn lifelong environmental issues	0.743	0.925
	Emphasis on problem-oriented education based on solving environmental problems	0.735	0.950
	Defining homework related to the environment to encourage students 'short-term achievements	0.726	0.916
	Providing the opportunity for students to respond to environmental challenges in nature	0.722	0.980
	Introducing and teaching the use of green technologies	0.719	0.865

Table 3 continued...

Continued of Table 3.

Key competencies of education for the 21st century	Green curriculum characteristics	CRV	CVI
	Focus on changing students' behavior to achieve sustainability in society	0.716	0.895
	Explaining sustainability in various industrial processes	0.700	0.975
	Explaining sustainability in various social processes	0.641	0.925
	Empowering students for addressing real environmental issues or concerns	0.600	0.975
	Emphasizing the necessity of environmental protection in the green curriculum	0.850	0.948
	Encouraging students to consider sustainability in economic activities	0.478	0.923
Learning to be	Teaching a critical attitude towards behaviors and activities that affect the environment	0.692	0.975
	Enabling the students to consider the consequences of personal behaviors and paying attention to the direct effects of these behaviors	0.789	0.925
	Enabling the students to consider the consequences of personal behaviors and paying attention to the indirect effects of these behaviors	0.700	0.925
	Teaching the sustainability skills to students (students' ability to combine the principles of sustainability with all aspects of life)	0.750	0.950
	Emphasis on creating a positive attitude toward environmental issues among students	0.800	.948
	Using teaching-learning approaches based on interaction in order to promote the spirit of empathy in solving environmental hazards among learners	0.850	0.948
Learning to live together	Creating an opportunity for students to exchange their experiences related to environmental management	0.700	0.973
	Creating a positive attitude toward environmental protection among students	0.750	0.925
	Considering social responsibility in the content of the green curriculum	0.600	0.947
	Emphasis on minimizing environmental risks	0.794	0.925
	Emphasis on systemic thinking and attention to the relationship between environmental components	0.641	0.950
	Emphasizing public interest repeatedly in the curriculum	0.550	1
	Considering topics related to personal adaptation in the content of the green curriculum	0.692	0.925
	Considering the issue of social justice (rights of the next generation) in the content of the green curriculum	0.692	1
	Considering the issue of environmental culture in the content of the green curriculum	0.794	0.950
Learning to transform oneself and society	Encouraging students to pursue environmentally friendly trans-sectoral activity	0.600	0.950
	Paying attention to the education of citizenship or global citizen in the content of the green curriculum	0.743	1
	Developing a curriculum based on the ecological needs of the society	0.743	0.923
	Encouraging students to consider future consequences of various activities	0.794	0.973



relevant literature. These characteristics were meticulously aligned with the key competencies essential for the 21st-century education. The validity of each characteristic was rigorously assessed using the CVR and CVI as per the Lawshe method. These findings provide a robust framework for the development of a green curriculum that is both contemporary and educationally sound.

DISCUSSION

The growing importance of sustainability necessitates that higher education systems integrate environmental principles into their curricula. Traditionally, universities have focused on sustainability in the physical environment, but the influence of higher education on human resources is equally significant. In today's world, the role of human resources in driving societal progress cannot be underestimated. Therefore, competencies such as learning to know, learning to do, learning to live together, learning to be, and learning to transform oneself and society are essential (Shaeffer, 2007). This study classifies the characteristics of a green curriculum based on these dimensions.

Learning to Know

This competency involves expanding learners' knowledge to create a well-informed, proactive, and engaged population capable of addressing global environmental challenges. For example, empowering learners to identify environmental problems is a key characteristic of the green curriculum, as confirmed by previous research (Pe'er *et al.*, 2007; Otto and Pensini, 2017). This competency includes providing environmental knowledge, encouraging lifelong learning, and introducing reliable sources for acquiring knowledge. Supporting researchers in environmental and curriculum development fields and integrating sustainability content

across all academic disciplines are recommended. Additionally, the green curriculum should empower learners to identify, recall, explain, discuss, and evaluate environmental issues, concerns, values, and problems at different levels from local to global society.

Learning to Do

This competency emphasizes the application of knowledge in everyday life, leading to the consolidation of learning and societal benefits from trained human resources. In green learning, which empowers individuals to fulfil their citizenship roles, problem-oriented education is crucial. Teaching through problem-solving methods, as highlighted by various researchers (Gabrys *et al.*, 2020; Avvisati and Borgonovi, 2020; Rebello *et al.*, 2017), involves students in environmental issues and encourages them to find solutions. This approach should be integrated into curriculum design, with practical assignments and projects that have tangible environmental management outcomes. For instance, arranging scientific short trips to areas impacted by human activities can enhance practical learning.

Learning to Live Together

This competency prepares individuals for a better life by empowering them to make informed decisions and understand the consequences of their behaviors on the environment. Critical thinking and the ability to evaluate personal behaviors are essential. Educational systems should provide opportunities for learners to apply sustainability principles in practical projects and share their achievements with others. Emphasizing responsibility, empathy, systemic thinking, and minimizing environmental risks are key characteristics of the green curriculum. For example, teaching the consequences of personal

behaviors and fostering a critical view towards activities affecting the environment are crucial.

Learning to Transform Oneself and Society

This highest level of learning involves seeking positive changes in oneself and society. In the context of environmental and sustainability issues, this means encouraging learners to engage in environmentally friendly extracurricular activities and collaborate with various societal sectors. The green curriculum should prepare students for interdisciplinary and trans-organizational work, fostering self-transformation and societal improvement. For instance, training students to work across sectors and value interdisciplinary thinking can significantly contribute to societal transformation.

CONCLUSIONS

This study identified and validated 48 key characteristics of the green curriculum aligned with UNESCO's five critical competencies for 21st-century education. The findings confirm that sustainability education should extend beyond environmental infrastructure to focus on human capacity building in higher education. The proposed framework provides practical guidance for integrating sustainability principles into curricula and for developing students' critical, creative, and transformer competencies.

The main limitation of our study was the reliance on the opinions of Iranian experts due to constraints in time, budget, and access to international researchers. Despite these constraints, we ensured the comprehensiveness and validity of our findings by reviewing publications from global experts. This approach enriched our study with diverse perspectives, enhancing its overall quality and relevance.

Future Research Directions

Future research should focus on expanding international collaboration and conducting longitudinal studies to track the long-term impact of green curriculum initiatives. Interdisciplinary approaches and technology integration can enhance curriculum delivery, while policy impact analysis and cultural context studies can identify the best practices. Exploring practical applications of green curriculum principles, methods to increase student engagement, and new assessment methods are also crucial. Additionally, involving the community in green curriculum initiatives can provide valuable insights for curriculum developers. These suggestions aim to build on the current findings and further promote sustainability and environmental awareness.

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Appendix 1: Explanation of the green curriculum characteristics.

Creating a positive attitude: Encouraging students to develop a favourable view of environmental issues.
Increasing awareness: Educating students about current global environmental problems.
Teaching critical attitude: Instilling a critical perspective on behaviours impacting the environment.
Sustainability-minded educators: Utilizing teachers who prioritize sustainability.
Social responsibility: Integrating social responsibility into the green curriculum.
Sustainability in evaluation: Including sustainability principles in curriculum assessments.
Responding to challenges: Allowing students to address environmental challenges in nature.
Ecological needs-based curriculum: Designing curriculum based on societal ecological needs.
Interactive approaches: Promoting empathy through interactive teaching methods.
Problem-oriented education: Focusing on solving environmental problems through education.
Operational principles: Teaching sustainability principles relevant to professional work.
Creativity in challenges: Enhancing creativity in solving environmental issues.
Necessity of protection: Emphasizing the importance of environmental protection.
Minimizing risks: Focusing on reducing environmental risks.
Systemic thinking: Encouraging understanding of the interconnections within the environment.
Global citizenship: Including global citizenship education in the curriculum.
Sustainability in design: Considering sustainability in curriculum design.
Auxiliary resources: Introducing supplementary sustainability resources.
Main resources: Providing primary sustainability resources.
Non-curriculum resources: Offering non-curricular sustainability resources.
Direct consequences: Teaching students to consider the direct effects of their behaviours.
Indirect consequences: Teaching students to consider the indirect effects of their behaviours.
Sustainable development: Integrating sustainable development aspects into the curriculum.
Sustainability skills: Teaching students to apply sustainability principles in life.
Social justice: Addressing social justice in the curriculum.
Environmental culture: Incorporating environmental culture into the curriculum.
Lifelong learning: Encouraging lifelong learning about environmental issues.
Behaviour change: Focusing on changing behaviours to achieve societal sustainability.
Educational resources: Using environment-related educational materials.
Political relations: Informing students about political factors in environmental risk management.
Experience exchange: Creating opportunities for students to share environmental management experiences.
Personal adaptation: Including personal adaptation topics in the curriculum.
Public interest: Repeatedly emphasizing public interest in the curriculum.
Trans-sectoral activity: Encouraging environmentally friendly activities across sectors.
Economic sustainability: Promoting sustainability in economic activities.
Implementation phase: Considering sustainability in curriculum implementation.
Environmental symbols: Using environmental symbols in the learning environment.
Future consequences: Encouraging consideration of future impacts of activities.
High-quality research: Providing knowledge from high-quality environmental research.
Addressing real issues: Empowering students to tackle real environmental concerns.
Practical guidelines: Developing practical guidelines for environmental education.
Positive attitude: Creating a positive attitude toward environmental protection.
Green concepts: Integrating green concepts into the curriculum.
Philosophy and concepts: Explaining the philosophy and basic concepts of the green curriculum.
Optional courses: Offering optional courses for environmental protection expertise.
Monitoring skills: Equipping students with skills to monitor environmental issues.
Environment-related homework: Assigning environment-related homework for short-term achievements.
Industrial sustainability: Explaining sustainability in industrial processes.
Social sustainability: Explaining sustainability in social processes.
Green technologies: Introducing and teaching the use of green technologies.

تبیین و اعتبارسنجی ویژگی‌های برنامه درسی سبز مبتنی بر شایستگی‌های حیاتی آموزش برای قرن بیست و یکم

مریم حسینی لارگانی، حسین تیمور، و مهسا سعدوندی

چکیده

هدف این پژوهش، تبیین و اعتبارسنجی ویژگی‌های برنامه درسی سبز بر اساس شایستگی‌های حیاتی مورد نیاز آموزش برای قرن بیست و یکم است. پژوهش با رویکرد ترکیبی (کیفی-کمی) انجام شد. در بخش کیفی، متخصصان دارای تجربه در حوزه پایداری در آموزش عالی ایران از طریق نمونه‌گیری گلوله‌برفی انتخاب و داده‌ها از راه مصاحبه‌های نیمه‌ساختاریافته گردآوری و با روش تحلیل محتوا تحلیل شدند. ویژگی‌های به‌دست‌آمده در بخش کمی با استفاده از روش لاوشه اعتبارسنجی شدند. بدین منظور، ۴۰ نفر از اعضای هیئت علمی و دانشجویان دکتری علوم تربیتی به‌صورت تصادفی برای ارزیابی تناسب این ویژگی‌ها انتخاب شدند. نتایج نشان داد از میان ۵۰ ویژگی شناسایی‌شده، ۴۸ ویژگی تأیید شدند. همچنین هر یک از پنج شایستگی آموزش برای قرن بیست و یکم (یادگیری برای دانستن، یادگیری برای انجام دادن، یادگیری برای بودن، یادگیری برای باهم‌زیستن، و یادگیری برای دگرگونی خود و جامعه) با چندین ویژگی برنامه درسی سبز هم‌راستا هستند. بر اساس یافته‌ها، برای عملیاتی‌کردن این شایستگی‌ها پیشنهاد می‌شود مدیریت محیط‌زیست در برنامه‌های آموزشی گنجانده شود، پروژه‌های عملی برای دانشجویان طراحی گردد، و تعامل آنان با سازمان‌های محیط‌زیستی و جوامع پژوهشی جهانی تقویت شود.

Identifying the Dimensions of Empowerment and Their Impacts on Food Security in Rural Women

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ABSTRACT

The present study was conducted to identify the empowerment dimensions of female-headed households in rural areas and their effects on food security in Iran. Based on the data collected from the Iranian Statistical Center, the population of rural women-headed households of Tehran Province included 495 individuals, from which 216 were selected using the stratified random sampling method with proportional allocation. A questionnaire was designed by researchers and its validity and reliability was confirmed by confirmatory factor analysis and Cronbach method, respectively. The data were collected from November 2020 to July 2021. Structural equation modeling was used to analyze and estimate the relationships among multiple variables. The results confirmed the dimensions of empowerment as grouping work and communication skills, creativity and solving problem, commitment and responsibility, information and specific knowledge, technical skills and operational work, including psychological, social, political, economic, managerial, and educational factors and their effects on food security. The highest effects on food security belonged to commitment and responsibility, economic factors, and grouping work, respectively. Job empowerment predicted 75% variance in food security. It is suggested to consider job empowerment of female-headed households in rural areas to decrease food insecurity.

Keywords: Female-headed households, Gender and food security, Social and economic empowerment.

INTRODUCTION

Poverty is a global challenge that mainly influences human societies in rural areas, especially in non-developing countries and rural women-headed households (Abrar ul haq *et al.*, 2019). Women-headed households are usually faced with several challenges and are one of the most vulnerable groups in different societies in terms of poverty and food security (Daoud *et al.*, 2019; Dunga, 2020). Food security is defined as the permanent physical, social and economic access to sufficient, safe and nutritious food to supply dietary requirements and food preferences for an

active and healthy life (Galiè *et al.*, 2019). It is estimated that 800 million people are undernourished across the globe who do not receive adequate nutritional content and calories (Pakravan-Charvadeh *et al.*, 2020). Women-headed households are more vulnerable to food insecurity compared to men-headed households due to low presence in the labor market and productive activities, housekeeping, and child care (Mallick and Rafi, 2010). A major portion of women-headed households lives in developing countries and in rural regions (Van Eerdewijk *et al.*, 2017). Social and cultural factors and expectations have prevented rural women from participating in the

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development programs (Sarani *et al.*, 2013). Despite the key role of rural women-headed households as suppliers of food security, income earners, and caretakers of households and children, they have been disempowering in society. Empowerment might be a key factor in improving food security in rural women-headed households in developing countries.

Empowerment is defined as controlling decision-making about his and/or her life and giving the ability to intervene in all life challenges (Baig *et al.*, 2018). This conception not only comprises extrinsic control but it also involves the development of intrinsic capabilities (Abrar-ul-Haq *et al.*, 2018). It initiates foundations for understanding available opportunities to women. Notably, most studies have emphasized the positive effects of empowerment in increasing food security and decreasing food insecurity (Asitik and Abu, 2020; Galiè *et al.*, 2019; Ntenkeh *et al.*, 2022; Sharaunga *et al.*, 2016), factors affecting empowerment are not the same in other countries. It is essential to identify factors empowering women in each country. Rural women in Iran mostly perform housekeeping, care of children, farming, tailoring, carpet weaving, and work at home. It was recently reported that 32% of Iranian families faced with food insecurity (Pakravan-Charvadeh *et al.*, 2020).

Although previous studies have emphasized the role of empowerment in increasing food security, they lack specific focus on the challenges and empowerment dimensions for women-headed households in rural areas, especially in countries like Iran. This study aimed to identify the unique empowerment dimensions affecting food security specifically for rural women-headed households in Iran, contributing to a localized understanding of the issue.

The research brings an innovative approach by considering not just economic, but other possible dimensions to women's empowerment and food security in rural areas. The study aimed to identify empowerment dimensions affecting food

security in rural women-headed households in Iran.

MATERIALS AND METHODS

Empowering Factors

Empowerment is a multi-dimensional factor and it can influence food security. It comprises intrinsic capabilities such as empowerment factors and job skills and communications that develop empowerment (Asitik and Abu, 2020; Galiè *et al.*, 2019; Ntenkeh *et al.*, 2022; Sharaunga *et al.*, 2016). Economic factors are one of the most important factors in the life of the women-headed households in rural areas (Sharma, 2019). Income generation, financial independence, and control over finances directly improve food security. Another factor affecting women's empowerment is social factors, which allow women to develop their lives in a holistic framework. These factors help women to (Sharma, 2019). Participation in social networks and community involvement enhances access to resources and support, and better food security. Psychological factors are another component of empowerment. It was reported that the increase in feelings of self-efficacy among people help to identify intrinsic empowerment (Muduli and Pandya, 2018). Self-efficacy, resilience, and adaptability lead to proactive behavior and greater food security. Political factors may influence women's empowerment. The political empowerment of women is a result of awakening at the individual and social levels to enable women who live with dignity (Sharma, 2020). Involvement in political processes helps women advocate for better governance and policies affecting food access. Women's education and using educational systems is an important strategy for empowering women (Savari *et al.*, 2020). Education and skills development provide better employment opportunities, indirectly improving food security. Women with management ability may have a better

feeling of self and their empowerment. Decision-making and resource management strengthen women's ability to ensure household food security.

H1: Economic, social, psychological, political, educational and managerial factors (empowering factors) are dimensions of empowerment and can independently affect food security of women-headed households in rural areas.

Job Skills and Communications

Participation of women in grouping works and using other experiences and opinions may empower rural women. The ability to communicate with others seems to be a strategy for empowering women. Other factors associated with empowerment may be creativity and solving problems. Creativity is the production of new and profitable ideas by persons in a working environment. Empowered people prefer to solve their problems and use creative solutions. Commitment and responsibility are important factors that may influence empowerment. Responsible and committed women try to maintain values in a working environment, correctly perform their tasks, show their interest in learning new subjects and have a positive view of working environments. It was reported that communication channels create several jobs in rural areas for Iranian women (Savari *et al.*, 2020). Thus, access to information and specific knowledge may empower rural women and indirectly food security. Women with technical skills can produce jobs for themselves. Such skills can decrease injuries and damage in working environments. They perform their job tasks in minimum time and decrease their costs.

H2: Creativity and solving problems, communication skills and grouping work, commitment and responsibility, information and specific knowledge and practical work and technical skills (Job skills and communications) are dimensions of empowerment and can independently affect

food security of women-headed households in rural areas.

We hypothesized that job skills and communications and empowering factors influence food security.

H3: Empowerment influences food security of women-headed households in rural areas.

A study investigated the effects of women empowerment in rural areas in South Africa and showed that female-headed households had better economic conditions, physical capital empowerment, psychological empowerment and farm financial management skills had better food security (Sharaunga *et al.*, 2016). An original study in Iran investigated the role of educational channels in improving household food security in Iranian rural women (Savari *et al.*, 2020). Recently, a study showed a positive relationship between women's empowerment and food security in Cameroon (Ntenkeh *et al.*, 2022). Another study investigated determinants of food security among female-headed households in South Africa and showed that age, race, income and size of the household had significant effects on food security (Dunga, 2020). Positive relation was reported between women's empowerment and food security and emphasized social, cultural, economic and educational factors for improving empowerment (Meti and Sathish, 2016). Another study found a significant positive relationship between the economic dimension of empowerment and food security in communities in Tanzania (Galiè *et al.*, 2019). It was reported that empowered women enhance household food security (Asadullah and Kambhampati, 2021). A positive link has been found between women's empowerment and food security (Aziz *et al.*, 2022). It has been reported that socio-economic factors play significant roles in women's food security (Clement *et al.*, 2019). The current study investigates comprehensive factors affecting empowerment in Iranian women that have not been previously investigated in female-headed households in rural areas.

**MATERIALS AND METHODS****Measurements****Statistical Population, Sample and Sampling Method**

This applied descriptive study aimed to explore the empowerment dimensions of female-headed households in Tehran Province, Iran, and their effects on food security. The statistical population comprised 495 women, based on data from the Statistical Center of Iran. Tehran Province was divided into ten rural districts, each treated as a separate stratum. A stratified sampling method with proportional allocation was used to ensure appropriate representation from each district. The sample size was determined using Cochran's formula, and 216 women were selected to participate in the study.

The indicators used in the two self-constructed questionnaires for empowerment and food security are presented in Table 1.

Additionally, demographic variables such as age, education, employment status, family size, and annual income were collected to provide context for the analysis. The data were collected from November 2020 to July 2021.

Validity and Reliability of the Questionnaires

To ensure the validity and reliability of the instruments, Confirmatory Factor Analysis (CFA) was conducted on all theoretical constructs. CFA was employed to validate the measurement models of both empowerment and food security, following the guidelines of previous studies (Magnier-Watanabe *et al.*, 2020; Yang and Hsu,

Table 1. The indicators used in the two self-constructed questionnaires.

Indicators	Number of items	Scoring	References
Empowerment		Likert scale (1-5)	
Grouping work and communication skills	7		Authors
Creativity and solving problem	6		Authors
Commitment and responsibility	6		Authors
Information and specific knowledge	5		Authors
Technical skills and operational work	5		Naseri <i>et al.</i> , (2020)
Psychological factors	9		Naseri <i>et al.</i> , (2020)
Social factors	9		Naseri <i>et al.</i> , (2020)
Political factors	5		Naseri <i>et al.</i> , (2020)
Economic factors	6		Naseri <i>et al.</i> , (2020)
Managerial factors	7		Authors
Educational factors	7		Authors
Food security		Likert scale (0-5)	
Accessibility	6		FAO (2016) WFP (2018)
Availability	6		Coates <i>et al.</i> , (2007) FAO (2016) USDA (2020)
Utilization	7		WHO (2019)
Stability	4		FAO (2016) WFP (2018)

2018). Reliability was assessed using Cronbach's alpha, ensuring internal consistency of the scales.

Data Analysis

Structural Equation Modeling (SEM) was used to analyze the relationships between the empowerment dimensions and food security. Both CFA and SEM were conducted using AMOS software (version 24). SEM allowed for the estimation of direct and indirect effects among multiple variables, providing a comprehensive understanding of how different dimensions of empowerment influence food security outcomes.

RESULTS

Descriptive Statistics

The results indicated that the average age of female-headed households in rural areas was 48.28 years, with a standard deviation of 11.55 years. The majority of the women were between 31 and 60 years old. Most had only primary education (41.20%), while a smaller proportion had attained a high school diploma or higher education. A significant portion of the women were unemployed (24.53%), with the remainder engaged in various occupations, including service work (18.51%), tailoring (15.27%), peddling (13.88%), carpet weaving (11.57%), farming (2.31%), and other jobs (13.93%). The average annual income of these households was 85 million IRR. It is also noteworthy that unemployed women were under the supervision of supporting institutions.

The means and standard deviations for the constructs were as follows: grouping work and communication skills (2.77 ± 0.88), creativity and solving problem (3.01 ± 0.90), commitment and responsibility (3.26 ± 1.02), information and specific knowledge

(2.94 ± 0.94), technical skills and operational work (3.22 ± 1.00), psychological factors (3.16 ± 0.86), social factors (3.08 ± 0.78), political factors (2.63 ± 1.01), economic factors (2.24 ± 0.86), managerial factors (2.75 ± 0.81), educational factors (2.70 ± 0.99), access (2.23 ± 0.84), availability (2.42 ± 0.80), utilization (2.69 ± 1.10) and stability (2.57 ± 0.74).

Table 2 displays the correlations between these constructs. The analysis revealed positive correlations among all variables.

Analysis of the Measurement Models

The validity and reliability of the individual measurement models were assessed following the methodologies outlined by Yang and Hsu (2018). The results are summarized in Table 3. CFA and model fit indices confirmed that all items appropriately fit their respective constructs. According to previous research (Magnier-Watanabe *et al.*, 2020), the recommended fit indices are as follows: normed chi-square less than 3.00, Root Mean Square Residual (RMR) less than 0.09, Normed Fit Index (NFI) greater than 0.90, and IFI and TLI greater than 0.95. Hair *et al.* (2010) suggest that the values for each construct should fall between 0.5 and 0.9, with reliability values exceeding 0.7. The obtained values in this study were all above 0.7, confirming the internal consistency and reliability of each scale.

Structural Equation Modeling

To avoid ambiguity and complexity, we considered the mean of constructs and did not use items for SEM. A SEM was built and the results are shown in Figure 1. The results for model-fitting showed that empowerment and food security had a good fit for the data with fit indices ($\chi^2/df = 1.86$, CFI = 0.98; NFI = 0.98; IFI = 0.97; TLI = 0.98; RMR = 0.032; RMSEA = 0.036).

The results in Figure 2 for model-fitting showed that empowerment dimensions and

Table 2. Correlation between constructs.^a

GC	CS	CR	IS	TS	PF	SF	POF	EF	MF	EDF	ACC	AVA	UTI	ST
GC	0.893 ^{***}	0.836 ^{***}	0.798 ^{***}	0.792 ^{***}	0.506 ^{***}	0.509 ^{***}	0.298 [*]	0.363 ^{***}	0.389 ^{***}	0.360 ^{***}	0.373 ^{***}	0.133 [*]	0.523 ^{***}	0.383 ^{***}
CS		0.873 ^{***}	0.807 ^{***}	0.773 ^{***}	0.505 ^{***}	0.520 ^{***}	0.272 ^{***}	0.325 ^{***}	0.506 ^{***}	0.369 ^{***}	0.379 ^{***}	0.135 [*]	0.532 ^{***}	0.269 ^{***}
CR			0.886 ^{***}	0.832 ^{***}	0.529 ^{***}	0.520 ^{***}	0.265 ^{***}	0.373 ^{***}	0.538 ^{***}	0.378 ^{***}	0.350 ^{***}	0.150 ^{***}	0.557 ^{***}	0.506 ^{***}
IS				0.865 ^{***}	0.533 ^{***}	0.533 ^{***}	0.269 ^{***}	0.335 ^{***}	0.531 ^{***}	0.532 ^{***}	0.530 ^{***}	0.139 [*]	0.537 ^{***}	0.533 ^{***}
TS					0.512 ^{***}	0.372 ^{***}	0.238 ^{***}	0.333 [*]	0.523 ^{***}	0.510 ^{***}	0.505 ^{***}	0.133 [*]	0.536 ^{***}	0.518 ^{***}
PF						0.765 ^{***}	0.518 ^{***}	0.566 ^{***}	0.725 ^{***}	0.685 ^{***}	0.372 ^{***}	0.179 [*]	0.508 ^{***}	0.533 ^{***}
SF							0.593 ^{***}	0.325 ^{***}	0.675 ^{***}	0.629 ^{***}	0.376 ^{***}	0.163 [*]	0.533 ^{***}	0.512 ^{***}
PO								0.503 ^{***}	0.363 ^{***}	0.352 ^{***}	0.263 ^{***}	0.166 [*]	0.313 ^{***}	0.360 ^{***}
F									0.563 ^{***}	0.306 ^{***}	0.317 ^{***}	0.215 ^{**}	0.338 ^{**}	0.336 ^{***}
EF										0.739 ^{***}	0.512 ^{***}	0.195 [*]	0.372 ^{***}	0.386 ^{***}
MF											0.501 ^{***}	0.130 [*]	0.390 ^{***}	0.526 ^{***}
ED														
F														
AC												0.339 ^{***}	0.693 ^{***}	0.555 ^{***}
C														
AV													0.333 ^{***}	0.332 ^{***}
A														
UT														
I														0.659 ^{***}

^a Grouping work and Communication skills (GC), Creativity and Solving problem (CS), Commitment and Responsibility (CR), Information and Specific knowledge (IS), Technical Skills and operational work (TS), Psychological Factors (PF), Social Factors (SF), Political Factors (POF), Economic Factors (EF), Managerial Factors (MF), Educational Factors (EF), Accessibility (ACC), Availability (AVA), Utilization (UTI) and Stability (ST). Superscripts *, and *** show significant correlation at P<0.05 and P<0.0001, respectively.

Table 3. Constructs and reflective indicators.

Constructs and reflective indicators	Loading
Empowerment ($\chi^2=42.47$, $df=24$; CFI= 0.93; RMR= 0.031; IFI= 0.96; TLI= 0.97)	
Grouping work and communication skills (Cronbach's $\alpha=0.759$; CR= 0.865; AVE= 0.623)	
Reflection of facts in presenting feedback is common in my work environment	0.523
I express my opinion in relation to job issues	0.598
I receive a good reaction along with patience from others.	0.502
I am interested in the transformation of information and experiences to others.	0.523
I have a devotion to solving challenges in the working environment.	0.589
Coordination and integrity are found between my colleagues.	0.569
I use my supervisor's ideas and my colleague's for solving problems.	0.567
Creativity and solving problem (Cronbach's $\alpha=0.796$; CR= 0.802; AVE= 0.551)	
I solve working issues through data collection and analysis.	0.509
I consider various aspects of a problem.	0.521
I use opportunities for creating positive changes in my life.	0.598
I am interested in new experiences and experiments	0.595
I present new strategies for job issues.	0.569
I suggest new strategies for performing job tasks.	0.567
Commitment and responsibility (Cronbach's $\alpha=0.899$; CR= 0.815; AVE= 0.665)	
I am on time in the working environment.	0.595
I try to maintain values in the working environment.	0.502
I correctly conduct working tasks.	0.685
I am interested to increase knowledge and job skills.	0.672
I have a positive view of the working environment.	0.621
I have actively participation in educational periods for improving technical skills.	0.512
Information and specific knowledge (Cronbach's $\alpha=0.752$; CR= 0.785; AVE= 0.515)	
I have sufficient information for equipment and tools in working environment.	0.526
I have obtained general knowledge for my job.	0.612
Job purposes are achieved by required knowledge.	0.570
I need a presence in educational periods for improving information and specific knowledge.	0.572
I have sufficient information for quality and activity standards.	0.525
Technical skills and practical work (Cronbach's $\alpha=0.717$; CR= 0.709; AVE= 0.589)	
I correctly use equipment in the working environment.	0.599
I have standardized job skills.	0.597
Damages and injuries have decreased in the working environment.	0.598
I perform job tasks in minimum time and for improving working quality.	0.602
I use raw materials in a true way.	0.707
Psychological factors (Cronbach's $\alpha=0.717$; CR= 0.741; AVE= 0.576)	
I feel myself to be a valuable human.	0.712
I feel to have several good characteristics.	0.511
I can well conduct several works.	0.541
I have a good view of myself.	0.539
I have abilities for the expression of opinions in family meetings.	0.614
My member family uses my opinions.	0.647
I am a determiner of interactions of my member family with others.	0.615
I have abilities for changing the conditions of my life based on current possibilities.	0.523
I am independent in solving problems.	0.516
Social factors (Cronbach's $\alpha=0.802$; CR= 0.773; AVE= 0.562)	
I am interested in participation in grouping works.	0.506
I have interactions with kinfolk and neighbors.	0.501
I participate in different meetings.	0.605
I consult others for different problems.	0.712
I participate in community-oriented educational classes.	0.597
I have the ability for finding new friends.	0.522
Political factors (Cronbach's $\alpha=0.739$; CR= 0.752; AVE= 0.562)	

Table 3 continued



Continued of Table 3. Constructs and reflective indicators.

Constructs and reflective indicators	Loading
The services given by the village council are efficient for improving my job.	0.553
Political decisions influence my life.	0.552
I participate in elections.	0.551
Decisions of local agents for rural regions influence my life.	0.514
I participate in meetings of people agents and managers.	0.595
Economic factors (Cronbach's α = 0.702; CR= 0.717; AVE= 0.645)	
I have access to facilities and a bank loan.	0.516
I participate in the microfinance credits fund.	0.667
I participate in activities of consumers' co-operative.	0.547
I provide the required equipment and facilities for myself and member family.	0.702
I decide on financial resources and ways for spending them.	0.502
I feel to be valuable women activities in society.	0.540
Managerial factors (Cronbach's α = 0.751; CR= 0.820; AVE= 0.598)	
I have abilities for handling my job.	0.589
I participate in local meetings.	0.597
I have enough ability for supplying local products.	0.606
My job is affecting society.	0.641
I can manage crises in my life.	0.578
I manage economic issues in my life.	0.641
I can manage my assets.	0.529
Educational factors (Cronbach's α = 0.796; CR= 0.824; AVE= 0.591)	
I feel rural women appreciate educational periods.	0.532
I feel that potential trainers educate us.	0.541
Educational contents are in agreement with my requirements.	0.537
It is possible to combine science and practice	0.536
All skills and educations are various.	0.541
I have the ability for learning professional skills.	0.546
I feel educational classes are in agreement with my requirements.	0.527
Food security (χ^2 = 43.12, df= 24; CFI= 0.98; RMR= 0.033; IFI= 0.97; TLI= 0.98)	
Access (Cronbach's α = 0.741; CR= 0.736; AVE= 0.565)	
My required food is in access.	0.632
My required food for my children is in access.	0.541
Various foods are in access to us.	0.571
Food supplier centers are in access.	0.569
Food supplier centers supply enough food.	0.502
Food supplier centers supply high-quality foods.	0.622
Availability (Cronbach's α = 0.741; CR= 0.751; AVE= 0.598)	
I have enough income for purchasing the required foods for my body.	0.571
I have enough income for purchasing the required foods for my children.	0.533
I have enough income for providing dietary diversity.	0.625
Price fluctuations influence dietary diversity.	0.593
My income is one important factor in purchasing interesting foods.	0.576
My saving is affected by purchasing in an emergency condition.	0.588
Utilization (Cronbach's α = 0.912; CR= 0.755; AVE= 0.717)	
Knowing quality affects food utilization.	0.555
Knowing calories affects food utilization.	0.575
Foods with low waste influence their utilization.	0.632
Knowing diets influence food utilization.	0.509
Knowing food benefit influences food utilization.	0.707
An appropriate food program for family members influences food utilization.	0.812
Having an appropriate food program for children influences food utilization.	0.589
Stability (Cronbach's α = 0.763; CR= 0.751; AVE= 0.613)	
Required foods are constantly supplied in the market.	0.645
Foods are scarce in undetermined and unpredictable times.	0.512
Suppliers immediately supply scarce foods.	0.507
Precise mechanisms are considered and performed for keeping stability.	0.596

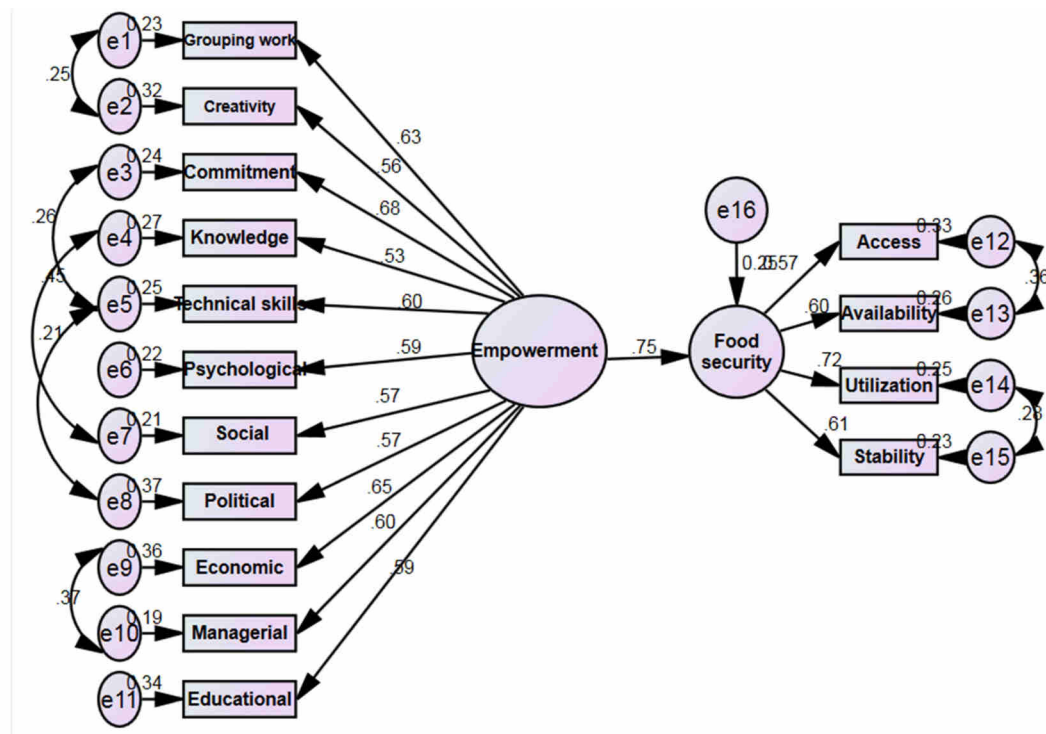


Figure 1. Results of the structural equation modeling for the effect of empowerment on food security.

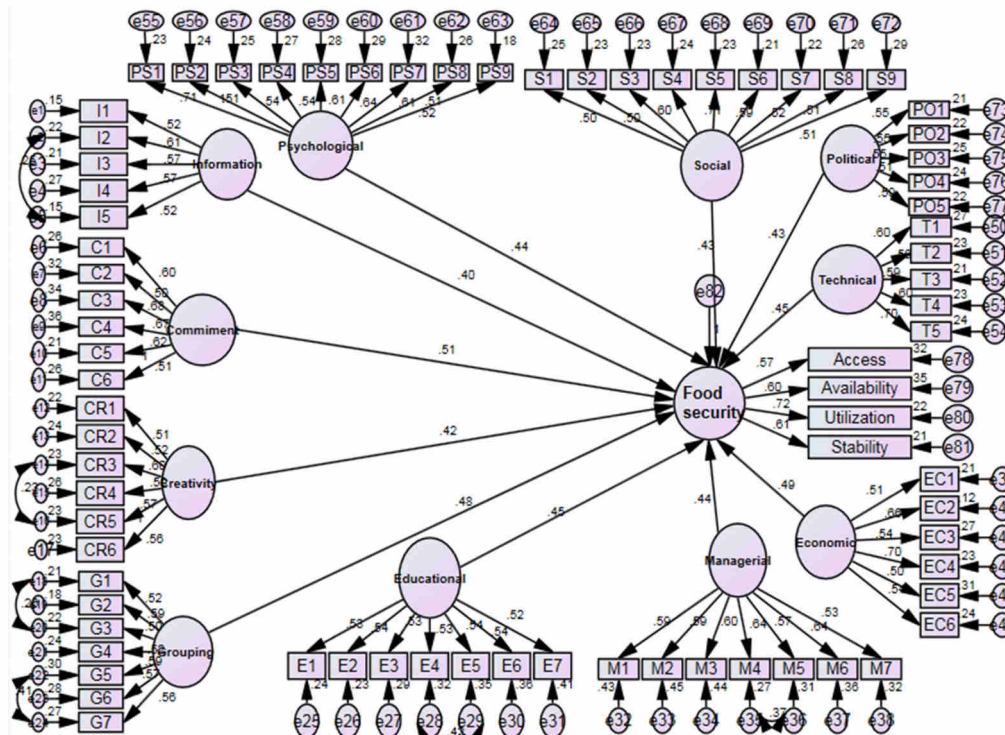


Figure 2. Results of structural equation modeling for the effect of empowerment dimensions on food security.



food security had a good fit for the data with fit indices ($\chi^2/df= 1.71$, CFI= 0.96; NFI= 0.95; IFI= 0.96; TLI= 0.97; RMR= 0.036; RMSEA= 0.041).

The results for the SEM of the effects of empowerment and its dimensions on food security are shown in Table 4, which show that empowerment predicts 75% of the variance in food security. To investigate the hypotheses, we ran another model comprising items, and the results are shown in Table 4 and Figure 2.

The results of the effects of the empowerment construct on food security are shown in Table 4, which confirmed all the hypotheses. All the constructs predicted food security. Commitment and responsibility, economic factors and grouping work predicted 51.00%, 49.00% and 48.00% of the variance of food security, respectively. The results also showed that job skills and communications and empowering factors predicted 61% and 58% of the variance of food security, respectively. The model was run twice. The first run assessed the effects of factors as dimensions of empowerment, which allowed for an evaluation of the overall empowerment effect.

DISCUSSION

The empirical role of empowerment and its dimensions as independent variables in enhancing food security is revealed by this

study. In line with previous research, the findings demonstrate that empowerment accounts for 75.00% variance in food security. (Clement *et al.*, 2019; Sharaunga *et al.*, 2016).

Group work and communication abilities predicted differences in food security and empowerment by 48.00 and 63.00%, respectively. Through improved decision-making, negotiation, and leadership, communication promotes the sharing of information and experiences, thereby increasing empowerment (Mishra and Mishra, 2020). The ability to articulate ideas clearly enhances women's self-esteem and strengthens their contributions to family or community food security plans. Working in groups offers individuals the opportunity to solve problems and exchange experiences. These social partnerships can promote collective empowerment by addressing issues collaboratively, transforming women's individual abilities into community-based solutions for food security while also providing emotional and practical support.

Food security and empowerment are influenced by creativity and problem-solving. Women with creative minds can develop innovative ways to raise living standards in rural areas, such as launching new businesses or adopting sustainable farming methods. Women who apply their creativity are better equipped to leverage local resources, reduce risks, and identify new sources of income, thereby

Table 4. The results of SEM for the effects of constructs on food security**.

Relationship	Estimates	C.R.	P-value
Empowerment→Food	0.75	6.92	0.001
Grouping work→Food	0.48	4.42	0.001
Creativity→Food	0.42	3.87	0.001
Commitment →Food	0.51	4.70	0.001
Information→Food	0.40	3.68	0.001
Technical skills →Food	0.45	4.14	0.001
Psychological→Food	0.44	4.05	0.001
Social→Food	0.43	3.96	0.001
Politicial→Food	0.43	3.95	0.001
Economic→Food	0.49	4.51	0.001
Managerial →Food	0.44	4.05	0.001
Educational→Food	0.45	4.15	0.001

strengthening their ability to provide food for their families.

Food security and empowerment were most impacted by commitment and responsibility ($\beta=0.68$). Commitment represents a strong intrinsic drive to provide sufficient food for families, particularly in female-headed households. Women's sense of duty to their families and children motivates them to take the initiative in developing their skills and ensuring food security by seeking reliable sources of income, improving agricultural yields, or securing high-quality food products.

Disparities in food security and empowerment were predicted by specific knowledge and information to be 40.00 and 53.00%, respectively. Women with access to timely and relevant information are better equipped to make informed decisions about nutrition, food production, and household management. Another important factor is women's participation in training programs or knowledge-sharing networks, which help them progress into more empowered and financially stable roles.

Technical skills and practical work predicted 60.00 and 45.00% of the variations in empowerment and food security, respectively. These abilities provide women with opportunities to work in occupations that can increase income and improve living standards. Women who possess technical skills in business, crafts, or agriculture are evidently better able to empower themselves and contribute to their households' food security.

Both food security ($\beta=0.44$) and empowerment ($\beta=0.59$) were significantly influenced by psychological factors. Long-term food security relies on women's ability to manage risks and seize opportunities, both of which are strengthened by psychological well-being (Ahmed and Malik, 2019). When faced with obstacles, psychologically empowered women are more likely to persist, whether through education, starting a business, or adopting improved farming methods.

Social and political factors alone predicted 43.00% of the variance in food security and 57.00% in empowerment. When women participate in community organizations, cooperatives, or political systems they gain platforms for advocacy, resource access, and mutual support. Especially in rural regions, women can influence decisions that shape food security policies through political engagement. Participation in social groups enhances women's agency and voice, opening opportunities for collective action that can improve both community-wide food security and individual empowerment.

Economic considerations had a significant impact on both food security ($\beta=0.49$) and empowerment ($\beta=0.65$). These findings align with documented research on the influence of economic factors on food security (Ali *et al.*, 2019; Oni *et al.*, 2010). Food security improves directly when women have access to economic resources, such as land ownership, credit, and financial capital, allowing them to invest in productive assets like business or farming equipment. Additionally, 60% of the variance in empowerment was explained by managerial factors, which enable women to manage resources effectively and balance economic activities with domestic responsibilities, thereby supporting food security.

Food security was significantly impacted by educational characteristics, similar to other factors. Education provides women with the technical know-how, social skills, and critical thinking abilities necessary for managing food production, finding employment, and participating in community decision-making. Educational initiatives, particularly those emphasizing employable, and real-world skills can greatly aid in reducing food insecurity.

There are limitations to this study. The exclusive emphasis on a specific rural group may restrict the broader applicability of the results to other cultural or regional contexts. Significant differences in socioeconomic and cultural factors influencing empowerment and food security in rural



developing nations compared to urban areas or other regions may limit the generalizability of the findings. To better understand how various groups of women perceive empowerment, future research should consider intersectional aspects such as age, ethnicity, class, and disability. Although the study discusses several empowerment-related aspects, it falls short in addressing external factors that can directly impact food security in rural areas, such as market access, government policy, and climate change.

CONCLUSIONS

In summary, empowerment and its components significantly impacted food security individually. To enhance women's empowerment and food security, it is essential to educate them about the largely internal factors involved. We propose that local institutions, NGOs, and government agencies collaborate to establish community-based skill development centers specifically designed for rural women to improve food security and empowerment in these areas. In addition to partnering with local media for educational outreach, they must implement practical technical training that incorporates safety precautions. The centers should also support women-led cooperatives by facilitating peer learning and access to microfinance. Furthermore, strong monitoring and evaluation procedures must be established to track progress and make the necessary program adjustments.

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شناسایی ابعاد توانمندسازی و تأثیر آن بر امنیت غذایی زنان روستایی

کیوان شجاع چاغروند، علیرضا پورسعید، و مریم امیدی نجف آبادی

چکیده

این مطالعه با هدف شناسایی ابعاد توانمندسازی خانوارهای زنان سرپرست در مناطق روستایی و تأثیرات آنها بر امنیت غذایی در ایران انجام شد. بر اساس داده‌های جمع‌آوری‌شده از مرکز آمار ایران، جمعیت زنان سرپرست خانوار در مناطق روستایی استان تهران شامل ۴۹۵ نفر بود که از میان آنها ۲۱۶ نفر با استفاده از روش نمونه‌گیری تصادفی طبقه‌بندی‌شده با تخصیص تناسبی انتخاب شدند. پرسشنامه‌های توسط پژوهشگران طراحی شد و اعتبار و پایایی آن به ترتیب با استفاده از تحلیل عاملی تأییدی و روش کرونباخ تأیید گردید. داده‌ها از نوامبر ۲۰۲۰ تا ژوئیه ۲۰۲۱ جمع‌آوری شد. برای تحلیل و برآورد روابط بین متغیرهای متعدد از مدلسازی معادلات ساختاری استفاده شد. نتایج، ابعاد توانمندسازی را شامل کار گروهی و مهارت‌های ارتباطی، خلاقیت و حل مسئله، تعهد و مسئولیت‌پذیری، اطلاعات و دانش تخصصی، مهارت‌های فنی و کار عملیاتی نشان داد که شامل عوامل روانی، اجتماعی، سیاسی، اقتصادی، مدیریتی و آموزشی میشوند و تأثیر آنها بر امنیت غذایی را مشخص کرد. بیشترین تأثیر بر امنیت غذایی به ترتیب متعلق به تعهد و مسئولیت‌پذیری، عوامل اقتصادی و کار گروهی بود. توانمندسازی شغلی توانست ۷۵٪ واریانس امنیت غذایی را پیشبینی کند. پیشنهاد میشود برای کاهش ناامنی غذایی، توانمندسازی شغلی خانوارهای زنان سرپرست در مناطق روستایی مورد توجه قرار گیرد.

Investigating Agricultural Ecosystem Functions and Services in Northern Iran

Sareh Hosseini^{1*}, and Fahimeh Karimpour²

ABSTRACT

Agricultural ecosystem provides various functions and services for humans. Therefore, investigating their role and importance in agricultural land programming and management is one of the research goals. In this research, we used Common International Classification of Ecosystem Services (CICES) for identification of the Agricultural Ecosystem Functions and Services (AEFS). Also, Multi-Criteria Decision-Making (MCDM) models were used for weighting and prioritizing of the AEFS like Stepwise Weight Assessment Ratio Analysis (SWARA) for calculating their weight, and Simple Additive Weighting (SAW), Additive Ratio Assessment (ARAS), and Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) for prioritizing them. The research data was extracted using field survey, random sampling, and completing the Delphi questionnaire of 40 agricultural experts in the north of Iran. Also, the R^2 coefficient was used to compare the AEFS prioritization models. The results of SWARA technique showed that provisioning, regulation, and cultural functions with weights of 0.0298, 0.0286 and 0.0250 had the highest weight, respectively. Also, the results indicated that the SAW model with the $R^2 = 0.90$ was chosen as the appropriate prioritization model. Provisioning, regulation, and cultural functions with marginal weights of 0.6319, 0.5448, and 0.5092 were ranked the first to third priority, respectively. Also, food supply, employment, genetic material supply, and educational and research services were important positive services of the agricultural ecosystem compared to the other services. It is suggested that more appropriate programming and more research be done by relevant organizations for the sustainable management of agricultural ecosystems in northern Iran.

Keywords: Agricultural Ecosystem Functions and Services, Common International Classification of Ecosystem Services, Weighting assessment.

INTRODUCTION

A set of ecosystem services that human life depends on them is provided by agriculture (Heinze *et al.*, 2022). Also, due to the increasing growth of the world population, there is more pressure on agricultural prospects to receive different services (Azaiez *et al.*, 2020). Based on this, a series of factors such as climate, geology, ecology, as well as management methods, technology and skills affect the provision of landscapes ecosystem

services. In fact, agriculture ecosystems are both a recipient and a provider of services. Therefore, the sustainability of agricultural ecosystems requires their ability to simultaneously provide services in a balance between the provision and consumption of services. However, the main management approach is based on the preservation of the services for the use of future generations, and the balance between services compared with other agricultural ecosystems (Altieri, 2018). Therefore, agricultural ecosystem managers

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are trying to integrate ecosystem services in agricultural ecosystem policies and management by using a set of methods including evaluation of dependencies and effects of ecosystem services, valuation of ecosystem services, scenario creation and other interventions that can become the main basis for resolving conflicts and establishing a compromise between development and nature, and guaranteeing the stability of both (Xu and Peng 2022). Therefore, access to quantitative and qualitative information about the positive services of the agricultural ecosystem is of particular importance to achieve sustainable agriculture (Jia *et al.*, 2021).

Among the diverse ecosystems, the agricultural ecosystem with different functions and services have direct and indirect roles in the economic and human livelihoods (FAO, 2018), whose maintenance should be the main goal of human activities. Therefore, five classifications including the study of Costanza *et al.*, 1998, De Groot *et al.* (2012), Millennium Ecosystem Classification (MEA, 2005), The Economics of Ecosystems and Biodiversity (TEEB) (2010), and Common International Classification of Ecosystem Services (CICES) (2018) emphasized the classification of ecosystem services (Haines-Young and Potschin 2013). CICES (2018) is the latest classification of ecosystem functions and their services that was developed by the European Environment Agency (EEA) with the aim of providing a standard for the systematic nomenclature, description, and classification of ecosystem services. This classification includes three main groups of provision, regulating, and cultural functions (EEA, 2016).

Based on CICES classification, provisioning services are products and energy outputs obtained from goods and products. The regulating services include all the ways in which ecosystems can manage the environment where people live or depend on in some way, and benefit from them in terms of their health or safety. Finally, the cultural services category refers to all the non-material aspects of an ecosystem that contribute to or are important

for humans' mental or intellectual wellbeing. Cultural services are intangible benefits that contribute to human development and culture, including the functioning of local, national, and international cultural ecosystems. It is included dissemination of knowledge and ideas, and interaction with nature (music, art, architecture) creativity emerges from dialogue, and entertainment (CICES, 2018).

These functions and services are not free and have hidden economic value. If these services are considered free, the agricultural ecosystem will be destroyed (Dick *et al.*, 2018). Various pressures arising as economic purposes have caused their decline and destruction, and we are witnessing their destruction in everywhere in the world. For this reason, the identification of the Agricultural Ecosystem Functions and Services (AEFS) has become very important. Obviously, this issue requires the participation of stakeholders and finding out about their preferences for positive services of the agricultural ecosystem, especially the Agricultural Ecosystem of Northern Iran (AENI) (Dumont *et al.*, 2019). So far, different models have been proposed for ranking and valuation functions and services, but few studies have been done about defining them. Some of the most important ones are mentioned here.

Jia *et al.* (2021) surveyed agricultural ecosystem services in arid and semi-arid regions of western China based on the equivalent factor method. The study results showed that the factor evaluation method is an accounting tool for the evaluation of ecosystem services. Also, 9 agricultural ecosystem services were analyzed in this evaluation. The findings showed that the agricultural environmental services value in Gansu Province increased from 2008 to 2017. Also, ecological services are the most important agricultural ecosystem services in arid and semi-arid areas. Sun *et al.* (2021) assessed agricultural services in North China and predicted their changes under different land use scenarios. The results indicated that agricultural ecosystem services play an

important role in the economic and social conditions of the society. Also, Wang *et al.* (2022) assessed the ecological value of China's conventional agricultural ecosystem services in the framework of Energy-Based Life-Cycle Assessment. The findings showed that the importance of agricultural ecosystem provisioning services ecosystem is much higher than the production services provided by them. In this regard, Heinze *et al.* (2022) investigated farm diversity and its ecosystem services in different land use scenarios of southeastern Mexico. The results indicated that farms provide different services, and provisioning services are more important compared to other services and should be considered in different management methods.

A review of the previous sources showed that despite the existence of research related to the AEFS evaluation with different approaches, no study has been done about the identification, weighting, and prioritization of AEFS. Therefore, this has been tried according to the followings:

- a. The importance of the AENI and highlighting its values to the society.
- b. The tensions resulting from the change of agricultural land use in the north of Iran.
- c. The possibility of the agricultural lands' drought of northern Iran due to the lack of

water resources and the phenomenon of climate change in recent years.

d. The important role of agricultural ecosystem services in the comprehensive management of water resources.

Also, the three main provisioning, regulating, and cultural services and the AENI based on the CICES are identified and prioritized for their optimal management.

MATERIALS AND METHODS

Study Area

The agricultural ecosystem has an important role in Iran's economy. The Iranian agricultural land area is 16.5 million hectares, of which 14.7 million hectares are under field crops and the rest are gardens. Crops production in the Northern Iran was about 8417436 tons in 2017-2018, which was almost a ninth of the countries (Figure 1).

To carry out this research, the three provinces of Mazandaran, Guilan and Golestan were selected. Currently, the cultivated area of agricultural lands in Mazandaran is 476 thousand ha with an annual production of more than 3.574 million tons. Important characteristics of

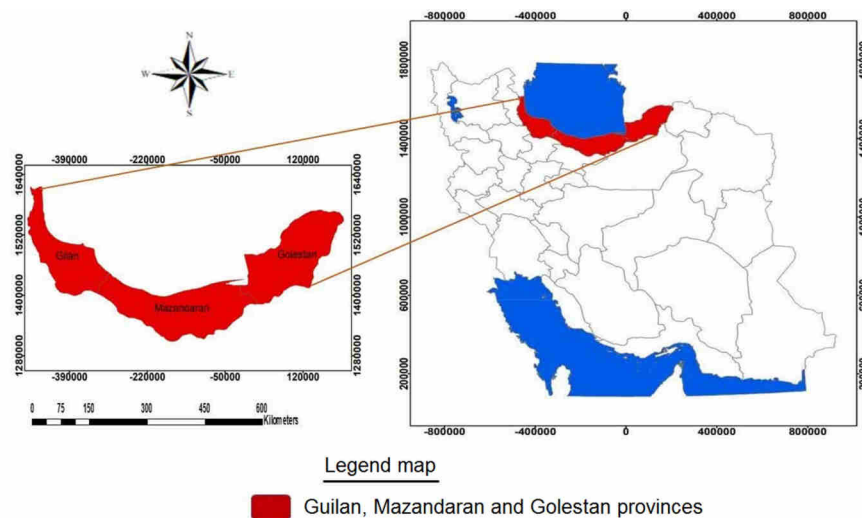


Figure 1. The location of the case study.



Mazandaran is the high cultivation coefficient (1.4) compared to the total of Iran (0.7). It has made Mazandaran as the largest producer of rice in Iran, and it has many capacities in increasing the quality and quantity in this regard. Also, it has caused the annual cultivated land of this province to more than 600000 ha. Notably, there are more than 45 types of cultivated crops in Mazandaran, of which the most important ones are rice, wheat, barley, soy bean, rapeseed, corn, fodder plants, vegetables and summer vegetables. Each of these products provide many services to the society. Also, the area of arable land of Golestan Province is 850000 ha, of which the agricultural land area is 710000 ha (250,000 ha of irrigated land and 460,000 ha of dry land). Also, the products of the agricultural ecosystem are very diverse, and some of the products are of special value and importance on a national scale in Guilan Province. Therefore, agriculture in Guilan has both nutritional and commercial value for its producers. The agricultural ecosystem is about 30% of Guilan area. The proportion of irrigated and dry lands in this province is 82% and 18%, respectively (<https://maj.ir/>).

Methodology of the Study

In this research, in order to weigh and prioritize the AEFS in northern Iran, firstly, the AEFS were identified and compiled based on the most CICES. Then, the research data was extracted in the form of field survey, random sampling, and by completing the Delphi questionnaire and face-to-face interviews with 40 experts of agricultural ecosystem management. Then, the question was asked which of the positive

AEFS in northern Iran were more important for the optimal and sustainable management of agricultural ecosystem? Based on the answers, one of the five degrees of importance of the Likert scale were selected: Unimportant= 1, Little importance= 2, Important= 3, Great importance= 4, and Very important= 5 (Hosseini *et al.*, 2021). Also, if there were new services, they were added to the questionnaire. Finally, among the 40 questionnaires gathered, 10 questionnaires were removed due to the incompleteness of the information, and the data of 30 questionnaires were used to analyze the information (Table 1).

In order to check the reliability of the Delphi questionnaire, Cronbach's alpha coefficient of reliability technique was used (Mengual-Andrés *et al.*, 2016). According to the value of this coefficient ($\alpha = 0.91$), the reliability of the questionnaire was confirmed.

In this study, to weigh and prioritize each of AEFS, we used the Multi-Criteria Decision-Making (MCDM) models such as the Stepwise Weight Assessment Ratio Analysis (SWARA) in order to calculate the weight of AEFS (Debnath *et al.*, 2023), we used the Simple Additive Model (SAW) (Hosseini *et al.*, 2021), the Additive Ratio Assessment (ARAS) (Ben Amor *et al.*, 2022), and Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) and to prioritize functions and services (Ramón-Canul *et al.*, 2021). Finally, the curve slope (R^2) was used for comparing and choosing the suitable models for prioritizing the AEFS in the northern Iran (Figure 2).

Spss16 software was used to process and statistically analyze the questionnaire data such as calculating the questionnaire

Table 1. Delphi members to identify the positive functions and services of the northern Iran agricultural ecosystem.

Row	Delphi members	Education	Number
1	Faculty members of agricultural universities in Iran	Ph.D	15
2	Ministry of Agricultural Jihad of Iran	MSc, Ph.D	10
3	Land Affairs Organization of Iran	BC, MSc	5

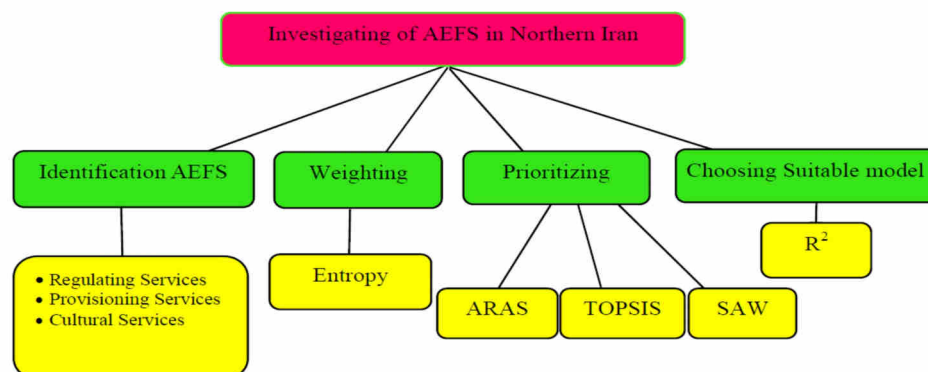


Figure 2. Methodology steps for investigating the agricultural ecosystem functions and services in northern Iran.

reliability with Cronbach's alpha test. Also, Excel software was used for weighting and prioritization models analysis.

Step-Wise Weight Assessment Ratio Analysis (SWARA)

The most important advantages of the SWARA method is its ability to evaluate the accuracy of experts' opinions about weight criteria, simple implementation, and no need for high volume of comparisons (Ayan *et al.*, 2023). The steps to implement this method are as follows:

- First Step: Sorting Criteria (Services)

At first, the criteria are written based on their importance. The most important criteria are placed in higher categories and less important criteria in lower categories (Debnath *et al.*, 2023).

- Second Step: Determining the Relative Importance of Each Criterion (S_j)

In this step, the relative importance of each criterion was compared with the previous criteria. This value represented using S_j .

- The Third Step: Calculating the Coefficient K_j

The coefficient K_j is a service of the relative importance of each criterion that is calculated using Equation (1):

$$K_j = S_j + 1 \quad (1)$$

- Fourth Step: Calculating the Initial Weight of Each Services

The initial weight (recalculated weight) of criteria (Q_j) is calculated with Equation (2). In this regard, it should be noted that the weight of the first criterion (the most important criterion) is considered equal to one (Majeed and Breesam, 2021; Zolfani and Saparaukas, 2013).

$$Q_j = x_j - 1 / K_j \quad (2)$$

- Step Five: Calculate the Final Normal Weight

In the last step, the final weight of the evaluation criteria is calculated by Equation (3). Normalization is done by simple linear method (Yücenur *et al.*, 2021).

$$W_j = Q_j / \sum_k^n Q_j \quad (3)$$

Additive Ratio Assessment Method (ARAS)

The ARAS method was proposed by Zavadsakas *et al.* (2010). This method is one of the best MCDM models to choose the best option. The best option is to have the greatest distance from negative factors and the least distance from the positive factors (Ben Amor *et al.*, 2022). The implementation section of this method is as follows:



Formation of the Decision Matrix

The first step in this technique is to create a decision matrix. A decision matrix is a matrix for evaluating a number of options based on a number of criteria. That is, a matrix in which each option is scored based on a number of criteria. The decision matrix is denoted by x and each term is denoted by x_{ij} (Equation 4) (Fan *et al.*, 2021).

$$X = \begin{bmatrix} x_{11} & x_{12} & x_{1n} \\ x_{21} & x_{22} & x_{2n} \\ x_{m1} & x_{m2} & x_{mn} \end{bmatrix} \quad (4)$$

Creation of Normal Decision Matrix

Normalization or descaling is the second step in solving all MCDM models (Equation 5) (Prayogo *et al.*, 2019).

$$N = \begin{bmatrix} n_{11} & n_{12} & n_{1n} \\ n_{21} & n_{22} & n_{2n} \\ n_{m1} & n_{m2} & n_{mn} \end{bmatrix} \quad (5)$$

Formation the Normal Weighted Decision Matrix

In the third step of the ARAS technique, the created normal decision matrix should be weighted. For this purpose, each criterion weight is multiplied in all the regions under the same criterion. The criteria weight should be determined in advance (Equation 6). The SWARA technique is usually used for this purpose (Jaukovic Jovic *et al.*, 2020).

$$V = \begin{bmatrix} v_{11} & v_{12} & v_{1n} \\ v_{21} & v_{22} & v_{2n} \\ v_{m1} & v_{m2} & v_{mn} \end{bmatrix} \quad (6)$$

Calculate the Utility of Each Option

The desirability of each option is calculated by the desirability service in the

fourth step of the ARAS technique. The best option is the one that has greater utility. Finally, the degree of desirability must be calculated. The total desirability of each option is represented by S_i that it calculated with Equation (7):

$$S_i = \sum V_{ij} \quad (7)$$

The degree of desirability of the option (K_i) is calculated based on the comparison with an optimal value (S_o) using Equation (8). The optimal value can be obtained based on the opinion of experts or the best weighted matrix values (Hosseini *et al.*, 2025)

$$K_i = S_i / S_o \quad (8)$$

Simple Additive Weighing (SAW)

In order to use the SAW model for prioritizing AEFS, first, the completed decision matrix was scaled using the linear scaling method. Then, weight is calculated by the SWARA technique multiplying in the unscaled matrix. In this method, taking into account the AEFS weight is calculated by the SWARA technique. The score of each Service (S_i) is calculated by the weighted average of their values in all services based on Equation (9) (Hosseini *et al.*, 2021).

$$S_i = \sum_j n_{ij} \cdot w_j \quad (9)$$

Where, W_j is Weight of each service and n_{ij} is score of each service.

Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS)

In this method, m options are evaluated by n indicators and the options are ranked based on their similarity to the ideal solution (Ramón-Canul *et al.*, 2021). The technique basis is based on the concept that the selected option should have the smallest distance with the positive ideal solution and the largest distance with the negative ideal solution. The steps of this method are as follows (Zavadskas and Turskis, 2010):

First Step: Converting the Existing Decision-Making Matrix into a Matrix (Unscaled) Using Equation (10):

$$n_{ij} = \frac{r_{ij}}{\sqrt{\sum_{i=1}^m r_{ij}^2}} \quad (10)$$

Where, n_{ij} : normalized matrix, and r_{ij} : score of each criterion.

The Second Step: Creating the Weight Matrix Assuming the Vector Was Input to the Algorithm Equation (11):

$$W = \{W_1, W_2, \dots, W_n\} \quad (11)$$

Where, W is Weight of each criterion.

So that ND is a matrix in which the criteria scores are dimensionless and comparable,

and $W_{n \times n}$ is a diagonal matrix in which only the main diagonal elements will be non-zero Equation (12).

$$V = ND \cdot W_{n \times n} = \begin{bmatrix} v_{11} & v_{12} & v_{1n} \\ v_{21} & v_{22} & v_{2n} \\ v_{m1} & v_{m2} & v_{mn} \end{bmatrix} \quad (12)$$

Where, V is weight matrix (dimensionless).

The Third Step: Specifying the Positive Ideal Solution (A^+) and the Negative Ideal Solution (A^-) Based on Equation (13):

$$\begin{aligned} A^+ &= \{(max V_{ij} | j \in J), (min V_{ij} | j \in J') | i = 1, 2, \dots, m\} = \{V_1^+, V_2^+, \dots, V_j^+, \dots, V_n^+\} \\ A^- &= \{(min V_{ij} | j \in J), (max V_{ij} | j \in J') | i = 1, 2, \dots, m\} = \{V_1^-, V_2^-, \dots, V_j^-, \dots, V_n^-\} \end{aligned} \quad (13)$$

$J = \{j = 1, 2, \dots, n | j \in \text{benefit}\}$, $J' = \{j = 1, 2, \dots, n | j \in \text{Cost}\}$

Step 4: Calculate the Distance between the i_{th} Option and Ideals (d_i) Using the Euclidean Method Based on Equation (14):

$$\begin{aligned} d_{i+} &= \sum_{j=1}^n (V_{ij} - V_j^+)^2)^{0.5}; i = 1, 2, \dots, m \\ d_{i-} &= \sum_{j=1}^n (V_{ij} - V_j^-)^2)^{0.5}; i = 1, 2, \dots, m \end{aligned} \quad (14)$$

The Fifth Step: Calculating the Relative Proximity of A_i to the Ideal Solution (cl_{i+}) Using Equation (15):

$$cl_{i+} = \frac{d_{i-}}{(d_{i+} + d_{i-})}; 0 \leq cl_{i+} \leq 1; i = 1, 2, \dots, m \quad (15)$$

The Sixth Step: Ranking the Options Based on cl_{i+} Descending.

RESULTS

The research results include two parts of identifying and determining the AEFS in northern Iran using the Delphi method and prioritizing them with MCDM models. The findings of each part are presented separately below:

Identifying the AEFS of the Northern Iran

In this research, the AEFS of northern Iran identified using CICES (Table 1). Then, the questionnaire containing them was distributed among the members of the Delphi group (experts in the field of agricultural ecosystem management with at least 15 years of experience) in order to score based on the Likert scale. In our research, 30 people formed a Delphi group and expressed their opinions regarding the identification of positive AEFS at each stage (Table 1).

At the end of the first stage of the Delphi method, using the opinions of experts and some specialist expert in this field (Delphi method designer and analyst team), the positive AEFS of northern Iran were modified, integrated, and adjusted. Then, three functions and 23 services were determined for the agricultural ecosystem in northern Iran (Table 2).

In this research, in order to investigate the reliability of the questionnaire questions, the Cronbach's Alpha coefficient was used. Cronbach's alpha coefficient obtained was $\alpha = 0.97$, which was confirmed.

Weighing and Prioritizing the AEFS in Northern Iran

After collecting and analyzing the questionnaires, in order to determine the weight of the AEFS, SAW, ARAS, and TOPSIS method, the SWARA technique was used for AEFS priority. The finding models are presented below.

Determining the Weight of the AEFS of

**Table 2.** The Agricultural Ecosystem Functions and Services (AEFS) of northern Iran.

Functions	Services	Description
Regulating services	Local and regional climate regulation	The plants of the agricultural ecosystem can create a more additive microclimate by creating shade and lowering the temperature.
	Improve air quality	Carbon storage by plants causes reduction of greenhouse gas and consequently improves air quality.
	Hydrological cycle and groundwater maintenance (including regulation of surface water flow; groundwater recharge; basin drainage)	In the agricultural ecosystem, the high rate of water infiltration causes the regulation of surface flows and maintaining the flow of underground water.
	Regulating water quality (Water purification)	The vegetation of the agricultural ecosystem causes its filtration by breaking down and removing nutrients and other water pollutants.
	Pollination and seed dispersal	Wind causes seeds to disperse by moving plants in the agricultural ecosystem.
	Pest and disease control (Biological pest control)	Some agricultural plants help to regulate and control the abundance of pathogens.
	Smell reduction, noise reduction, visual screening	Vegetation reduces noise pollution in addition to creating visual appeal and creating a pleasant smell, agricultural.
	Natural hazard regulation	Agricultural vegetation prevents soil erosion and landslides and prevents floods by absorbing rain.
	Soil erosion control	Vegetation increases resistance to erosion; It also prevents soil erosion by keeping sediments.
	Soil formation	Agricultural vegetation facilitates soil formation by depositing organic matter.
	Regulating soil moisture and maintaining soil fertility	Agricultural vegetation regulates soil moisture and maintains soil fertility.
	Ecosystem connectivity	The agricultural ecosystem provides the migration paths of plants and animals to other ecosystems and provides ecosystem connectivity.
	Nutrient cycle	The living organisms in the agricultural ecosystem play an important role in the decomposition of plant and animal organic matter and the cycle of carbon, oxygen, nitrogen, etc. elements.
	Role in food webs and prey/predator relationships	Agricultural ecosystem connects several food chains. It also causes communication between different species (such as coexistence, competition and hunting and hunter).
	Providing and maintaining habitats (Biodiversity)	The agricultural ecosystem provides suitable habitats for the life, reproduction of all kinds of plant and animal species, invertebrates and vertebrates.
	Primary production	The consumption of carbon dioxide by plants in the process of photosynthesis causes the production of organic substances, which in addition to plant growth, also produces oxygen.

Table 2 continued

Functions	Services	Description
Regulating services	Local and regional climate regulation	The plants of the agricultural ecosystem can create a more additive microclimate by creating shade and lowering the temperature.
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Table 2 continued



Table 2 continued

Functions	Services	Description
Provisioning services	Water supply	Water supply systems are very important for the proper functioning of communities. It can be achieved with various engineering projects such as wells or reservoirs.
	Food supply	Commercial and subsistence production of food and crops
	Energy production (Renewable)	Production of fuel energy
	Fiber, fuel, fodder	Providing renewable and extractable raw materials for fuel and fiber, including plant stumps, shrubs and fodder and wood (fuel wood); providing fiber from plants (water hyacinth, straw, etc.); Charcoal production from the processing of many plants.
	Biological materials (Biotics)	The use of agricultural plants as building materials, the production of various secretions such as gum, resin, handicrafts, etc.
	Providing genetic materials, natural medicines and biochemistry (Biochemical)	Including the extraction of genetic material from plants in the agricultural ecosystem for biomass production, biochemical, industrial and pharmaceutical processes (such as drugs, fermentation, detoxification), breeding programs (examination of genes for resistance to plant pathogens)
	Creating a green belt (Protective walls)	The agricultural ecosystem with diverse vegetation plays a very important role in beauty and reducing the amount of air pollution and preventing floods and soil erosion, etc.
	Carbon sequestration	The agricultural ecosystem with diverse vegetation reduces the concentration of carbon dioxide in the atmosphere.
	Fauna and Flora habitat and shelter	The agricultural ecosystem is home to some small rodents that feed on invasive and non-native plants.
Cultural services	Spiritual, religious and therapeutic services	Agricultural ecosystem has spiritual and religious value in many religions, some plant species have spiritual importance.
	Recreation and ecotourism	The agricultural ecosystem provides opportunities for recreational activities such as hiking, hunting, observing plant and animal species, recreational camps, nature watching, etc.
	Cultural heritage values and sense of place	The agricultural ecosystem represents the culture and civilization of many years of indigenous communities located around it.
	Conservation values	Endangered native species are preserved in the agricultural ecosystem and its margins.
	Aesthetic, inspiring culture, art and design	The existence of spectacular landscapes is one of the aesthetic aspects of the agricultural ecosystem.
	Health and Mental Well-being	Reducing stress by spending time near the agricultural ecosystem, enjoying recreational activities such as group camps in the vicinity of the agricultural ecosystem.
	Education and Research	Agroecosystem can be used to develop many research and education (educational ecosystem services mean formal and informal educational opportunities created by access to particular ecosystems such as providing condition for education and research about ecosystem services such as biotechnology research, thesis research, toxicology research on the ecosystem services and etc).
	Existence values	People feel pleasure and satisfaction from the plant and animal species in and around it.
	Employment (Creating job)	The agricultural ecosystem directly by creating employment in field of agricultural products, crops, livestock, fish, and aquaculture and indirectly by attracting investments and businesses that support tourism and eco-tourism to help contributes to the economy of the region
	Meetings and social relations	Agricultural ecosystem connects people, places and other forms of life and causes social interaction. Also, agricultural ecosystem is a suitable place for holding ceremonies.

Table 3. Calculating the AEFS weight in northern Iran using the SWARA technique.

Functions	Services	W _j
Regulating services	Local and regional climate regulation	0.0305
	Improve air quality	0.0309
	Hydrological cycle and groundwater maintenance	0.0306
	Regulating water quality (Water purification)	0.0279
	Pollination and seed dispersal	0.0311
	Pest and disease control (Biological pest control)	0.0252
	Smell reduction, noise reduction, visual screening	0.0267
	Natural hazard regulations	0.0270
	Soil erosion control	0.0319
	Soil formation	0.0286
	Regulating soil moisture and maintaining soil fertility	0.0267
	Ecosystem connectivity	0.0248
	Nutrient cycle	0.0275
	Role in food webs and prey/predator relationships	0.0288
	Providing and maintaining habitats (Biodiversity)	0.0290
	Primary production	0.0301
Provisioning services	water supply	0.0163
	food supply	0.0397
	Energy production (Renewable)	0.0304
	Fiber, fuel, fodder	0.0290
	Biological materials (Biotics)	0.0238
	Providing genetic materials, natural medicines and biochemistry (Biochemical)	0.0367
	Creating a green belt (Protective walls)	0.0338
	Carbon sequestration	0.0346
Cultural services	Fauna and Flora habitat and shelter	0.0239
	Spiritual, religious and therapeutic services	0.0154
	Recreation and ecotourism	0.0221
	Cultural heritage values and sense of place	0.0236
	Conservation values	0.0236
	Aesthetic, inspiring culture, art and design	0.0323
	Health and mental well-being	0.0161
	Education and research	0.0365
	Existence values	0.0260
	Employment	0.0384
	Meetings and social relations	0.0257
	Security	0.0148

Northern Iran with the SWARA Technique the results of AEFS weighting are indicated in Table 3. The weighing findings showed that the food supply, employment, supply of genetic materials, and educational and research services have the highest weight, respectively.

According to the results of Table 4, the provisioning function has the highest weight among other functions of the agricultural ecosystem in northern Iran (Table 4).

Determining the Priority of the AEFS in Northern Iran

The results obtained from the implementation of ARAS, TOPSIS and SAW models to determine the priority of the AEFS are presented in Tables 5 and 6.

The final weight of the agricultural ecosystem functions with ARAS, TOPSIS and SAW models is indicated in Table 7.

**Table 4.** Calculating the weight of agricultural ecosystem functions in northern Iran, using the SWARA.

Function	W_i	Rank
Provisioning	0.0298	1
Regulating	0.0286	2
Cultural	0.0250	3

Table 5. Final weights of the AEFS in northern Iran.

Code	Services	Models		
	Regulating services	ARAS	SAW	TOPSIS
A ₁	Local and regional climate regulation	0.7679	0.6091	0.5422
A ₂	Improve air quality	0.7782	0.7252	0.5114
A ₃	Hydrological cycle and groundwater maintenance	0.7713	0.6179	0.5493
A ₄	Regulating water quality (Water purification)	0.7048	0.5198	0.5000
A ₅	Pollination and seed dispersal	0.7850	0.6772	0.5449
A ₆	Pest and disease control (Biological pest control)	0.6365	0.4392	0.4661
A ₇	Smell reduction, noise reduction, visual screening	0.6724	0.4586	0.4551
A ₈	Natural hazard regulations	0.6809	0.4590	0.4661
A ₉	Soil erosion control	0.8055	0.6226	0.4551
A ₁₀	Soil formation	0.7201	0.5369	0.5061
A ₁₁	Regulating soil moisture and maintaining soil fertility	0.6741	0.4705	0.4696
A ₁₂	Ecosystem connectivity	0.6246	0.3864	0.3965
A ₁₃	Nutrient cycle	0.6945	0.5013	0.4878
A ₁₄	Role in food webs and prey/predator relationships	0.7270	0.5593	0.5228
A ₁₅	Providing and maintaining habitats (Biodiversity)	0.7304	0.5561	0.5174
A ₁₆	Primary production	0.7594	0.5782	0.5199
Provisioning services		ARAS	SAW	TOPSIS
B ₁	Water supply	0.4113	0.2487	0.3754
B ₂	Food supply	1.0000	1.1024	0.6667
B ₃	Energy production (Renewable)	0.7662	0.6138	0.5469
B ₄	Fiber, fuel, fodder	0.7304	0.5503	0.5124
B ₅	Biological materials (Biotics)	0.6007	0.3764	0.4120
B ₇	Providing genetic materials, natural medicines and biochemistry (Biochemical)	0.9249	0.8950	0.7509
B ₈	Creating a green belt (Protective walls)	0.8515	0.7227	0.4871
B ₉	Carbon sequestration	0.8737	0.7623	0.5135
B ₁₀	Fauna and Flora habitat and shelter	0.6024	0.4157	0.4690
Cultural services		ARAS	SAW	TOPSIS
C ₁	Spiritual, religious and therapeutic services	0.3891	0.2314	0.3696
C ₂	Recreation and ecotourism	0.5580	0.4149	0.4815
C ₃	Cultural heritage values and sense of place	0.0141	0.4369	0.4706
C ₄	Conservation values	0.0141	0.3826	0.4313
C ₅	Aesthetic, inspiring culture, art and design	0.0263	0.6772	0.4255
C ₆	Health and mental well-being	0.0065	0.2376	0.3512
C ₇	Education and research	0.0337	0.8917	0.4824
C ₈	Existence values	0.0170	0.4522	0.4645
C ₉	Employment	0.0371	1.0207	0.5796
C ₁₀	Meetings and social relations	0.0167	0.6456	0.6772
C ₁₁	Security	0.0055	0.2102	0.3392

Table 6. Prioritization of agricultural ecosystem services in northern Iran.

ARAS	SAW	TOPSIS
B ₂	B ₂	B ₇
B ₇	C ₉	C ₁₀
B ₉	B ₇	B ₂
B ₈	C ₇	C ₉
A ₉	B ₉	A ₃
A ₅	A ₂	B ₃
A ₂	B ₈	A ₅
A ₃	C ₅	A ₁
A ₁	A ₅	A ₁₄
B ₃	C ₁₀	A ₁₆
A ₁₆	A ₉	A ₁₅
A ₁₅	A ₃	B ₉
B ₄	B ₃	B ₄
A ₁₄	A ₁	A ₂
A ₁₀	A ₁₆	A ₁₀
A ₄	A ₁₄	A ₄
A ₁₃	A ₁₅	A ₁₃
A ₈	B ₄	B ₈
A ₁₁	A ₁₀	C ₇
A ₇	A ₄	C ₂
A ₆	A ₁₃	C ₃
A ₁₂	A ₁₁	A ₁₁
B ₁₀	A ₈	B ₁₀
B ₅	A ₇	A ₈
C ₂	C ₈	A ₆
B ₁	A ₆	C ₈
C ₁	C ₃	A ₇
C ₉	B ₁₀	A ₉
C ₇	C ₂	C ₄
C ₅	A ₁₂	C ₅
C ₈	C ₄	B ₅
C ₁₀	B ₅	A ₁₂
C ₄	B ₁	B ₁
C ₃	C ₆	C ₁
C ₆	C ₁	C ₆
C ₁₁	C ₁₁	C ₁₁

Table 7. The final weight and priority of the agricultural ecosystem functions in northern Iran.

Functions	Final weight			Priorities		
	TOPSIS	SAW	ARAS	TOPSIS	SAW	ARAS
Provisioning	0.5260	0.6319	0.7512	1	1	1
Regulating	0.4944	0.5448	0.7208	2	2	2
Cultural	0.4611	0.5092	0.1016	3	3	3



The finding showed that the provisioning functions have gained more weight among other functions at the three models (Table 7).

Statistical Analysis of Selecting the Appropriate Model for Prioritizing the AEFS in Northern Iran.

In order to compare the models for prioritizing the AEFS in northern Iran, the

curve slope (R^2) of the factor weight was used in the three models (Figure 3). The slope curve of the relative proximity of the weights in the SAW model is a descending exponential function with an explanatory degree of 0.90, which indicates the obvious difference between the AEFS in northern Iran.

The R^2 in the SAW model is higher and

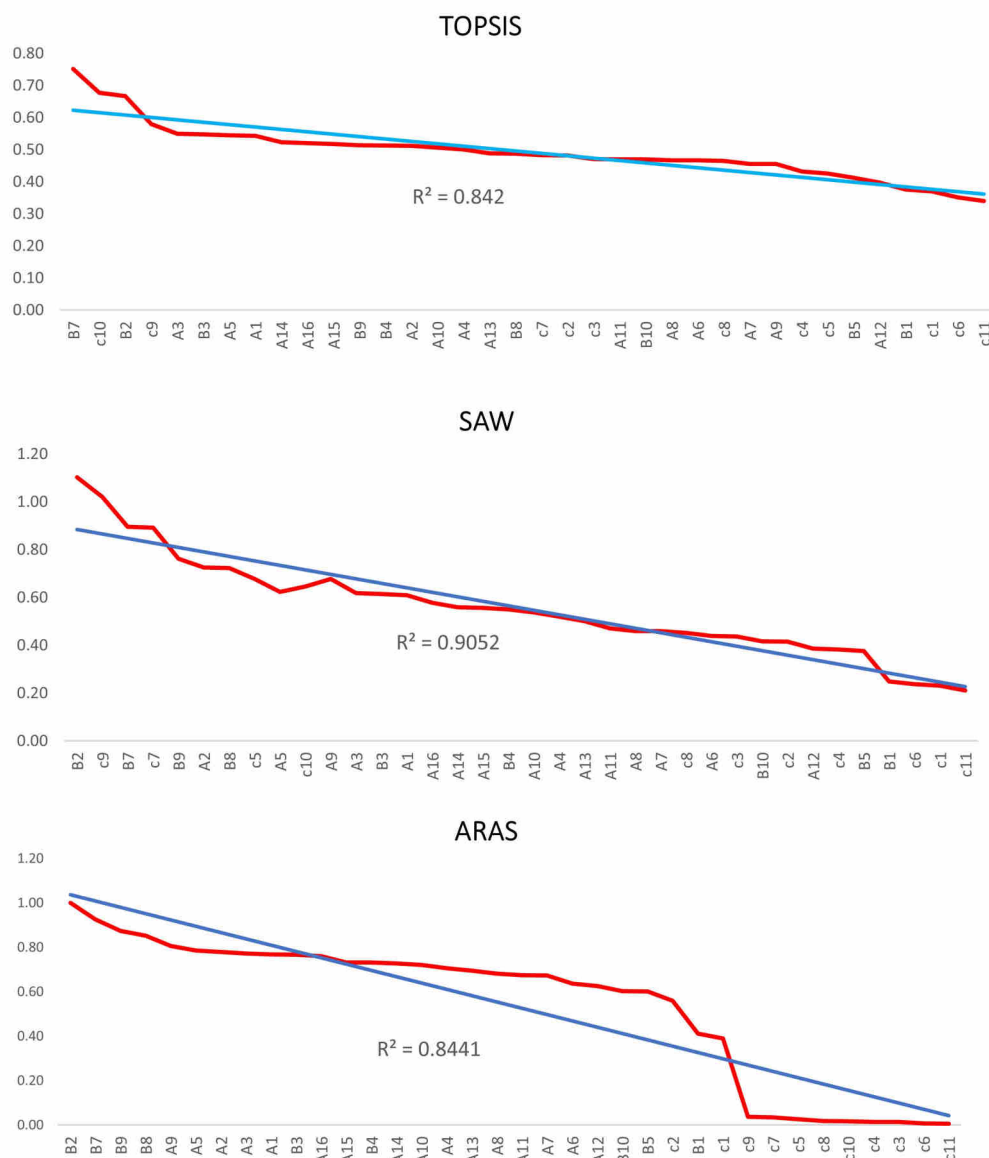


Figure 3. Curve slope (R^2) in ARAS, TOPSIS and SAW models.

closer to one than the other models. Based on the findings and the consensus of some experts, the result prioritization of the AEFS in northern Iran in the SAW model is closer to reality. Therefore, the SAW model is suggested as a suitable model for prioritizing the AEFS in northern Iran.

-Prioritizing the AEFS in Northern Iran Based on Suitable Model (SAW Model)

Based on the results of the best model for prioritizing the AEFS in northern Iran (SAW model), provisioning, regulating and cultural functions are, respectively, the most

important functions of the agricultural ecosystem in that area (Figure 4).

The findings of prioritizing agricultural ecosystem services in northern Iran with the SAW model are presented in Figures 5, 6, and 7. The results indicated that food supply, employment, air quality improvement services of provisioning, and cultural regulating functions had, respectively, the first priority compared to other agricultural ecosystem services in the north of Iran.

DISCUSSION

Although the primary goal of agriculture is

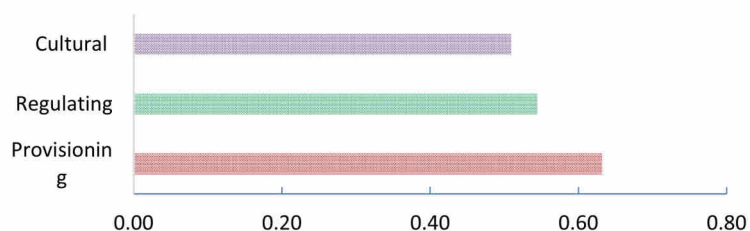


Figure 4. The priority of the functions of agricultural ecosystem in northern Iran with SAW model.

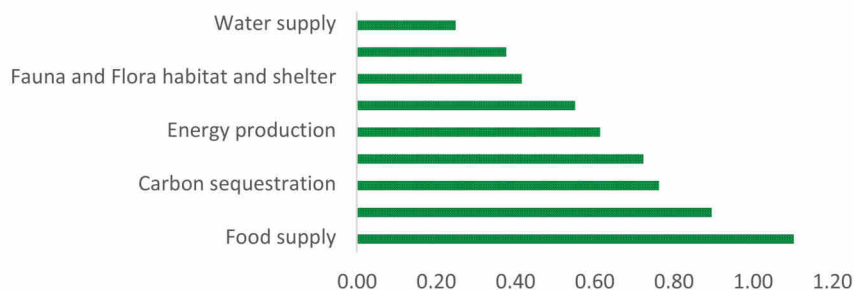


Figure 5. The priority of the provisioning services.

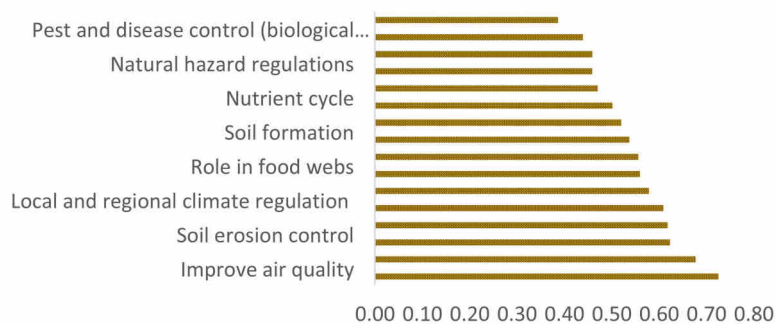


Figure 6. The priority of regulating services.



to produce food, the importance of agriculture is beyond the production of crops (Swinton *et al.*, 2015). And a set of ecosystem services that human life depends on is provided by agriculture (Rabbinge and Bindraban, 2012). The knowledge and skills of farmers in managing agro ecosystems can play an essential role in improving the balance between ecosystem services. Based on this, the management approach of each agro ecosystem is very important, so that sustainable agro ecosystems are involved with ecosystem services. However, the management approach plays a fundamental role in any agroecosystem, and in sustainable agroecosystems, management focuses on

maintaining ecosystem services for the benefit of future generations (Altieri, 2018).

The AEFS prioritization results in northern Iran using MCDM models indicated that the provisioning and regulating functions have the first priority among all the prioritization models. In other words, provisioning and regulating functions are the most important functions of the agricultural ecosystem in northern Iran. According to the agricultural experts' opinions in northern Iran, the higher priority of the provisioning function is due to the fact that the agricultural ecosystem in northern Iran was one of the richest ecosystems in terms of providing food, genetic material, carbon sequestration, creating a green belt, etc., each of which has

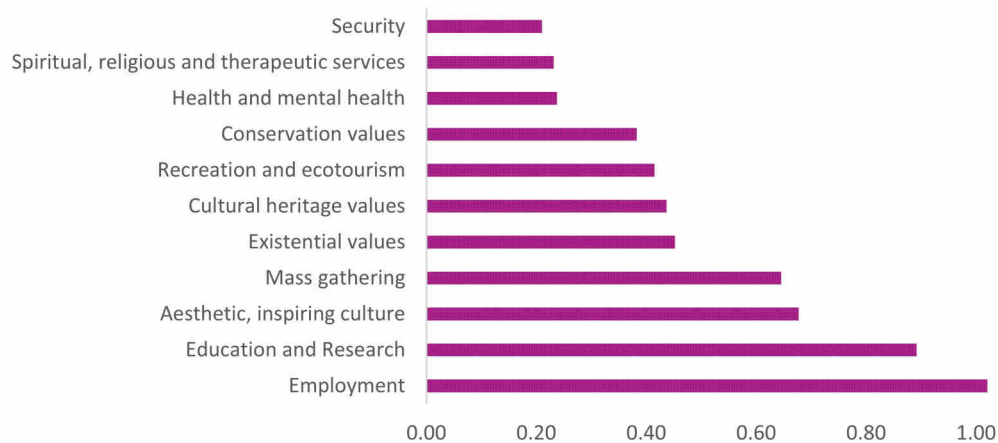


Figure 7. The priority of cultural services.

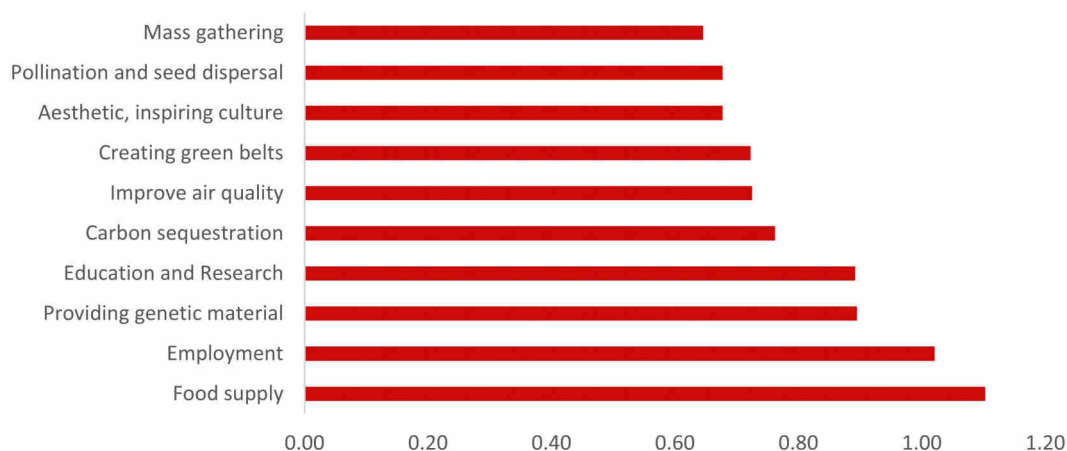


Figure 8. The most important agricultural ecosystem services in northern Iran.

many benefits for the local communities (Figure 8). The result is in accordance with the findings of Jia *et al.* (2021) and Heinze *et al.* (2022). Also, De Groot *et al.* (2012) stated regulating services include maintaining essential ecological processes and environmental protection systems. The study results showed that the agricultural ecosystem regulating services such as air quality improvement, pollination and seed dispersal, and soil erosion control have the highest priorities compared to the other services in that area.

Cultural services provide opportunities for spiritual, aesthetic, educational and scientific enrichment. In this regard, the results obtained from the prioritization of agricultural ecosystem services in the north of Iran indicated that the services of creating employment, education and research are the most important agricultural ecosystem cultural services. In other words, the agricultural ecosystem of northern Iran has created many employment, educational and research opportunities for various academic researchers. Also, the presence of beautiful landscapes on the edge of the agricultural ecosystem of northern Iran has provided a suitable potential for tourism and ecotourism. The improvement of recreational conditions and tourism facilities provided tourism income for investment in this area, which is one of the reasons for getting higher priority of employment creation services from the point of view of communities on the edge of the agricultural ecosystem. These results confirm Sohrabi's *et al.* (2021) in Iran and Assandri's *et al.* (2018) in Trentino, Italy. The findings showed that the cultural function was one of the most important functions of the agricultural ecosystem.

Unfortunately, the lack of information and insufficient recognition of the positive services of the agricultural ecosystem in northern Iran has caused increase in the amount of damage to the ecosystem and decrease in its habitat desirability. Meanwhile, most of the economic researches published in developing countries

are focused on the direct benefits of the agricultural ecosystem. The lack of proper understanding of these functions and the services produced by them is considered a serious danger for the society. Therefore, it is suggested to inform the communities about the importance of the positive services of the agricultural ecosystem in northern Iran in order to protect them.

As seen, the current research has been done at a relatively limited level. Therefore, it is necessary to pay attention to the agricultural ecosystem services in a large area. In addition to the opinions of experts, the opinions of native and non-native communities should be considered in determining priority. Because knowing, classifying, and prioritizing the services will be the guidance for policy making, management, and how to use the agricultural ecosystem (De Groot *et al.*, 2010). Also, it is necessary for future researchers to pay more attention to the role and importance of the functions and services and to survey the environmental behaviors of people in relation to the northern areas in Iran AEFS. Because the concept of agricultural ecosystem services, including all social, economic, and ecological dimensions, is a suitable framework for integration in the planning and management of these areas.

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بررسی کارکردها و خدمات اکوسیستم کشاورزی در شمال ایران

ساره حسینی، و فهیمه کریمپور

چکیده

اکوسیستم کشاورزی کارکردها و خدمات مختلفی را برای انسان فراهم می‌کند. لذا بررسی نقش و اهمیت آنها در برنامه‌ریزی و مدیریت اراضی کشاورزی یکی از اهداف تحقیق می‌باشد. برای شناسایی کارکردها و کالاها و خدمات اکوسیستم کشاورزی از طبقه‌بندی مشترک بین‌المللی خدمات اکوسیستمی (CICES) استفاده گردید. همچنین جهت وزندهی و اولویت‌بندی کارکردها و خدمات اکوسیستم کشاورزی از تکنیک‌های تصمیم‌گیری چند معیاره شامل تحلیل نسبت ارزیابی وزندهی تدریجی (SWARA) به منظور محاسبه وزن کارکردها و خدمات، و مدل‌های مجموع ساده وزین (SAW)، ارزیابی نسبت جمعی (ARAS) و تکنیک ترجیحات بر اساس مشابهت به راه حل ایده‌آل (TOPSIS) برای اولویت‌بندی آنها استفاده شده است. در این مطالعه داده‌های پژوهش به صورت پیمایش میدانی، نمونه‌گیری تصادفی و با تکمیل پرسشنامه دلفی توسط ۴۰ نفر از خبرگان کشاورزی در شمال ایران استخراج گردید تا نمایان شود کدامیک از کارکردها و خدمات مثبت اکوسیستم کشاورزی دارای اهمیت بیشتری در مدیریت بهینه آن می‌باشند. همچنین برای مقایسه مدل‌های اولویت‌بندی از ضریب R^2 استفاده شد. یافته‌های تکنیک SWARA نشان داد که کارکردهای تأمینی، تنظیمی و فرهنگی به ترتیب با کسب وزن‌های ۰/۲۹۸، ۰/۲۸۶ و ۰/۲۵۰ بیشترین وزن را به خود اختصاص داده‌اند. همچنین نتایج نشان داد که مدل SAW با کسب $R^2 = ۰/۹۰$ به عنوان مدل مناسب انتخاب گردید. طبق نتایج اولویت‌بندی این مدل، کارکردهای تأمینی، تنظیمی و فرهنگی با وزن‌های ۰/۶۳۱۹، ۰/۵۴۴۸ و ۰/۰۵۰۹۲ به ترتیب در اولویت اول تا

سوم جهت مدیریت بهینه اکوسیستم کشاورزی شمال ایران قرار گرفتند. همچنین در میان خدمات اکوسیستمی، خدمات تامین غذا، اشتغال، تامین مواد ژنتیکی و خدمات آموزشی و پژوهشی جزء خدمات مهم و مثبت اکوسیستم کشاورزی شمال ایران نسبت به سایر خدمات می باشند. لذا پیشنهاد می شود برنامه ریزی و تحقیقات مناسب توسط سازمان های ذیربط جهت مدیریت پایدار اکوسیستم های کشاورزی صورت پذیرد.

واژگان کلیدی: کارکردها و خدمات اکوسیستم کشاورزی، طبقه بندی مشترک بین المللی خدمات اکوسیستم، ارزیابی وزنی.

Investigating the Effects of Microcredit on Food Security of Rural Households: Evidence from Zehak County, Iran

Alireza Sani Heidary¹, Mahmoud Daneshvar Kakhki^{1*}, Mahmoud Sabouhi Sabouni¹, and Hosein Mohammadi¹

ABSTRACT

Microcredit plays a vital role in rural households' food security. However, the effects of microcredit on improving the food security of households have not yet been well studied and understood in Iran. Thus, the purposes of this research was to analyze the success of microcredit programs on enhancing the food security of rural households in Zehak County, Iran, using the propensity score matching method and bootstrap algorithm. Therefore, two food security indices, including Food Consumption Score (FCS) and Household Food Insecurity Access Scale (HFIAS) were used. The results revealed that 100% of the households faced food insecurity. The prevalence of food insecurity was 20.0, 42.5, and 37.5% for mild, moderate, and severe food insecurity, respectively. In addition, 30% of households were in poor status of food consumption. Our findings emphasize the positive and significant role of microcredit in improving food security and demonstrated that microcredit decreased the HFIAS index of the recipient households by 24.3-27.8% and increased the FCS index by 25.8-31.4%. Therefore, policy- and decision-makers should promote and strengthen governmental and non-governmental organizations providing microcredit. It is also recommended to provide information and reduce collateral restrictions to increase households' access to microcredit.

Keywords: Bootstrap algorithm, Food consumption score, Household food insecurity access scale, Propensity score matching.

INTRODUCTION

Since food security is important for human well-being, its realization is one of the most important goals of development plans at the national and international levels (World Bank, 2008; Dehbidi *et al.*, 2022; Bahiru *et al.*, 2023). Food security means that all people can obtain sufficient, safe, and nutritious food materially and economically at any time to meet their dietary needs and food preferences and live an active and healthy life. Therefore, food availability, food accessibility, food utilization, and stability over time are four important components to food security (Dehbidi *et al.*, 2022).

Food insecurity has been one of the major global problems in the last two decades, especially in developing countries. Food security is affected by climate change and extremes (Schillerberg and Tian, 2023; Kandel *et al.*, 2024), resource consumption (Chowdhury *et al.*, 2017; Liu *et al.*, 2020), land degradation (Gomiero, 2016), population growth (Liu *et al.*, 2020), and urbanization (Boltana *et al.*, 2023). Among them, climate change seems to have a significant impact on activities related to food security in agriculture-dependent countries. The agricultural sector plays a vital role in food supply, i.e. food production, and is strongly influenced by climate variability (Ghalibaf *et al.*, 2023).

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For this reason, the destructive effects of climate change are greater for the rural community and lead to an increase in food insecurity. In the long term, the adverse effects of climate change and other factors will pose major challenges to the nutrition and food security of rural communities (Ehtesham Majd *et al.*, 2019; Salman *et al.*, 2023).

Therefore, ensuring food security, especially in vulnerable rural areas, requires changing systems through government institutions, regional development institutions, and non-governmental organizations (Boltana *et al.*, 2023). In this regard, microcredit is one of the key measures proposed to reduce food insecurity (Salima *et al.*, 2023).

Microcredit is a form of microloans that are granted to poor rural households who usually lack collateral, verifiable credit history, and steady employment. In addition, there are micro-loans specifically intended for the creation and development of income-generating rural businesses.

Microcredit has a high potential to enhance food security, improve living standards, and reduce poverty by supporting entrepreneurship and creating income-generating activities (Bakare *et al.*, 2023).

In Iran, a new approach to microfinance was developed by the United Nations International Fund for Agricultural Development to provide access to formal and informal loans for the poor and low-income rural groups to create and develop rural businesses, empower them to cope with many shocks, improve livelihoods and food security, reduce vulnerability, and break out of the cycle of poverty. These programs have great potential to improve household food security by diversifying rural income-generating activities (Ministry of Cooperatives, Labour, and Social Welfare, 2018).

As a developing country, Iran faces the challenge of food insecurity, particularly in rural areas. Based on FAO, IFAD, UNICEF, WFP, and WHO (2022), 42.4% of Iran's population is affected by moderate or severe

food insecurity. For this reason, ensuring food security has become one of the most important goals of Iran's national development plans in the last two decades. In this regard, various measures have been taken to improve rural households' food security, of which microcredit is one of the most important ones.

However, few studies investigated the effects of microcredit on household food security. These studies are divided into three groups. First, most of the studies revealed that microcredit increases the per capita consumption of calories, increases the number of meals and increases the access to food, which results in improving the food security of households (Islam *et al.*, 2016; Devereux, 2016; Berhanu *et al.*, 2021; Boltana *et al.*, 2023), particularly female-headed households (Hamad and Fernald, 2012; Bocher *et al.*, 2017; Haque, 2021; Kianersi *et al.*, 2021; Wongnaa *et al.*, 2023). Secondly, a small number of studies did not find a significant effect of the role of microcredit on improving households' food security, and they stated that receiving credit was not successful in improving households' food security (Banerjee *et al.*, 2015; Seng, 2018; Mahmud *et al.*, 2022; Salima *et al.*, 2023). Thirdly, a limited number of studies showed that excessive debt, loan repayment pressure, women's lack of control over the use of loans, and frequent loans with high-interest rates lead to food insecurity in households, especially with female heads (Ahmed *et al.*, 2001; Develtere and Huybrechts, 2005; Aromolaran, 2010; Ganle *et al.*, 2015; Namayengo *et al.*, 2018).

In general, this study can contribute to the literature on the effects of microfinance programs on improving household food security in three ways. Firstly, this study investigates the effects of implementing an effective economic program (such as microcredit) on reducing household vulnerability to food insecurity. Considering household food security is subject to change, it is necessary to examine the effects of food security improvement programs such as microcredit to predict future shocks and

understand how households respond to food insecurity. Secondly, this study can help to understand why microcredit has positive and negative effects in different situations or times by generating empirical evidence and documenting the evaluation of its effects. Thirdly, the studies show that there is no consensus or global pattern on the effects of microcredit, and to find if it can be beneficial or harmful, local policy-makers and decision-makers must see evidence of the effects of its implementation in a specific region.

In this regard, this study can help local policy-makers gain a clearer picture and better understanding of the effects of implementing microcredit programs on improving the food security of rural households and take them into account when defining and changing their policies and programs.

Therefore, this study seeks to answer three key questions. First, what is the food security situation of the target rural households? Secondly, what factors influence the access of target households to microcredit? And thirdly, has the microcredit program improved the food security of the target households or not?

MATERIALS AND METHODS

Study Area and Data

Zehak County is a poor county, which is

located in the north of Sistan and Baluchestan Province, Iran, and consists of two districts: Central and Jazinak, and four rural districts: Zehak, Khajeh-Ahmad, Jazinak, and Khamak (Figure 1). There are 20,055 households in this county, of which 16,817 are rural (Statistical Center of Iran, 2016). Rural households in Zehak face problems such as lack of financial resources, poverty, high vulnerability, and food insecurity. In addition, this county suffers from climatic events such as drought, excessive heat, low rainfall, and 120-day winds. Considering the high poverty and deprivation in this county, climate disasters have increased the vulnerability and food insecurity of the households, particularly rural ones. The food security situation of rural households in this county indicates that a high proportion of households are in a state of food insecurity and use the most difficult strategies to cope with this situation (Okati *et al.*, 2020). According to the document on economic development and employment generation in rural areas of Sistan and Baluchestan Province, one of the effective measures to reduce household vulnerability, create employment, improve food security, and diversify economic and production activities is to support the establishment and development of microfinance funds to increase rural households' access to microcredit (Ebrahimzadeh and Paidar, 2019). This county's most important organizations providing microfinance services include the Agricultural Bank, the Welfare Organization

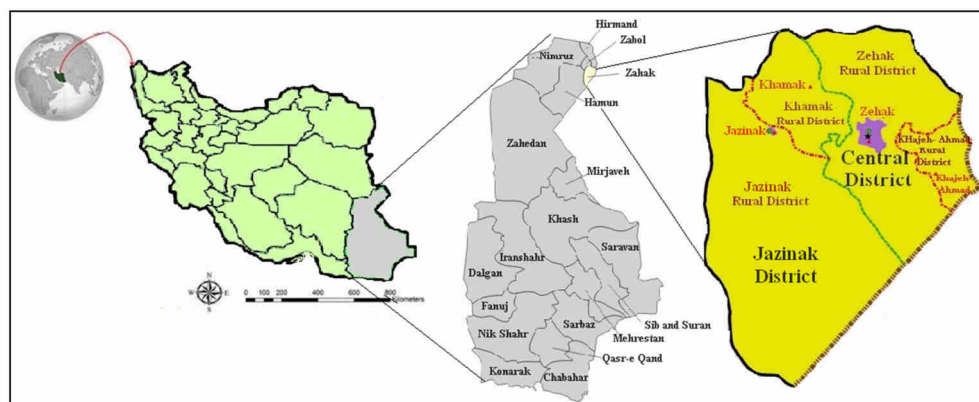


Figure 1. Geographical location of the study area.



and, the Kara System (governmental organizations), the Barkat Foundation, and the Alavi Foundation (non-governmental organizations). The total microcredit payments to rural households from 1397 to 1400 was about 1459 billion rials, of which about 3 percent was paid by the government organizations and 97 percent by the non-governmental organizations. Therefore, conducting this study in Zehak County can be a suitable platform for evaluating the performance of microcredit programs on rural households' food security for appropriate and well-functioning local policymaking and planning.

To obtain the needed data, Stratified Random Sampling was applied. According to Cochran's formula, the sample size was estimated to be 376 rural households. A multidimensional questionnaire and semi-structured interview were used to obtain the necessary data to calculate food security indicators, socio-economic, and demographic characteristics, farm and livestock characteristics, and experiences of shocks.

Food Security Index

Two food security indices, including the Household Food Insecurity Access Scale (HFIAS) and Food Consumption Score (FCS), were used to understand households' food security status in this study. The HFIAS index was developed by the Food and Nutrition Technical Assistance II (FANTA) project between 2001 and 2006 (Coates *et al.*, 2007; Salman *et al.*, 2023). This index is measured based on a short questionnaire that determines the behavioral and psychological characteristics of households from access to food insecurity in 30 days (Kolog *et al.*, 2023). The questionnaire consists of two types of questions: there are nine "occurrence" questions and nine "frequency of occurrence". The respondent is first asked whether he or she has experienced a certain situation (0= No, 1= Yes) and if so, how

often it has been experienced (1= Rarely, 2= Sometimes, 3= Often). To calculate the HFIAS index, each of the nine Questions (Q_i) is given a score between 0 and 3, and finally the scores of all questions are summed together using Equation (1). The calculated HFIAS score for each household ranges from 0 to 27, which indicates the degree of food insecurity experienced by the households (Coates *et al.*, 2007).

$$HFIAS_{score} = \sum Q_i a, \quad i=1,2,\dots,9 \quad (1)$$

Where, the HFIAS questionnaire provides information on the Domains and Prevalence of household food insecurity (Appendix 1).

The FCS index was developed by the World Food Programme (WFP) in 1996. This index measures diet quality and food intake (Baumann *et al.*, 2013). The respondent reports the frequency of household consumption of 8 different food groups (X_i) (Appendix 2) during a 7-day reference period. The frequency of consumption of each food group is

multiplied by an assigned weight (α_i) for each group and the resulting scores are summed to calculate the FCS using Equation (2) (Jones *et al.*, 2013):

$$FCS = \sum \alpha_i X_i, \quad i = (1, 2, \dots, 8) \quad (2)$$

The households are classified into three groups of food consumption: poor, borderline, and acceptable. The maximum score for a household is 112. This score can only be reached if a household consumes food from each food group every day (Baumann *et al.*, 2013).

Propensity Score Matching

This study used the propensity score matching algorithms to investigate the effect of microcredit on food security indices. This method is included in the group of methods for assessing the impact of an action or policy on two groups, affected and unaffected. In other words, Propensity Score Matching (PSM) is an intuitive approach to estimating the effects of implementing an

action or policy that broadly evaluates its success. This method has attracted the attention of researchers to evaluate the effect of development programs such as microcredit on households' food security and living standards (Berhanu *et al.*, 2021; Mahmud *et al.*, 2022; Boltana *et al.*, 2023; Wongnaa *et al.*, 2023).

The PSM method is one of the methods that can eliminate the problem of selection bias due to observed factors in the framework of observational data without functional and distributional assumptions (Gitonga *et al.*, 2013). This method is based on the assumption that selection bias due to the observed factors can be eliminated by matching each recipient household with one or more non-recipient households that are similar in observable characteristics. The PSM method identifies a causal relationship between microcredit receipt and outcome variables by comparing the means between recipient households (treatment group) and non-recipient households (control group) based on the Wilcoxon rank test (Gitonga *et al.*, 2013; Luan and Bauer, 2016). This method does not require time series data to evaluate the success of a policy or action and can be estimated only with data from a single point in time (Sani Heidary *et al.*, 2020). One of the important limitations of PSM is that it cannot exploit selection bias caused by unobserved factors (latent bias). In this regard, in the PSM method, the degree of sensitivity of the results to latent bias should be determined using the sensitivity analysis proposed by Rosenbaum (2002).

The matching method was conducted through two main stages to investigate the effect of microcredit on food security. The first stage is to determine the factors influencing the household decision to use microcredit using the logit model. This model is usually preferred over the probit model for reasons such as: (a) Simple interpretability of estimated coefficients; (b) greater flexibility in fitting data, and (c) Being resistant to outliers and providing more stable results (Greene, 2012).

A household may apply for microcredit based on its expected costs and benefits (Luan and Bauer, 2016; Boltana *et al.*, 2023). The logit model can be written as follows:

$$AMC_i^* = \beta X_i + \varepsilon_i, \forall i = 1, 2, \dots, N \quad (3)$$

Where, AMC_i^* is the microcredit status of the household. AMC_i equals one if the household took at least one microcredit in the previous 36 months, and otherwise zero. X_i is a set of independent variables (Appendix 3), and ε_i is an error term following the normal distribution, and N is the Number of households.

In the second step, recipients and non-recipients of microcredit were matched by their propensity scores using three matching estimators, including nearest neighbor, kernel, and radius matching. In the nearest neighbor method, each household in the control group is matched to the nearest household in the treatment group. $C(P_i)$ represents the set of households in the Control group matched to households in the treatment group, which have Propensity scores P_j and P_i , respectively. Therefore, the nearest neighbor matching algorithm is defined as follows (Becker and Ichino, 2002):

$$C(P_i) = \min_j \|P_i - P_j\| \quad (4)$$

In the radius method, households in the control and treatment groups are matched within a certain distance of the Propensity score of the treatment group household (P_i). Therefore, matching based on the radius method is defined as follows (Becker and Ichino, 2002):

$$C(P_i) = \{p_j \mid \|p_i - p_j\| < r\} \quad (5)$$

Where, all propensity scores of the control group households are matched with unit i of the treatment group household at a distance r from p_i .

In core matching, each treatment group household is matched with a weighted average of each control group household



that has a similar propensity score; but more weight is given to the households with a closer propensity score. Assuming that T and C are the sets of Treatment and Control group households, respectively, and Y_iT and Y_jC are the observed outcomes for their groups, core matching algorithms are defined in standard terms as follows (Becker and Ichino, 2002):

$$TK = \frac{\sum_{j \in C} Y_j^C G\left(\frac{p_j - p_i}{h_n}\right)}{\sum_{k \in C} G\left(\frac{p_k - p_i}{h_n}\right)} \quad (6)$$

Where, $G(\cdot)$ is a kernel function and h_n is a bandwidth parameter.

The effect of microcredit on food security indices is estimated by the Average Treatment Effects on the Treated (ATT), which is expressed as follows (Luan and Bauer, 2016; Boltana *et al.*, 2023):

$$ATT = E(Y^1|D=1) - E(Y^0|D=1) \quad (7)$$

Where, $E(Y^1|D=1)$ and $E(Y^0|D=1)$ denote outcomes for microcredit accessed households and the hypothetical outcome that would have resulted if the accessed household had not taken microcredit, respectively.

The degree of sensitivity of the results to the bias caused by unobserved factors was investigated using the sensitivity analysis (Boltana *et al.*, 2023).

This analysis can determine to what extent the existence of latent bias in the study will have no effect on the results (Rosenbaum, 2002). The odds ratio of two identical households i and j to receive the credit is defined as Equation (8) (Guo and Fraser, 2014):

$$\frac{1}{\Gamma} \leq \frac{P_i(1-P_j)}{P_j(1-P_i)} \leq \Gamma \quad (8)$$

Where, $P_i/(1-P_i)$ and $P_j/(1-P_j)$ represent the odds of households i and j receiving the credit, Γ denotes the degree of a study's bias to latent bias. Sensitivity analysis at different values of Γ examines how changes in Γ lead to changes in the outcome of the participation effect in microcredit. A study is

sensitive if values of Γ close to 1 can lead to very different inferences from the obtained results (i.e., the probability level at $\Gamma=1$ is significant). If larger values of Γ are required to change the inference, the study is insensitive (Guo and Fraser, 2014).

Additionally, the bootstrap algorithm was also used to improve the standard error of the PSM method (Austin and Small, 2014).

RESULTS AND DISCUSSION

Descriptive Statistics

Table 1 provides the descriptive statistics of variables for microcredit recipients and non-recipients. Of the 376 households' heads, 177 (47%) were microcredit recipients and 199 (53%) were non-recipients. Compared to non-recipient households, microcredit recipients were younger, more educated, had more people who could help them in times of crisis, had larger families, lower dependency ratios, more social interactions (in terms of membership in social groups and the number of visits to agricultural extension services), had a higher level of awareness and access to information about strategies for adapting to climate change, greater access to local markets, and lower savings. Recipient households had larger agricultural land compared to non-recipients. They had experienced more shocks and, consequently, were more exposed to various shocks; therefore, they had suffered greater losses in agricultural and livestock products. These households were located at a shorter distance from microcredit disbursing institutions.

Households' Food Security Status

Table 2 shows the results of the HFIAS and FCS indices. Our findings show that 100% of the households experienced anxiety and uncertainty related to food insecurity. Also, approximately 94.7% of the households experienced insufficient and poor quality food, and about 80.0% suffered

Table 1. Descriptive statistics of variables, measurements, and expected signs.

Variables	Unit	Non-recipient	Recipient	Expected sign
Age of household heads	Years	63.784	53.243	+/-
Membership of the head of the household in social groups	Number	1.829	2.960	+
Education of household heads	Years	4.864	6.740	+
Household size	Persons	4.719	6.198	+
The number of people known who could be asked for help	Persons	13.890	19.158	+
The contacts with agricultural extension	Number	4.055	7.881	+
Saving	Million Rials (IRR)	18.658	13.073	-
Dependency ratio: The ratio of household members without income to household income earners	(%)	0.423	0.278	-
Total land size	Hectare	3.262	6.090	+
Experience of various natural shocks in the last three years	Number	10.302	11.616	+
Cropshock: The value of losses of agricultural products due to various shocks	Million Rials	98.719	118.446	+
Animals shock: Livestock lost due to various shocks	Number	4.025	6.616	+
Awareness of adaptation strategies	Quality: Score from 1 to 3	1.714	2.678	+
Access to information on climate change	1= Yes; 0= No	0.428	0.718	+
Distance to the lending institution	Minutes	32.281	20.232	-
Access to the local market	1= Yes; 0= No	0.745	0.802	+
Number of observations	-	199	177	

Table 2. Summary information on household food insecurity access (a) domains, (b) prevalence, and FCS.

Index	Categories	Frequency	Percentage
HFIAD	Insufficient food intake and physical consequences	301	80
	Insufficient quality	356	94.7
	Anxiety and uncertainty	376	100
HFIAP	severely food insecure	141	37.5
	moderately food insecure	160	42.5
	mildly food insecure	75	20
	food secure	0	0.0
FCS	Acceptable	97	26.8
	Borderline	166	44.1
	Poor	113	30.1

Source: research findings

from insufficient food intake and its physical consequences. In addition, the results reveal that 100% of the households are food insecure, and 37.5% of them suffer from severe food insecurity. The results of the FCS index indicate that 44.1 and 30.1% of the total households are at borderline and poor food consumption levels, respectively. Table 3 presents the regional analysis of food security. The results show that food insecurity is more in the central district than Jazinak district. Among the rural districts,

food insecurity is more severe in Zehak Rural District.

Propensity Score Matching

Table 4 provides the logit model results. The findings reveal that 1% increase in the age of the head of the household decreases the probability of access to microcredit by 6.1%. (Luan and Bauer, 2016; Sani Heidary *et al.*, 2020), which shows that older household heads have less access to

**Table 3.** Summary of the estimated values for HFIAS and FCS indices.

Region	Villages (Number)	Households (Number)	HFIAS	FCS
Central District	28	278	17.10	24.00
Zehak Rural District	20	212	17.80	23.00
Khajeh-Ahmad Rural District	8	66	16.30	25.00
Jazinak District	16	98	13.75	27.75
Khamak Rural District	9	53	13.30	28.50
Jazinak Rural District	7	45	14.20	27.00
Average scores of total observations	44	376	15.85	25.36
minimum scores of total observations	44	376	5.00	17.00
maximum scores of total observations	44	376	23.00	75.50

Source: research findings

innovations and financial information. However, this finding contradicts the results by Akotey and Adjasi (2016) for Ghana. A 1% increase in household savings leads to decrease the probability of access to microcredit by 8.5%. Similarly, the studies by Luan and Bauer (2016) in Vietnam and Sani Heidary *et al.* (2020) in Iran revealed that household savings were used to invest in future productions and meet essential needs. A 1% increase in the dependency ratio of households reduces the probability of their access to microcredit by 0.330%. Households with more dependents are generally exposed to more credit constraints. These findings is consistent to the results of Thanh *et al.* (2019) and inconsistent with the results of Li *et al.* (2011). The probability of receiving microcredit by the households with high awareness of climate change adaptation strategies is 12.9% more than the households with low awareness. This result is consistent with the findings of the previous research (Luan and Bauer, 2016; Ojo *et al.*, 2019), which indicated that microcredit is a critical tool for improving adaptation strategies. However, our findings do not confirm the results obtained by Bakare *et al.* (2023).

A 1% increase in the household head's education increases the probability of access to microcredit by 1.4%. Similarly, the studies by Thanh *et al.* (2019) and Berhanu *et al.* (2021) revealed that educated household heads were more willing to receive microcredit to reduce the financial imbalance. The results reveal that 1%

increase in households' contacts with agricultural extension institutions increases the probability of microcredit access by 2.1%. In addition, 1% increase in the number of helpers increases the probability of access of microcredit by 9.5%. These findings are similar to previous research (Luan and Bauer, 2016; Sani Heidary *et al.*, 2020; Berhanu *et al.*, 2021), which indicated that increasing the social connections of households through their connections with institutions leads to an increase in their information about important rural issues, particularly financial resources, and influences their demand for access to credit. Additionally, increasing the number of people, who can help households in critical situations such as loan repayment, leads to an increase in their demand for credit, and can even be considered as social guarantors of households for credit-paying institutions. The results of these two variables emphasize the effective social communications and interactions of households that facilitate their access to the necessary resources, particularly credit.

A 1% increase in household size increases the probability of access to microcredit by 4.5%. The studies by Akotey and Adjasi (2016) in Ghana and Berhanu *et al.* (2021) in Ethiopia revealed that larger households have sufficient labor force to participate in rural micro-businesses, which increases the need for household credit to establish businesses. In addition, larger households have greater food needs for sustainable consumption and, therefore, require more

Table 4. The results of logit model.

Variable		Coefficients (std. err)	Z-value	Marginal Effect
Age of household heads		-0.244 (0.118)	-2.07**	-0.061**
Saving		-0.339 (0.176)	-1.93**	-0.085**
Distance to the lending institution		-0.636 (0.772)	-0.82	-0.158
Dependency ratio		-1.326 (0.695)	-1.91**	-0.330**
Awareness of adaptation strategies	Medium	-0.196 (0.244)	-0.81	-0.049
	High	0.521 (0.241)	2.16**	0.129**
Education of household heads		0.055 (0.026)	2.15**	0.014**
The contacts with agricultural extension		0.084 (0.041)	2.03**	0.021**
The number of people known who could be asked for help		0.384 (0.151)	2.53**	0.095**
Household size		0.182 (0.085)	2.14**	0.045**
Total land size		0.205 (0.057)	3.59***	0.051***
Crops shock		0.049 (0.021)	2.31**	0.013**
Animals hock		0.094 (0.044)	2.13**	0.023**
Experience of various natural shocks		0.090 (0.096)	0.94	0.022
Membership in social groups		0.031 (0.082)	0.38	0.008
Access to information on climate change		0.488 (0.256)	1.90*	0.120*
Access to the local market		0.311 (0.293)	1.20	0.077
Intercept		-3.492 (1.615)	-2.16**	-
LR chi2 (17)		100.80***		
Pseudo R2		0.194		
Correctly classified (%)		76.06		
Hosmer–Lemeshow chi2 (8)		5.55		
Prob > Hosmer–Lemeshow chi2		0.236		
Number of observations (No credit)		199		
Number of observations (Credit accessed)		177		
Number of observations (All sample)		376		

*** Significant at $P < 0.01$; ** Significant at $P < 0.05$; * Significant at $P < 0.05$.

financial resources for sustainable household food consumption, which microcredit can meet.

A 1% increase in household farm size increases the probability of access to microcredit by 5.1%. This result is consistent with previous studies (Luan and Bauer, 2016; Sani Heidary *et al.*, 2020),

demonstrating that access to larger agricultural land increases the use of key inputs, and, consequently, increasing the need for capital and credit. A 1% increase of losses in the production of agricultural products and loss in livestock populations increases the probability of households' access to microcredit by 1.3 and 2.3%,

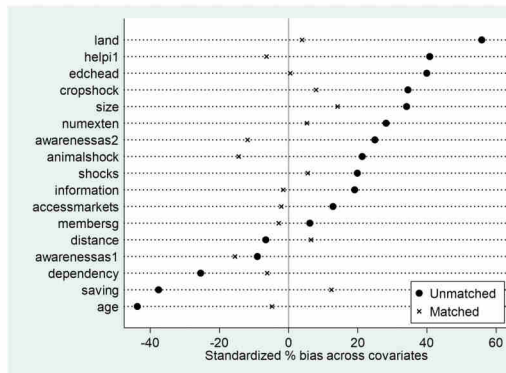


Figure 2. Bias % of covariates before and after matching.

respectively. The greater the losses caused by various shocks, the more the households use microcredit as an immediate tool to increase coping ability (Luan and Bauer, 2016; Berhanu *et al.*, 2021).

The results show that the mean of bias decreased and covariates became insignificant after matching. The bias percentage of covariates after matching has been significantly reduced compared to before. (Figure 2). Visual inspection of propensity score distributions showed that the common support condition was met, indicating a high overlap between microcredit recipients and non-recipients (Figure 3). This shows that the matching of the two groups is appropriate and the PSM results are highly reliable. In Figure 3, “On support” refers to the households that were present in the compliance of the two treatments and the control groups, and “Off support” refers to the households that were not present in the compliance of the two treatments and the control groups.

Table 5 provides the effects of microcredit on the HFIAS and FCS indices using three matching algorithms (nearest neighbor, kernel, and radius). The findings demonstrate that microcredit has a negative and significant effect on HFIAS for the three matching estimators. Households receiving microcredit have lower food insecurity scores (9.80-9.99) than non-recipient households (12.95-13.83). Microcredit has reduced the HFIAS score by 24.3-27.8% for the recipient households compared to the

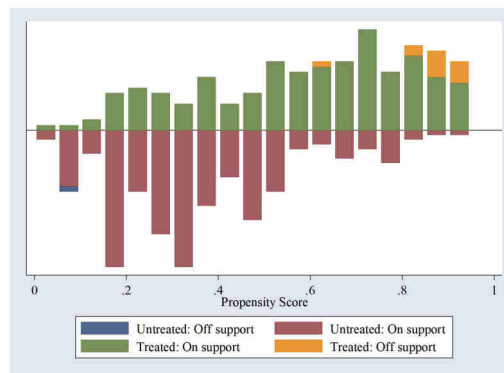


Figure 3. The distribution of Propensity Scores (PS) and common support for estimating PS.

non-recipient households. The findings indicate that microcredit has a significant positive effect on FCS for all matching algorithms. The FCS score is higher for recipient households (45.82-46.23) than non-recipient households (35.17-36.56). This means microcredit has increased the FCS score by 25.9-31.4% for recipient households compared to the non-recipient households. These results are consistent with the existing literature (Hamad and Fernald, 2012; Islam *et al.*, 2016; Devereux, 2016; Bocher *et al.*, 2017; Kianersi *et al.*, 2021; Haque, 2021; Berhanu *et al.*, 2021; Bahiru *et al.*, 2023; Woleba *et al.*, 2023; Kolog *et al.*, 2023; Wongnaa *et al.*, 2023; Boltana *et al.*, 2023). They reveal that microcredit help to enhance food security of households through investing in income-generating activities, creating diverse income streams and safe networks, reducing vulnerability to health shocks, and improving the flow of information on household health and nutrition programs. However, some studies showed that microcredit had no significant effect on food security (Banerjee *et al.*, 2015; Seng, 2018; Mahmud *et al.*, 2022; Salima *et al.*, 2023). In addition, other studies have shown that microcredit may push households into food insecurity situations by creating excessive debt and loan repayment pressure (Develtere and Huybrechts, 2005; Aromolaran, 2010; Ganle *et al.*, 2015; Namayengo *et al.*, 2018; Ahmed *et al.*, 2021).

Table 5. Impact of microcredit accessed on HFIAS and FCS indices.^a

Outcome	Matching	Controls	Treated	ATT	Bootstrap SE	T-stat	Matched observations		
							All sample	Credit accessed	No Credit
HFIAS	Neighbor	13.538	9.848	-3.690	0.649	-5.686***	369	171	198
	Kernel	12.954	9.797	-3.157	0.460	-6.864***	376	177	199
	Radius	13.834	9.986	-3.847	0.580	-6.635***	376	177	199
FCS	Neighbor	35.871	45.819	9.947	2.733	3.640***	369	171	198
	Kernel	36.564	46.023	9.458	2.530	3.738***	376	177	199
	Radius	35.168	46.229	11.061	1.587	6.970***	376	177	199

^a Bootstrap SE: Bootstrap Standard Error with 1000 times simulations. *** Significant at $P < 0.01$.

Table 6. Sensitivity analysis of outcome variables.

Gamma	HFIAS		FCS	
(Γ)	Significant-	Significant+	Significant-	Significant+
1	0.00	0.00	0.00	0.00
1.2	0.00	0.00	0.00	0.00
1.4	0.00	0.00	0.00	0.00
1.6	0.00	0.00	0.00	0.00
1.8	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
2.2	0.00	0.00	0.00	0.00
2.4	0.00	0.00	0.00	0.00
2.6	0.00	0.00	0.00	0.00
2.8	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00

^a Γ : Log odds of unobserved differential assignment; Significant-: Lower bound significance level, Significant+: Upper bound significance level.

Sensitivity Analysis for Hidden Bias

Table 6 shows the results of checking hidden bias by sensitivity analysis. Our findings reveal that the effect of microcredit interventions on HFIAS and FCS indices does not change, and the households are allowed to differ in their odds of treatment by 200% $[(3-1) \times 100 = 200]$ at $\Gamma = 3$ in terms of unobserved covariates in both groups. Therefore, it can be concluded that the results of ATT for all output variables are not sensitive to unobserved hidden bias, and the estimated effect is a pure effect of using microcredit. This finding is consistent with the results of Berhanu *et al.* (2021) and Boltana *et al.* (2023).

CONCLUSIONS

This study seeks to answer how microcredit plans lead to enhance the households' food security by developing the

PSM method through the bootstrap algorithm. The findings emphasize the positive role of microcredit in reducing the HFIAS and increasing the FCS. However, a large number of target households are food insecure and do not have a good condition in terms of food consumption; because a significant number of households did not have access to or did not receive microcredit for various reasons.

The results showed that the access of households to microcredit was positively influenced by high awareness of adaptation strategies, access to climate change information, the household head's education, the number of helpers, the number of household contacts with agricultural extension institutions, household size, agricultural land size, the value of crop losses and the number of lost livestock. However, the household head's age, households' savings, and dependence ratio have a negative effect on the access of households to microcredit.



Based on these findings, this study proposes the following policy implications. First, non-governmental organizations and local social associations should be further promoted and strengthened to increase households' access to rural microcredit. In addition, they should be flexible in accepting natural guarantees, such as agricultural land and household livestock, and social guarantees, such as membership in social groups, to increase the level of households' access to microcredit. Secondly, non-governmental and governmental organizations providing microcredit should emphasize the organizing effective training programs to increase households' knowledge and skills. This leads to improved households' food security through individual development and collective participation. These organizations should target educated rural youth with suitable incentive programs. Educated youths have high capacities for correctly using microcredit in income-generating activities, which can provide a basis for improving food security in rural communities.

Although this study has provided several new insights about the effect of microcredit on food security, some limitations need to be considered in the future research. First, future studies can expand the subject of this study using other food security indices such as the Household Dietary Diversity Score (HDDS) (Wongnaa *et al.*, 2023; Borku *et al.*, 2024), and other methods like the Endogenous Switching Model (Salima *et al.*, 2023). Secondly, considering the limited data availability, this study uses a cross-section sample. Future research can achieve more comprehensive findings using panel data (Islam *et al.*, 2016).

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Appendix 1. Measurement of HFIAD and HFIAP.

Index	Category	Calculation ^a
HFIAD	Insufficient food intake and physical consequences	Number of respondents to Q5 to Q9= 1
	Anxiety and uncertainty	Number of respondents to Q2 to Q4= 1
	Insufficient quality	Number of respondents to Q1= 1
HFIAP	Severely food insecure	Number of respondents to Q5a= 3 or Q6a= 3 or Q7a= 1 or 2 or 3; or Q8a= 1 or 2 or 3; or Q9a= 1 or 2 or 3.
	Moderately food insecure	Number of respondents to Q3a= 2 or 3; or Q4a= 2 or 3; or Q5a= 1 or 2; or Q6a= 1 or 2.
	Mildly food insecure	Number of respondents to Q1a= 2 or 3; or Q2a= 1 or 2 or 3; or Q3a= 1 or Q4a= 1.
	Food secure	Number of respondents to Q1a=0 or 1.

^a Q1 to Q9 denotes occurrence questions, while Q1a to Q9a represents their frequency. Source: Coates *et al.* 2007.

Appendix 2. Summary of weights for each food group.

Food group	Weight
Staple foods	2
Meat and fish	4
Fruit and vegetables	1
Dairy products	4
Pulses	3
Oil and sugar	0.5

Appendix 3. Demographic background of the sampled households.

Variables	Group	Frequency	Percentage
Age of the household head	35-45	70	19
	46-55	100	27
	56-65	118	31
	65>	88	23
Education level of household heads	Not able to read and write	133	35
	primary education	84	22
	secondary education	102	27
	higher education	57	15
Sex of household head	Female	74	20
	Male	302	80
Economic activities of households	Farming	144	38
	Livestock farming	148	39
	shopkeeper	24	6
	Handicrafts	47	13
	Employee	13	3
Fields of microcredit receipts of households	Consumption	38	10
	Working capital	56	15
	Agriculture	113	30
	Livestock	169	45
Loan size of households [Million Rials (IRR)]			
Consumption	150-300	38	10
Working capital	350-500	56	15
Agriculture	450-700	113	30
Livestock	700-1000	169	45



ارزیابی اثرات اعتبارات خرد بر وضعیت امنیت غذایی خانوارهای روستایی: مطالعه تجربی از روستاهای شهرستان زهک، ایران

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چکیده

اعتبارات خرد نقش حیاتی در امنیت غذایی خانوارهای روستایی دارد. با این حال، اثرات اعتبارات خرد بر بهبود امنیت غذایی خانوارها هنوز در ایران به خوبی مورد مطالعه و درک قرار نگرفته است. لذا این پژوهش با هدف بررسی موفقیت برنامه‌های اعتبارات خرد در ارتقای امنیت غذایی خانوارهای روستایی شهرستان زهک با استفاده از روش تطبیق امتیاز گرایش و الگوریتم بوت استرپ انجام شده است. برای این منظور از دو شاخص امنیت غذایی شامل مقیاس دسترسی به ناامنی غذایی خانوار (HFIAS) و امتیاز مصرف غذا (FCS) استفاده می‌شود. نتایج نشان داد که 100 درصد خانوارها با ناامنی غذایی مواجه هستند. شیوع ناامنی غذایی برای ناامنی غذایی خفیف، متوسط و شدید به ترتیب 20، 42.5 و 37.5 درصد بود. علاوه بر این، 30 درصد خانوارها در وضعیت نامناسب مصرف مواد غذایی قرار دارند. یافته‌های ما بر نقش مثبت و قابل توجه اعتبار خرد در بهبود امنیت غذایی تاکید می‌کند. نتایج نشان داد اعتبار خرد امتیاز شاخص HFIAS خانوارهای دریافت‌کننده را بین 24.31-27.81 درصد کاهش و امتیاز شاخص FCS را بین 25.78-31.45 درصد افزایش داده است. بنابراین، سیاست‌گذاران و تصمیم‌گیران باید سازمان‌های دولتی و غیردولتی ارائه دهنده اعتبارات خرد را ترویج و تقویت کنند. همچنین ارائه اطلاعات و کاهش محدودیت‌های وثیقه برای افزایش دسترسی خانوارها به اعتبارات خرد توصیه می‌شود.

Predicting Farmers' Behavioral Intentions towards Adoption of Essential Oil Extraction Practices Using Structural Equation Modeling

Fouzia Anjum¹, Sher Muhammad^{1*}, Badar Naseem Siddiqui², Farhat Ullah Khan¹, Muhammad Yaseen³, and Muhammad Shahbaz Anjum⁴

ABSTRACT

Smallholder farmers in northern Punjab struggle to adopt sustainable practices like essential oil extraction, despite their potential to improve livelihoods. Core elements from theory of planned behavior, technology acceptance model, and innovation diffusion theory are amalgamated to develop an adoption model that is subsequently analyzed using structural equation model. The results unveil significant mediating effects involving attitudes (perceived usefulness, easiness), normative concerns (social influence), and indicating maximum variation (R²) regarding by-product preparation (0.76) and steam distillation (0.65). The model successfully accounts moderating effects of socioeconomic variables, indicating a robust association among latent variables. Hence, improving the adoption behavior among smallholders necessitates a focus on socio-psychological and socioeconomic factors.

Keywords: Aromatic growers, Decision-making, Innovation Diffusion.

INTRODUCTION

Worldwide demand for essential oils is increasing due to growing interest and commercial importance. Currently, Pakistan heavily relies on imported essential oils, with over 90% of local industry demand being met through imports. Research by Riaz *et al.*, (2021) shows imports (\$9.2 million) exceeding exports (\$3.2 million) threefold, indicating a need for local production initiatives. Favorable climatic conditions in Pakistan make it conducive for high-value essential oil production, with potential benefits for both awareness and education (Khalid *et al.*, 2020). Utilizing essential oils alongside herbal and

agro-based materials presents an eco-friendly and cost-effective approach to co-composting (Greff *et al.*, 2021). Among many, *Eucalyptus globules*, the most prevalent species used for Essential Oil Extraction (EOE), possesses insect repellent properties, offering innovative applications in bio-pesticides and composting (Dhakad *et al.*, 2018). Eucalyptus leaves, often discarded, hold potential for Essential Oil Extraction (EOE), serving domestic and industrial purposes (Barbosa *et al.*, 2016). Steam distillation efficiently extracts oils, preserving their properties to a minimum (Ndiaye *et al.*, 2018). Steam-distilled eucalyptus oil enriches composting and improves soil fertility. Moreover, it acts as a bio-pesticide, against garden pests (Regnault-

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Roger *et al.*, 2012).

The pace of agricultural technology adoption among end users in developing nations remains sluggish, driven by economic potential, but not always profit maximization (Ikram *et al.*, 2021). Analyzing farmers' perceptions aids decision-making (Liu *et al.*, 2018). Socio-psychological factors, often analyzed through the Theory of Planned Behavior (TPB), shape adoption behavior (Dessart *et al.*, 2019). Understanding communication channels improves integration and predicts agricultural technique adoption, including EOE (Mohd Israfi *et al.*, 2022). Hence, this study aims to grasp eucalyptus growers' behavioral intentions to promote EOE practices, focusing on (i) Steam distillation, and (ii) Byproduct preparation like bio-pesticides and bio-compost.

The present study contributes significantly in several ways. Recent research has integrated TPB/Technology Acceptance Model (TAM) with Structural Equation Modeling (SEM), or combined both methodologies, to understand agricultural technology adoption and model farmers' behavior towards implementing good agricultural practices (Dong *et al.*, 2022). However, these studies often overlook the economic potential of cultivating aromatic plants among smallholders. Firstly, this study fills this gap by employing a combination of TPB, TAM, Innovation Decision Theory (IDT), and SEM to assess aromatic crop growers' intention towards adopting EOE practices. Secondly, besides mediation analysis using Partial Least Square (PLS)-SEM, moderation analysis, incorporating socioeconomic variables, predicts the relationship direction between exogenous and internal variables. Thirdly, the study also examines direct effects of latent and observed variables on behavioral intention (Bi) and, finally, evaluates predictive efficacy using PLS-SEM to enhance model robustness.

Theoretical Background

To deepen understanding of innovation adoption, Rogers' Innovation Decision Theory (IDT) elucidates the link between farmers' adoption and their knowledge-based perception of an innovation (Bakkabulindi, 2014). Despite EOE technology being perceived as new among aromatic crop growers due to limited knowledge, its global adoption and economic efficacy from aromatic plants are well-documented (Chhetri *et al.*, 2021). An empirical model integrating TPB (Ajzen, 1991), Rogers' IDT (Miller, 2015), and TAM by Silva (2015) (Figure 1) was developed to examine the cause-and-effect relationships among variables influencing adoption behavior.

While no universally accepted model exists, TPB and TAM are commonly applied in agricultural technology adoption (Marangunić and Granić, 2015). This study's model explains eucalyptus growers' intention through attitude into Perceived usefulness (Pu) and ease of use (Peou) from TAM, and Perceived compatibility (Pc) from Rogers' IDT, perceived control into self-efficacy and Perceived resources (Pr), and normative concerns, further enriched with elements from Social media (Sm), influence (Si), Technical training (Tt), and Extension services (Es) to capture social pressure and communication source (Momani, 2020).

Generation of Hypotheses for the Proposed Adoption Model

H₁= Attitude, perceived control, and normative concerns have significant and positive effects on the growers' intentions toward EOE practices.

H₂= All observed variables (Pu, Peou, Pc, Tt, Si, Sm, Es, Pe, Pr) have significant and positive effects on the growers' Bi concerning EOE practices.

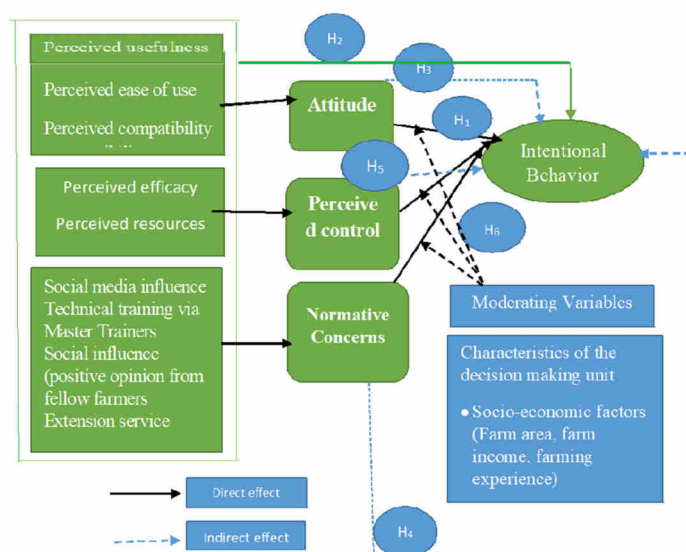


Figure 1. Extended proposed adoption model of the study (conceptual framework for behavioral intention of eucalyptus growers towards EOE practices). The proposed adoption model for EOE practices is a fusion of three different theories; Theory of Planned Behavior (TPB) by (Ajzen, 1991), Technology Acceptance Model (TAM) by (Davis, 1989), and Innovation Diffusion Theory by (Rogers, 2003).

H₃= Attitude facilitates the positive effects of perceived usefulness, ease of use, and compatibility on EOE adoption intentions.

H₄= Normative concerns mediate the effects of social media, technical training, and extension services on the growers' intentions towards EOE practices.

H₅= Perceived control mediates self-efficacy and resources positively on growers' intended behavior towards EOE adoption.

H₆= Socioeconomic factors moderate positive relationships between variables towards EOE adoption.

MATERIALS AND METHODS

Study Area

The universe of study was Pothwar Region of Punjab Pakistan (Northern Punjab) with longitude 73.07° E, latitude 33.6° N and elevation of 517 m from mean sea level located between the Indus and Jhelum rivers comprising four main districts, namely,

Chakwal, Attock, Jhelum and Rawalpindi (Figure 2). The study focuses on steam distillation and by-product preparation for essential oil extraction, as they are practical, widely used, and easier to adopt, offering greater benefits to farmers' livelihoods.

Selection of Sample Size and Data Collection

A list of 942 registered eucalyptus growers was obtained from the Director of Agriculture (Extension and Adaptive Research), Rawalpindi and Punjab Forestry Department. A sample size of 274 was obtained by using the table developed by Krejcie and Morgan (1970) from a homogenous finite population using simple random sampling from each district (Table 1). Initially, master trainers were used to disseminate the targeted information about EOE practices among the sampled respondents during training sessions. Further, the data were collected through personal (face-to-face) interviews of the



respondents using a structured research instrument.

Implementation of an Extended Proposed Adoption Model of Study

The proposed adoption model was employed to test the aligned hypotheses. The model was analyzed using PLS-SEM, which was further subdivided into measurement and structural model.

Structure of the Adoption Model (Research Instrument)

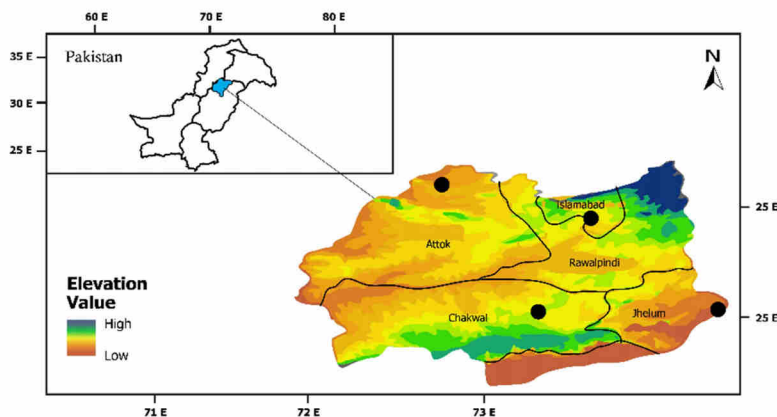
The variables examined were latent (unobserved) and assessed through observable statements. About 84 statements were used in the questionnaire for both EOE practices. The statements were loaded into 13 factors. The factors including Bi, At, Nc, Pct, self-efficacy, and Pr were weighted by six, four, four, three, two, and two recorded statements, respectively, while the

remaining factors were loaded by three statements under each practice.

Partial Least Squares-Structural Equation Modeling (Assumptions and Estimation)

Structural Equation Modeling (SEM), a statistical method blending factor analysis and multiple regression, analyzes cause-effect relationships among latent variables. These variables, not directly measured, are inferred from observed variable responses. However, the variance-based PLS-SEM approach was chosen to assess the adoption model for the following several reasons (Leguina, 2015):

- (i) Maximizing explained variability (R^2) in the criterion variable
- (ii) Flexibility regarding data structure normality
- (iii) Accommodating underlying variables with few items
- (iv) Favoring prediction over theory



Map of Pothwar Plateau, Pakistan

Figure 2. Map of study area.

Table 1. Estimation of sample size from each selected district of the study area.

District	Population	Percentage	Sample size (n)
Attock	349	36.81	101
Chakwal	277	29.22	80
Jhelum	210	22.15	61
Rawalpindi	112	11.81	32
Total	948	100.00	274

testing

This methodological choice allows for a comprehensive examination of relationships in the adoption model, prioritizing practical relevance and prediction accuracy. The PLS-SEM adoption model underwent evaluation in two steps using SMART-PLS. First, assessing the relationship between indicators and inferred variables (measurement model), and second, examining causal relationships among inferred variables (structure model) (Khoi and Van Tuan, 2018). Hence, Each PLS path item is a linear function with errors:

$$X_j = \Lambda_j \xi_j + \varepsilon \quad (\text{exogenous latent variable } \xi_j) \quad (1)$$

$$X_l = \Lambda_l \xi_l + \delta \quad (\text{endogenous latent variable } \xi_l) \quad (2)$$

Here, ξ_j = Set of the exogenous (external) underlying variables

ξ_l = Set of the endogenous (internal) underlying variables.

X_j and X_l = Associated set of indicators ($X_1, \dots, X_j$; $X_1, \dots, X_l$) of the external variable ξ_j and internal variable ξ_l , respectively.

Λ_j and Λ_l = Loading coefficient matrices ($\Lambda_1, \dots, \Lambda_m$), k = No. of indicators (items)

ε and δ = Set of error terms for the items

Furthermore, indicator reliability, convergent validity, and discriminant validity using Heterotrait-Monotrait (HTMT) were assessed for the measurement model. Factor loadings should surpass 0.7, Average Variance Extracted (AVE) > 0.5, Composite Reliability (CR) > 0.7 (Annex A).

Before proceeding to the structure model estimation phase, multicollinearity issues were assessed using Variation Inflation Factors (VIF) for each variable (Annex B). An iterative sequence of least square regressions was then utilized to estimate model parameters, maximizing explained variance (R^2) (Monecke and Leisch, 2012). Hence, the structure model links the internal (ξ_k) and external (ξ_j) implied variables and can be expressed as:

$$\xi_k = B \xi_k + \Gamma \xi_j + \zeta \quad (3)$$

Where, B = Coefficient matrix indicating the causal effects between internal implied variables, Γ = Coefficient matrix of the causal effects of the external implied variable ξ_j on the internal inferred variable ξ_k . In the structural model, an inferred endogenous variable can also act as an exogenous variable for another endogenous variable, with ζ representing the residuals or error terms. A bootstrapping process with 5,000 subsamples calculated P-values and effect sizes (f^2 - value) (Purwanto, 2021). Furthermore, moderating variables like farm area, farming experience, and farm income were assessed through bootstrap multi-group analysis (Tian *et al.*, 2021). Predictive relevance Q^2 for the endogenous variable (B_i) was also estimated using PLS-Predict (Hossan *et al.*, 2020).

RESULTS

Estimation Results of the Extended Proposed Adoption Model of the Study

Measurement Model

Annex-A results indicate no Heterotrait-Monotrait (HTMT) ratio surpassing the critical level of 0.9 for each variable under each EOE practice. Table 2 displays mean and factor loading results for each variable. CR values above 0.7 denote favorable internal consistency within the adoption model for each practice. The lowest CR value, 0.724, was observed for extension services in the use of a steam distillation unit for EOE. The highest CR value, 0.895, was found for the implied variable 'Bi' in by-product preparation. Cronbach's alpha (α) for each variable was also assessed, with the highest values (0.863 and 0.798) for 'Bi' in by-product preparation and usage of a steam distillation unit, respectively. Convergent validity, measured by AVE, exceeded 0.5 for each inferred variable. Each variable can account for over 50% of the indicator variance. The latent variable "self-efficacy

**Table 2.** Results for the measurement model concerning first practice (Usage of steam distillation unit for essential oil extraction) and second practice (By-products preparation) in the study.^a

Variable	Statements (Indicator)	1st practice					
		Mean	α	AFL	CR	AVE	Cramer-von Mises P value
Behavioral intentions	6	3.525	0.772	0.769	0.887	.579	0.000
Attitudes	4	3.511	0.772	0.756	0.843	0.607	0.000
Perceived controls	4	3.240	0.779	0.841	0.879	0.602	0.000
Normative concerns	4	3.324	0.794	0.782	0.859	0.640	0.000
Perceived usefulness	3	3.136	0.725	0.715	0.779	0.574	0.000
Perceived ease of use	3	3.229	0.750	0.794	0.837	0.710	0.000
Perceived compatibility	3	3.949	0.745	0.730	0.783	0.614	0.000
Perceived efficacy	2	2.934	0.777	0.701	0.776	0.550	0.000
Perceived resources	3	2.951	0.760	0.734	0.779	0.508	0.000
Social influence	3	3.128	0.787	0.755	0.799	0.512	0.000
Social media	3	3.897	0.756	0.732	0.779	0.508	0.000
Technical training	3	3.694	0.798	0.778	0.861	0.675	0.000
Extension service	3	2.656	0.795	0.796	0.724	0.513	0.000
Variable	Statements (Indicator)	2nd practice					
		Mean	α	AFL	CR	AVE	Cramer-von Mises P value
Behavioral intentions	6	3.725	0.863	0.770	0.895	0.695	0.000
Attitudes	4	3.446	0.756	0.756	0.843	0.673	0.000
Perceived controls	4	3.582	0.793	0.841	0.879	0.707	0.000
Normative concerns	4	3.556	0.783	0.726	0.859	0.606	0.000
Perceived usefulness	3	3.305	0.775	0.734	0.779	0.543	0.000
Perceived ease of use	3	4.066	0.708	0.845	0.837	0.632	0.000
Perceived compatibility	3	4.176	0.709	0.856	0.783	0.656	0.000
Perceived efficacy	2	4.130	0.701	0.705	0.774	0.677	0.000
Perceived resources	3	2.044	0.796	0.838	0.708	0.626	0.000
Social influence	3	3.882	0.723	0.746	0.799	0.570	0.000
Social media	3	4.024	0.794	0.803	0.779	0.545	0.000
Technical training	3	3.997	0.804	0.878	0.826	0.618	0.000
Extension service	3	2.848	0.704	0.685	0.776	0.575	0.000

under 1st practice" had the lowest AVE (0.512), confirming convergent validity. VIFs below 5 indicate no multicollinearity for these variables (Annex B).

Structure Model

Table 3 displays SEM outcomes, including path coefficients, p-values, and effect sizes (f^2 -value). Behavioral intention predicts approximately 79% for by-product preparation and 65% for using a steam distillation unit. Attitude, perceived control, and normative concerns are significant predictors of Pothwar Region growers' EOE adoption intention. Respondents with

positive attitudes, indicated by standardized coefficients, tended to show stronger intentions towards adopting by-product preparation ($\beta = 0.48$) and steam distillation usage ($\beta = 0.23$) compared to others. The variance (R^2) of normative concerns extracted by assigned variables was 50% for by-product development and 40% for steam distillation usage. The latent variable 'perceived controls' encompassed both personal efficacy and perceived resources, predicting 84% (by-product preparation) and 40% (steam distillation usage) of available variance. The findings supported the Hypotheses (H_1), indicating favorable At, Pc, and Nc significantly influence Pothwar growers' adoption intentions.

Table 3. PLS-SEM results for the structure model for 1st practice (usage of steam distillation unit for essential oil extraction) and 2nd practice (by- product preparatory methods) (n= 274).^a

Model	Variable's path	Path-coefficient β	P-value	F-square ^a	Path-coefficient β	P-value	F-square ^a
		1st practice			2nd practice		
Behavior intention (<i>Bi</i>)	At -> Bi	0.23	0.000	0.15	0.48	0.000	0.34
	Nc-> Bi	0.53	0.003	0.24	0.55	0.004	0.64
	Pct-> Bi	0.17	0.000	0.17	0.21	0.000	0.15
	Perceived usefulness	0.224	0.000		0.721	0.000	
	Perceived ease of use	0.258	0.000		0.34	0.031	
	Perceived compatibility	0.097	0.015		-0.248	0.228	
	Technical training	0.256	0.000		1.709	0.006	
	Social media	0.314	0.000		0.319	0.037	
	Social influence	0.229	0.001		0.031	0.017	
	Extension service	-0.029	0.663		-0.272	0.070	
	Personal efficacy	0.177	0.009		0.168	0.048	
Attitude (<i>At</i>)	Perceived resources	0.105	0.395		0.302	0.000	
	Perceived usefulness-> Attitudes	0.28	0.009	0.07	0.31	0.006	0.24
	Perceived ease of use-> Attitudes	0.17	0.048	0.13	0.19	0.003	2.70
	Perceived compatibility-> Attitudes	0.22	0.008	0.15	0.20	0.035	0.11
Normative concerns (<i>Nc</i>)	Technical training -> Nc	0.34	0.000	0.66	0.83	0.027	2.17
	Social media-> Nc	0.28	0.046	0.20	0.13	0.049	0.06
	Social influence-> Nc	0.15	0.244	0.02	0.12	0.040	0.18
	Extension service-> Nc	0.04	0.695	0.03	-0.10	0.308	0.04
Perceived controls (<i>Pct</i>)	Personal efficacy-> Pct	0.14	0.071	0.12	0.17	0.009	0.02
	Perceived resources-> Pct	0.19	0.039	0.28	0.22	0.001	0.03

^a **1st practice:** Bi ($R^2 = 0.65$), At ($R^2 = 0.43$), Nc ($R^2 = 0.44$), Pc ($R^2 = 0.40$). **2nd practice:** Bi ($R^2 = 0.79$), At ($R^2 = 0.64$), Nc ($R^2 = 0.77$), Pc ($R^2 = 0.37$). Bi: Behavior intention; At: Attitude; Nc: Normative concern; Pct: Perceived control.

Results show perceived usefulness and ease of use positively affect farmers' intended behavior, while perceived compatibility negatively impacts intentions for the 2nd practice (-0.248). Normative concerns, including technical training, social media, and social influence, have significant direct effects on growers' intentions for both practices. However, extension services have negative indirect effects on adoption behavior. Perceived controls, like perceived resources and self-efficacy, significantly affect intentions, except for resources in the first practice ($p = 0.395$). These findings support H2, suggesting positive direct effects of observed variables on intentions, except for perceived resources and extension services.

Mediation Analysis

Table 3 illustrates the mediation results of inner or structure model PLS-SEM for both EOE practices. Attitude explains approximately 64 and 43% of available variance (R^2) in respondents' attitudes towards the second and first practices, respectively. Attitudes improved by 19% and 17% for by-products and steam distillation usage, respectively. Thus, the findings support the H₃, indicating attitudes mediate the positive effect of Pu and Peou on growers' intentions for both EOE practices.

Normative concerns, reflecting peer groups and external factors, show significant



positive effects on each EOE practice, except for extension services. Technical training had a notably higher coefficients ($\beta = 0.83$) for by-product preparation than for the other practice. However, extension services lacked a substantial effect on farmers' normative concerns regarding either practice. Thus, H_4 , stating positive and significant mediating effects of technical training and social media influence on growers' intentions through normative concerns was supported for both practices, while extension services did not demonstrate a positive mediation effect on intentions.

Positive and significant indirect effects of perceived resources influence individuals' intention towards EOE practices, mediated by perceived control. Self-efficacy demonstrates positive indirect effects, except for the first practice's non-significant effect. Hypothesis (H_5) proposed perceived control mediates positive effects of personal willingness and resources on intentions, except for self-efficacy's non-significant effect in the second practice. Moreover, large-sized effects were observed for Peou on At ($f^2 = 2.70$) (2nd practice), Tt on Nc ($f^2 = 0.66$) (1st practice), and Nc on Bi ($f^2 = 0.64$) (2nd practice).

Moderation Analysis

Table 4 illustrates results from bootstrap multi-group analysis, indicating socio-economic variables' moderation effects for both EOE practices. Notably, normative concerns exhibit the most positive and significant path coefficient ($\beta = 0.545$) on intention towards adopting steam distillation usage under medium-level farm income (PKR, Official Currency of Pakistan) 40,001-120,000). Similarly, a positive standardized path coefficient ($\beta = 0.389$) is observed for normative concerns on intended behavior under high-level farm income (PKR 120,001 and above) in by-product preparation. The model also predicts significant coefficients ($\beta = 0.664$) for normative concerns on growers' intentions

with medium farm area (9-24 kanal) for steam distillation usage. Additionally, ' $\beta = 0.378$ ' is significant for perceived control on intentions in by-product preparation with high farming experience (11-15 years). These findings support H_6 , indicating socio-economic variables moderate positive and significant effects between exogenous and endogenous latent variables.

PLS-SEM Model Predict

The Q^2 value for all predicted measured variable 'Bi' surpassed zero, indicating adequate predictive relevance. Errors in the PLS-SEM_MAE model were fewer than in the linear model (LM_MAE) for all Bi indicators, demonstrating high predictive power for by-product preparation (1st practice), with medium prediction power for steam distillation (1st practice) (Annex C).

DISCUSSIONS

The estimation results under the measurement scale reflect significant values concerning reliability and validity as supported by Henseler *et al.* (2015) that the discriminant validity of the model must not exceed a value of 0.9 for all constructs. Furthermore, internal consistency, as measured by CR, indicates the extent to which items effectively measure an underlying variable and should surpass a threshold of 0.7 (Mohd Dzin and Lay, 2021). Further, Cronbach's alpha presents another estimate of internal consistency similar to CR value, but less precise than CR measured under PLS-SEM (Hair *et al.*, 2019). The results are also in line with Cheah *et al.* (2018) that AVE must surpass a value of 0.5 depicted convergent validity of the model. VIF values over 5 indicate significant collinearity in the formative model, requiring evaluation to avoid indicator insignificance (Wong, 2013).

Table 4. PLS-SEM results for the moderation effect for 1st practice (usage of steam distillation unit for essential oil extraction) and for 2nd practice (By-products preparation) (n= 274).^a

Moderating Variable	Path	Coefficient β (Low)	Coefficient β (Medium)	Coefficient β (High)	P-value (Low)	P-value Medium	P-value (High)
1st practice							
Income (PKR)	At -> Bi	0.197	0.189	0.339	0.060	0.045*	0.001***
	Nc -> Bi	0.467	0.545	0.485	0.000***	0.000***	0.000***
	Pc-> Bi	-0.002	0.220	0.313	0.988	0.028*	0.002***
Farm area (Kanal)	At -> Bi	0.067	0.276	0.133	0.716	0.000***	0.196
	Nc -> Bi	0.454	0.664	0.483	0.032*	0.000	0.000***
	Pc-> Bi	0.085	0.236	0.212	0.467	0.002	0.149
Farm experience (Year)	At -> Bi	0.109	0.220	0.367	0.131	0.003	0.001***
	Nc -> Bi	0.429	0.491	0.453	0.000***	0.000***	0.001***
	Pc-> Bi	0.148	0.214	0.327	0.266	0.011***	0.001***
2nd practice							
Income (PKR)	At -> Bi	0.181	0.397	0.233	0.008	0.001***	0.000***
	Nc -> Bi	-0.031	0.156	0.381	0.882	0.226	0.000***
	Pc-> Bi	0.348	0.593	0.622	0.001	0.000***	0.000***
Farm area (Kanal)	At -> Bi	0.162	0.223	0.293	0.303	0.005***	0.000***
	Nc -> Bi	0.372	0.251	0.292	0.149	0.199	0.003***
	Pc-> Bi	0.240	0.359	0.440	0.297	0.016**	0.001***
Farm experience (Year)	At -> Bi	0.311	0.246	0.458	0.000	0.000***	0.001***
	Nc -> Bi	0.120	0.101	0.543	0.199	0.120	0.000***
	Pc-> Bi	0.125	0.367	0.378	0.678	0.035*	0.007***

^a Bi: Behavior intention; At: Attitude; Nc: Normative concern; Pct: Perceived control. Farm income (PKR) (Low =< 40,000/-; Medium= 40,001–120,000/-; High=> 120,001 and above). Farm area (*kanal*) (Low= 1-8 and 9-24; Medium= 25-44; High=> 45). Farm experience (Year) (Low=< 5; Medium= 6-10; High= > 11 and above). P< 0.001 (P< 0.01; P< 0.05) is inferred by *** (**, *).

The proposed adoption model yielded positive and significant results from mediation-moderation analysis among specific variables derived from Rogers' IDT, TAM by Ajzens' TPB. The reported R^2 values of 0.67, 0.33, and 0.19 for PLS-SEM indicate substantial, modest, and weak explanatory power, respectively (Kock and Hadaya, 2018). Thus, the R^2 of the proposed model can be characterized as substantial and modest for all inferred variables. Additionally, Hair *et al.* (2019) reported that f^2 -values exceeding 0.02, 0.15, and 0.35 indicate small, medium, and large effects of the external on the internal variable, respectively, while Q^2 values surpassing 0 indicate adequate predictive relevance of the proposed model (Shmueli *et al.*, 2019). Furthermore, a high R^2 in the PLS-SEM indicates the model effectively captures key

factors influencing respondents' decisions in adopting EOE practices. Farmers' intentions to adopt EOE practices are shaped by attitudes, perceived control, and normative concerns. These findings align with Riaz *et al.* (2021), who found that perceived usefulness, ease of understanding, and low complexity influence farmers' intentions toward sustainable and aromatic plant cultivation. These factors matter in both developed countries and aromatic plant farming in developing ones. The adoption of EOE interventions, such as combining eucalyptus farming and essential oil extraction, is likely influenced by technical training provided to growers through master trainers. Thus, training and social influence, including positive opinions from peers, may alleviate growers' uncertainty about the economic potential of cultivating high-value



crops (Roussy *et al.*, 2017). Similarly, this applies to the preparation of by-products like bio-compost and bio-pesticides derived from distillation waste of aromatic plants (Lalthazuali and Mathew, 2017; Zaccardelli *et al.*, 2021). While extension services are often considered significant in improving farmers' perceptions towards adopting innovative practices (Labarthe and Laurent, 2013). However, in this study, they showed a non-significant effect on normative concerns about eucalyptus growers' intentions towards both EOE practices. This could be due to the limited access of extension personnel to potential technology users (Gatdet, 2022), preferably during the growing season of the targeted crop, or lack of field expertise within the particular research area. Additionally, an increase in external resources is associated with an increase in perceived behavioral control, suggesting that barriers such as shortage of economic resources may impede practice adoption. These findings are consistent with those of some authors (Dessart *et al.*, 2019; Zeweld *et al.*, 2017) who argued that resource conditions perspectives, and compatibility greatly impact technology adoption.

CONCLUSIONS

This study examines the adoption of two Essential Oil Extraction (EOE) practices—steam distillation and by-product preparation—for eucalyptus. Key drivers include socio-psychological factors, particularly attitudes and normative concerns, which enhance intentions to adopt these practices. Attitudes improve perceptions of usefulness and ease of use, while normative concerns influence the effects of training and social support. Perceived resources do not significantly affect adoption intentions, and socio-economic factors such as farm size, experience, and income moderate the adoption, as confirmed by the PLS-SEM model. This study has few limitations and

recommends future research on alternative extraction methods beyond steam distillation and by-product preparation. Employing covariance-based CB-SEM and exploring similar agro-climatic regions could enhance the model, while broader sampling may improve predictions of the relationship between intention and actual adoption.

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پیش‌بینی نیت‌های رفتاری کشاورزان نسبت به پذیرش شیوه‌های استخراج اسانس با استفاده از مدل‌سازی معادلات ساختاری

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چکیده

کشاورزان خرده‌پا در شمال پنجاب، با وجود استعداد بالای این روش‌ها برای بهبود معیشت، در اتخاذ شیوه‌های پایدار مانند استخراج روغن‌های ضروری با مشکل مواجه هستند. عناصر اصلی نظریه رفتار برنامه‌ریزی‌شده، مدل پذیرش فناوری و نظریه انتشار نوآوری برای توسعه یک مدل پذیرش که متعاقباً با استفاده از مدل معادلات ساختاری تجزیه و تحلیل می‌شود، ادغام شده‌اند. نتایج آزمون، اثرات واسطه‌ای قابل توجهی را شامل نگرش‌ها (سودمندی ادراک‌شده، سهولت)، نگرانی‌های هنجاری (نفوذ اجتماعی) و نشان دادن حداکثر تغییر (R^2) در مورد آماده‌سازی محصول جانبی (۰.۷۶) و تقطیر با بخار (۰.۶۵) آشکار کرد. این مدل با موفقیت اثرات تعدیل‌کننده متغیرهای اجتماعی-اقتصادی را در نظر می‌گیرد و نشان‌دهنده ارتباط قوی بین متغیرهای پنهان است. از این رو، بهبود رفتار پذیرش در بین کشاورزان خرده‌پا مستلزم تمرکز بر عوامل اجتماعی-روانشناختی و اجتماعی-اقتصادی است.

Strategies for Enhancing Water Security in Iran's Agricultural Sector under Climate Change

Majid Gholami¹, Bahareh Heidary^{1*}, Maryam Afkhami¹, and Mohammad Ali Kiani²

ABSTRACT

The issue of climate change and its associated water security challenges has become a growing concern for Iran, particularly in its agricultural sector. Increasing population, rising demand for agricultural products, and the need for food security exacerbate these challenges. This study highlights the risks posed by reduced precipitation, rising temperatures, and inefficient water management practices, including heavy reliance on groundwater and outdated irrigation systems. It emphasizes the urgent need for modern irrigation technologies, such as water recycling (NEWater), and robust governance reforms to improve water use efficiency, analyzed through the HES framework. The study concludes that adopting a comprehensive, long-term strategy, incorporating technological innovations, localized water management practices, and enhanced governance can mitigate the impacts of climate change and ensure the sustainable use of water resources in Iran's agricultural sector.

Keywords: Agriculture, Climate change, HES analysis, Iran, NEWater, Water security.

INTRODUCTION

It is undeniable that climate change and water security are fundamental global challenges for sustainable development and human security. Water is essential for life and is a crucial aspect of the goals and challenges of sustainable development. Moreover, climate change can exacerbate water tensions and lead to a scarcity crisis, provoking both positive and negative shifts globally (Zhou *et al.*, 2021). Scholars, including Patrick (2022), have documented the impact of these twin challenges on human security and development. In Iran, these challenges are further intensified by multiple vulnerabilities, such as population growth, poverty, governance deficiencies, and the effects of economic sanctions (Farzanegan and Habibpour, 2017; Pourezzat *et al.*, 2018; Shahriyari *et al.*, 2018; Abdoli, 2020). Similarly, Biswas and Tortajada's (2022) study on the estimated

economic losses caused by climate-related disasters shows that economic losses as a percentage of GDP are significantly higher for low-income countries compared to high-income. This disparity may exacerbate inequality both between rich and poor nations and within low- and middle-income countries (Biswas and Tortajada, 2022). Reports from Iran indicate that a 1% increase in the temperature across the country's provinces could lead to a 0.12% decrease in GDP growth, contributing to a climate-induced reduction in Iran's overall economic growth (Salehi Komroudi and Abounoori, 2019).

The escalating population growth in Iran presents significant challenges, particularly in meeting the increasing demand for essential resources such as food, water, and energy. With the population projected to reach 200 million by 2050, the strain on existing resources will intensify, further complicating efforts to tackle climate

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change. As the population grows, pressure on agricultural systems to produce more food increases, leading to higher water consumption and energy use. In the context of climate change, this demand becomes even more critical, as rising temperatures and decreasing precipitation threaten the availability of these vital resources. Moreover, the relationship between climate and agriculture is inherently bidirectional. Human activities, particularly intensive agricultural practices, contribute to greenhouse gas emissions, accelerating climate change. In turn, these climatic shifts exacerbate agricultural vulnerabilities by increasing water requirements, reducing crop yields, and diminishing overall productivity. This reciprocal relationship highlights the need for sustainable solutions that address both climate change mitigation and adaptation in the agricultural sector.

The growing emphasis on sustainable pathways toward improving water security led Grey and Sadoff (2007) to define water security as "the availability of an acceptable quantity and quality of water that is essential to health, livelihoods, ecosystems, and production, and at the same time the extent of the risks that water poses to people, the environment, and the economy". This definition underscores that water is not only vital for human survival but also serves as the economic foundation for millions of enterprises, farms, power plants, and industries, all of which rely on dependable water quality and availability (Gunda *et al.*, 2019).

In this regard, some researchers argue that the scope of social challenges in achieving and maintaining sustainable water security is influenced by several factors, including the followings (Grey and Sadoff, 2007):

1. The hydrological environment, which is a natural heritage;
2. The socio-economic environment, reflecting the economic structure and behavior of its actors, as well as the natural, cultural, and political heritage;
3. Future environmental changes, notably climate change.

Consequently, addressing water security concerns requires not only policymaking, comprehensive planning, technological innovations, and sectoral collaboration but also consideration of their profound impacts on both natural and social environments. Even if complete water security cannot be fully achieved, policy instruments should be expanded to enhance water security. These tools may include governance strategies, institutional reforms, market-based approaches, adaptive capacity-building, and information exchange (World Bank, 2015; OECD, 2013; United Nations University, 2013).

Given the interconnectedness of these vulnerabilities and the dynamic nature of the challenges Iran faces, managing these concerns becomes increasingly complex. By consuming natural resources, we generate more greenhouse gases, which contribute to global warming and further climate change through various pathways. These issues increase the range of secondary problems that can seriously affect food production, energy needs, usage patterns, and water management.

This has often been a recurring issue, where solutions to one problem can create significant challenges in other areas. As such, it is essential to ensure that solutions deemed effective for addressing one major problem do not create issues in other contexts. Instead of focusing solely on isolated problems, it is crucial to develop solutions that consider and evaluate the interconnected challenges.

This research fills a significant gap in the literature by exploring the interplay between climate change and water security in Iran's agricultural sector—a topic that has received limited scholarly attention. While existing studies often address climate change or water security separately, this research uniquely examines their combined effects within the context of Iran, focusing on the sector-specific challenges of agriculture. It

identifies the lack of localized strategies tailored to Iran's unique climatic, socio-economic, and governance realities as a key research gap (Mansouri Daneshvar *et al.*, 2019; Mirzaei *et al.*, 2019).

Additionally, the study highlights the underexplored potential of integrating recycled water (NEWater) (highly treated reclaimed wastewater through microfiltration, reverse osmosis and ultraviolet radiation by brand name of NEWater) technologies into Iran's agricultural practices, drawing on international examples such as those implemented in Singapore and Namibia (Tortajada and van Rensburg, 2019). By doing so, it bridges the gap between global best practices and local applicability. Furthermore, the research incorporates socio-economic and policy dimensions, addressing gaps in the governance and planning frameworks that currently hinder optimal water resource management in Iran (Jamali Jaghdani and Kvartiuk, 2021). This comprehensive approach positions the study as a critical contribution to the discourse on sustainable water management in arid and semi-arid regions.

Climate change has had significant impacts on Iran, manifested in rising temperatures, altered precipitation patterns, increased frequency of droughts, sudden floods, and intensified dust storms. Over the past three decades, the average temperature in Iran has increased by approximately 1°C per decade, with projections indicating a further rise of 2.6°C by the end of the century. This steady increase in temperature has accelerated evaporation rates, exacerbating water shortages nationwide.

Precipitation patterns have also undergone significant shifts. Around 67% of climate stations in Iran report decreasing annual rainfall, with regions in the northern and northwestern parts of the country experiencing declines of up to 15% in yearly precipitation. Conversely, short-term, intense rainfall events have increased in arid and semi-arid regions, leading to flash floods. Recent data reveals that 50% of

monitored stations have recorded an increase in 24-hour maximum precipitation, causing devastating floods that affect urban infrastructure and agricultural productivity (Salehi *et al.*, 2020).

Droughts have become more frequent and prolonged, impacting over 90% of the country to varying degrees. Between 2001 and 2022, Iran experienced an unprecedented reduction in groundwater reserves, losing approximately 130 billion cubic meters, primarily due to unsustainable agricultural practices. This decline has placed additional strain on food security and rural livelihoods (Barati *et al.*, 2023). These reductions in precipitation, groundwater, and renewable resources underscore the urgent need for targeted climate adaptation strategies. Addressing these challenges will require a multidimensional approach that integrates advanced water management practices, effective governance, and community-level interventions.

This article explores the challenges of climate change and water security in Iran's agricultural sector, aiming to identify optimal strategies for managing water consumption in the face of escalating climate change and water insecurity. As climate change is expected to result in decreased rainfall and increased temperatures in the coming years, implementing effective water management strategies in agriculture. To achieve this, the article first introduces the concept of water security, followed by an examination of its implications within the context of climate change in Iran. It then highlights the significance and extent of water consumption in Iran's agricultural sector. Finally, the article discusses key strategies for enhancing water resource management in the country.

MATERIALS AND METHODS

This research adopts a comprehensive and innovative methodology to address the challenges of water security and climate



change within Iran's agricultural sector. A qualitative approach is utilized, combining systematic review, discourse analysis, scenario modeling, and stakeholder analysis to provide a multidimensional perspective.

During the data collection phase, both primary and secondary data are gathered from various sources. A systematic review of academic articles and reports is conducted to understand the relationship between Human-Environment Systems (HES) and water security in the context of climate change. In total, 68 articles were reviewed and analyzed to understand the interplay between HES and water security in the context of climate change, with 22 of these specifically exploring how human activities, such as agricultural practices, groundwater extraction, and governance frameworks, affect environmental feedback loops and the sustainability of water resources. The review also emphasizes the role of advanced irrigation technologies, governance reforms, and climate-resilient agricultural practices in improving water security under changing climatic conditions. By synthesizing these perspectives, the study establishes the HES framework as a conceptual foundation for exploring adaptive, resilient, and context-specific water resource management strategies. Additionally, semi-structured interviews were conducted with experts in agriculture, climate change, and water policy to gather specialized insights and indigenous knowledge. Quantitative and statistical data—including temperature fluctuations, precipitation patterns, and agricultural water consumption—were sourced from national and international organizations, providing a solid empirical foundation for the study.

In the analysis phase, discourse analysis was applied to policy documents, academic literature, and media reports, revealing patterns, contradictions, and thematic trends related to water security and agriculture in Iran. Scenario modeling was employed to simulate the impacts of climate change on water productivity and agricultural practices, with projections for temperature increases and reduced precipitation. Moreover,

stakeholder network analysis examined the interactions and influence of key actors, such as government agencies, farmers, and the private sector, to understand their roles in water management.

The final stage of the research focuses on the development of practical and sustainable solutions. A policy framework is proposed to optimize water resource management in agriculture, emphasizing the adoption of advanced technologies, modern irrigation systems, and water recycling methods such as NEWater. These solutions are validated through expert consultations and feedback from key stakeholders. To enhance resilience, adaptive decision-making tools are developed to assist policymakers in responding to rapidly changing climatic conditions.

This research is innovative in several ways. First, it integrates multiple analytical methods to offer a holistic understanding of the challenges. Secondly, it bridges global best practices, such as NEWater technologies, with localized solutions tailored to Iran's specific context. Thirdly, it adopts a participatory approach by incorporating the perspectives and interactions of various stakeholders. By addressing current challenges and proposing forward-looking strategies, this study makes a significant contribution to the discourse on sustainable water management in arid and semi-arid regions.

A scoping review was seen as a method for synthesizing evidence-based research, focusing on identifying research priorities and gaps to inform policy reviews and future studies (Hosea and Khalema, 2020). This approach allows complex issues or under-examined topics to be treated as specific projects (Gutierrez-Bucheli *et al.*, 2022). The scoping review led to the compilation of grey literature, studies, and available online reviews on "climate change," "water security," and "Iranian agriculture," sourced from Scopus and other scholarly search engines. Using these keywords, the search revealed 460,847 articles related to climate change, of which 120,165 discussed both

climate change and water. Of these, only 5,252 articles addressed the intersection of water security and climate change. When focusing specifically on Iran, just 68 articles covered both climate change and water security in the Iranian context. Furthermore, 24 of these articles incorporated an agricultural dimension in their discussion of climate change and water security in Iran (See <https://www-scopus-com>). A purposive sampling technique was employed to ensure the inclusion of high-quality, contextually relevant studies. Articles were selected based on their geographical focus on Iran, methodological rigor, and relevance to the themes of climate change, water security, and agricultural practices. Additionally, local studies and reports were incorporated to capture region-specific insights and challenges.

RESULTS

Climate Change in Iran

Temperature and precipitation are two of the most critical climatic parameters influencing food production in Iran. Among

countries in the west Asia, Iran is projected to experience a 2.6°C rise in mean temperatures and a 35% decline in precipitation over the coming decades (Mansouri Daneshvar *et al.*, 2019). Evidence shows that Iran, like many other countries, has witnessed rapid warming in recent decades. Using meteorological data from fifteen ground stations across Iran over a 63-year period (1951–2013), Alizadeh-Choobari and Najafi (2017) examined minimum, maximum, and daily near-surface air temperatures. Their findings indicated that annual minimum, maximum, and average near-surface air temperatures have all increased in most regions of Iran. Thus, it can be concluded that Iran, like most countries, has been warming rapidly over the past few decades. In particular, temperatures in many regions of Iran began to show a significant shift in the 1980s or 1990s, with average temperatures rising by approximately 1.2°C after these turning points (Alizadeh-Choobari and Najafi, 2017).

As a result of this warming, Iran has experienced a downward trend in annual precipitation. The decrease in precipitation, coupled with rising temperatures, suggests

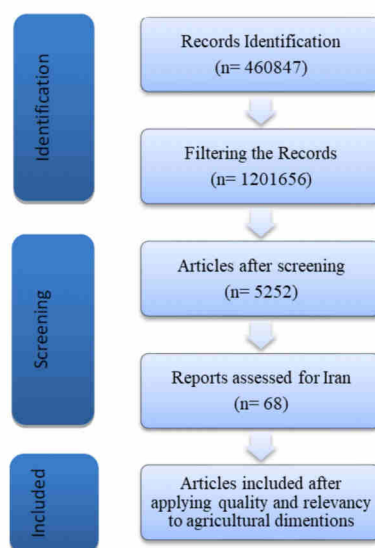


Figure 1. Preferred Reporting Items for Systematic Reviews flow chart for the systematic literature review.



that Iran has become drier and more vulnerable to droughts in recent decades (Alizadeh-Choozari and Najafi, 2017). Additionally, Bazrkar *et al.* (2015) predicted an increase in monthly temperatures for Iran in the coming years, based on the IPCC's SRES scenarios.

When considering precipitation, several critical parameters influence food production, such as the quantity and variability of rainfall. In Iran, annual precipitation is declining at 67% of climate stations, while 50% of the stations are experiencing an increase in the 24-hour maximum precipitation (Salehi *et al.*, 2020; Bazrkar *et al.*, 2015). The decline in annual rainfall is most prominent in northern and northwestern regions, while the increase in maximum 24-hour rainfall is observed mainly in arid and semi-arid areas. Although regional variations in annual precipitation are substantial, they are insufficient to compensate for the increasing 24-hour rainfall events. These changing precipitation patterns began in the 1970s across most climate stations, signaling the initial stages of climate change in Iran.

The decreasing annual precipitation could eventually lead to significant changes in Iran's water supply, particularly increasing demand for agricultural and urban water in arid and semi-arid regions. Conversely, the increasing intensity of 24-hour maximum rainfall poses a risk of accelerating soil degradation, which could contribute to desertification in these already vulnerable areas.

Recent studies highlight the growing prevalence of rainfall variability and climate change in Iran, which has resulted in more frequent floods and droughts, the two most significant climate-related challenges affecting food production (Vaghefi *et al.*, 2019). Abeysekera *et al.* (2015) observed substantial increases in rainfall variability in the dry zones during both cultivation seasons. These changes led to fluctuations in moisture conditions during the reproductive stage of crops, impacting both the quantity and quality of crop yields. A recent study

also showed an increase in extreme rainfall during the cropping season, which can result in excessive humidity during critical stages of crop development, ultimately affecting yield quality and quantity (Abeysekera *et al.*, 2015).

Extreme precipitation events have become critical factors in managing erosion and flood risks. Maleksaeidi *et al.* (2021) note that, over the past years (2013–2017), extreme weather events with varying impacts on crop production have become more frequent. Rice, a staple crop that accounts for 15% of Iran's agricultural production, has been particularly affected. A comparison between agricultural production data from 2011–2012 and 2018–2019 reveals a troubling trend: agricultural production in Iran has stagnated, and the agricultural sector's share of the GDP is expected to decrease significantly. The studies conducted in western Iran further indicate water scarcity and low productivity, with environmental and climate-related disasters identified as the major concerns of participants—two of which are directly linked to climate change (Maleksaeidi *et al.*, 2021).

According to Iran Meteorological Organization, the average surface temperature in Iran has risen by 1 to 1.5°C over the past 30 years, with an average increase of approximately 0.05°C per year. Each 1°C increase in temperature results in a 5–7% rise in evapotranspiration. Iran currently has around 106 billion m³ of renewable water, with 75% lost to evapotranspiration, 16% as runoff, and 8% percolating into aquifers. However, only 30% of this renewable water is accessible, amounting to around 31 b.m³. Over the next 50 years, annual precipitation is projected to decline from approximately 357 to 218 b m³. Groundwater and renewable water resources are expected to decrease significantly, from 45.7 and 106 b m³, respectively, to 8.64 and 37.9 b m³. This disparity between the projected 38.9% decline in precipitation and the 81 and 64% reductions in groundwater and renewable resources indicates a future

intensification of water scarcity (Barati *et al.*, 2023; Cline, 2007; Mansouri Daneshvar *et al.*, 2019; Babaeian *et al.*, 2015). Figure 2 illustrates the predicted climate change scenarios.

The implications of this decline in precipitation, coupled with rising temperatures, cannot be overstated. Water insecurity is becoming increasingly probable (Patrick, 2021). Consequently, the effects of climate change on food production and agricultural sector at large are emerging as critical policy and security issues.

Water Security in Iran

Water security is defined in various ways across cultural, academic, and practical contexts. At the Second World Water Forum in March 2000, held in The Hague, water security was characterized as the enhancement and protection of freshwater and coastal ecosystems, promoting sustainable development and political stability. It also involves ensuring safe and affordable water for all and protecting vulnerable populations from water-related

hazards.

The Centre for Water Security defines water security as the ability of communities to maintain access to sufficient, quality water for human and ecosystem health, while efficiently protecting lives and property from water hazards (Centre for Water Security, 2014). Similarly, the UN (United Nations, 2013) emphasizes the importance of water security in ensuring sustainable livelihoods, promoting socio-economic development, preserving ecosystems, and ensuring stability. The UN's definition focuses on adequate water access to sustain livelihoods while safeguarding against pollution and water-related disasters.

Despite these varying definitions, they all aim to ensure access to safe and quality water for both social and economic needs. However, achieving consensus on water security at the transnational level remains challenging due to the lack of authoritative international legal frameworks and competing national interests. The diverse uses of water—along with the significance of local context and cultural perspectives—

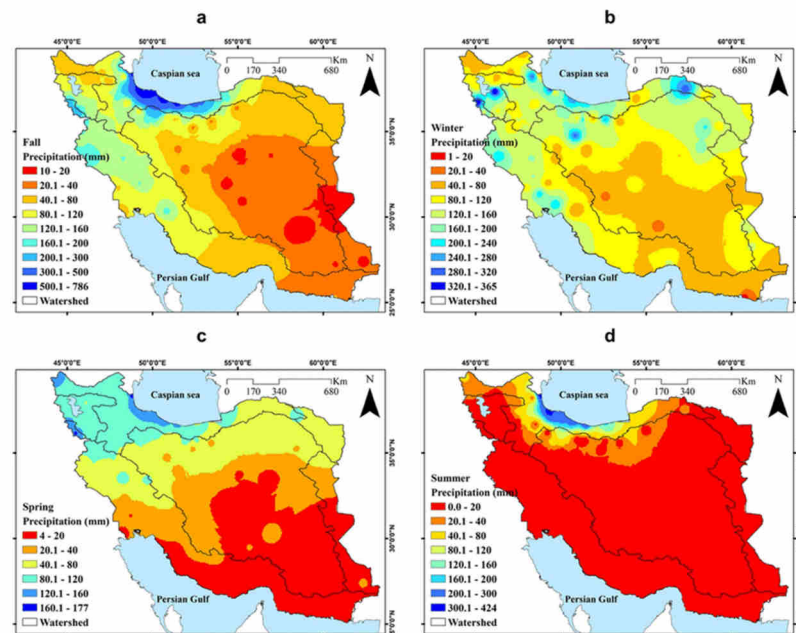


Figure 2. Long-term (2020 - 2050) mean precipitation in Iran (Behzadi *et al.*, 2022).



further complicate this understanding. Consequently, a comprehensive approach to defining and achieving water security is essential.

The Middle East, situated in an arid and semi-arid region, faces significant water security challenges exacerbated by the effects of climate change (Sowers, Vengosh and Weinthal, 2011; Lelieveld *et al.*, 2012; Osman *et al.*, 2017; Mansouri Daneshvar *et al.*, 2019; Nazemi *et al.*, 2020). Researchers have linked drought-induced water scarcity to political unrest and social instability, particularly in Syria (Kelley *et al.*, 2015; Almer *et al.*, 2017), as well as in Afghanistan and Iran (Dehghan *et al.*, 2014). Also, researchers determined the water security indicators and the situation of water security in Iran and their main watersheds (Zakeri *et al.*, 2022). Several studies have examined the complex interplay between water scarcity, drought, and conflict in the region, highlighting the potential for water shortages to escalate tensions and trigger conflicts (Gleick, 2014; Michel, 2020; Czulda, 2022).

Iran faces severe water shortages and significant climate change impacts, which are further exacerbated by challenges in water management and increasing consumption (Danaei *et al.*, 2019; Mirzaei *et al.*, 2019; Gürsoy and Jacques, 2014). Poor management practices, including excessive groundwater extraction, dam overflows, and inadequate wastewater treatment have brought Iran closer to the brink of "water bankruptcy" (Mirzaei *et al.*, 2019). These challenges threaten national security, as rising water stress may heighten the potential for conflicts (Farinosi *et al.*, 2018).

Iran's agricultural self-sufficiency projects, initiated as a response to economic sanctions, present a dilemma for water security. While these projects are essential for ensuring food independence, they place significant strain on the country's renewable water resources due to excessive consumption driven by heavily subsidized water use (Jamali Jaghdani and Kvartiuk, 2021). Policymakers must find a balance

between achieving agricultural independence and maintaining sustainable water use. This balance will require reforms that promote advanced agricultural technologies, reduce water consumption, and optimize crop production.

Historically, water in Iran has been used for agriculture, industry, and domestic purposes. Biswas and Tortajada (2022) argue that ensuring water security requires addressing the long-term needs of all these sectors. This article reviews the historical context of water consumption in Iran, focusing particularly on agricultural use and the role of climate change in shaping water security challenges.

Water security is a multifaceted concept, encompassing a variety of indicators that measure the availability, quality, efficiency, and sustainability of water resources. This section explores the key water security indicators, their calculation methodologies, and their implications for water management in Iran, as well as providing comparative insights from both developing and developed countries.

Key Indicators and Their Methodologies

Per Capita Water Availability

This indicator measures the total renewable freshwater resources divided by the population. In Iran, per capita water availability has decreased from 7,000 m³ in 1956 to less than 1,400 m³ in recent years, crossing the water stress threshold of 1,700 m³ per capita (UNESCO, 2021). This sharp decline is attributed to rapid population growth and overexploitation of water resources.

Agricultural Water Use Efficiency

Defined as the ratio of water effectively used by crops to the total water applied, this indicator highlights irrigation inefficiencies.

In Iran, irrigation efficiency averages 35%, significantly lower than in the developed countries such as Australia and the United States, where efficiencies range between 70–90% due to the adoption of modern technologies like drip and precision irrigation (Mirzaei *et al.*, 2019).

Groundwater Depletion Rates

Groundwater resources are crucial for Iran, accounting for over 50% of its agricultural water supply. Between 2001 and 2021, Iran lost approximately 130 B.m³ of groundwater due to unsustainable extraction (Nazari *et al.*, 2020). By comparison, developed countries have implemented strict regulations and monitoring systems to control groundwater usage, reducing depletion rates significantly.

Water Recycling and Reuse

This indicator reflects the percentage of wastewater treated and reused for agricultural, industrial, or domestic purposes. Iran recycles less than 10% of its wastewater, whereas countries like Singapore have achieved recycling rates of over 30% through technologies like NEWater, ensuring a sustainable water supply (Tortajada and van Rensburg, 2019). Developing countries like Iran face significant challenges in achieving water security compared to developed nations. The key differences lie in the followings:

- **Technological Integration:** Developed countries widely adopt advanced technologies such as precision irrigation, desalination, and water recycling. In contrast, developing countries struggle with limited financial resources and access to such innovations.
- **Policy and Governance:** Developed nations have established robust governance frameworks to regulate

water use and enforce sustainability practices, whereas developing countries often face fragmented policies and weak enforcement mechanisms.

- **Climate Resilience:** Developed countries have invested in adaptive measures to combat climate change impacts, while developing nations like Iran are more vulnerable due to inadequate infrastructure and limited financial support.

These comparisons underscore the need for tailored approaches in addressing water security. For Iran, improving irrigation efficiency and implementing wastewater recycling programs can bridge the gap, while effective governance reforms can create an enabling environment for sustainable water management.

Water Consumption in Iran's Agricultural Sector

Globally, water consumption in Iran's agricultural sector is among the highest, accounting for over 90% of the country's freshwater resources (Nazari *et al.*, 2018). Rural households are heavily dependent on agriculture, which remains largely traditional and is supported by government subsidies for inexpensive water. Despite agriculture contributing only about 10% of Iran's GDP, it remains the dominant user of water, far exceeding the global average for renewable water resources. However, Iran's annual rainfall is less than one-third of the global average, resulting in unsustainable groundwater extraction across all provinces (Golian *et al.*, 2021).

This growing groundwater depletion, often referred to as a sign of "water bankruptcy," poses a significant threat to Iran's long-term food security (Mirzaei *et al.*, 2019). Groundwater storage has dramatically declined, with some regions losing up to –4,400 Mm³ between 2002 and 2017 (Safdari *et al.*, 2022). The situation is



exacerbated by increased agricultural water use, which reached 103 B.m³ in 2021, far surpassing the national water consumption estimate of 88.5 B.m³ (Yousefi *et al.*, 2021).

Although the agricultural sector's share of total water consumption has been gradually decreasing, the absolute demand continues to rise. This trend, which began around 2013, is evident in Figure 3, which shows that despite attempts to curb overall consumption, agricultural water demand remains on an upward trajectory. With 90% of Iran's freshwater allocated to agriculture and an irrigation efficiency of only 35%, Iran lags behind developed countries, where irrigation systems typically achieve efficiencies between 70 and 90%. This inefficiency is a major challenge for Iran, especially when compared to international standards (Nazari *et al.*, 2018; FAO, 2016). Currently, only 2.4 M.ha of Iran's total 16.5 M.ha of agricultural land benefit from modern irrigation systems.

The inefficiency of Iran's irrigation practices underscores the urgent need for modernization in agricultural water management. Critics, including Mirchi *et al.* (2010), and Islam and Madani (2017) point to significant failures in Iran's water management systems, which lack comprehensive planning that accounts for the ecological context of water use. Improved water management practices could allow Iran

to achieve similar agricultural outputs with far less water consumption. Without substantial improvements, Iran faces a future where water scarcity—exacerbated by climate change and poor management—could severely affect its agricultural productivity and overall socio-economic stability.

NEWater, Continuous Return of Water to the Recycling Cycle

Technological advances illustrate that optimal water management can effectively mitigate water scarcity by recycling this vital resource (Tortajada and van Rensburg, 2019). Through efficient collection, treatment, and reuse of wastewater, treated water can be cycled back into consumption, including drinking water, without limitations on quality or quantity. NEWater refers to high-grade reclaimed water produced through advanced purification processes, including microfiltration, reverse osmosis, and ultraviolet disinfection. This technology, pioneered in Singapore, recycles wastewater into potable water, significantly reducing dependency on traditional freshwater sources. In the context of this study, NEWater serves as a potential model for addressing water scarcity in Iran through wastewater recycling. Successful examples

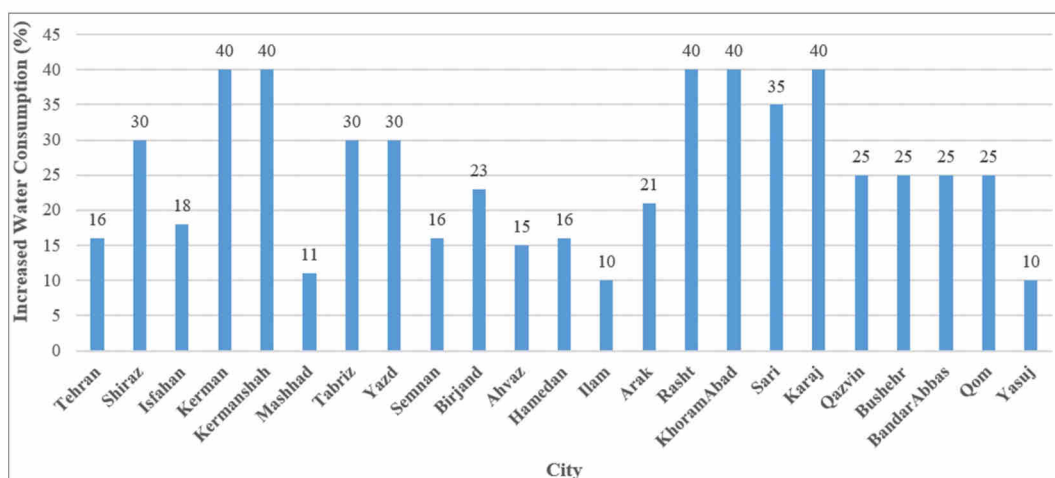


Figure 3. Increased water consumption in Iran in different states (Iran's Energy Balance, 2020).

of this practice exist worldwide, one notable case being Windhoek, Namibia, where innovative management of domestic wastewater has resolved long-standing drinking water issues over the past 50 years. Despite Namibia's arid conditions, its citizens have reported no health problems from using recycled drinking water (Tortajada and van Rensburg, 2020).

While Namibia may not be well-known in Iran, its leadership in recycled water use offers valuable lessons. Singapore is another exemplary case, with over 30% of its water demand met through recycled sources. Countries like Japan, Germany, and US (California) have also adopted similar strategies (Voulvoulis, 2018; EPA, 2017). Despite these successes, public acceptance of recycled water, especially NEWater, which is perceived as superior to regular tap water, remains a challenge due to psychological barriers (Bai *et al.*, 2020; Tortajada and Buurman, 2017). This reluctance has generated significant opposition, ultimately causing the U.S. government to halt major recycled water initiatives (Hartley, 2006).

In Iran, the total municipal wastewater generated is 6.5 b m^3 annually, with only 42% treated and recycled, raising

environmental and public health concerns. The conventional activated sludge process dominates this treatment, and operational costs average \$0.20 per cubic meter (FAO, 2017). With total water withdrawal in Iran estimated at 93.3 billion cubic meters per year, treating wastewater could fulfill 6% of the nation's water needs.

Reducing water consumption positively impacts the environment by lowering energy use and greenhouse gas emissions, which is particularly crucial in the context of climate change. While it is challenging to quantify the precise effects of a 6% reduction in water usage, it is evident that this strategy is vital for ensuring sustainable water supplies and mitigating climate impacts in Iran.

Importance of Human-Environmental System in Increasing Water Security against Climate Change

Human-Environmental Systems (HES) are complex, paired systems that require specialized methods and interdisciplinary approaches to understand and manage. Human-environmental interactions represent the difference between harmful and beneficial interactions (Pahl-Wostl, 2015). Figure 4 shows the HES framework for water security. This framework and its relevant principles are designed to facilitate

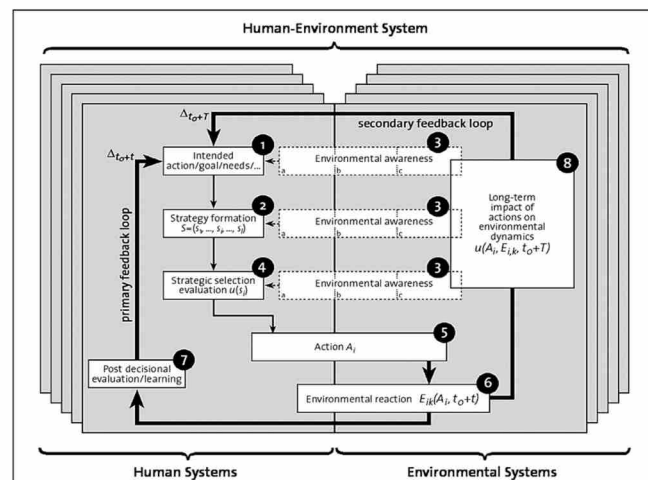


Figure 4. HES framework of water security.



research on and address complex human-environmental issues. Typically, in the early stages of tackling a complex environmental problem, the issue often appears unstructured and cannot always be clearly defined. The principles identified in the HES framework (denoted by numbers) serve as key elements for understanding and transforming the water security issue.

The framework adopts a hierarchical view of the human system. At each hierarchical level, different regulatory mechanisms are in place concerning the environmental system. Understanding these mechanisms helps identify intervening regulatory mechanisms. The framework also focuses on the conceptualization of human behavior through goal setting, strategy selection, action, and learning, with particular attention to immediate (primary) and delayed (secondary) responses to the environmental system.

In this context, and according to the definition of water security, awareness of human and environmental stimuli, along with the primary and secondary feedback loops between the human and environmental systems, results from the interaction between ecosystem services, such as the production and supply of water—and environmental hazards, such as floods and droughts. These interactions ultimately influence the strategies adopted to ensure water security in the basin (Scholz, 2011).

The research findings provide a structured foundation that logically leads to the model presented in Figure 4, illustrating the interconnectedness of human-environmental systems (HES) and their relevance to water security in Iran's agricultural sector. The findings identify critical challenges such as the impacts of climate change, including rising temperatures and reduced precipitation, water insecurity, inefficient irrigation practices, and governance shortcomings. These challenges highlight the complexity of interactions between human and environmental factors, which is central to the HES model.

The study demonstrates how human activities, such as over-extraction of groundwater and reliance on traditional agricultural methods, exacerbate environmental stress. These interactions are captured in the HES model, which links human goals (e.g., achieving food security) with environmental constraints (e.g., water scarcity). Furthermore, the research underscores the need for holistic solutions, such as improving irrigation efficiency, adopting water recycling technologies like NEWater, and reforming governance structures. These solutions align closely with the principles of the HES model, which emphasizes feedback loops and regulatory mechanisms between human and environmental systems.

By proposing actionable strategies, such as better water management, technological adoption, and farmer education, the findings align with the hierarchical structure and feedback-based approach of the HES model. The review also highlights the critical role of the human-environmental system (HES) in addressing water security challenges in the context of climate change. Human activities, such as unsustainable irrigation practices and groundwater over-extraction, have intensified environmental stressors, thereby reducing water availability. Conversely, the implementation of advanced technologies (e.g., precision irrigation and water recycling systems) demonstrates how human interventions can mitigate these impacts.

The reviewed studies highlight feedback loops within the HES framework, where climate changes exacerbate agricultural water demand, and inefficient human responses further degrade the environment. For instance, 75% of the analyzed articles identified groundwater depletion as a direct consequence of unregulated extraction, while 60% emphasized the potential of governance reforms to create adaptive water management systems. These findings underscore the importance of integrating human-environmental systems into water resource management strategies to enhance resilience against climate change.

DISCUSSION

This study examines the critical challenges of water security in Iran's agricultural sector, particularly in the context of climate change. The findings highlight the significant risks posed by the rising air temperatures, reduced precipitation, and inefficient water management practices. Climate change exacerbates existing vulnerabilities in the agricultural sector, including heavy reliance on groundwater and outdated irrigation methods, which collectively account for over 90% of freshwater consumption in Iran.

The research emphasizes the urgent need for adopting modern irrigation systems to address these inefficiencies, as Iran's current irrigation efficiency is only 35%, well below global standards. Technological solutions, such as water recycling (e.g., NEWater) and the expansion of greenhouse cultivation, offer promising strategies to reduce water demand while maintaining agricultural productivity. However, implementing these solutions requires robust governance reforms to regulate water usage, curb illegal activities like unregulated well drilling, and optimize resource allocation.

Additionally, the study stresses the importance of localized approaches to water resource management, considering Iran's diverse regional climates and socio-economic conditions. Addressing these challenges necessitates collaborative efforts from policymakers, farmers, and the private sector to implement sustainable practices, enhance farmer education on advanced techniques, and foster innovation in agriculture.

In conclusion, this research underscores the interconnected nature of climate change and water security challenges in Iran's agricultural sector. By adopting a holistic approach, incorporating technological advancements, governance reforms, and sustainable practices, Iran can mitigate the

impacts of climate change and ensure the long-term sustainability of its agricultural sector. These actions are essential for maintaining food security and preserving vital water resources for future generations. A conceptual summary of the research findings is presented in Table 1.

Also, some of the most important effective ways to improve water security in Iran's agricultural sector in the current water shortage situation can be introduced as follows:

- Lack of a specific cultivation pattern in the country based on the National Agricultural Plan,
- Quantitative and qualitative development of greenhouse cultivation,
- Transferring the growing season of some agricultural products from spring to autumn and winter,
- Increase the use of modern irrigation systems and educate farmers in this regard,
- Repair of canals,
- Preventing the drilling of illegal wells
- Consolidation of agricultural lands in one area,
- Modification of the traditional pattern of agricultural water consumption,
- Prevent contamination of surface and groundwater resources,
- Attention to climate diversity in water resources management,
- Utilizing operational research in order to achieve the goal of reducing the level and increasing agricultural production,
- Paying attention to the production of strategic products for the country's self-sufficiency,

**Table 1.** Comprehensive summary of research findings.

Dimension		Findings	Implications
Climate Impact	Change	- Mean temperature to rise by 2.6°C by 2100. - Precipitation decline of 35%, leading to intensified droughts and floods.	- Reduced water availability for agriculture. - Increased vulnerability of crops to extreme weather.
Water Usage in Agriculture		- 90% of water is consumed in agriculture. - Groundwater depletion at alarming rates (e.g., -4400 Mm ³ between 2002-2017).	- Threatens long-term food security. - Risks of desertification and land subsidence.
Irrigation Efficiency		- Current efficiency is ~35% vs. 70-90% in developed nations. - Only 2.4 million hectares use modern methods out of 16.5 million hectares of farmland.	- High water wastage. - Immediate need for adoption of advanced irrigation techniques.
Governance Challenges		- Lack of specific cultivation patterns aligned with National Agricultural Plans. - Weak enforcement of water usage laws and excessive subsidies.	- Unsustainable agricultural practices persist. - Potential for socio-economic conflicts.
Technological Gaps		- Recycling municipal wastewater only meets 6% of national water needs. - Low adoption of technologies like NEWater.	- Missed opportunities for sustainable water management.
Socio-Economic Factors		- Limited private sector investment due to government price controls. - Farmers lack knowledge in advanced agricultural methods.	- Stagnation in productivity and innovation. - Inefficiency in resource allocation.

- Quantitative and qualitative development of conversion industries in the agricultural sector,
- Improving the quality and nutritional value of products produced,
- Use of intelligent methods to store water in dry areas and
- Use of soilless or hydroponic cultivation methods.

This article underscores the critical importance of effective water resource management in ensuring water security in Iran's agricultural sector. It emphasizes that improving water management practices is directly linked to Iran's ability to secure adequate water for its agricultural needs. The article also highlights the potential of strategies such as NEWater, a water recycling initiative, to enhance water management in the agricultural sector.

Moreover, the article stresses the necessity for long-term planning and a sustained commitment from government officials to prioritize water security within agriculture.

While short-term and medium-term solutions are essential, the article argues that long-term plans are crucial to address water security effectively. This requires a shift from seeking immediate responses to embracing a more sustained strategic approach.

In addition to long-term planning, the article advocates for promoting a culture of optimal water consumption within the agricultural sector. This includes improving the cultural infrastructure around water usage and conducting national research to develop cultivation models better suited to Iran's diverse climatic conditions. By integrating these measures, along with strengthening law enforcement, the article posits that water security in Iran's agricultural sector can be achieved in the future.

CONCLUSIONS

Based on the research findings, several recommendations are put forward for future studies aimed at tackling water security

challenges in Iran's agricultural sector. Future research should focus on regional water management, recognizing the varied climatic and agricultural conditions across Iran. Comparative studies could help develop tailored strategies for sustainable water use in different regions. Additionally, research into the impact of modern irrigation technologies, such as drip and sprinkler systems, on water use efficiency and crop yields is essential. Field trials and case studies would provide valuable insights into the feasibility and scalability of these methods. Integrating renewable energy sources like solar and wind power into water recycling and desalination processes is another critical area for investigation. This could improve the sustainability of water management systems while reducing reliance on conventional energy. Furthermore, future studies should include economic analyses of agricultural water subsidies to evaluate their effects on water consumption, agricultural productivity, and farmers' incomes. This would provide a foundation for potential policy reforms aimed at improving water use efficiency. Understanding farmers' attitudes toward adopting advanced technologies and sustainable practices is equally important. Research could assess the effectiveness of training programs and identify barriers to behavioral change. Additionally, developing and testing climate-resilient crop varieties that require less water and are better suited to Iran's changing climate is a promising area for innovation. Time series analyses studies on the implementation of water recycling technologies, such as NEWater, would provide insights into their environmental, economic, and health impacts over time. Moreover, evaluating the effectiveness of existing water governance frameworks and proposing integrated models involving local, regional, and national stakeholders could strengthen policy and management systems. These recommendations address the gaps identified in the study and offer valuable directions for advancing both knowledge and practical

solutions to ensure sustainable water security in Iran's agricultural sector.

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راهبردهای افزایش امنیت آب در بخش کشاورزی ایران در شرایط تغییر اقلیم

مجید غلامی، بهاره حیدری، مریم افخمی، و محمدعلی کیانی

چکیده

موضوع تغییرات اقلیمی و چالش‌های امنیتی مرتبط با آن به نگرانی فزاینده‌ای برای ایران به‌ویژه در بخش کشاورزی تبدیل شده است. افزایش جمعیت، افزایش تقاضا برای محصولات کشاورزی و نیاز به امنیت غذایی این چالش‌ها را تشدید می‌کند. این مطالعه خطرات ناشی از کاهش بارندگی، افزایش دما، و شیوه‌های مدیریت ناکارآمد آب، از جمله وابستگی شدید به آب‌های زیرزمینی و سیستم‌های آبیاری قدیمی را برجسته می‌کند. این بر نیاز فوری به فن‌آوری‌های آبیاری مدرن، مانند بازیافت آب (NEWater)، و اصلاحات قوی حکمرانی برای بهبود کارایی مصرف آب، که از طریق چارچوب HES تحلیل می‌شود، تأکید می‌کند. این مطالعه نتیجه‌گیری می‌کند که اتخاذ یک استراتژی جامع و بلندمدت، ترکیب نوآوری‌های فن‌آوری، شیوه‌های مدیریت محلی آب و حکمرانی تقویت‌شده، می‌تواند اثرات تغییر اقلیم را کاهش داده و استفاده پایدار از منابع آب را در بخش کشاورزی ایران تضمین کند.

Effect of Dietary Energy Source and Level on the Performance, Antibody Titers and the Relative Expression of *IL-2* and *IL-6* Genes in Broilers under Heat Stress

Nematollah Dayani¹, Mohammad Chamani^{1*}, Parvin Shawrang², Asa Ebrahimi³, and Ali Asghar Sadeghi¹

ABSTRACT

This study aimed to determine the effects of energy levels and sources on growth performance, antibody titers, and the gene expression of pro-inflammatory cytokines in broilers exposed to heat stress. A total of 450 one-day-old Ross chickens were assigned to six dietary treatments and five replicates in a completely randomized design. Chickens received diets differentiated by the main energy source (corn grain and soybean oil) and energy level (equal, 3% or 6% lower or higher than Ross 308 recommendation). Treatments were as follows: corn grain and equal as Control (CON), 3% lower corn grain (T1), 6% lower corn grain (T2), corn grain and soybean oil, equal (T3), 3% higher corn grain and soybean oil (T4), 6% higher corn grain and soybean oil (T5). The room temperature was increased to 34°C (6-h daily) from day 12 to 42 of age to induce heat stress. The highest corticosterone level was observed in T1, T2, and T5 groups. The lowest antibody titers were observed in T2 group and the highest expression levels of pro-inflammatory cytokines genes were in chickens receiving T5 diet. The highest Feed Conversion Ratio (FCR) during the grower and finisher periods was observed in T2, and the lowest in T3 and T4. It was recommended to feed Ross broiler with a diet containing oil instead of a part of grain based on energy recommended by the strain recommendation.

Keywords: Chicken, Corticosterone, Inflammation, Interleukin, Ross strain.

INTRODUCTION

The major broiler farms exist in subtropical and tropical regions of the world (Kpomasse *et al.*, 2021). In these regions, farmers have to use various strategies to control the temperature of their houses, to reduce the negative effects of heat stress on the health and performance of broilers (Costantino *et al.*, 2018). After exposing broilers to high ambient temperatures, some toxic mechanisms may be induced in the

body, including the generation of reactive oxygen species, which finally results in oxidative stress. Oxidative stress could affect the metabolic pathways liver and small intestine health, which reduce the nutrient digestion and absorption, and the merit of substrates for metabolism (Mancinelli *et al.*, 2023). Various management techniques, such as cooling systems, have been used to reduce the negative effects of heat stress on broiler chickens (Fisinin and Kavtarashvili, 2015). The cost of cooling broiler houses is high in

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many regions; hence, some researchers focused on nutritional management (Daghir, 2009). The manipulation of dietary energy level and source has been considered as a useful method in broiler farms to overcome the negative effects of heat stress (Daghir, 2009; Raghebian *et al.*, 2016). Seifi *et al.* (2018) reported that feeding a high-fat diet could improve the heat tolerance in broiler chickens, and dietary inclusion of palm oil improved the growth performance and survivability of heat stressed broiler chickens (Zulkifli *et al.*, 2007).

Moreover, Kim *et al.* (2019) reported that fat supplementation had preventative effects on weight loss for hens raised under heat stress. In contrast, Rafiei-Tari *et al.* (2021) reported that feeding oils containing n-6 fatty acids had detrimental effects on the health of broilers exposed to heat stress. On the other hand, when chickens were fed with low energy diets, deviations from physiological homeostasis occurred, leading to impaired bird welfare (Cheng and Jefferson, 2008) and significant reductions in production capabilities (Jariyahatthakij *et al.*, 2018). In an interesting study, Raghebian *et al.* (2016) reported that high energy in a broiler diet could enhance heat resistance and improve performance parameters.

Today, the effect of nutrition on gene expression is very important (Goel *et al.*, 2021). The effect of energy level and source on the expression of genes related to heat resistance has been investigated (Raghebian *et al.*, 2016), but its effect on gene expression of interleukins, as related to the immune response, was not evaluated. The Interleukin-2 (IL-2) and Interleukin-6 (IL-6) are pro-inflammatory cytokines that play an important role in the inflammatory response in the body of broiler chickens under heat stress (Goel *et al.*, 2021) and prolong inflammation responses cause tissue damage, especially in the liver and immune system tissues (Helwig and Leon, 2011; Goel *et al.*, 2021). Finding the relationship between the level and energy source of the diet with the relative expression of genes of

these two cytokines helps to understand better the cause of the effects observed in the body of broiler chickens.

In the literature, the effects of energy source and level on the antibody titers and relative expression of pro-inflammatory cytokine genes (IL-2 and IL-6) in chickens under heat stress have not been completely evaluated (Ndlebe *et al.*, 2023). Taleb *et al.* (2017) reported that antibody titers decreased in broiler chickens (Cobb 500 strain) raised under hot environmental conditions receiving soybean oil. In contrast, Sadeghi *et al.* (2013) reported that including soybean oil could enhance the immune response in broiler chickens (Ross 308). It is unclear what effects the soybean oil inclusion and the dietary energy concentration have on the expression of genes related to the immune system and the antibody titer.

It was hypothesized that in the heat stress condition, including soybean oil and formulation of high-energy diet could enhance health, immune responses, and performance compared to a diet containing the main energy source from a carbohydrate or low-energy diet. In the present study, low and high levels of dietary energy were considered factors that cause metabolic stress in the body to mimic the conditions chickens face in different breeding centers.

Therefore, the present study aimed to assess the effects of energy source and level on the growth performance, liver health, immune responses, and the relative expression of IL-2 and IL-6 genes in broiler chickens exposed to heat stress.

MATERIALS AND METHODS

Chickens Management

A total of 465 one-day-old male Ross 308 broiler chickens (average weight of 40 g) were purchased from a local hatchery and allocated randomly to thirty one floor pens (200×180 cm) covered with wood shaving. Chicks were randomly assigned to 6 dietary

treatments with 5 replicates and 15 chicks per each. Except ambient temperature, chicks were raised under controlled conditions, lighting program, and feed recommendations based on Ross 308 broiler guides. Chickens ($n=450$) were exposed to heat stress from day 12 to 42 of age, with the relative humidity of 65%. During heat stress, temperatures were raised daily to $34\pm1^{\circ}\text{C}$ for 6 hours from 08:00 to 14:00 and then decreased to $24\pm1^{\circ}\text{C}$. Fifteen chicks were kept in a room at normal temperature to assess whether experimental chicks were exposed to heat stress. These chickens received corn grain and energy density based on Ross 308 recommendation (3100 and 3200 kcal/kg during grower and finisher periods, respectively). Blood samples were taken from these chickens to measure corticosterone levels as a biological marker of heat stress. All chickens had access *ad libitum* to feed and fresh water, especially throughout the heat challenge period. Chickens were vaccinated with the Newcastle Disease (ND) vaccine and Infectious Bursal Disease (IBD) vaccine. In the experiment's initial and end, the amount of feed intake and body weight were measured, and the Feed Conversion Ratio (FCR) was calculated. Dead chicken was weighed and the weight was included in the calculations of FCR.

Experimental Design

Dietary treatments were included in the Control group (CON), chickens receiving the main energy source from corn grain and energy density based on Ross 308 recommendation. Treatments included the followings:

T1: Chickens receiving the main energy source from corn grain and 3% lower energy density than Ross 308 recommendation;

T2: Chickens receiving the main energy source from corn grain and 6% lower energy density than Ross 308 recommendation;

T3: Chickens receiving the main energy source from corn grain and soybean oil and

energy density based on Ross 308 recommendation;

T4: Chickens receiving the main energy from corn grain and soybean oil and 3% higher energy density than Ross 308 recommendation,

T5: Chickens receiving the main energy from corn grain and soybean oil and 6% higher energy density than Ross 308 recommendation.

Metabolizable energy levels of diets were balanced using starch or washed sand. Chickens were raised at three feeding periods: starter (days 1 to 10), growers (days 11 to 24), and finishers (days 25-42) periods.

Sample Collection and Measurements

On days 24 and 42 of age, blood samples (6 mL) were collected using sterile Venoject directly from the heart of two chickens in each replicate. The serum of the blood sample was separated using a centrifuge ($1,500\times g$, 15 minutes) and stored at -20°C until further analysis. Two days after sampling, antibody titers against viruses of ND and IBD were determined in all serum samples. Biochemical measurements were done on samples taken on day 42 of age.

On day 24, immediately after blood sampling, chicks were sacrificed by cervical dislocation, then the spleen and liver were removed and sampled. Five spleen samples from each treatment were collected to analyze the relative expression of the *IL-2* and *IL-6* genes. Spleen tissues were transferred in a cry-protectant tube, snap-frozen in liquid nitrogen, and stored at -70°C until RT-PCR analysis.

Blood Sample Analysis

Serum corticosterone level was measured enzymatically using an enzyme-linked immunosorbent assay kit (Enzo Life Sciences, NY, USA). Serum concentrations of glucose, total protein, albumin, creatinine, and uric acid were measured using the photometric method



by auto-analyzer (BS-120 model, Minbray Co., USA) and commercial kits (Pars Azmon Co., Tehran, Iran).

Serology

The titers of antibodies against Newcastle disease virus were measured by hemagglutination-inhibition test (Allan and Gough, 1974) and against Infectious Bursal Disease virus by ELISA kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA). The value of antibody titers was transformed to $\log_2(x)$ before statistical analysis.

Analysis of the Gene Expression of *IL-2* and *IL-6*

The relative abundances of *IL-2* and *IL-6* mRNA were determined by the RT-PCR technique described by Paraskeuas and Mountzouris (2019) and Long *et al.* (2019). The frozen spleen sample was crushed in a sterile mortar, and the powder was applied for total RNA extraction using a suitable kit (Bioneer Co., Seoul, South Korea). Then, each gene's cDNA was synthesized using a suitable kit using the reverse transcription technique (Bioneer Co., Seoul, South Korea). Quantitative PCR was performed with specific primer pairs for *IL-2* (Paraskeuas and Mountzouris, 2019) and *IL-6* (Long *et al.*, 2011) using Quanti Fast SYBER Green PCR kit (QIAGEN, Cat. No. 204052). GAPDH was chosen as a housekeeping gene. The relative gene expression of *IL-2* and *IL-6* as target genes was normalized to the GAPDH gene using the method previously described by Livak and Schmittgen (2001). Quantification for each treatment group was performed in triplicates.

Statistical Analysis

Statistical analyses were done using the General Linear Model procedure of the SAS

for Windows version 9.1 (SAS Institute Inc., Cary, NC) appropriate for a completely randomized design. To evaluate the normal distribution of data, the Kolmogorov-Smirnov test was done. Duncan multiple range tests were used to compare the means. Effects between the control and experimental groups were considered significant when $P < 0.05$.

RESULTS

Effect on Serum Corticosterone Level and Biochemical Measurements

Table 1 shows the serum corticosterone levels and biochemical parameters of broilers receiving different dietary energy levels and sources. The highest corticosterone level was observed in the T1, T2, and T5 groups, and no difference was observed among other treatments with the control group. Broilers in the T5 group had the highest serum glucose level, and those in the T2 group had the lowest. Broilers receiving the T4 diet had the highest albumin, globulin, and total protein, and broilers in the T2 group had the lowest protein sections. The highest concentration of creatinine and uric acid was observed in the T2 group, while broilers of CON, T3, and T4 had the lowest.

Effect on Antibody Titers and the Relative Expression Levels of Interleukins

Table 2 shows the effect of dietary energy level and source on antibody titers against Newcastle disease virus and infectious bursa disease virus determined on days 24 and 42 of age. There was no difference among treatments for ND titer on day 24, but differences were observed for ND titer on day 42. At day 42 of age, the lowest ND titer was observed in the T2 group and the highest in the T3 and T4 groups. On day 24, the IBD titer was the highest in the T3 and T4 groups and the lowest in the T2 group.

Table 1. Effect of dietary energy level and source on serum corticosterone level and biochemical parameters in broiler chickens under heat stress.

Item ^a	CON	T1	T2	T3	T4	T5	SEM	P value
Corticosterone (ng mL ⁻¹)	20.61 ^b	29.84 ^a	30.37 ^a	21.26 ^b	22.54 ^b	27.33 ^a	1.03	0.01
Glucose (mg dL ⁻¹)	200.09 ^{bc}	195.12 ^{cd}	189.45 ^d	204.72 ^{bc}	210.58 ^{ab}	219.21 ^a	3.50	0.01
Albumin (mg dL ⁻¹)	1.13 ^b	1.13 ^b	1.02 ^c	1.15 ^{ab}	1.23 ^a	1.07 ^{bc}	0.027	0.02
Globulin (mg dL ⁻¹)	1.35 ^b	1.27 ^c	1.17 ^d	1.33 ^b	1.51 ^a	1.17 ^d	0.021	0.03
Total protein (mg dL ⁻¹)	2.48 ^b	2.40 ^b	2.19 ^c	2.49 ^b	2.73 ^a	2.24 ^c	0.039	0.01
Creatinine (mg dL ⁻¹)	2.54 ^c	2.73 ^{bc}	3.74 ^a	2.53 ^c	2.51 ^c	3.01 ^b	0.103	0.02
Uric acid (mg dL ⁻¹)	4.22 ^c	4.58 ^b	4.94 ^a	4.20 ^c	4.24 ^c	4.83 ^a	0.023	0.01

^{a-d} Means within a row with different superscripts are significantly different ($P < 0.05$). ^a CON: Control, energy based on Ross standard diet with main energy from corn; T1: Chickens receiving 3% lesser energy than Ross standard diet with energy from corn; T2: Chickens received 6% lesser energy than Ross, T3: Chicken receiving Ross standard diet with main energy from corn grain and soybean oil, T4: Chicken receiving 3% upper energy than Ross standard diet, and T5: Chicken receiving 6% upper energy than Ross standard diet.

Table 2. Effect of dietary energy level and source on antibody titers against viruses of Newcastle Disease (ND) and Infectious Bursal Disease (IBD) on days 24 and 42.

Item ^a	CON	T1	T2	T3	T4	T5	SEM	P value
Day 24 of age								
ND (log 2)	4.67	4.35	4.00	3.65	3.34	4.00	0.45	0.369
IBD (log 2)	419.31 ^{ab}	414.25 ^{ab}	402.56 ^b	434.97 ^a	432.08 ^a	416.98 ^{ab}	9.20	0.016
Day 42 of age								
ND (log 2)	5.35 ^{abc}	5.09 ^{bc}	4.67 ^c	7.00 ^a	7.15 ^a	6.65 ^{ab}	0.55	0.021
IBD (log 2)	3177.30 ^a	3007.60 ^b	3017.09 ^b	3106.02 ^a	3187.72 ^a	3125.34 ^a	50.6	0.050

^{a-c} Means within a row with different superscripts are significantly different ($P < 0.05$). ^a CON and T treatments as defined in the main text and Table 1.

On day 42, the highest IBD titer was observed in the CON, T3, T4, and T5 groups, and the lowest titer was found in T1 and T2.

Figure 1 shows the relative expression level of *IL-2* and *IL-6* genes in the spleen of broiler chickens under heat stress receiving different energy densities and sources. Significant differences were observed among treatments for the relative expression of *IL-2* and *IL-6* genes. The highest relative expression of the *IL-2* gene was observed in chickens receiving T5, then, in T4 diets, and the lowest expression was observed in chickens receiving CON, T1, T2, and T3 diets. Chickens receiving the T5 diet had the highest relative gene expression of *IL-6*, and the lowest expression was observed in chickens receiving CON, T1, and T2 diets.

Effect on Performance Parameters

The performance parameters of broiler chickens are presented in Table 3. There were no differences among treatments for performance parameters during the starter period. These differences appeared at grower and finisher periods. The daily gain of broilers in the T3 and T4 was higher than in T1 and T2. The lowest daily gain during grower and finisher periods was observed in T2 and the highest daily gain was observed in broilers receiving T3 diet. Feed intake of broilers during grower and finisher periods was the highest in the T2, and there was no difference in feed intake among other treatments. The highest FCR during the grower and finisher periods was observed in T2, and the lowest FCR was observed in the T3 and T4. Broilers in the T5 group had the same FCR as T3 and T4.

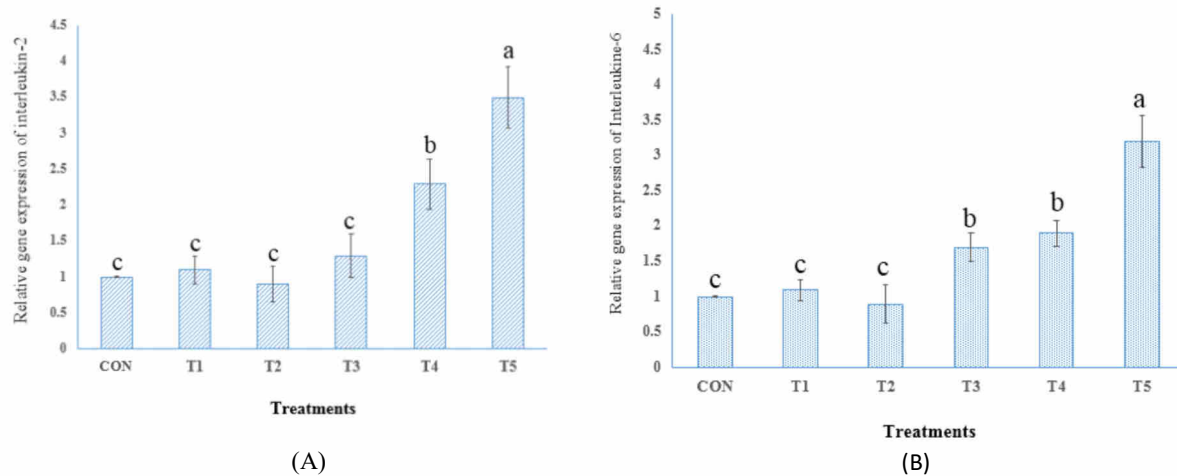


Figure 1. The relative expression level of *IL-2* (A) and *IL-6* (B) genes in heat-stressed broilers receiving different energy level and source. CON and T treatments as defined in the main text and Table 1.

Table 3. Effect of dietary energy level and source on performance parameters of broiler chickens under heat stress.

Item ^a	CON	T1	T2	T3	T4	T5	SEM	P value
Starter phase								
Gain (g d ⁻¹)	17.21	17.42	17.15	17.02	17.00	17.06	0.209	0.70
Feed intake (g d ⁻¹)	24.10	24.30	24.20	24.00	23.90	23.90	0.161	0.29
FCR	1.40	1.41	1.41	1.41	1.40	1.40	0.023	0.98
Grower phase								
Gain (g d ⁻¹)	51.05 ^{ab}	48.22 ^{bc}	46.41 ^c	52.05 ^a	53.12 ^a	50.72 ^{ab}	0.681	0.01
Feed intake (g d ⁻¹)	87.95 ^{ab}	89.67 ^{ab}	90.45 ^a	85.47 ^b	85.32 ^b	86.32 ^{ab}	1.301	0.01
FCR	1.72 ^{bc}	1.86 ^{ab}	1.95 ^a	1.64 ^{bc}	1.60 ^c	1.70 ^{bc}	0.044	0.01
Finisher								
Gain (g/d)	80.41 ^{bc}	77.52 ^{cd}	73.67 ^d	87.37 ^a	85.75 ^{ab}	83.87 ^{ab}	0.985	0.01
Feed intake (g/d)	160.21 ^b	163.63 ^b	172.12 ^a	161.71 ^b	160.80 ^b	160.3 ^b	1.53	0.03
FCR	1.99 ^{bc}	2.11 ^b	2.33 ^a	1.85 ^d	1.87 ^{cd}	1.91 ^{cd}	0.033	0.01

^{a-c} Means within a row with different superscripts are significantly different ($P < 0.05$). ^a CON and T treatments as defined in the main text and Table 1.

DISCUSSION

In the present study, serum corticosterone levels were high in broilers receiving the control and experimental diets (heat stress condition) compared to the level of corticosterone in chickens kept in normal temperature conditions (6.78 ng mL^{-1}). Consistent with our results, previous studies have reported that acute heat stress elevates corticosterone levels in the serum of broiler chickens (Quinteiro-Filho *et al.*, 2010; Soleimani *et al.*, 2011). In contrast to our findings, broilers' exposure to heat did not show to influence serum corticosterone

levels (Mack *et al.*, 2013; Xie *et al.*, 2015). Possible reasons for the discrepancies among the results of various studies might be differences in temperature and humidity set, time of blood sampling, and chicken genotypes. Increases in corticosterone levels in broilers' serum are linked to the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis controls the adaptability of broilers in response to various stressors (He *et al.*, 2018).

In the present study, broilers receiving diets with energy restriction (T1 and T2) showed higher corticosterone levels than the control group. In stressful conditions, a change in the energy density of the diet

causes additional metabolic stress in the body of chicks and may increase the generation of free radicals (Raghebian *et al.*, 2017; He *et al.*, 2018). Based on reports (Emami *et al.*, 2021, Rafiei-Tari *et al.*, 2021), chickens receiving a diet with restricted energy experience higher protein turnover, and those receiving a diet with surplus energy experience higher metabolic rate, which both processes increase the heat production and expose body to intense heat stress. Chicken receiving diets containing soybean oil (T4 and T5) had no difference in corticosterone concentration compared to the control group. Also, chicken in T3 and T4, which received soybean oil instead of a part of starch from corn grain, showed lower corticosterone levels than T1 and T2, which may be related to lower heat increment. In a previous study (Sadeghi *et al.*, 2013), a shift from starch to lipid during heat stress decreased heat increment. Many researchers recommend replacing soybean oil with starch (Yaqoob, 2004; Cherian, 2015) to reduce the heat increment and the negative effects of heat stress on the animal body.

The serum glucose level of chickens in heat stress was higher than those raised in normal conditions (185 mg/mL), which might be an adaptation for survivability and tolerance. In agreement with our finding, Bogin *et al.* (1996) reported that chickens that survived under intense heat stress had higher blood glucose levels than the non-surviving chickens. The reductions in serum albumin, globulin, and total protein levels in the chicken receiving low dietary energy (T1 and T2) and high dietary energy (T5) compared to the control group can be linked to elevation of serum corticosterone levels. Corticosterone can change metabolic pathways, reduce protein synthesis (Sadeghi *et al.*, 2013), and increases the catabolism of proteins to use as fuel in broilers receiving low dietary energy (Kitaysky *et al.*, 1999). In broilers receiving high dietary energy (T5), reduced total protein in the serum may be linked to liver inflammation. The result of a previous study (Özbey *et al.*, 2004) is

consistent with the reductions observed in our study after the heat challenge.

The marked increase in the serum uric acid of broilers receiving low dietary energy (T2) may be linked to an increase of protein turnover and, in those receiving surplus energy (T5), linked to liver inflammation and oxidative stress. Previous studies reported increases (Özbey *et al.*, 2004), reductions (Bogin *et al.*, 1996), and no alteration (Xie *et al.*, 2014) in the serum levels of uric acid after heat stress and energy restriction or surplus. The discrepancies in responses among various studies may be related to the differences in metabolic rates and physiological states and also signify protein catabolism for energy generation in energy-restricted birds resulting from increased corticosterone levels (Vandana *et al.*, 2021).

Chicken receiving T1, T3, T4, and T5 had the same performance parameters, but the chickens in the T2 group had higher feed intake and FCR than the control group. To compensate for the energy dilution of the diet, chickens receiving the T2 diet try to feed more. As feed intake increased, the activity of eating and the digestive tract increased, resulting in increased heat production (Herd and Arthur, 2009). In the heat stress condition, heat dissipating from the body decreases, and the animal body is exposed to oxidative stress (Teeter and Belay, 1996). Chickens exposed to oxidative stress could not grow perfectly and showed a lower feed conversion ratio than CON, T3 and T4 groups. Consistent with our finding, Classen (2017) and Azizi *et al.* (2011) reported that chickens increased feed intake in response to dietary energy dilution. However, Yuan *et al.* (2008) reported that the weight gain of chickens was not altered by dietary energy level, in contrast with our results.

Antibody titers against ND and IBD were the highest in the T3 and T4 diet formulated with soybean oil, and the lowest in the T1 and T2 diet formulated with low energy density, which is inconsistent with the findings of Taleb *et al.* (2017). They



reported that an increase in soybean oil level in the Cobb strain diet resulted in lower antibody titers against ND and IBD. In the current study, an increase in the level of soybean oil in the Ross broiler diet had no negative effect on the antibody titers against ND and IBD.

In broilers receiving low-energy diets, the effect of metabolic stress caused by energy level on the high corticosterone level and low blood glucose level may play an important role in reducing the immune response and antibody production (Yang *et al.*, 2015; Aami Azghady *et al.*, 2014). The factors above cause disturbances in the process of growth and maturation of T and B cells in primary and secondary lymphoid tissues, which ultimately causes numerous immune abnormalities in broiler chickens (Hirakawa *et al.*, 2020).

Pro-inflammatory cytokines such as *IL-2* and *IL-6* have been found to play an active role in the inflammatory response under stressful conditions (Helwig and Leon, 2011). In the literature, limited information exists concerning energy level's effect on the gene expression of pro-inflammatory cytokines. A striking finding in the present study was the low expression of genes involved in inflammation in broiler chickens' diets with low energy density (T1 and T2). This finding agrees with some studies (Trayhurn and Wood, 2004; Higami *et al.*, 2006) that reported low energy diet resulted in low inflammation and gene expression of pro-inflammatory cytokines in laboratory animals. In contrast, high-energy diets (T4 and T5) increased the relative gene expression of *IL-2* and *IL-6*. In T4 and T5 groups, high corticosterone levels may be influenced by the expression of pro-inflammatory cytokines as it could increase the proliferation of lymphocytes and macrophages (Hirakawa *et al.*, 2020; Goel *et al.*, 2021). In energy-dense diets, soybean oil is included, and higher expression of these genes may be related to oil inclusion. Previous studies (Mu *et al.*, 2018) revealed that dietary soybean oil significantly increased the gene expression of pro-

inflammatory cytokines. The results observed for fasting glucose level and *IL-2* gene expression in the present study are consistence with the finding of Kochumon *et al.* (2020), who reported that the level of *IL-2* expression was associated positively with fasting blood glucose.

CONCLUSIONS

The results of the present study indicate that energy restriction and surplus negatively affect the immune response and performance of chickens raised under heat stress. Surplus energy negatively affects the relative expression levels of pro-inflammatory cytokines genes (*IL-2* and *IL-6*), and energy restriction results in higher protein catabolism (higher uric acid and creatinine), which reduces broiler performance and immune responses. The inclusion of soybean oil in the diet positively affected immune response and performance. It was recommended to feed Ross broiler chickens under heat stress with a diet containing oil instead of a part of grain based on the energy recommended by the strain recommendation.

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اثر منبع و سطح انرژی جیره بر عملکرد، تیر آنتی بادی و بیان نسبی ژن های
اینترلوکین ۲ و ۶ در جوجه های گوشتی تحت تنش گرمایی

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چکیده



این مطالعه با هدف تعیین تأثیر سطوح و نوع منبع انرژی بر عملکرد رشد، تیتراکتیویتی و بیان ژن های سیتوکین های پیش التهابی در جوجه های گوشتی در معرض تنش گرمایی انجام شد. ۴۵۰ قطعه جوجه راس یک روزه در قالب طرح کاملاً تصادفی در شش جیره آزمایشی و پنج تکرار قرار گرفتند. جوجه ها جیره های متمایز شده بر اساس منبع اصلی انرژی (دانه ذرت و روغن سویا) و سطح انرژی (برابر، ۳ یا ۶ درصد کمتر یا بالاتر از توصیه (Ross 308) دریافت کردند. تیمارها به شرح زیر بود: دانه ذرت و انرژی برابر با شاهد (CON)، دانه ذرت، ۳ درصد کمتر (T1)، دانه ذرت، ۶ درصد کمتر (T2)، دانه ذرت و روغن سویا، برابر (T3)، دانه ذرت و روغن سویا، ۳ درصد بیشتر (T4)، دانه ذرت و روغن سویا، ۶ درصد بیشتر (T5)، دمای سالن از روز ۱۲ تا ۴۲ درجه پرورش به ۳۴ درجه سلسیوس (۶ ساعت در روز) افزایش یافت تا تنش گرمایی ایجاد شود. بالاترین سطح کورتیکوسترون در گروه های T1، T2 و T5 مشاهده شد. کمترین تیتراکتیویتی بادی در گروه T2 و بالاترین سطح بیان ژن های سیتوکین های پیش التهابی در جوجه های دریافت کننده جیره T5 مشاهده شد. بیشترین ضریب تبدیل خوراک (FCR) در طول دوره رشد و پایانی در T2 و کمترین FCR در گروه T3 و T4 مشاهده شد. تغذیه جوجه های گوشتی راس با جیره غذایی حاوی روغن به جای بخشی از غلات بر اساس انرژی پیشنهاد شده در کاتالوگ سویه توصیه می شود.

Effectiveness of *Bacillus thuringiensis* (Shigetane) Commercial Products against Tomato Leaf Miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

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ABSTRACT

The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is one of the most important pests causing significant economic losses in plant species belonging to the Solanaceae family. The preferred management method for *T. absoluta* currently involves insecticide application. However, beside the undesired effects of insecticides, chemical treatments can also negatively impact the efficiency of Integrated Pest Management programs (IPM). *Bacillus thuringiensis* (Shigetane 1902) (Bacillales: Bacillaceae) (Bt) is a pathogen with formulations used as host-specific bio-insecticides. These formulations decompose quickly in the environment, thereby reducing non-target effects and residue problems compared to chemical pesticides. In this study, the effectiveness of six commercial *Bt* products, belonging to *aizawai* and *kurstaki* strains, against *T. absoluta* was assessed under laboratory conditions, using manufacturer-recommended doses. The efficacy of the *Bt* products varied between 70 and 97.5%. The lowest and highest mortalities were recorded in *B. thuringiensis* var. *aizawai* and *B. thuringiensis* var. *kurstaki* products, respectively. Mortality reached 100% within three days following insecticide treatments, whereas peak mortality in *Bt* applications was noted after a post-treatment period of 15 days. These findings highlight the potential of certain *Bt* products as effective components of IPM programs for *T. absoluta*, suggesting the need for further field studies to optimize their use in agricultural practices.

Keywords: Development time, Host-specific bio-insecticides, IPM programs, Mortality.

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), originating from South America, stands as one of the most economically detrimental pests affecting a range of plant species within the Solanaceae family (Miranda *et al.*, 1998; Garzia, 2009). Initially reported in Spain in 2006, the pest subsequently spread throughout Europe and the Mediterranean countries (Urbaneja *et al.*, 2007; Arno *et al.*, 2009). In Turkey, following its first appearance in 2009, it rapidly proliferated and emerged as a prominent pest in both greenhouse and field tomato cultivation (Kılıç, 2010; Karut *et al.*, 2011). *T. absoluta*

larvae feed between the two epidermal layers of tomato leaves, creating irregular transparent galleries that eventually turn brown, causing complete leaf desiccation. Furthermore, the larvae also feed on tomato fruits, and their excrement fosters an environment conducive to decay and the development of secondary microorganisms. Collectively, these damages result in significant losses in fruit quality and yield (Korycinska and Moran 2009; Desneux *et al.*, 2010).

Among the existing practices, the predominant method for controlling *T. absoluta* involves insecticide application (Trobea *et al.*, 2012; Roditakis *et al.*, 2018). However, due to the limited penetration of

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insecticides into plant tissues and the rapid development of resistance attributed to *T. absoluta*'s high reproductive capacity, chemical control alone often fails to yield the desired results (Biondi et al., 2018; Buragohain et al., 2021). Moreover, the indiscriminate and intensive use of insecticides poses adverse effects on human and environmental health. Consequently, alternative control methods, such as biological and biotechnical control, have gained preference for the better management of the pest (Lietti et al., 2005; Gonzales-Cabrera et al., 2011; Desneux et al., 2022).

Numerous natural enemies of *T. absoluta* from Hymenoptera and Hemiptera group of insects have been identified (Miranda et al., 1998; Marchiori et al., 2004; Luna et al., 2007; Bajonero 2008; Cabello et al., 2009; Kabiri et al., 2010; Doğanlar and Yiğit 2011). In addition to predators and parasitoids, microorganisms are also employed for pest control (Buragohain et al., 2021). *Bacillus thuringiensis* (Shigetane 1902) (Bacillales: Bacillaceae) (*Bt*) is a unique soil-dwelling bacterium utilized in the biological control of *T. absoluta* (Palma et al., 2014; Dammak et al., 2016; Biondi et al., 2018). Commercial products derived from various subspecies of *Bt* are deployed in managing insect species across different families. While *B. t* var. *kurstaki* is effective against lepidopteron larvae, *B. t* var. *israelensis* and *B. t* var. *tenebrionis* are used to control mosquitoes and coleopteran pest species, respectively (Gelernter, 2004; Palma et al., 2014; Dammak et al., 2016).

Studies investigating the efficacy of *Bt* products against on *T. absoluta* commenced with *B. t* var. *kurstaki* (Btk), sourced from South America in the early 2000s. Giustolin et al. (2001) demonstrated *Btk* induced mortality across all developmental stages of *T. absoluta* larvae. Subsequently, there has been a notable increase in research assessing the efficacy of *Bt* products in managing the pest (Niedmann and Meza-Basso, 2006; Gonzalez-Cabrera, 2011; Sarr et al., 2021). Niedmann and Meza-Basso (2006) revealed that two indigenous strains of *Bt* exhibited

lethal effects against *T. absoluta* in Chile. Gonzalez-Cabrera (2011) reported that the impact of *T. absoluta* could be significantly diminished by exclusively applying *B. t*-based formulations, obviating the need for chemical insecticides. Sarr et al. (2021) demonstrated a reduction in the proportion of damaged fruits and an improvement in tomato yield, particularly with the application of *Bt* products. Furthermore, it has been revealed that more favorable outcomes in pest management could be achieved by combining *Bt* with various bio-control agents (Gonzalez-Cabrera et al., 2011; Alsaedi et al., 2017; Jamshidnia et al., 2018; Asma et al., 2018).

Environmentally friendly agents such as *Bt* strains are essential for a sustainable Integrated Pest Management (IPM) program against tomato pests. Therefore, this study aimed to evaluate the effects of specific *Bt* commercial products with the potential to be used in biological control programs against *T. absoluta*.

MATERIALS AND METHODS

Host Plant Rearing

Tomato (*Lycopersicon esculentum* L.) cultivar Soray was used as a host plant in this study. The production of tomato plants was carried out in the specialized rearing room adjusted at 25±2°C and 70±5% humidity with long day lighting (16 Light: 8 Dark) hours. The plants were grown in pots (15 x15 cm) containing potting soil.

Tomato Leaf Miner Rearing

The initial population of *T. absoluta* was obtained from tomato fields of Adana, and bioassay studies were completed at Cukurova University, Faculty of Agriculture, Department of Plant Protection, Laboratory of Insect Molecular Genetics and Biotechnology. The production was carried out in three fully grown tomato plants in net

cages. The cages, each measuring 70×70×150 cm, were placed in the rearing room adjusted to 25±2°C and 70±5% humidity, with long-day lighting (16 Light:8 Dark) hours. To maintain the *T. absoluta* production, dead tomato plants were replaced with new healthy plants during mass rearing period.

***Bacillus thuringiensis* Products**

In this study, six registered *Bt* products in Turkey were tested. In addition to those products, two commercial insecticides, spinetoram 120 g L⁻¹ (Radiant™, Dow AgroSciences, Istanbul, Turkey), and spinosad 480 g L⁻¹ (Laser™, Dow AgroSciences, Istanbul, Turkey), widely preferred in pest control by growers, were used as positive controls. The features and recommended doses of the products are given in Table 1. Except for Dacron, all products were registered against *T. absoluta*.

Bioassay Experiment

Leaves obtained from the upper half of 40 cm tall tomato plants were used in the experiments. The recommended doses of the products, given in Table 1, were prepared using distilled water, and were applied to the tomato leaves by leaf dipping method. In the process, the leaves were dipped in the prepared solution for three seconds, then, allowed to dry on a paper for 30 minutes under laboratory conditions. The petiole of the tomato leaves were wrapped in wet cotton to provide moisture and keep the leaves alive during the experiments. The leaves were placed in rectangular transparent plastic containers of 12×6×6 cm, where the lids were covered with nets for ventilation. One newly hatched first instar of *T. absoluta* larvae was transferred to each leaf with the help of a fine-tipped paint brush. The first instar larvae were obtained from *T. absoluta* eggs kept in cabinet adjusted to 25±1°C and 70±5% humidity. The larvae released on

leaves treated with distilled water were considered as the controls. The prepared units were placed in a cabinet adjusted to 25±1°C, 70±5% humidity, and long-day lighting (16 h Dark :8 h Light). Experimental units were checked daily, and the number of live/dead larvae and the development of the larvae that remained alive were recorded. The stages of the larvae were determined depending on the head capsules they left after each molting. A total of ten individuals were used per replicate, and each treatment was set up with 10 replicates (100 individuals) in bioassay experiments. The mean development time of larval instars was determined from individuals that remained alive and completed the immature development (Kandil *et al.*, 2020). To determine adult longevity, individuals reaching the adult stage were carefully transferred to separate containers, and provided with honey as a regular consistent food source. These containers were kept under controlled environmental conditions, including a temperature of 25±1°C, relative humidity of 70±5%, and a photoperiod of 16 h light/8 h dark. Each adult was observed daily, and their survival was recorded until death.

Statistical Analyses

Corrected mortality rates of 3, 7, 10, and 15 days after application and cumulative mortality were calculated using the Abbott formula (Abbott, 1925). Before conducting the analysis, we assessed the normality using the Shapiro–Wilk test and checked for homogeneity of variances using Levene’s test. In case of violation of assumptions, the data were transformed using Log10(X+1) and arcsin for homogeneity of variances. The original data are presented in the results. Data were analyzed using the One-Way ANOVA, followed by separation of means using the Tukey test. All analyses were conducted using SPSS 25.0 (Chicago, IL, USA).

**Table 1.** Characteristics of *Bacillus thuringiensis* products and insecticides used in the experiments.

Name	Formulation	<i>Bt</i> products			Bacteria density	Recommended rate
		Strain	Isolate			
Agree 50	WG	<i>B. thuringiensis</i> spp. <i>aizawai</i> + <i>kurstaki</i>	GC-91	%50		100 g 100 L ⁻¹
Dacron	WP	<i>B. thuringiensis</i> <i>berliner</i> var <i>kurstaki</i>	Serotype 3a 3b, SA- 11 5300	32000 IU mg ⁻¹		100 g 100 L ⁻¹
Delfin	WG	<i>B. thuringiensis</i> <i>berliner</i> var <i>kurstaki</i>	Serotype 3a 3b, SA- 11	32000 IU mg ⁻¹		100 g 100 L ⁻¹
Dipel DF	WG	<i>B. thuringiensis</i> <i>subsp</i> <i>kurstaki</i>	ABTS-351	32000 CLU mg ⁻¹		100 g 100 L ⁻¹
Florbac	WG	<i>B. thuringiensis</i> var. <i>aizawai</i>	ABTS-1857	35000 DBM mg ⁻¹		150 g 100 L ⁻¹
Rebound	WP	<i>B. thuringiensis</i> var. <i>kurstaki</i>	-	16000 IU mg ⁻¹		200 g 100 L ⁻¹
Insecticides						
Laser	SC	Active ingredient				25 mL 100 L ⁻¹
Radiant	SC	480 g L ⁻¹ Spinosad 120 g L ⁻¹ Spinetoram				50 mL da ⁻¹

RESULTS

Effects of *B. thuringiensis* Products on Mortality of *Tuta absoluta*

The insecticides spinosad and spinetoram exhibited the highest cumulative mortality rates, both reaching 100%, indicative of their potent lethal effects. Delfin and Dacron, belonging to *Bt* category, followed with mortality rates of 97.5 and 92.5%, respectively, and statistically fell within the same group [$F(7, 79) = 9.74, P = 0.0001$]. The remaining *Bt* products demonstrated mortality rates ranging from 86.2 to 70.0%, showcasing variability in their effectiveness (Figure 1).

On the third day after application, 100% mortality was observed for spinosad and spinetoram, while Dipel exhibited a low rate of 1%. Mortality rates increased for all products by day 7, ranging from 9% for Agree to 50% for Delfin, signifying varied responses to the treatments. On day 10, all products, except Delfin (67%), exhibited mortality rates below 50%, indicating sustained but varied efficacy across

treatments. On the 15th day, the highest and lowest mortality rates were observed in Delfin (94.2%) and Agree (55.2%), highlighting the durability and variability of the treatments (Table 2).

In the first instar larvae, the highest mean number of dead individuals was detected for Delfin (3.6), followed by Rebound (1.9) and Dipel (1.6). The three products differed statistically from the control experimental unit [$F(6, 69) = 10.1, P = 0.0001$]. No mortality was observed in Agree, which belonged to the same category as the control group. In the second instar larvae, the highest average numbers of dead individuals were found in Dacron (4.6), followed by Florbac (4.5). Other products showed varied mean mortality values between 0 and 2.8, and the differences were statistically significant [$F(6, 69) = 16.08, P = 0.0001$]. In the third instar larvae, the mean numbers of dead individuals were close to each other, with the highest in Agree and Dacron (2.8). All the products were statistically different from the control, but showed no difference between each other [$F(6, 69) = 8.27, P = 0.0001$]. In the fourth instar larvae, the

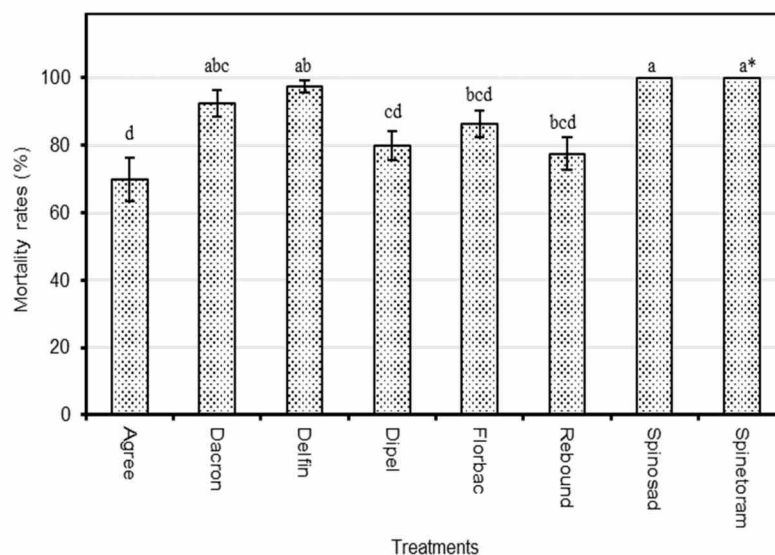


Figure 1. Cumulative mortality rates ($\pm$ SE) of *Tuta absoluta* caused by different *Bacillus thuringiensis* products and two insecticides (Spinosad and Spinetoram). * Values with different letters denote statistically significant difference (Tukey; $P < 0.05$).



highest mean mortality values was determined as 2 for both Agree and Florbac. The values varied between 0.6 and 1.5 for the other products. In the pupal stage, the highest and lowest mean numbers of dead individuals were recorded for, respectively, Rebound (0.8), and Delfin (0.1). The statistical difference, however, was not significant [$F(6, 69) = 1.40$, $P = 0.226$]. In the sum of the first and second instars, the mean number of dead individuals exceeded approximately 50% for the three products, i.e. Dacron, Delphin and Florbac (Table 3).

Effects of *B. thuringiensis* Products on Development and Longevity of *Tuta absoluta*

In the first instar, statistically significant differences were observed in mean development times. The longest and shortest times were recorded for Dipel (4.37 days) and the control (3.33 days), respectively [$F(5, 161) = 2.72$, $P = 0.02$] (Table 4). In the second instar, the mean development times varied between 2.77 and 5.00 days. In the third instar, a statistically significant

Table 2. Corrected mortality rates ($\pm$ SE) of commercial *Bacillus thuringiensis* (Bt)-based products and two insecticides (Spinetoram and Spinosad) after post-treatment period of 3, 7, 10 and 15 days.^a

Products	Days			
	3	7	10	15
Agree	0.00 $\pm$ 0.00 ^{b*}	9.00 $\pm$ 3.48 ^c	13.00 $\pm$ 3.34 ^b	55.25 $\pm$ 4.38 ^c
Dacron	0.00 $\pm$ 0.00 ^b	16.00 $\pm$ 4.00 ^{bc}	44.00 $\pm$ 6.86 ^a	64.50 $\pm$ 7.00 ^{bc}
Delfin	0.00 $\pm$ 0.00 ^b	50.00 $\pm$ 7.60 ^a	67.00 $\pm$ 9.07 ^a	94.25 $\pm$ 3.07 ^a
Dipel	1.00 $\pm$ 1.00 ^b	32.00 $\pm$ 4.16 ^{ab}	46.00 $\pm$ 7.18 ^a	78.50 $\pm$ 4.47 ^{abc}
Florbac	0.00 $\pm$ 0.00 ^b	21.00 $\pm$ 4.33 ^{bc}	45.00 $\pm$ 7.49 ^a	76.25 $\pm$ 6.57 ^{abc}
Rebound	0.00 $\pm$ 0.00 ^b	26.00 $\pm$ 6.15 ^{abc}	37.00 $\pm$ 6.15 ^{ab}	82.25 $\pm$ 6.17 ^{ab}
Spinetoram	100 $\pm$ 0.00 ^a	-	-	-
Spinosad	100 $\pm$ 0.00 ^a	-	-	-

^a Means within the same column with different letters denote statistically significant difference (Tukey; $P < 0.05$).

Table 3. Mean ($\pm$ SE) numbers of mortality of *Tuta absoluta* individuals at different larval instars treated with commercial *Bacillus thuringiensis* (Bt)-based products.^a

Products	Larval instars and pupa				
	I	II	III	IV	Pupa
Agree	0.0 $\pm$ 0.00 ^{c*}	2.0 $\pm$ 0.36 ^{bc}	2.8 $\pm$ 0.44 ^a	2.0 $\pm$ 0.61 ^a	0.7 $\pm$ 0.30 ^a
Dacron	0.5 $\pm$ 0.30 ^{bc}	4.6 $\pm$ 0.76 ^{ab}	2.8 $\pm$ 0.46 ^a	1.2 $\pm$ 0.38 ^a	0.3 $\pm$ 0.30 ^a
Delphin	3.6 $\pm$ 0.61 ^a	2.8 $\pm$ 0.44 ^{abc}	2.0 $\pm$ 0.55 ^a	1.4 $\pm$ 0.26 ^a	0.1 $\pm$ 0.10 ^a
Dipel	1.6 $\pm$ 0.54 ^{ab}	2.5 $\pm$ 0.54 ^{abc}	2.6 $\pm$ 0.30 ^a	1.5 $\pm$ 0.37 ^a	0.2 $\pm$ 0.13 ^a
Florbac	1.0 $\pm$ 0.29 ^{bc}	4.5 $\pm$ 0.63 ^a	2.4 $\pm$ 0.26 ^a	0.6 $\pm$ 0.26 ^a	0.3 $\pm$ 0.15 ^a
Rebound	1.9 $\pm$ 0.62 ^{ab}	1.5 $\pm$ 0.16 ^c	2.0 $\pm$ 0.59 ^a	2.0 $\pm$ 0.53 ^a	0.8 $\pm$ 0.29 ^a
Control	0.0 $\pm$ 0.00 ^c	0.0 $\pm$ 0.00 ^d	0.1 $\pm$ 0.10 ^b	0.6 $\pm$ 0.22 ^a	0.4 $\pm$ 0.22 ^a

^a Means within the same column with different letters denote statistically significant difference (Tukey; $P < 0.05$).

Table 4. Mean ($\pm$ SE) development time (day) of different larval stages, and adult longevity of *Tuta absoluta* calculated from the larvae do not dead and completed development after *Bacillus thuringiensis* treatment.^a

Products	Larval instars and pupa							Longevity
	n	I	II	III	IV	Pupa	Total	
Agree	24	3.45 $\pm$ 0.20 ^{ab*}	4.16 $\pm$ 0.48 ^{ab}	4.54 $\pm$ 0.37 ^a	3.54 $\pm$ 0.24 ^{ab}	8.12 $\pm$ 0.06 ^a	23.83 $\pm$ 0.63 ^{ab}	12.33 $\pm$ 1.13 ^{ab}
Dacron	6	4.33 $\pm$ 0.42 ^{ab}	4.83 $\pm$ 0.30 ^a	3.50 $\pm$ 0.22 ^{ab}	3.66 $\pm$ 0.33 ^{ab}	8.16 $\pm$ 0.18 ^a	24.50 $\pm$ 0.42 ^a	15.16 $\pm$ 0.60 ^a
Dipel	16	4.37 $\pm$ 0.32 ^a	3.62 $\pm$ 0.32 ^{ab}	3.37 $\pm$ 0.28 ^{ab}	3.56 $\pm$ 0.47 ^{ab}	8.12 $\pm$ 0.17 ^a	23.06 $\pm$ 0.50 ^{ab}	13.68 $\pm$ 0.76 ^a
Florbac	12	3.91 $\pm$ 0.28 ^{ab}	5.00 $\pm$ 0.68 ^a	3.33 $\pm$ 0.28 ^{ab}	2.83 $\pm$ 0.40 ^{ab}	7.58 $\pm$ 0.19 ^a	22.66 $\pm$ 0.93 ^{abc}	8.66 $\pm$ 1.00 ^b
Rebound	18	3.66 $\pm$ 0.19 ^{ab}	2.77 $\pm$ 0.26 ^c	2.88 $\pm$ 0.25 ^b	4.22 $\pm$ 0.40 ^a	7.94 $\pm$ 0.17 ^a	21.50 $\pm$ 0.49 ^{bc}	14.66 $\pm$ 0.94 ^a
Control	86	3.33 $\pm$ 0.13 ^b	3.50 $\pm$ 0.14 ^{bc}	2.74 $\pm$ 0.09 ^b	2.72 $\pm$ 0.13 ^b	7.88 $\pm$ 0.07 ^a	20.27 $\pm$ 0.26 ^c	11.04 $\pm$ 0.32 ^{ab}

^a Means within the same column with different letters denote statistically significant difference (Tukey; $P < 0.05$).

difference in mean development times was observed, exceeding those of the control [F(5, 161)= 9.62, $P = 0.0001$]. In the pupal stage, mean development times were close to each other and did not show statistically significant differences [F(5, 161)= 1.83, $P = 0.10$]. Total mean development times ranged between 20.27 and 24.5 days, with statistically significant differences observed [F(5, 161)= 12.7, $P = 0.0001$]. Except for Florbac, adult longevities in all treatments were longer and statistically different from the control [F(5, 161)= 6.42, $P = 0.0001$] (Table 4).

DISCUSSION

Although there were statistical differences, the effectiveness of *B. thuringiensis* (Bt) products, manifested by mortality rates exceeding 70.0%, was confirmed in this study. Similarly, the effectiveness of Bt products on larval mortality of *T. absoluta* was confirmed under laboratory and greenhouse conditions (Hafsi *et al.*, 2012; Birgücü *et al.*, 2014; Jallow *et al.*, 2019; Kandil *et al.*, 2020; Sandeep Kumar *et al.*, 2020a, b; Buragohain *et al.*, 2021; Sarr *et al.*, 2021). Although the application method is different, Hafsi *et al.* (2012) also found an average of 72.5% larval mortality seven days after the

treatment of the Bt product (Bt 32000) under laboratory conditions. Jallow *et al.* (2019) reported 55–65% mortality when second-instar *T. absoluta* larvae were exposed to tomato leaves treated with Bt (Dipel).

It can be suggested that the high mortality rate in the first two larval stages, with over 50% mortality in three Bt products (Delfin, Florbac, and Dacron), could increase the success in the biological control of tomato leaf miner. Similar results were reported in different studies, and mortality in the first and second larval stages was found to be higher than the other larval stages (Giustolin *et al.*, 2001; Gonzalez-Cabrera *et al.*, 2011; Hashemitassuji *et al.*, 2014). Coelho and França (1987) argued that this was because the new larva that emerged from the egg was feeding by chewing the leaf surface to reach the mesophyll layer. This behavior increases the chance of getting bacterial toxins into the digestive system of the larvae.

B. thuringiensis products prolonged the larval development period in infected individuals that survived and completed their development. These results were aligned with other researchers who demonstrated the effect of Bt products on *T. absoluta* larvae, reporting a significant increase in larval and pupal development periods (Kandil *et al.*, 2020). Similar results were also reported for other lepidopteron pests. Yang *et al.* (2008) determined that the



Bt YL17 isolate disrupted the development of the 3rd larval stage of *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae), and prolonged the total immature development. Erb *et al.* (2001) reported that *Bt* had a sub-lethal effect on *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantridae) fourth instar larvae and prolonged the development period. Barker (1998) reported 12.4 days longer total immature development time for *Bt* treated *Cochylis hospes* (Walsingham, 1884) (Lepidoptera: Tortricidae) larvae compared to the control group. Similarly, Huarong *et al.* (2005) found that, after *Bt* applications, larval development of *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Pyralidae) was prolonged when compared to the control.

The mortality rates of the *Bt* products were found to be close to the that of the insecticides. However, the highest mortality rate (100%) was reached on the 3rd day in insecticide treatments, while it was reached on the 15th day in *Bt* treatments. This could be due to the different modes of action of the insecticide and *Bt*. While insecticides lead to immediate death after application, mortality in *Bt* applications may occur after a few hours or weeks (Perez *et al.*, 2015). *B. thuringiensis* strains generate toxins during both the initial sporulation phase and the growth stage, resulting in the formation of para-sporal crystalline inclusions. Upon ingestion by insects, these toxins dissolve within the midgut. Subsequently, midgut proteases trigger the toxins through proteolysis, binding them to precise receptors on the insect cell membrane. This binding results in cell disruption, ultimately leading to the death of the insect (Schnepf *et al.*, 1998; Palma *et al.*, 2014).

In this laboratory study, we demonstrated that *Bt* products are, at least, as effective as insecticides, but require more time to achieve the maximum mortality rate. Therefore, for a successful IPM program in greenhouses, these products should be applied repeatedly at specific time window (one week) supported with supplemental

application of other natural enemies, such as predators or parasitoids.

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اثربخشی محصولات تجاری (*Bacillus thuringiensis* (Shigetane) علیه مینوز برگ گوجه فرنگی، (*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae))

بورچین چیچک، محمود مته کاراجا، و کامیل کاروت

چکیده

مینوز برگ گوجه فرنگی، (*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae))، یکی از مهمترین آفاتی است که خسارات اقتصادی قابل توجهی را در گونه‌های گیاهی متعلق به خانواده Solanaceae ایجاد می‌کند. در حال حاضر، روش مدیریت ترجیحی برای *T. absoluta* شامل استفاده از حشره کش است. با این حال، علاوه بر اثرات نامطلوب حشره کش‌ها، درمان‌های شیمیایی می‌توانند بر کارایی برنامه‌های مدیریت تلفیقی آفات (IPM) نیز تأثیر منفی بگذارند. باسیلوس تورینجینسیس (شیگتان 1902) (Bacillales: (Bt) Bacillaceae) یک پاتوژن است که فرمولاسیون‌های آن به عنوان حشره کش‌های زیستی (bio-insecticides) مخصوص میزبان استفاده می‌شود. این فرمولاسیون‌ها به سرعت در محیط تجزیه می‌شوند و در نتیجه اثرات غیرهدف (non-target) و مشکلات غیرهدف (non-target problems) را در مقایسه با آفت کش‌های شیمیایی کاهش می‌دهند. در این مطالعه، اثربخشی شش محصول تجاری *Bt*، متعلق به سویه‌های *aizawai* و *kurstaki*، علیه *T. absoluta* در شرایط آزمایشگاهی و با استفاده از دوزهای توصیه شده توسط سازنده ارزیابی شد. اثربخشی محصولات *Bt* بین ۷۰٪ و ۹۷.۵٪ متغیر بود. کمترین و بیشترین میزان مرگ و میر به ترتیب در محصولات *B. thuringiensis* var. *kurstaki* و *B. thuringiensis* var. *aizawai* ثبت شد. مرگ و میر ظرف سه روز پس از تیمار با حشره کش به ۱۰۰٪ رسید، در حالی که اوج مرگ و میر در



کاربردهای *Bt*، ۱۵ روز پس از آن مشاهده شد. این یافته‌ها، استعداد برخی از محصولات *Bt* را به عنوان اجزای مؤثر برنامه‌های IPM برای *T. absoluta* برجسته می‌کند و نیاز به مطالعات میدانی بیشتر برای بهینه‌سازی استفاده از آنها را در فعالیتهای کشاورزی نشان می‌دهد.

Fitness Enhancement by Crosses between Two Populations of *Trissolcus vassilievi* (Hymenoptera: Scelionidae)

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ABSTRACT

Trissolcus vassilievi (Mayr) (Hymenoptera: Scelionidae) is one of the most important egg parasitoids of the Common Sunn Pest (CSP), *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) in Iran. In this study, the fitness of two populations of *T. vassilievi* was studied on two populations of hosts in terms of life history parameters. Two populations of both *T. vassilievi* and the host, CSP were selected: (1) Tabriz (as a temperate area), and (2) Varamin (as a subtropical area), for CSP. Moreover, regarding that outcrossing between populations can produce progeny with superior characteristics, the progeny of reciprocal crosses between original populations also were examined on a single host. The crosses between the two populations caused 13.9-18.5% higher net fecundity than maternal populations, which suggests fecundity to be a function of maternal phenotype. The intrinsic rate of increase showed minor differences among treatments, which varied between 0.291 ± 0.003 to 0.305 ± 0.003 . The partial advantage of Varamin wasps over Tabriz, and the crosses over the original populations was obvious. Such differences may be used to obtain more efficient parasitoids in augmentation programs.

Keywords: Intrinsic rate of increase, Life expectancy, Net reproduction rate, Parasitoid wasp, Reciprocal crosses.

INTRODUCTION

The biological fitness of a living organism is the relative ability of an organism to survive and pass on its genes to the next generation (Krebs and Davies, 1993). This is possible through birth (transferring more proportion of genes to the next generation) in the shortest possible time (high speed of gene transcription) and the ability of progeny to survive (persistence of the genes). Therefore, the developmental rate, the mortality rate at all stages of life, and fecundity are relevant features of fitness and the ability of a living organism to compete with other species. These characteristics can

be examined as life tables and stable population growth models (Lotka, 1907a, b; Portilla *et al.*, 2014). So far, several studies have investigated the stable population growth parameters in egg parasitoids of Common Sunn Pest (CSP), *Eurygaster integriceps* Puton (Hem., Scutelleridae), and related stinky bugs. These are dominantly on *Trissolcus* spp. and *Telenomus* spp. (Hymenoptera: Scelionidae) (Asgari and Kharrazi Pakdel, 1998; Laumann *et al.*, 2008; Amir-Maafi and Parker, 2011; Nozad-Bonab *et al.*, 2014; Bazavar *et al.*, 2015; Benamolaei *et al.*, 2015a, b; Abdi *et al.*, 2017; Teimouri *et al.*, 2019), and *Ooencyrtus* spp. (Hymenoptera: Encyrtidae)

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(Ahmadpour et al., 2013; Mele et al., 2024).

There is little information about intra- and inter-specific variation of survival value and reproductive potential of egg parasitoids of CSP, and it is limited to few studies. In the 1970s, several species of CSP's egg-parasitoids, collected from different regions of the world, were transferred to the former Soviet Union and compared with native species, especially *Trissolcus grandis* (Thomson), in laboratory and field conditions. The overall result of this study showed that native species had superiority and it was due to the adaptation of native species to climatic and seasonal conditions (Nouri et al., 2011). Awan et al. (1990) compared three geographical populations of *T. basalis* (Wollaston) collected from France, Italy, and Spain regarding biological and behavioral characteristics. The emergence rate of adult wasps from *Nezara viridula* (L.) eggs was significantly higher in the French population than in the Italian and Spanish ones. The development of immature stages of the Italian population was significantly longer than the other two populations. Taghadosi et al. (1993) and Nozad Bonab et al. (2014) observed differences among populations of *T. grandis* of Tehran-Alborz-Qazvin and East Azarbaijan provinces, respectively. A comparison of biostatistics of Scelionidae by Amir-Maafi (2010) during 2004-2006 in different provinces of Iran revealed significant differences between species and populations of the wasps. Fecundity, oviposition period, and gross and net reproductive rates differed between species or populations. The net fecundity of *T. vassilievi* (Mayr) from Lorestan and Tehran was 240.8 and 227.5 eggs, respectively, which was 2.5 times the other populations. The highest intrinsic rate of increase of *T. grandis*, *T. semistriatus* (Nees), and *T. vassilievi* was recorded for Golestan, West Azarbaijan, and Tehran provinces, respectively.

It can be seen from the above reports that inter-specific and intra-specific differences in the parasitism rate and population growth

rate of the parasitoid wasps are sometimes very considerable. Therefore, in this study, we attempted to study the differences between two populations of *T. vassilievi* with temperate (Tabriz) and subtropical (Varamin) origins simultaneously. On the other hand, since the parasitoid spends all immature stages within the host body, it can be affected by the host's quality, so the host population was also included as a second variable. Finally, the hypothesis was tested to find if crosses between populations could enhance the fitness of populations. Therefore, crosses as Tabriz-female×Varamin-male (T×V) and Varamin-female×Tabriz-male (V×T) were conducted to evaluate the possibility of obtaining populations with superior or intermediate characteristics.

MATERIALS AND METHODS

Cultures of *Eurygaster integriceps* Puton

Adult bugs were collected on several occasions at the end of the winter from mountains around Tabriz and Varamin before leaving the resting sites. Collecting of specimens were continued in wheat fields during post diapause phase. The collected insects were transferred to a greenhouse unit of the Department of Plant Protection, Faculty of Agriculture, University of Tabriz. Transparent rectangular cubic plastic containers (20×30×9 cm) equipped with a mesh cap for ventilation were used for the rearing of both populations. Dry wheat grain was used as a foodstuff and soaked cotton balls as a water source. The paper strips folded fan-like to serve as an oviposition substrate. These insects were exposed to 25±2°C, 40±10% RH, and 16:8 h L:D photoperiod (Iranipour et al. 2015).

Cultures of *Trissolcus vassilievi* (Mayr)

In this study, two original populations of *T. vassilievi*, namely, one from Tabriz (1360

AMSL, 46°E, 38°N) and the other from Varamin (918 AMSL, 51°E, 35°N) were examined. To collect egg parasitoids, host egg traps (yellow cardboards, 5×15 cm, folded twice to construct a Δ -shaped structure) were used (Safavi, 1973). The traps were tied to wheat ears and removed after one week. Then, the parasitized eggs were transferred to glass vials (1.5×10 cm) and kept in a growth chamber (Iran Khodsaz Co., IKH.RH model) under constant conditions (26±1°C, 50±5% RH and 16:8 h L:D photoperiod). The emerged wasps were identified by the identification key of Kozlov and Kononova (1983). After rearing for one generation, males and females of the second generation from the same population were randomly coupled and each pair was transferred to a similar vial supplied by the host eggs of either population. Small drops of honey were used to feed the wasps.

Fecundity-Life Table Studies

Ten clutches of 24-hour-old CSP eggs (14 eggs per clutch) from each population were exposed to 48-hour-old mated *T. vassilievi* females of the second-generation of each population. After 24 hours, females were removed and the host eggs were kept as a life table cohort in the so-called growth chambers to determine their fate. The experiment was conducted as a factorial experiment in a completely randomized design framework, with two factors including wasp and host populations, respectively; each one in two levels that were represented as TT, TV, VT, and VV (original populations); where, the first letter delineates the parasitoid origin, and the second one the host origin (T= Tabriz, V= Varamin). Twenty pairs of third-generation wasps from each population were coupled randomly, and five clutches of 24-hour-old CSP eggs were offered daily until death. The fate of host eggs was followed by daily checks, and the date of emergence was recorded separately for males and females.

In the next step, the progeny of the third generation of the two populations was reciprocally crossed to the other population.

Thus, two kinds of the cross were present: Tabriz females×Varamin males (T×V) and Varamin female×Tabriz males (V×T). Considering the non-significant effect of the host, the outcrossed wasps were studied only on CSP eggs of Varamin in the same manner described for original populations.

Measures of Stable Population Growth Parameters

The method described by Carey (1993) was used to calculate life table parameters and entropy (Equation 1).

$$H = \frac{\sum_{x=0}^{\omega} e_x d_x}{e_0} \quad (1)$$

e_0 , e_x , and d_x represent life expectancy at age 0, and life expectancy and mortality distribution at age x , respectively, summed over ages 0 to ω (the last death event), and H denotes entropy.

The entropy values less than, equal to, and greater than 0.5 represent the survivorship curves of type I (convex), type II (straight line), and type III (concave), respectively. Stable population growth parameters, including Gross Reproduction Rate (GRR), net Reproduction rate (R_0), mean generation Time (T), Doubling Time (DT), intrinsic rate of increase (r_m), finite rate of increase (λ), intrinsic birth rate (b), and intrinsic death rate (d) were estimated. The r_m -value was calculated by solving the Lotka equation using iterative calculations of the Newton-Raphson method as follows:

$$\sum_{x=0}^{\omega} e^{-r_m x} l_x m_x = 1 \quad (2)$$

l_x and m_x represents survival rate and fecundity at age x respectively, r_m denotes intrinsic rate of increase, and $\sum$, ω , and e are symbols of summation, last event of cohort and natural logarithm base respectively.

To determine the standard error of the above statistics, we used bootstrap methods in 1000 replicates (Meyer *et al.*, 1986). Estimation of the parameters was carried out by a program in Excel (Iranipour, 2018).



Statistical Analyses

Statistical analysis was performed using SPSS software. Since the host-effect was non-significant in all parameters, this factor was excluded from analysis and comparison between main populations and crossed populations was done by One-Way ANOVA. The means were compared by Tukey's test at 0.05 significance level. Bootstrap estimates of r_m and the other stable population growth parameters were also compared among the main populations and crosses. Pairwise comparisons between those treatments were done by the random pairing of estimates, and the differences of randomly paired values were ranked from the smallest to the largest value. Then 25 boundary values from both ends were excluded and 950 median values were considered as 95% Confidence Interval (CI). If 95% CI included zero, no significant difference between the two treatments was interpreted.

RESULTS

Life History Parameters of *T. vassilievi*

In life table studies, two populations of *T. vassilievi* were compared on two host populations (Table 1), and due to the insignificant effect of the host and their interactions, the host effect was excluded from the analysis. The wasps from the crosses on the Varamin host were compared with their parental populations only on the Varamin host.

Analysis of variance showed that there was a significant difference in the longevity of both females and males when crosses were included

as well (Tabriz population, Varamin population, T×V, and V×T crosses; $F_{3,76} = 3.62$, $P = 0.017$ for female longevity, and $F_{3,76} = 3.12$, $P = 0.031$ for male longevity). The highest longevity of females and males was observed in Tabriz and Varamin wasps, respectively (Table 2).

Female wasps from the crosses showed intermediate longevity of the two populations, so, their differences were not significant with either population. However, the male progeny of the crosses had a shorter lifespan than their parents (Table 2). Females lack a pre-oviposition period. The mean oviposition period of *T. vassilievi* females was significantly higher in the Varamin population than in Tabriz and cross populations ($F_{3,76} = 4.36$, $P = 0.007$). The total fecundity of *T. vassilievi* females was significantly higher in cross populations than those of parental populations ($F_{3,76} = 22.38$, $P < 0.001$). Females of crosses laid 8–29% more eggs than both populations. The highest fecundity was observed in T×V. Almost in all cases, maximum oviposition occurred on the first day of life and declined to zero with a non-linear trend in less than three weeks (Figure 1). The highest fecundity was obtained in T×V, and V×T crosses, respectively, followed by the original populations (Table 2). The age-specific sex ratio (proportion of females) declined at senescence (Figure 2).

Life Tables and Survivorship Curves of *T. vassilievi*

Age-specific mortality (q_x) of *T. vassilievi* in all treatments increased by age. The results showed that mortality seldom occurs during and prior to oviposition. The survivorship

Table 1. Analysis of variance of life history components of the two populations of *T. vassilievi* on two populations of sunn pest eggs.^a

Parameter	Wasp		Host		Wasp × Host	
	F	P	F	P	F	P
Female longevity	14.61	< 0.001	1.74	0.191	0.42	0.519
Male longevity	3.43	0.068	0.00	1.000	0.01	0.939
Oviposition period	29.41	< 0.001	0.31	0.580	0.17	0.678
Post-oviposition period	36.66	< 0.001	1.54	0.219	0.31	0.578
Total fecundity	24.16	< 0.001	1.80	0.183	1.58	0.212
Average daily fecundity	0.00	0.949	2.37	0.128	0.84	0.364
Sex ratio	0.04	0.847	0.01	0.932	0.22	0.641

^a df for all treatments= 1,76.

Table 2. Reproductive parameters of two *T. vassilievi* populations on two CSP populations and crosses as T×V and V×T.^a

Treatments	TT	VT	TV	VV	T×V	V×T
Female longevity (d)	31.80±1.19A	28.25±1.14B	31.05±1.15Aa	26.05±0.86Bb	29.90±1.50ab	28.10±1.23ab
Male longevity (d)	19.60±0.49A	20.75±0.55A	19.55±0.41Aab	20.80±0.95Aa	18.30±0.32b	18.75±0.32ab
Oviposition period (d)	18.50±0.41A	20.60±0.37B	18.45±0.39Ab	20.25±0.23Ba	18.40±0.48b	18.60±0.53b
Post oviposition period (d)	13.30±1.08A	7.65±1.15B	12.60±1.02Aa	5.80±0.84Bb	11.50±1.39a	9.50±1.01ab
Life time fecundity	216.90±5.36A	249.30±5.07B	230.55±6.02Ac	249.75±4.30Bb	280.40±2.77a	269.50±4.80a
Daily egg	11.80±0.34A	12.13±0.23A	12.63±0.49Ab	12.34±0.20Ab	15.46±0.47a	14.67±0.43a
Sex ratio	0.82±0.03A	0.82±0.02A	0.82±0.02Aa	0.82±0.02Aa	0.78±0.05a	0.86±0.01a

^a Means bearing the same letter in a row are not significantly different (Tukey's HSD, $\alpha=0.05$). Capital letters are for comparison between original populations and lower cases are for comparison between original populations and reciprocal crosses reared on the Varamin host.

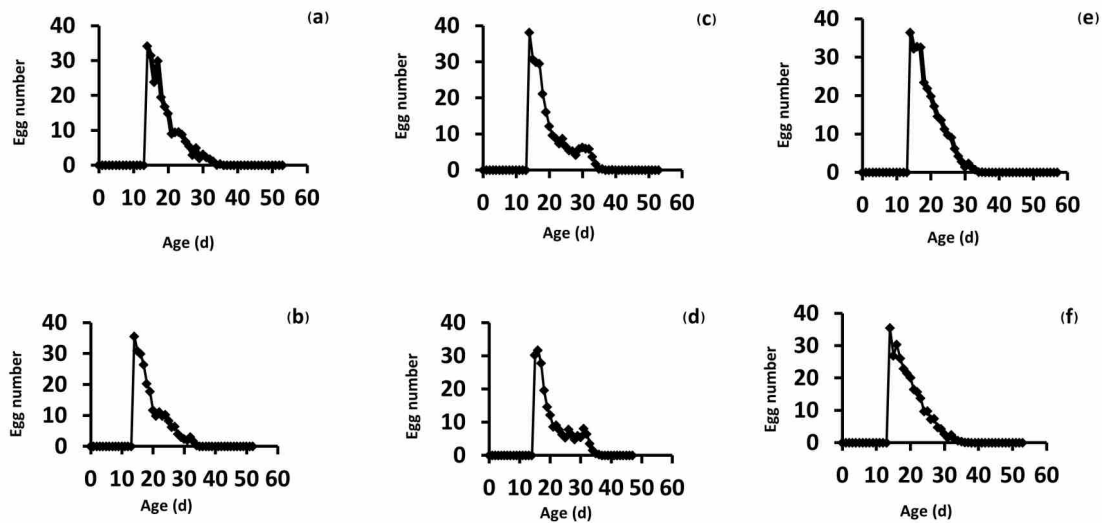


Figure 1. The trend of Oviposition of two *T. vassilievi* populations on two CSP populations and their reciprocal crosses: (a) TT, (b) TV, (c) VT, (d) VV, (e) T×V, and (f) V×T.

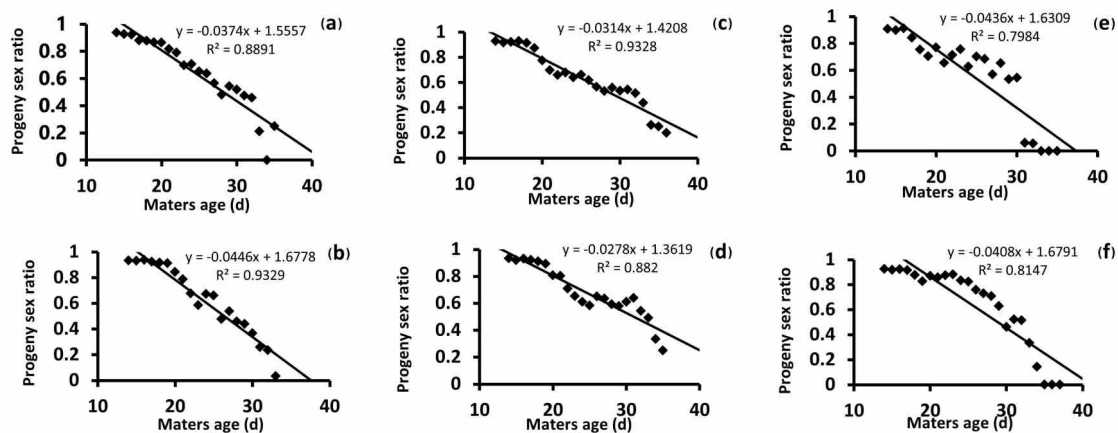


Figure 2. Age-specific sex ratio of two *T. vassilievi* populations on two CSP populations and their reciprocal crosses: (a) TT, (b) TV, (c) VT, (d) VV, (e) T×V, and (f) V×T.

curve of *T. vassilievi* was from type I ($H < 0.5$) in all treatments (Figure 3, Table 3). The life expectancy (e_x) decreased linearly from birth to death (Figure 4). The life expectancy of *T. vassilievi* at birth and emergence, as well as under the curve area of e_x and N_x are shown in Table 3.

Reproduction Tables for *T. vassilievi*

The reproductive parameters of *T. vassilievi* on CSP eggs are shown in Table 4. Varamin population has higher gross and net fecundity and fertility rates than Tabriz population. The crosses had higher values of these parameters than the main populations, with the highest value in T×V. The emergence rate of wasps was very high,

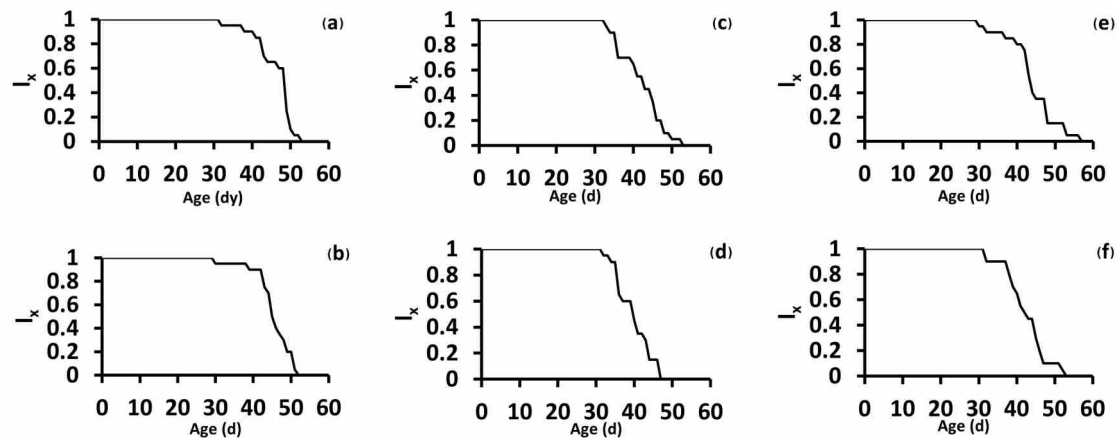


Figure 3. The survivorship curve of two *T. vassilievi* populations on two CSP populations and their reciprocal crosses: (a) TT, (b) TV, (c) VT, (d) VV, (e) T×V, and (f) V×T.

Table 3. Life table statistics of two *T. vassilievi* populations on two CSP populations and their reciprocal crosses under laboratory conditions.

Treatments	TT	TV	VT	VV	T×V	V×T
Entropy	0.078	0.090	0.114	0.097	0.119	0.112
Life expectancy at birth	45.9	45.1	41.8	39.55	43.75	42.05
Life expectancy at adult emergence	31.9	31.1	27.8	25.55	29.75	28.05
Under curve area of N_x (Insect-day)	928	912	846	801	885	851
Under curve area of life expectancy e_x	1108.42	1070.47	945.75	832.91	1050.67	956.47

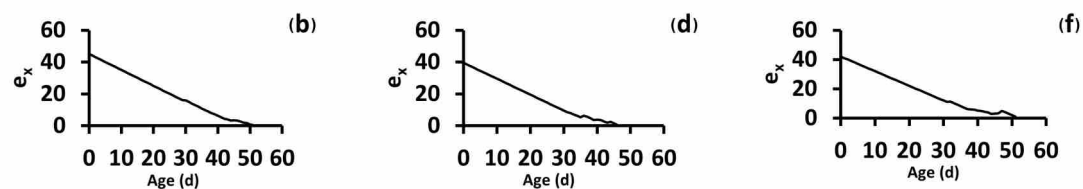


Figure 4. The life expectancy of two *T. vassilievi* populations on two CSP populations and their reciprocal crosses: (a) TT, (b) TV, (c) VT, (d) VV, (e) T×V, and (f) V×T.

ranging from 92% to 94%. The mean age of emergence was lower in Tabriz wasps and higher in Varamin than in the other treatments. The mean reproductive age for *T. vassilievi* was around 19 days. Other variables are shown in Table 4.

Stable Population Growth Parameters of *T. vassilievi*

Bootstrap estimates of stable population growth parameters of *T. vassilievi* on CSP eggs and their statistical comparisons are shown in Table 5. No significant difference was observed between values in columns 1

**Table 4.** Reproductive parameters of *T. vassilievi* on sunn pest eggs.

Treatments	TT	TV	VT	VV	T×V	V×T
Gross fecundity rate	201.53	212.20	229.53	232.56	262.21	250.98
Net fecundity rate	201.39	211.80	229.05	231.80	261.76	250.67
Gross fertility rate	187.20	195.60	211.89	216.52	245.29	234.19
Net fertility rate	187.09	195.26	211.48	215.85	244.97	233.96
Gross hatch rate	0.93	0.92	0.92	0.93	0.94	0.93
Mean age hatch (d)	24.67	24.23	25.97	25.28	24.70	24.95
Mean age gross fecundity (d)	19.17	19.30	19.72	19.82	19.15	19.40
Mean age net fecundity (d)	19.16	19.28	19.69	19.78	19.13	19.38
Mean age gross fertility (d)	19.04	19.11	19.53	19.61	19.01	19.22
Mean age net fertility (d)	19.03	19.09	19.50	19.57	18.99	19.21
Mean egg per day	5.17	5.58	5.89	7.05	6.24	6.44
Mean fertile egg per day	4.80	5.15	5.43	6.56	5.84	6.00
Eggs/Female/Day	6.31	6.81	8.24	9.07	8.95	5.96
Fertile eggs/Female/day	5.86	6.28	7.61	8.45	8.37	5.56

and 2, neither between columns 3 and 4. These comparisons refer to host populations and indicate the non-significant effect of the host. In contrast, comparisons between parasitoids of original populations (either between columns 1 and 3 or between columns 2 and 4) indicate significant differences in some parameters. For example, the parasitoid of Varamin population exhibited a higher level of reproduction (both GRR and R_0) compared to Tabriz population. On the other hand, *T. vassilievi* from Varamin origin exhibited a higher rate of population increase, finite population increase, birth rate, and shorter doubling time. Crosses showed a higher reproduction rate than both original populations, with a minor advantage of V×T. In addition, their r_m -values were slightly higher than Tabriz parasitoids. Crossing between the two populations resulted in a 13.9 to 18.5% higher net reproduction rate than the maternal populations and 7.7 to 25.2% than the paternal ones. It seems that the reproductive phenotype of the progeny of outcrosses followed the maternal phenotype. The other statistics are also presented in Table 5. Estimates of stable age distribution (C_x) showed that adults make about 1% of a stable population.

DISCUSSION

In the present study, two geographical populations of *T. vassilievi* were compared in terms of life history parameters by considering two host populations. Outcrosses between populations with the aim of improving parasitoid fitness and breeding parasitoids with superior characteristics were also carried out. Overall, it can be stated that the parasitoids of Varamin population had a minor advantage over the Tabriz wasps. The most significant difference was in their reproductive rate, which was 8-15% higher in Varamin wasps. Increased reproduction was due to 2-days longer oviposition period in Varamin wasps. However, the daily fecundity rate was similar in both groups and did not differ significantly. Therefore, it can be concluded that wasps increase their reproduction by lengthening the oviposition period rather than increasing daily fecundity. Hence, the best way to improve the efficiency of these wasps is to select wasps with longer reproductive periods. Higher fecundity, on the other hand, reduced female longevity: Varamin females lived four days less, while the males lived one day more than the Tabriz population. The above statements were not true for crosses. The daily and total fecundity of the cross wasps increased independent of the oviposition period, as

Table 5. Population growth parameters of *T. vassilievi* on two populations of sunn pest and their reciprocal crosses under laboratory conditions.^a

Treatments	TT	TV	VT	VV	T×V	V×T
GRR (Female/Generation)	168.21±7.24C	172.00±5.06C	188.10±5.93B	190.34±4.03B	208.38±11.06AB	216.38±4.85A
R ₀ (Female/Generation)	168.14±7.23C	172.00±5.06C	187.95±5.96B	189.86±4.04B	208.23±11.13AB	216.22±4.90A
r _m (d ⁻¹)	0.291±0.0033C	0.296±0.002BC	0.302±0.0023AB	0.303±0.0020AB	0.305±0.0032A	0.302±0.0025AB
λ (d ⁻¹)	1.337±0.0043C	1.345±0.0037BC	1.353±0.0031AB	1.354±0.0027AB	1.357±0.0044A	1.353±0.0033AB
T (d)	17.63±0.13AB	17.37±0.14BC	17.33±0.16BC	17.31±0.10C	17.48±0.13ABC	17.79±0.13A
DT (d)	2.31±0.04A	2.27±0.03AB	2.22±0.03BC	2.21±0.02BC	2.21±0.05C	2.22±0.03BC
b (d ⁻¹)	0.292±0.0033C	0.297±0.0028BC	0.303±0.0023AB	0.304±0.0020AB	0.307±0.0033A	0.303±0.0025AB
d (d ⁻¹)	0.0010±3.4×10 ⁻⁵ C	0.0011±3.02×10 ⁻⁵ BC	0.0012±2.6×10 ⁻⁵ AB	0.0012±2.3×10 ⁻⁵ A	0.0012±3.6×10 ⁻⁵ A	0.0012±2.8×10 ⁻⁵ AB

^a Means bearing the same capital letters in a row are not significantly different (Bootstrap's pairwise comparisons, $\alpha=0.05$).

they exhibited 20% and 10% higher fecundity compared to Tabriz and Varamin wasps, respectively, with similar reproductive periods. This has been achieved by the introduction of new genes into the genetic pool of the original populations. The reason that males were not affected by crossing may be due to receiving only one copy of their maternal genes. Female longevity is an important qualitative indicator of parasitoids in the field. The longer life span of a parasitoid, the higher encounter to hosts, thus a wasp will have more fortune to find and exploit hosts (Suh *et al.*, 2000). However, higher reproductive effort results in the exhaustion of females themselves and shortens their lifespan (Krebs and Davies, 1993). This can be deduced by comparing the longevity of the more fecund Varamin population compared to Tabriz one. As it can be seen, Varamin wasps had higher fecundity and, at the same time, lower life span than the Tabriz wasps, which may confirm the above statement.

The average fecundity recorded for *T. vassilievi* in this study was higher than the other telenomin species. It was 98.0, 22.4, 29.6, and 63.7 for *T. biproruli*, (James, 1988), *Telenomus calvus* Johnson, (Orr *et al.*, 1986), *T. podisi* Ashmead, and *Trissolcus euschisti* (Ashmead), respectively (Yeargan, 1982). Powell and Shepard (1982) reported 88.1-141.9 broods for different isolates of *T. basalis*. The average progeny number of *T. semistriatus* was reported 88 in Turkey (Kivan and Kilic, 2006), and 210 in Varamin, Iran (Asgari, 2002). Also, it was 85 for Tabriz (Nozad Bonab, 2009), and 200 for the Varamin population of *T. grandis* (Amir-Maafi, 2000). The difference between the two populations of *T. vassilievi* was minor and not comparable to the above species. Perhaps, one reason is that the physical conditions are quite similar for both populations in the present study, while it may be deeply different in the two separate studies on a single species in the above examples. A higher number of daily fecundities may benefit augmentation programs (van Driesche and Bellows, 1996).



The sex ratio of progeny can change as parasitoid get older (Bueno *et al.*, 2008; Amir-Maafi and Parker, 2011), because, sperm reserves of the female are depleting and, as a result, insemination and, consequently, female offspring decreases (Kivan and Kiliç, 2006; Amir-Maafi and Parker, 2011). Higher female progeny benefits the scelionids, because it reduces the competition between brothers for mating with sisters (Wilson, 1961; Safavi, 1968).

Among the stable population growth parameters of *T. vassilievi*, only the gross and net reproductive rates exhibited appreciable variation among treatments. In general, cross wasps had higher reproductive rates than the original populations, which may be due to the flowing new genes in their original pool. The gross and net reproductive rates of *T. grandis* (Amir-Maafi, 2000), and *T. semistriatus* (Asgari, 2002) were 136 and 130 daughters per generation, both less than the values obtained in this study.

The intrinsic rate of population increase (r_m) is a useful indicator of the fitness of a species or population in response to physical and/or nutritional conditions (Southwood and Henderson, 2000). This parameter can be used as a criterion for selecting natural enemies and predicting the success of bio-control agents (van Lenteren, 2003). The r_m -value of *T. vassilievi* in this study varied between 0.291 and 0.305 d^{-1} , which was slightly higher in Varamin and crossed populations compared to the Tabriz population. It refers mainly to their higher fecundity. Among *Trissolcus* species, the maximum value of r_m has been reported as 0.368 d^{-1} on *T. grandis* (Nozad Bonab, 2009). The r_m -value of *T. semistriatus* has been 0.226, and 0.227 d^{-1} for an Iranian and a Turkish population, respectively (Asgari, 2002, Kivan and Kilic, 2006). These three species are considered as the most effective species in controlling CSP. Based on R_0 and r_m -values we can rank them as *T. grandis* > *T. vassilievi* > *T. semistriatus*.

The generation Time (T) was 17.31-17.79 days for *T. vassilievi* in this study and 13.43 days for *T. grandis* (Amir-Maafi, 2000),

which can well explain why *T. grandis* is the most prevalent egg parasitoid species of CSP in Iran (Radjab and Amir Nazari, 1989). Life history data suggest the high importance of *T. vassilievi* for the biological control of CSP. A highly female-biased sex ratio, high attack rate, and longevity are positive properties for *T. vassilievi*. However, field data at different climatic conditions are essential to prove the role of *T. vassilievi* in large-scale inundation programs. Several field studies indicate the acceptable effect of some telenomins on target pests (Justo *et al.*, 1997; van Lenteren and Bueno, 2003; Asgari *et al.*, 2010; Bagheri Matin *et al.*, 2010; Asgari, 2011).

In conclusion, it can be stated that the host population had no significant effect on parasitism by *T. vassilievi*, however, fecundity was significantly higher in crosses compared to the original populations. This suggests increased fitness of progeny. Similar effects were observed on the thermal phenotypes of this species (Iranipour *et al.*, 2015). This has been demonstrated in some studies (e.g. Carson, 1968; Rasanen and Kruuk, 2007), and, nowadays, it is accepted as a scientific rule by most biologists and can be used for the artificial selection of parasitoids in the laboratory (Arakawa *et al.* 2004). The intrinsic rate of increase (r_m) of *T. vassilievi* was less variable among treatments of this study and lied between *T. grandis* and *T. semistriatus*. The results of this study revealed that we can benefit from the intra-populations diversity of *Trissolcus* species to obtain more advantageous parasitoids via out-crossing them.

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افزایش شایستگی با تلاقی بین دو جمعیت زنبور (*Trissolcus vassilievi* (Hymenoptera: Scelionidae))

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چکیده

Trissolcus vassilievi (Hymenoptera: Scelionidae) یکی از مهم‌ترین زنبورهای پارازیتوید تخم *Eurygaster integriceps* در ایران است. در این تحقیق شایستگی دو جمعیت زنبور *T. vassilievi* روی دو جمعیت میزبان در قالب جدول‌های زندگی زادآوری مورد مطالعه قرار گرفت. دو جمعیت *T. vassilievi* یکی تبریز



(با منشاء معتدله) و دیگری ورامین (با منشاء نیمه گرمسیری) هم برای زنبور و هم برای میزبان آن لحاظ گردید. به علاوه با توجه به این که انجام تلاقی های غیرخویشاوندی بین جمعیت ها می تواند منجر به ایجاد جمعیت هایی با خصوصیات برتر شود تلاقی بین دو جمعیت روی یک میزبان واحد بررسی شد. تلاقی بین دو جمعیت موجب بروز ۱۳.۹ تا ۱۸.۵% زادآوری خالص بیشتر نسبت به جمعیت های مادری شد که به نظر می رسد بیشتر تابع فنوتیپ مادری باشد. نرخ ذاتی افزایش جمعیت اختلاف جزئی نشان داد و بین 0.291 ± 0.003 تا 0.305 ± 0.003 در تیمارهای مختلف متغیر بود. برتری جزئی زنبورهای ورامین نسبت به تبریز و تلاقی ها نسبت به جمعیت های اصلی مشاهده شد. این تفاوت ها می توانند در پرورش انبوه این زنبورها برای به دست آوردن پارازیتوئیدهایی با خصوصیات برتر مورد توجه قرار گیرند.

Fruit Biochemical and Nutritional Properties of Some Asian and European Pears (*Pyrus* spp.) Grown under Tehran Environmental Conditions

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ABSTRACT

Pear is one of the most important pome fruits in the world fruit market with a high nutritional value. This study was performed to determine the phenolic compounds and some chemical properties of the fruit flesh and peel of 12 Asian and European pears. Chlorogenic acid and rutin were found as the important phenolic compounds in the fruit peel, which were measured using HPLC. Results showed fruit Titratable Acidity (TA, 0.17-0.53%), Total Soluble Solids (TSS, 13.33-17.33 °Brix), firmness (1.7-2.75, kg cm⁻²), and color parameters. The highest L* value was observed in KS7 (40.55), while the lowest was in KS12 (14.26) and KS13 (14.78). Additionally, the study assessed the nutrient and total phenol content of fruit samples. The 'Shahmiveh' cultivar displayed the highest total phenol content (638 mg 100 g⁻¹ FW), while the KS7 cultivar had the lowest (420 mg 100 g⁻¹ FW). Potassium was the most abundant nutrient (1.16 mg 100 g⁻¹ DW), followed by nitrogen and calcium contents. As the total phenol increased, so did the amount of rutin. Principal Components Analysis (PCA) of all data showed that the European and Asian pears studied cultivars and genotypes were different in terms of most of the studied biochemical traits, and significant relationships were observed between some traits. Besides, the obtained results help in the selection of the best pear cultivars or genotypes in terms of the highest phenolic content and nutrients, both for fresh consumption and in the juice industry.

Keywords: Rutin, Chlorogenic acid, Pear macronutrients, Pear micronutrients, Total phenol.

INTRODUCTION

Pyrus (*Pyrus* spp.) is the second most important crop following apple in the *Rosaceae* family and can be divided into two major groups of Asian and European type pears (Arzani, 2019; Wang and Arzani, 2019). European species (*Pyrus. communis* L.) have more than 5000 cultivars (Kadkhodaei *et al.*, 2021; Monte-Corvo *et al.*, 2001). Asian pears (*Pyrus pyrifolia*) are mainly cultivated in countries such as Korea, China, and Japan in East Asia. They have been cultivated in various parts of Asia for over 3,000 years. Currently, this species is

grown commercially in more than 50 countries under temperate climate regions. At least 22 early *Pyrus* species have been identified, all of which are native to Asia, Europe, and the mountainous regions of North America (Bell *et al.*, 1996). This crop is cultivated mainly due to its commercial value and desirable fruit taste (Arzani, 2002; Wang and Arzani, 2019; Arzani, 2019; Arzani, 2021).

Today, plants can be used to prevent and treat diseases (Jimenez-Garcia *et al.*, 2021; Fattahi *et al.*, 2021). Pears contain phenolic substances and have antioxidant and antimicrobial properties (Jennings *et al.*,

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2017; Tiwari *et al.*, 2023). Antioxidant supplements can be mentioned as an important non-pharmacological strategy against oxidative stress (Mota *et al.*, 2022). A self-incompatible, high heterogeneity and allelic diversity have been reported in this genus (Monte-Corvo *et al.*, 2001). In recent decades, numerous studies have been devoted to assessing the genetic diversity in various *Pyrus* species including morphological, biochemical, and DNA markers among European, Asian, and other species. Polyphenols are secondary metabolites (de Paulo Farias *et al.*, 2020). Plant secondary metabolites are a group of chemicals that play a major role in plant growth and survival (Singh *et al.*, 2021; Fattahi *et al.*, 2021). Polyphenols are compounds that occur naturally in fruits and vegetables and are important because of their healing properties and application in technology (de Araújo *et al.*, 2021), and as such, they have received much attention in recent years (de Paulo Farias *et al.*, 2020). Consumption of plants helps the supply of macro- and micro-nutrient elements and reduces the incidence of functional disorders in the body and human health (Li *et al.*, 2017). The role of nutrients in improving the quality of fruit and its other effects- in particular, respiratory failure- causes delay in ripening, increased fruit firmness, and improved fruit storage. Meanwhile, over-consumption of nutrients also impedes the production of quality fruit. Besides, nutrient imbalances cause numerous disorders that affect the quality and performance of pears (Dar *et al.*, 2015; Wang and Arzani, 2019; Arzani, 2019).

Phenolic compounds are chemicals found in most plant tissues of fruits and vegetables. Also, chlorogenic acid (5-Ocaffeoylquinic acid) is a secondary metabolite of phenolic acids and is found in many plants and has an obese anti-obesity mechanism (He *et al.*, 2021). Chlorogenic Acid (CGA) is a natural product that has medicinal properties such as anti-cancer, light protection, antioxidant, anti-inflammatory, hypoglycemic, and hypoglycemic effects. After absorption,

CGA is further metabolized into sulfate metabolites, glucuronic acid, and glycosides (Sanchez *et al.*, 2017; Nwafor *et al.*, 2022). Rutin is an important flavonoid also known as vitamin P and quercetin-3-o-rotnoside, and has a protective role against liver and gastrointestinal problems (Hosseinzadeh and Nassiri-Asl, 2014), with anti-inflammatory, anti-tumor, antioxidant, and neuro-protective effects (Muvhulawa *et al.*, 2022; Song *et al.*, 2014). Recently, eight phenolic compounds have been identified in the fruit peel of Asian pear (Lee *et al.*, 2011). Pear fruit peel has far higher and more varied phenolic contents than its flesh (Chen *et al.*, 2006). In addition, it is highly recommended that natural products are eaten with their peels, because if they reduce oxidation, they will be useful for well-being and disease reduction (Nazir *et al.*, 2020). Previous studies on pears have shown that they contain minerals (Brunetto *et al.*, 2015; Ozturk *et al.*, 2009), and pear fruit is rich in macro- and micro-nutrients (Nazir *et al.*, 2020). Mineral nutrients play an important role in plant growth and metabolic functions and are heavily involved in maintaining the health and proper functioning of an organism (Tewari *et al.*, 2021). There are human, plant, and animal diseases associated with micronutrient deficiencies. Also, efforts should be made to produce aggregating microelements of genotypes with an overexpression approach (bio-genetic enhancement) (Izydorczyk *et al.*, 2021). 'Shahmiveh' and 'Sebri' are native commercial European pears cultivars in Iran. Additionally, A95 promising chance seedling genotype showed superiority in some qualitative fruit characteristics (Wang and Arzani, 2019; Kadkhodaei *et al.*, 2021; Yadegari and Arzani, 2023).

The objective of this research was to explore the phenolic and biochemical compounds of 'Shahmiveh', 'Sebri' and A95 promising genotype compared with some commercial Asian pear cultivars that are grown under Tehran (Iran) environmental conditions.

MATERIALS AND METHODS

In this experiment, 9 Asian pear cultivars including KS6, KS7, KS8, KS9, KS10, KS11, KS12, KS13, and KS14 (Arzani, 2002), as well as 3 European pear cultivars, 'Shahmiveh', 'Sebri', and A95 promising genotypes (Najafzadeh, 2015; Wang and Arzani, 2019), were used. Trees were planted under Tehran environmental conditions at Tarbiat Modares University (TMU) Asian Pear Collection Orchard, with latitude: 35° 41' 39.80" N and longitude: 51° 25' 17.44" E. Besides, fruits were harvested at the commercial maturity harvest index (Arzani, 2019) mainly based on the fruit background color, flesh firmness, and Total Soluble Solids (TSS) for further assessments.

Determination of Total Phenolic Content

Fruit samples (fruit with the peel) were freeze-dried for 48 hours and then powdered. For the extraction of phenolic compounds, 2 g. of powdered pulp was used according to the method described by Lister *et al.* (1994) with slight modification. Then, 5 mL of extraction solvent consisting of 85% methanol and 15% acetic acid was added. The samples were placed at 4 °C for 24 h and centrifuged at 10,000 rpm for 10 min. About 1 mL of the supernatant of each sample was filtered using a 0.45 µm syringe filter.

The total phenol content of the extracts was measured by the Folin-ciocalteu method. The absorbance was measured using a spectrophotometer at 765 nm. Total phenol contents were expressed in terms of a milligram of gallic acid content per 100 g fruit fresh weight.

Determination of the Phenolic Content and Components

A water liquid chromatography apparatus consisting of a separations

module: Waters 2695 (USA) and a PDA Detector water 996 (USA) was used for the HPLC analysis. Data acquisition and integration were performed via Millennium32 software. The injection was performed by an auto-sampler injector equipped with chromatographic assay performed on a 15 cm×4.6 mm with pre-column, Eurospher 100-5 C18 analytical column provided by waters (Sunfire) reversed-phase matrix (3.5 µm) (waters). The elution was carried out in a gradient system with methanol as the organic phase and distilled water with a flow rate of 1 mL min⁻¹. Peaks were monitored at 195-400 nm wavelength. The injection volume was 20 µL and the temperature was maintained at 25°C.

Fruit Sample Preparation for TSS, TA, pH, Firmness, and Color

To determine Total Soluble Solids (TSS), a few drops of fruit flesh extract were poured onto the refractometer. For this purpose, Japan's portable refractometer Model 9703 was used (Bexiga *et al.*, 2017). For Titratable Acidity (TA) and pH of the fruit extract, 10 g of smashed fruit flesh was used, with the addition of about 30 mL of distilled water. The extract was centrifuged at 50°C for 30 minutes at 4000 rpm. To determine the TA with 0.1 normal solutions, it was titrated to reach pH 8.3 (pH meter Consort- model C860), after which the acidity was calculated based on a milligram of malic acid per 100 g of fruit tissue. To measure the flesh firmness of the fruit tissue, after removing a thin layer of fruit peel, an 8 mm diameter probe by penetrometer (Wagner) was used and fruit firmness was measured in kg cm⁻². The pear fruit peel color was measured via the Lutron RGB-1002 color analyzer and converted to L* (Lightness), a* (green to red), and, b* (blue to yellow).



Extraction to Measure Nutrients

Initially, the fruit samples (fruit with the peel) were washed with tap water, followed by 0.1 M hydrochloric acid (HCl), then, rinsed again with distilled water. The sample was dried in an oven at 70°C and powdered. The 0.5 mm sieve mesh was used for collecting the clean powdered samples. Extraction steps were performed as follows: Briefly, 2 g of the dried sample was heated in an oven at 550°C for 4 hours. The ash was slightly moistened with distilled water, and 10 mL of 2 M HCl was added. The final extract was delivered in a volume of 100 mL (Waling *et al.*, 1989). Distillation and sample titration were used to measure the percentage of plant nitrogen (Waling *et al.*, 1989). Phosphorus was measured using a colorimetric (Vanadate-Molybdate) method. The absorbance was measured via a spectrophotometer at 470 nm (Chapman and Pratt, 1962). Potassium and sodium were measured by atomic emission spectrometry.

The absorbance was read by a flame photometer with 766.5 nm for K and 589 nm for Na (Waling *et al.*, 1989). The Azomethine colorimetric method was employed to measure the amount of boron, and the spectrophotometer device was used at 430 nm. An atomic absorption device was utilized to measure the percentage of magnesium (285.2 nm) and calcium (422.7 nm) in the plant (Waling *et al.*, 1989). Measurement of microminerals (iron-manganese-zinc and copper) was performed by Atomic Absorption Spectrometry (AAS) method. Measurement of the resulting extraction was carried out by dry burning and use of HCl. The absorption rate of Fe, Mg, Zn, and Cu was measured at 248.3, 289.5, 213.9, and 324.7 nm, respectively (Elmer and Conn, 1982).

Statistical Analysis

The obtained data were initially checked for normality and analyzed using SAS (Ver. 9.3, SAS Institute, Cary, NC). The results

were statistically evaluated by Analysis Of Variance (ANOVA) and expressed as mean±Standard Error (SE). Biochemical data for Principal Component Analysis (PCA) and cluster analysis were used. PCA and cluster analysis were performed using Minitab software (Ver. 17). For the Heat map, R 3.5.3 software was used.

RESULTS AND DISCUSSION

Total Phenol

The highest total phenol amount was observed in the 'Shahmiveh' cultivar and the lowest in KS7 which were 638.01 and 420.02 (mg 100 g⁻¹ FW), respectively. Based on the results, the total phenol amount in different cultivars and the studied genotype showed significant differences (Table 1). The differences in the phenolic composition of different cultivars confirm the genetic role in the synthesis of phenolic compounds since the amount of polyphenols was affected by genotype, rootstock, and climatic conditions (Lin and Harnly, 2008; Mainla *et al.*, 2011; Maleki Asayesh *et al.*, 2023). Phenolic compounds may impair callus formation by affecting cell division, development, and differentiation (Bennett and Wallsgrove, 1994). The total phenolic compound in the fruit tissue of the KS13 was higher than KS6 and KS9 Asian pear cultivars (Maghdori *et al.*, 2015). In another study performed on several Australian pear cultivars, the highest amount of total phenol was observed in 'Beurre Bosc' (3.14 ± 0.02 a mg GAE g⁻¹) and the lowest in 'Winter Nelis' European pear (1.89 ± 0.03 a mg GAE g⁻¹) (Wang *et al.*, 2021).

Phenolic Compounds

The minimum amount of chlorogenic acid was observed in 'Sebri' and KS8, which were 3.48 and 3.55 (mg g⁻¹ FW), respectively, while its maximum was obtained in KS14 and was 9.48 (mg g⁻¹ FW).

Table 1. Total Phenole, Chlorogenic acid, Rutin, TSS, TA, and TSS/TA of pear cultivars.^a

Cultivar	Total Phenol (mg 100 g ⁻¹ FW)	Chlorogenic acid (mg g ⁻¹ FW)	Rutin (mg g ⁻¹ FW)	TSS °Brix	TA (%)	TSS/TA
KS6	466.93±9.19efg	7.8±0.0274b	0.08±0.000cde	14.67±0.033cde	0.24±0.003g	60.33±2.166b
KS7	420.02±14.6g	5.56±0.140gh	0.05±0.001fg	14.33±0.033de	0.38±0.015bc	37.82±1.478f
KS8	586.68±32.6ab	3.55±0.177i	0.04±0.000g	17.33±0.033a	0.32±0.011def	54.25±1.212bc
KS9	450.92±3.45fg	7.67±0.328bc	0.04±0.000g	16±0.577ab	0.33±0.003de	48.96±1.360cd
KS10	443.75±16.1fg	7.2±0.208cd	0.07±0.000def	13.67±0.333e	0.23±0.003g	58.57±1.260b
KS11	489.55±7.42def	7.71±0.158bc	0.11±0.006c	13.34±0.333e	0.29±0.005f	46.01±1.441de
KS12	487.35±7.82def	6.54±0.144ef	0.10±0.008cd	15.33±0.333bcd	0.33±0.005de	46.5±1.275cde
KS13	560.75±7.05bc	6.89±0.058de	0.06±0.002efg	14.66±0.333cde	0.17±0.006h	84.95±4.681a
KS14	473.55±8.88efg	9.46±0.088a	0.06±0.003efg	13.33±0.333e	0.33±0.012de	41.43±2.638def
A95	520.46±11.6cd	5.03±0.088h	0.66±0.003a	16.67±0.333ab	0.41±0.008b	40.39±1.618ef
Shahmiveh	638.01±18.2a	5.99±0.106fg	0.47±0.020b	16.33±0.333ab	0.35±0.003de	45.79±0.802de
Sebri	530.40±1.91cd	3.48±0.061i	0.06±0.008efg	15.33±0.333bcd	0.53±0.012a	29.32±0.774g

^a Values represent the mean±Standard Errors (SE). Different letters in the same column indicate significant differences between treatments at $P \leq 0.05$.

The amount of rutin was between 0.04 (KS8, KS9) and 0.66 (mg g⁻¹ FW) (A95) in different cultivars (Table 1).

Maghdori *et al.* (2015) reported a higher amount of chlorogenic acid and catechin in KS6 Asian pear fruit tissues that were grown under Tehran environment conditions. The highest amount of phenolic compounds among the two measured phenolic compounds belonged to chlorogenic acid. It is the most crucial derivative of cinnamic acid in fruits and is known as a disinfectant and radical modifier. These antifungal properties were also evaluated in vitro and the results were satisfying (Martínez *et al.*, 2017).

Pyrus pashia and *Pyrus pyrifolia* are two important sources of chlorogenic acid and rutin (Tiwari *et al.*, 2023). Rutin is a phenolic compound found in other plants, including peaches (Chang *et al.*, 2000). In this study, the amount of rutin in the A95 promising genotype, which is one of the European pears, was higher than in the others. Due to the role of phenolic compounds in human health, cultivars and genotypes with higher amounts of these compounds are important. Also, in another experiment, several phenolic compounds such as chlorogenic acid and rutin were measured in some popular pear cultivars. The highest and lowest values of

chlorogenic acid were 0.69±0.033 mg g⁻¹ as well as 0.32±0.005 mg g⁻¹ in 'Graboid' and 'Grabova' cultivars, respectively. Also, the highest and lowest routine values of 0.09±0.001 and 0.01±0.001 were reported in 'Patten' and 'Conference' cultivars (Liaudanskas *et al.*, 2017). It has been reported that the amount of phenolic compounds may vary among Asian and European pears (Lin and Harnly, 2008).

According to Figure 3, for every 484.34 mg 100 g⁻¹ FW of total phenol, the amount of rutin increased by 1 mg g⁻¹ FW, which showed a linear relationship.

pH, TSS, TA, Color, and Firmness

The results showed that the amount of the Total Soluble Aolids (TSS) was significant among the studied cultivars, and the highest amount was observed in cultivar KS8 (17.33 °Brix) while the lowest was observed in KS10 (13.67 °Brix), KS11 (13.34 °Brix), and KS14 (13.33 °Brix) cultivars (Table 1). TA was 0.53% in 'Sebri' and 0.17% in the KS13 cultivar. Also, according to the results, the amount of TSS/TA ratio was higher in KS13 (84.95%) and less in 'Sebri' (29.32%) than in other cultivars (Table 1). Three European pear cultivars had lower pH levels than Asian cultivars. KS13 (5.72) had the highest



pH among other cultivars. In general, firmness was higher in Asian pear cultivars than in European ones (Table 1). The highest amount of L^* was in KS7 (40.55) and the lowest in KS12 (14.26) as well as KS13 (14.78). The a^* value was higher in KS9 (11.65), KS12 (11.70), and KS13 (10.39) than in the others, and b^* was significantly higher in 'Sebri' (29.25) than in the other cultivars (Table 2).

The acidity concentration and pH extraction were among the studied traits and had a great influence on the aroma, taste, as well as quality of edibility, and fruit storage. It was shown in an experiment that the amount of pH pear fruit was within the range of 3.94 -4.28. Also, the pH in fruit depends on the cultivar and the condition of the planting location (Ozturk *et al.*, 2009). In this experiment, the pH was between 3.9 and 5.72. It has been reported that the Titratable Acidity (TA) of pear fruit was in the range of 0.5-0.21% in different parts of Turkey, which was consistent with the results of the present research (Ozturk *et al.*, 2009). In another published report, the range of 0.1 to 46% was mentioned (Chen *et al.*, 2007).

The aroma and taste of fruit are a

cultivars at maturity (Colaric *et al.*, 2007). Fruit skin color is one of the most important indicators of determining quality and maturity in pears. Previous experiments have shown a link between fruit ripening, L^* , a^* , and b^* (Kawamura, 2000). Also, L^* , a^* , and b^* are different in pear cultivars (Feng *et al.*, 2023). In addition, fruit tissue firmness is one of the most important traits of quality and physiology that directly affect the texture of the fruit. In many fruits, softening is a programmed process to ripen fruit. Much of this process is a consequence of the chemical alteration of the cell wall, which eventually results in variations in the fruit tissue at the time of maturity (Chen *et al.*, 2006; Ozturk *et al.*, 2009; Wang and Arzani, 2019; Arzani, 2019). Our results showed that pear firmness in the commercial maturity stage was 8.66 to 4.06 (kg cm^{-2}). Overall, Asian pear showed more firmness than European pear cultivars (Arzani, 2019). In the present research, 'Sebri', as one of the European pear cultivars, showed higher firmness within the studied cultivars.

In an experiment, the degree of fruit firmness in different pear genotypes was reported at 3.4 to 8 kg cm^{-2} (Tatari *et al.*,

Table 2. Fruit color L^* , a^* , b^* , firmness, and pH of the studied pear cultivars.^a

Cultivar	L^*	a^*	b^*	Firmness (kg cm^{-2})	pH
KS6	21.16±0.88g	7.99±0.39bc	20.68±0.99d	5.5±0.288de	5.17±0.088b
KS7	40.55±0.44a	5.75±0.53c	25.97±0.52bc	6.16±0.166d	4.77±0.033bc
KS8	17.00±0.38h	6.36±0.16c	14.26±0.07e	8.66±0.333a	4.63±0.033c
KS9	24.16±0.13f	11.65±1.28a	20.98±0.17d	5.26±0.145e	4.83±0.033bc
KS10	40.32±0.07ab	7.02±0.28bc	26.72±0.49ab	7.4±0.264bc	5±0.057bc
KS11	38.19±0.35c	6.39±0.21c	23.78±0.68c	7.9±0.208abc	5.17±0.033b
KS12	14.26±0.52i	11.70±2.17a	13.10±0.46e	7.33±0.202c	4.58±0.346c
KS13	14.87±0.58i	10.39±0.63a	14.04±0.51e	8.23±0.145ab	5.72±0.044a
KS14	31.67±0.27e	7.59±0.82bc	25.94±0.57bc	7.43±0.296bc	4.92±0.044bc
A95	36.36±0.11d	6.96±0.26bc	26.31±0.27bc	4.06±0.166f	3.9±0.054d
Shahmiveh	38.19±0.62c	7.41±0.59bc	28.28±1.35ab	4.76±0.066ef	4.1±0.057d
Sebri	35.47±0.44d	6.60±0.72c	29.25±0.46a	7.33±0.145c	3.93±0.033d

^a Values represent the mean±Standard Errors (SE). Different letters in the same column indicate significant differences between treatments at $P \leq 0.05$.

combination of the amount and type of sugars, organic acids, and aromatic substances. The standard titratable acidity varies depending on the cultivar and season. Malic acid is the main acid in most pear

2020). In this research, we found that by increasing 484.34 $\text{mg } 100 \text{ g}^{-1}$ FW total phenol of a pear, and rutin increased by 1 mg g^{-1} FW.

Also, by increasing the total phenol by 125.69 mg 100 g⁻¹ FW, one °Brix was increased in fruit TSS. It has been reported that the fruit TSS is an important indicator that is used for the proper time of harvest (Arzani *et al.*, 2008). According to the reported results of the experiments, the Total Soluble Solids (TSS) in different cultivars of pears was 12.5-14 (Bexiga *et al.*, 2017). Besides, TSS is one of the ways to control the quality of fruit, which is important in the grading of fruits in the agricultural industry. Also, the fruit TSS monitoring is a fast, easy, and cheap record used by the orchardist for considering as one of the good indicators for proper pear fruit harvest (Bexiga *et al.*, 2017; Wang and Arzani 2019; Arzani, 2019).

In another study, TSS was measured in 9 pear genotypes and was reported to be 7-13% (Tatari *et al.*, 2020). The ratio of TSS/TA is an indicator of fruit flavor. Natanz and Arbakhaj genotypes had the highest and lowest TSS/TA ratios with averages of 97.54 and 94.11, respectively (Rezaeirad *et al.*, 2013).

Fruit Nutrients

Nitrogen content in KS8 and 'Sebri' was 0.44 and 0.5 (mg 100 g⁻¹ DW), respectively, and significantly higher than in other cultivars. The KS13 (0.18 mg 100 g⁻¹ DW) cultivar had less N than other cultivars (Table 3). The highest phosphorus levels were observed in KS14 (0.24 mg 100 g⁻¹ DW) and the lowest levels were in 'Shahmiveh' as well as A95 promising genotype (0.14 and 0.16 mg 100 g⁻¹ DW). Potassium in KS14 (1.15 mg 100 g⁻¹ DW) and KS9 (1.16 mg 100 g⁻¹ DW) was higher than in other cultivars and had the highest potassium among pears cultivars (Table 3). KS8 (0.42 mg 100 g⁻¹ DW) showed the higher amount and 'Shahmiveh' (0.04 mg/100g DW) had the minimum amount of calcium. In KS7 (0.15 mg 100 g⁻¹ DW) and

KS13 (0.13 mg 100 g⁻¹ DW) Asian pears, the amount of magnesium was higher than in the other studied cultivars (Table 3). In the KS9 (100 mg kg⁻¹ DW) cultivar, Fe was higher than in the other cultivars. 'Shahmiveh' (29 mg kg⁻¹ DW) and KS9 (25.67 mg kg⁻¹ DW) had also less iron than other cultivars. The highest amount of Mn was found in cultivar KS9 (3.46 mg kg⁻¹ DW). KS12 (11.27 mg kg⁻¹ DW) had the highest amount of Zn while KS6 (7.26 mg kg⁻¹ DW) had the lowest amount of Zn compared to the other cultivars. The Cu was also higher in KS12 (18.5 mg kg⁻¹ DW) and lower in KS8 (8.5 mg kg⁻¹ DW), while the highest B was observed in the KS12 cultivar (96.53 mg kg⁻¹ DW) (Table 4).

Principal Component Analysis (PCA) and Heat Map

In this research, PCA showed two groups of pears: Asian pear (*Pyrus serotina* Rehd.) cultivars in one group and European pear (*Pyrus communis* L.) and A95 promising pear genotype in another (Figure 1). The heat map shows a positive (light color) and negative (dark color) relationship between all the measured traits in the study of the two groups. There is a positive relationship between total phenol and TSS, firmness, calcium, TA and Na, pH and TSS/TA, chlorogenic acid, K, and Ca (Figure 2).

Nutrients play an important role in increasing the quantity, quality, and shelf life and reducing fruit physiological disorders in European as well as Asian pears (Wang and Arzani, 2019; Arzani, 2019). The proper concentration of nitrogen can improve the color, taste, and size of the fruit (Brunetto *et al.*, 2015). Potassium affects fruit size, firmness, color, acidity, and TSS of fruit juice and its aroma. The imbalance in the K:Ca ratio in the plant could cause the cork spot in the 'D Anjou' cultivar (Brunetto *et al.*, 2015; Wang and Arzani, 2019). Many physiological

**Table 3.** Fruit macronutrient composition of different pear cultivars (mg 100 g⁻¹ DW).^a

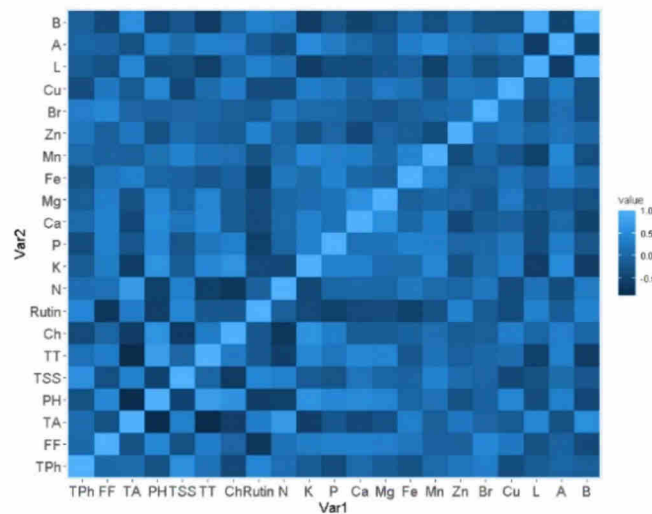
Cultivar	Mg	Ca	K	P	N
KS6	0.06±0.01bc	0.21±0.02bcd	1.13±0.008ab	0.23±0.02ab	0.28±0.008bc
KS7	0.15±0.01a	0.19±0.03bcde	1.07±0.008c	0.19±0.01abc	0.34±0.02b
KS8	0.12±0.02ab	0.42±0.04a	1.13±0.01ab	0.19±0.01abc	0.44±0.03a
KS9	0.06±0.005c	0.22±0.04bc	1.16±0.01a	0.22±0.01ab	0.26±0.02c
KS10	0.06±0.01bc	0.16±0.03bcdef	1.09±0.005bc	0.19±0.02abc	0.29±0.008bc
KS11	0.04±0.008c	0.21±0.05bcd	1.13±0.01ab	0.19±0.01abc	0.24±0.02cd
KS12	0.03±0.007c	0.07±0.01def	1.14±0.008ab	0.21±0.02abc	0.3±0.01bc
KS13	0.13±0.01a	0.25±0.06b	1.13±0.008ab	0.23±0.02ab	0.18±0.008d
KS14	0.04±0.01c	0.06±0.01def	1.15±0.01a	0.24±0.01a	0.30±0.01bc
A95	0.03±0.006c	0.08±0.01cdef	1.06±0.03c	0.16±0.01bc	0.30±0.01bc
Shahmiveh	0.03±0.008c	0.04±0.01f	1.07±0.008c	0.14±0.008c	0.29±0.02bc
Sebri	0.03±0.008c	0.05±0.01ef	1.03±0.01c	0.22±0.02ab	0.5±0.02a

^a Values represent the mean±Standard Errors (SE). Different letters in the same column indicate significant differences between treatments at $P \leq 0.05$.

Table 4. Fruit micronutrient composition of different pear cultivars (mg kg⁻¹ DW).^a

Cultivar	B	Cu	Zn	Fe	Mn
KS6	74.67±1.76cd	12±0.28cd	7.26±0.21f	35.57±1.68g	2.8±0.11b
KS7	56.67±0.88g	17.83±0.72a	10.06±0.47cd	51.77±1.47cd	1.67±0.17c
KS8	78±1.15bc	8.5±0.50f	8.33±0.32ef	43.97±0.84f	2.8±0.20b
KS9	52.7±1.56g	11.67±0.66cd	8.43±0.12e	100±1.53a	3.46±0.27a
KS10	73.4±0.83de	10±0.57def	8.9±0.11e	48.9±1.16def	1.7±0.15c
KS11	73.33±0.88de	16.5±0.28ab	8.13±0.34ef	56±1.53c	1.83±0.12c
KS12	96.53±0.74a	18.5±0.28a	11.27±0.21a	56.33±0.88c	2.26±0.23bc
KS13	68.33±1.36f	15.67±0.88b	11.13±0.13abc	50.66±1.20de	2.2±0.05bc
KS14	70.33±0.88ef	12.43±0.34c	10.5±0.50abcd	46.33±1.45ef	2.23±0.14bc
A95	56.67±0.66g	9.1±0.30ef	11.2±0.26ab	25.67±1.76h	1.7±0.15c
Shahmiveh	78.5±0.76bc	10.33±0.33def	10.13±0.08bcd	29±0.57h	2.3±0.05bc
Sebri	79±0.57b	11±0.57cde	10.03±0.08d	94.67±1.20b	2.2±0.15bc

^a Values represent the mean±Standard Errors (SE). Different letters in the same column indicate significant differences between treatments at $P \leq 0.05$.

**Figure 1.** Heat map of Spearman's correlations between chemical traits of the studied Asian and European pear cultivars.

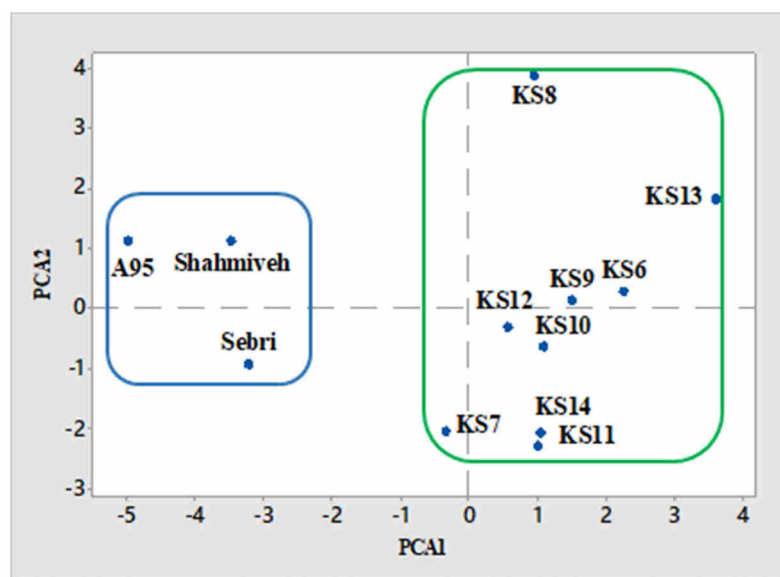


Figure 2. Principal component analysis of the studied Asian and European pear cultivars based on chemical traits. Each point represents one genotype and the surrounding green and blue lines are drawn to indicate the division of cultivars and genotypes into two large groups of the Asian and European pears, respectively.

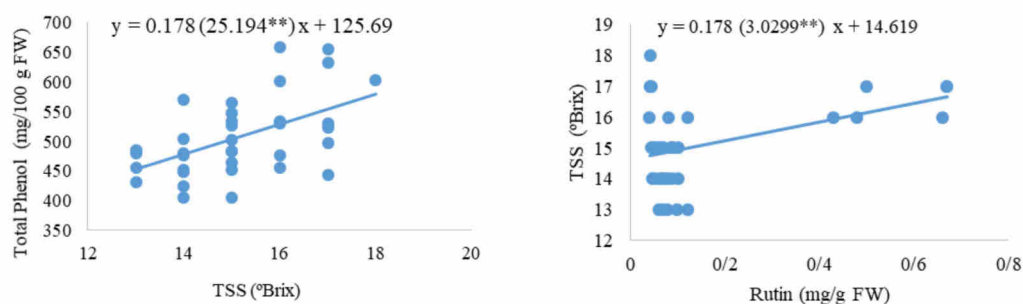


Figure 3. Linear regression between total phenol and rutin, total phenol and TSS, firmness and Ca, and TSS and rutin of the studied European, and Asian pear genotypes. The Pearson correlation coefficient between total phenol and rutin was 0.413*, total phenol and TSS 0.532*, firmness and Ca 0.369*, and TSS and rutin 0.426**. In correlations, * and ** were used to show the significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

disorders in pear fruits are closely related to calcium deficiency whose prevention requires adequate application of calcium fertilizers (Duan *et al.*, 2019; Wang and Arzani, 2019; Arzani, 2019). In this study, K was found to be the most abundant nutrient (Table 3). Also, previously published research results showed the amount of this element was higher than the other nutrients (Chen *et al.*, 2007). As reported, the presence of K under stress enhanced the function of photosystem II, the

biosynthesis of chlorophyll, and the antioxidant enzyme activity (Shahid *et al.*, 2019). Besides, Fe deficiency causes disturbances in the photosynthetic system, a decrease in chlorophyll content, and leads to the reduction of crop yield. Also, iron deficiency causes photosynthetic system abnormalities and diminished chlorophyll content (Pestana *et al.*, 2001). The amount of iron in pear fruit is low and has been reported between 20 and 35 mg Fe kg⁻¹ DW (Brunetto



et al., 2015). In our study, the amount of iron varied from 29 to 100 mg kg⁻¹ DW (Table 4). Manganese deficiency, which is more likely to occur under alkaline conditions, can markedly reduce the pear yield (Brunetto *et al.*, 2015). Zinc deficiency in the soil disrupts the growth of the plant and the fruit set conditions, accordingly, the fruits get miniaturized, and the yield decreases (Wójcik and Popińska, 2009). In another experiment, the amount of zinc among pears was reported from 14 to 27 (mg kg⁻¹ dry weight) (Arzani *et al.*, 2008). Also, boron plays an important role in pollen tube growth, pollen germination, fruit size, sourness, and early ripening (Wojcik and Wojcik, 2003).

In this research, we found a linear relationship between total phenol and rutin, total phenol and TSS, firmness, and Ca, and TSS and rutin (Figure 3). Due to the importance of Ca in the cell wall structure, which led to the increase in fruit storage and shelf life, it was observed that for the increase of 5.9249 kg cm⁻² of fruit firmness, one mg 100 g⁻¹ DW of Ca increased (Figure 3). Wang and Arzani (2019) reported that Ca deficiency in the pear fruit also causes disorders such as cork spot (bitter pit). Also, calcium treatment decreased the peak of ethylene production and decreased respiration rate during fruit storage (Han *et al.*, 2021).

We found in another linear relationship that by increasing 1 mg 100 g⁻¹ FW of total phenol in the pear, 125.69 TSS (°Brix) increased. It was also observed that for every increase of 14.61 TSS, the amount of rutin increased by 1 mg g⁻¹ FW (Figure 3).

CONCLUSIONS

In general, the results showed that the amounts of nutrients and biochemistry measurements in this study were different across pear cultivars. Important factors, including mineral compounds and sugars, affect the quality of pears. Identifying promising genotypes of native pears in the

world is important. Recently, A95 as a promising pear genotype has been identified through the breeding program at Tarbiat Modares University, Iran. Also, 'Shahmiveh' and 'Sebri' are native to Iran, belonging to the European pear group (*Pyrus communis* L.), and have been compared to Asian pear cultivars in terms of chemical characteristics. It is necessary to examine indigenous cultivars that also have commercial properties. The genotype of A95 could be one of them. In addition to genetics, factors such as climate, region, and orchard management also affect fruit quality. The highest amount of phenol was found in the 'Shahmiveh' cultivar, which is one of the important native cultivars of Iran. Chlorogenic acid and rutin were relatively high in KS14 and A95. However, in previous experiments, the highest amounts of TA, TSS, and pH were observed in KS8, 'Sebri', and KS13, respectively. Finally, pears can be considered important fruits in terms of minerals and phenolic compounds, and their role in human health is important. Our experiment, which was conducted among 12 European and Asian pear genotypes, will help us choose the best pear genotype in terms of the highest phenolic content and nutrients, both for fresh consumption and in the juice industry.

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ویژگی‌های بیوشیمیایی و تغذیه‌ای میوه برخی از گلابی‌های آسیایی و اروپایی (*Pyrus spp.*) کشت شده در شرایط آب و هوایی تهران

سمیه کدخدایی، و کاظم ارزانی

چکیده

گلابی یکی از مهمترین میوه‌های دانه‌دار در بازار جهانی میوه با ارزش غذایی بالا است. این مطالعه به منظور تعیین ترکیبات فنلی و برخی خواص شیمیایی گوشت و پوست میوه ۱۲ گلابی آسیایی و اروپایی انجام شد. اسید کلروژنیک و روتین به عنوان ترکیبات فنلی مهم در پوست میوه یافت شدند که با استفاده از HPLC اندازه‌گیری شدند. نتایج نشان داد که اسیدیته قابل تیتراسیون میوه (۰.۵۳-۰.۱۷٪ TA)، مواد کل جامد محلول (۳۳.۱۳-۳۳.۱۷ درجه بریکس TSS)، سفتی (۷۵.۲-۷۰.۱ کیلوگرم بر سانتی‌متر مربع) و پارامترهای رنگ افزایش یافت. بالاترین مقدار L* در KS7 (40.55) مشاهده شد، در حالی که کمترین مقدار در KS12 (14.26) و KS13 (14.78) بود. علاوه بر این، این مطالعه میزان مواد مغذی و فنل کل نمونه‌های میوه را ارزیابی کرد. رقم «شاه‌میوه» بالاترین میزان فنل کل (۶۳۸.۰۱ میلی‌گرم در ۱۰۰ گرم وزن تازه) را نشان داد، در حالی که رقم KS7 کمترین میزان (۴۲۰.۰۲ میلی‌گرم در ۱۰۰ گرم وزن تازه) را داشت. پتاسیم فراوان‌ترین ماده مغذی (۱.۱۶ میلی‌گرم در ۱۰۰ گرم وزن خشک) بود و پس از آن نیتروژن و کلسیم قرار داشتند. با افزایش فنل کل، مقدار روتین نیز افزایش یافت. تجزیه و تحلیل مؤلفه‌های اصلی تجزیه و تحلیل مؤلفه‌های اصلی (PCA) تمام داده‌ها نشان داد که ارقام گلابی اروپایی و آسیایی دسته‌بندی شده و در دو گروه مجزا قرار گرفتند. در نتیجه، ارقام و ژنوتیپ‌های مختلف گلابی اروپایی و آسیایی مورد مطالعه از نظر اکثر صفات بیوشیمیایی مورد مطالعه متفاوت بودند و روابط معنی‌داری بین برخی از صفات مشاهده شد. علاوه بر این، نتایج به‌دست‌آمده به انتخاب بهترین ارقام یا ژنوتیپ‌های گلابی از نظر بالاترین میزان فنول و مواد مغذی، هم برای مصرف تازه و هم در صنعت آبمیوه‌گیری، کمک می‌کند.

Study on Hemogram and the Effect of Thermal Stress on Hemocytes and Development in *Dacus ciliatus* (Diptera: Tephritidae)

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ABSTRACT

This study investigated the hemocyte profile, hemogram across all biological stages, and the morphological and frequency changes of hemocytes in the third instar larvae exposed to temperature stress. Cucumber fruits infected with insect larvae were collected and the third instar larvae were extracted from the fruit tissue. The hemolymph was then collected and, after staining with Giemsa solution, were identified under a light microscope. The hemogram analysis included DHC, THC, and blood volume across all biological stages. In the third instar larvae, plasmatocytes and granulocytes were the most abundant, comprising about 56% of the hemocyte population. In contrast, prohemocytes were most frequent in the first instar larvae, accounting for approximately 37%. THC was highest in the larvae, indicating a direct correlation between hemolymph volume and total hemocyte count. Temperature stress had a significant impact on hemocyte numbers. Heat stress, with temperatures up to 30 and 35°C, led to a notable increase in total cell count, granulocytes, and plasmatocytes. Conversely, cold temperatures resulted in a decrease in prohemocytes, plasmatocytes, granulocytes, and the total cell count compared to the control. Additionally, temperature stress induced hemocyte deformation, with plasmatocytes and granulocytes showing the most pronounced changes, including torn cell walls and loss of cell contents at 35°C. Cold stress had a greater effect on the shrinkage of prohemocytes than on the other cell types. Temperature stress also significantly affected the developmental characteristics of the fruit fly. Heat stress reduced the pupation length and emergence rates, while cold stress more prominently impacted birth rates. This study provides a foundation for further research into the physiological defense mechanisms of this pest.

Keywords: Developmental stage, *Dacus ciliatus*. DHC, Hemocyte identification, Temperature stress, THC.

INTRODUCTION

The Tephritidae family, commonly known as fruit flies, includes over 4,000 species worldwide. The larvae of this family feed on the seeds, fruits, and stems of various agricultural and horticultural crops, with approximately 30% of these species targeting the fruit tissue of truck crops (Norrbon, 2011). Among them, the

Ethiopian fruit fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae), is an oligophagous pest primarily affecting the Cucurbitaceae family. This pest is active in tropical and subtropical regions of Asia and Africa (Vayssières *et al.*, 2008, Abdallah *et al.*, 2012, EPPO, 2018) and is particularly destructive in Iran's fields and greenhouses (Barzkar *et al.*, 2017). The larvae of *D. ciliatus* cause significant damage to various cucurbit crops, including spring cucumbers,

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autumn cucumbers, Armenian cucumbers, pumpkins, melons, honeydew melons, and zucchini, leading to fruit spoilage and reduced market value. The damage begins when larvae penetrate the fruit tissue, leaving a visible entrance hole, and as they feed, they create tunnels that deform the fruit. Under severe infestation, a single fruit may contain multiple larvae at different developmental stages. Notably, *D. ciliatus* does not undergo obligatory diapause, allowing it to remain active year-round under favorable conditions, particularly in cucumber greenhouses, where it can devastate up to 90% of the crop yield if left unmanaged (Arghand, 1983, Paydar *et al.*, 2020, Mohammad, 2022).

Understanding the physiological characteristics, particularly the immunological aspects of insects, is crucial for developing effective strategies to combat the pests using chemical and microbial agents. The immune response of insects serves as a key indicator of hemolymph stress or contamination. Sensitivity and resistance to pathogenic agents vary across different insect species and developmental stages. A strong immune system in insects can prevent the development of infections during microbial challenges, with the outcome largely depending on the robustness of the insect's immune defenses (Washburn *et al.*, 2000, Kanost *et al.*, 2007). The first step in this field involves identifying hemocytes and their frequency across the insect's developmental stages (Valizadeh *et al.*, 2017, Go *et al.*, 2022).

The immune reactions of insects are influenced by various environmental and non-environmental factors, such as temperature changes, diapause, feeding, molting, starvation, and the entry of contaminants or infections into the hemolymph. These factors underscore the sensitivity of the circulatory system to stress and osmolality changes (Siva-jothy and Thompson 2002; Lee *et al.*, 2008). Osmolality, which refers to the concentration of solute particles in a solution, is a critical characteristic of

hemolymph, playing an essential role in blood circulation, gas exchange, metamorphosis, adult emergence, and wing expansion (Jiang *et al.*, 2023; Salcedo *et al.*, 2023). Upon identifying a foreign agent, hemocytes such as plasmatocytes and granulocytes, react by altering their shape, type, and density, followed by processes like phagocytosis and nodulation, which are vital for the insect's innate immune response (Pech and Strand, 2000, Black *et al.*, 2022).

Temperature is a significant environmental factor that influences insect growth, body size, molting, reproduction, abundance, survival, generation time, and immunity (Vogel *et al.*, 2022; Mutamisva *et al.*, 2023). Insects typically have an optimal temperature range for growth and development, with deviations from this range negatively affecting their survival (Foray *et al.*, 2014, Cui *et al.*, 2018). Exposure to high or low temperatures can significantly alter hemocyte density, depending on the insect's growth stage and species, thereby affecting the insect's resistance to control measures (Browne *et al.*, 2014; Vogel *et al.*, 2022). Various studies have documented the effects of temperature on hemocyte morphology and numbers in different insects, including *Phthorimaea operculella* Zeller (Lep: Gelechiidae), *Gromphadorhina coquereliana*, and *Megastigmus pistaciae* (Hym: Torymidae) (Pourali and Ajamhassani, 2018; Lubawy and Stocinska, 2020, Ajamhassani *et al.*, 2023).

Dacus ciliatus is a serious pest in fields and greenhouses, remaining active throughout the year. Environmental temperature fluctuations or temperature stresses in greenhouses can affect the growth and physiological activities of this insect. These environmental changes may also alter the insect's sensitivity to various pesticides or natural enemies (Zhu *et al.*, 2012). By studying how environmental changes affect the immune system of this fly, more effective control methods can be developed and implemented. Therefore, the purpose of this research was to identify the hemocytes,

assess the hemogram, and evaluate the effects of thermal stress on the hemocyte profile and some biological characteristics of *D. ciliatus*.

MATERIALS AND METHODS

Insect Rearing

Cucumbers infected with *D. ciliatus* larvae were collected from infested cucumber greenhouses of Semnan City (35.5767° North, 53.3949° East), Semnan Province, Iran, during 2022 growing season. They were transferred to the laboratory under controlled conditions in growth chamber (temperature 24±1°C, relative humidity 60%, and light-dark ratio 14:10 hours). Growth chamber condition was checked daily. Contaminated cucumbers were placed in plastic containers (40 cm length×40 cm width×40 cm height). The first, second, and third instar larvae were distinguished based on body length and head capsule width (Dyar, 1980) [Figures 1 (a-c)]. The characteristics of larval instars are shown in Table 1. Feeding third instar larvae were utilized to identify hemocytes and determine parameters related to hemogram. Subsequently, infested cucumbers, consumed by larvae and nearing spoilage, were substituted with healthy cucumbers, and the larvae were carefully transferred to the healthy cucumbers using a brush.

Hemocyte Identification

Hemocytes were identified by using Gupta keys and staining cells by Giemsa. Cells were observed using an Olympus BH2 light microscope at 40× magnification and

identified based on size and morphological characteristics (Gupta, 1985; Jones, 1962).

Hemogram

Differential Hemocyte Count (DHC)

The larvae fed on greenhouse cucumber were used for DHC calculations. Differential hemocytes of larvae, pupae, and adults were calculated. Following hemolymph collection using a sterile needle from the area between abdominal segments 3 and 4, the samples were placed on a slide, and a smear was prepared using another slide. A staining solution composed of Giemsa (Merck KGaA, Germany) and distilled water in a 9:1 ratio was added to the slide and allowed to stand for 5 minutes. Subsequently, the slide was washed in distilled water and briefly immersed for 5 seconds in a saturated lithium carbonate solution to fix the cell staining (Yeager, 1945). After another rinse, the underside of the slide was dried using filter paper. One hundred hemocytes were randomly selected at 40× magnification and differentially counted using an Olympus BH2 microscope. Twenty-five samples from each biological stage were examined.

Total Hemocyte Count (THC)

For THC, approximately 1 µL of hemolymph from two larvae was collected using a capillary tube and mixed with 10 µL of Tyson buffer as an anticoagulant solution (NaCl 72 Mm, Na₂SO₄ 9 Mm, glycerol 43 Mm, methyl violet 0.06 Mm, distilled water) (Mahmood and Yusaf, 1985). Hemolymph and Tyson solutions were placed on Neubauer slides (HBG, Germany), and

Table 1. Morphometric size (mean±SE) of different larval development of *Dacus ciliatus* (n= 20).

	Larval stages		
	First instar larvae	Second instar larvae	Third instar larvae
Body length (mm)	2.9±0.16	4.7±0.22	7.1±0.26
Head width (mm)	0.32±0.02	0.67±0.03	1.1±0.45

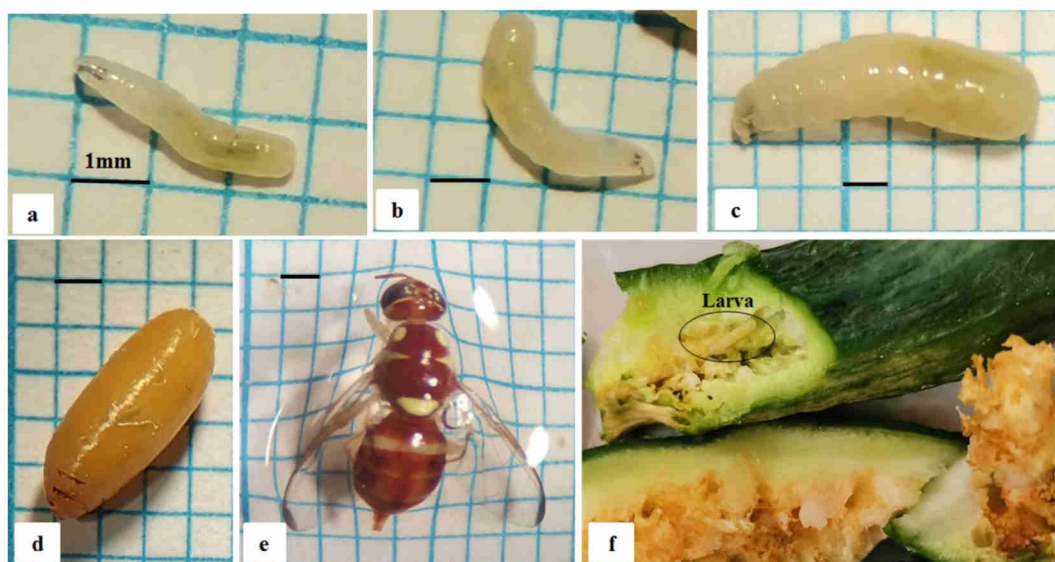


Figure 1. Developmental stages of *Dacus ciliates*: (a) First instar larva, (b) Second instar larva, (c) Third instar larva, (d) Pupa, (e) Adult (female), and (f) Damage of larvae on cucumber (original photo).

hemocytes were counted using the Jones formula and light microscopy at 40× magnification (Jones *et al.*, 1962).

$$\frac{\text{Hemocyte in } \times 1 \text{ mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

Dilution= 10 times, Depth factor of the chamber= 10, and No. of squares counted= 5

Hemolymph Volume (HV)

Hemolymph volume was directly determined by extracting hemolymphs from various developmental stages using micropipettes (Terra *et al.*, 1975, Ghasemi *et al.*, 2013). The weight of a piece of filter paper was measured when dry. Subsequently, a proleg was cut from the larval abdomen, and all the hemolymph was collected by using Hamilton syringe (10 µL, Switzerland), placed on filter paper and weighed. Sampling was also conducted for pupae and adults. The difference between the weights of wet and dry filter paper sheets was recorded and considered as hemolymph volume. Fifteen insects from each developmental stage were included.

Effect of Thermal Stress on Hemocytes Profile

The effect of temperature stress on the number of hemocytes comprised four treatments (5, 24±1, 30, and 35°C) and four repetitions. Based on previous observations, infected fruits with larval entrance holes and deformation due to larval activity were found to contain various larval instars. These fruits were divided into four groups and exposed to different conditions: controlled (24±1°C), cold stress (5°C), and heat stress (30 and 35°C). Hemocyte counts in the third instar larvae of *D. ciliatus* were assessed after 24 hours. The control group comprised larvae kept under growth chamber conditions (24±1°C). In each replicate, the hemolymph of three larvae (approximately 3 µL) was collected via a capillary tube and mixed with 20 µL of Tyson (anticoagulant solution). Cells in 3 µL of hemolymph were counted using a hemacytometer. To observe morphological changes in hemocytes under heat and cold stress, infected fruits were exposed to thermal stress (Pourali and Ajamhassani, 2018, Ajamhassani *et al.*,

2023). After 24 hours, hemocytes from the third instar larvae were stained with Giemsa and examined using a light microscope at 40× magnification. Hemocyte deformation was then recorded.

Effect of Thermal Stress on Pupae

Fruits infected with larvae were divided into four groups within rearing containers and were subjected to test temperatures (5, 24±1, 30, and 35°C). After 24 hours, the fruits were transferred to growth chamber conditions (for the control treatment, the fruits were kept under growth chamber conditions (temperature 24±1°C, relative humidity 60%, and light-dark ratio 14:10 hours) for 24 hours. The dead larvae were removed from the fruits and the alive third instar larvae were transferred to fresh fruits to complete their life cycle and become pupae and adults. These fruits were checked daily. New puparium usually has a light brown color and body length is 4.8±0.17 mm (Figure 1-d). New pupae were separated daily and 2-old-days pupa were weighed. The other characteristics such as the pupal period, percentage of adult emergence, and adult longevity were examined (40 third instar larvae were examined for each treatment). After emerging, adult flies were gently transferred to the falcon tubes and supplied with a solution of water and honey to determine their longevity.

Statistical Analysis

All data obtained from a complete randomized design were compared by one-way Analysis Of Variance (ANOVA) followed by Tukey's test when significant differences were found at $P \leq 0.05$ (SAS, 9.4). Differences between samplings ($n = 3$) were considered statistically significant at a probability less than 5%, and marked in figures and tables.

RESULTS

Identification of Hemocytes and Determination of Their Abundance

Five types of hemocytes were observed in the hemolymphs of *D. ciliatus* larvae, namely, prohemocytes, granulocytes, plasmatocytes, oenocytoids, and spherulocytes.

Prohemocytes are round and the smallest cells in terms of size (Table 2). They feature a large, central nucleus that occupies the majority of the cytoplasmic volume (Figure 2). The highest abundance of prohemocytes was observed in the first instar larvae ($37 \pm 2.2\%$), whereas their number decreased in subsequent stages, with the lowest abundant observed in the third instar larvae and pupae (Table 3) ($F = 56.3$, $df_{t,e} = 4, 14$, $P \leq 0.0001$).

Granulocytes with central or semi-central nuclei varied in sizes and were sometimes the largest cells (Figure 2). The cytoplasm surface contained numerous granules, which were visualized with Giemsa blue. The frequency of these cells was higher in the third instar larvae ($29 \pm 1.5\%$) compared to other stages and lowest in the first instar larvae ($16.5 \pm 2.3\%$) (Table 3) ($F = 84$, $df_{t,e} = 4, 14$, $P \leq 0.0001$). Plasmatocytes exhibited a spindle-shaped or eye-shaped morphology with varying sizes (Figure 2). The abundance of plasmatocytes was highest in the third instar larvae ($26.4 \pm 2\%$) and lowest in the first instar larvae ($21 \pm 1.6\%$) (Table 2), ($F = 55.4$, $df_{t,e} = 4, 14$, $P \leq 0.004$). Oenocytoids were egg-shaped with lateral nuclei and were similar but slightly larger in size compared to prohemocytes (Figure 2, Table 2). The frequency of these cells was lower than the previous cells (Table 3) ($F = 107$, $df_{t,e} = 4, 14$, $P \leq 0.0001$). Spherulocytes of medium to large sizes were observed in larval hemolymph (Table 2) (Figure 2). Small spherules around the nucleus occupied the cytoplasm surface and had the least frequency of cells (Table 3) ($F = 92.6$, $df_{t,e} = 4, 14$, $P \leq 0.0001$).

**Table 2.** Morphometric measurements of hemocytes in larvae of *Dacus ciliatus* (n= 20).

Hemocyte type	Size (μm) ^a	
	Length (mean $\pm$ se)	Width (mean $\pm$ SE)
Prohemocyte	3.1 $\pm$ 2.4b	3 $\pm$ 2.5b
Plasmatocyte	6.2 $\pm$ 2.6ab	2.4 $\pm$ 2.8bc
Granulocyte	8.2 $\pm$ 3.3a	6.2 $\pm$ 2.6a
Oenocytoid	3.2 $\pm$ 1.5b	2.9 $\pm$ 0.8b
Spherulocyte	6.5 $\pm$ 3.1ab	5.3 $\pm$ 2.8ab

^a Different letters in each column show significance using Tukey's test at $P < 0.05$.

Table 3. Frequency of hemocytes in developmental stages of *Dacus ciliatus* (n= 25).

Developmental stage	Frequency of hemocytes (%) ^a				
	Prohemocyte	Plasmatocyte	Granulocyte	Oenocytoid	Spherulocyte
1 st Instar larva	37 $\pm$ 2.2a	21 $\pm$ 1.6bc	16.5 $\pm$ 2.3c	11 $\pm$ 0.5b	6 $\pm$ 1.1a
2 nd Instar larva	30.3 $\pm$ 1.6b	23 $\pm$ 0.8b	20 $\pm$ 2.4b	14 $\pm$ 0.7a	8 $\pm$ 1.3a
3 rd Instar larva	24.1 $\pm$ 1.4cd	26.4 $\pm$ 2a	29 $\pm$ 1.5a	12 $\pm$ 0.5b	7 $\pm$ 1a
Pupa	24.2 $\pm$ 2.2cd	25.4 $\pm$ 1.6a	27 $\pm$ 0.8a	11.5 $\pm$ 1.1b	6.4 $\pm$ 0.2a
Adult	27 $\pm$ 1.3c	25.4 $\pm$ 1.4a	26 $\pm$ 1.3ab	8 $\pm$ 0.6c	6a

^a Different letters in each column show statistical differences among biological stages (Tukey's test, $P \leq 0.05$).

**Figure 2.** Light microscopy pictures of *Dacus ciliatus* hemocytes stained with Giemsa. PR (Prohemocyte), PL (Plasmatocyte), OE (Oenocytoid), GR (Granulocyte), SP (Spherulocyte), and Scale bar= 10 μm .

Hemogram

According to Table 3, the weight of the first and second instar larvae was lower than that of other stages ($F = 44.4$, $df_{t,e} = 4,14$, $P \leq 0.0001$). Due to the higher feeding of the third instar larvae, the weight of these larvae was higher significantly than that of the younger larvae. On the other hand, the amount of nutrition was also effective on the hemolymph volume; so, the hemolymph volume was higher in the third instar larvae,

pupae, and adults than in the early larval stages ($F = 87.7$, $df_{t,e} = 4,14$, $P \leq 0.0001$). Hemocyte number of adults (230.2 ± 21.4) cells mm^{-3}) decreased compared to the third instar larvae (314.4 ± 22.4) cells mm^{-3}) (Table 4), ($F = 35.5$, $df_{t,e} = 4,14$, $P \leq 0.0001$).

Effect of Thermal Stress on Hemocytes Profile

Significant changes were observed in the number of hemocytes of *D. ciliatus* larvae

Table 4. Body weight, Hemolymph Volum (HV), Total Hemocyte Count (THC), in developmental stages of *Dacus ciliatus*.^a

Developmental stage	Weight (mg)	HV (μ L)	THC (cell mm^{-3})
1 st instar larva	0.08 $\pm$ 0.01d	1 $\pm$ 0.33cd	85.5 $\pm$ 10d
2 nd instar larva	3 $\pm$ 0.2c	1.5 $\pm$ 0.2c	210 $\pm$ 34.3c
3 rd instar larva	17 $\pm$ 0.4a	3.1 $\pm$ 0.3a	314.4 $\pm$ 22.4a
Pupa	14 $\pm$ 0.4b	2.9 $\pm$ 0.3a	256 $\pm$ 16.4b
Adult	18 $\pm$ 1 a	2.2 $\pm$ 0.21b	230.2 $\pm$ 21.4bc

^a Different letters in each column show statistical differences among biological stages (Tukey's test, $P \leq 0.05$).

affected by cold and heat. The results showed that the total hemocyte count ($F = 84.2$, $df_{t,e} = 3,10$, $P \leq 0.0001$), granulocytes ($F = 102.5$, $df_{t,e} = 3,10$, $P \leq 0.0001$), plasmatocytes ($F = 109.35$, $df_{t,e} = 3,10$, $P \leq 0.0001$), and oenocytoids ($F = 104$, $df_{t,e} = 3,10$, $P \leq 0.0001$) of larvae subjected to heat (30 and 35°C) were significantly higher than those of the control larvae. In all the aforementioned cases, except for oenocytoids, a significantly lower number of hemocytes in larvae experienced cold stress compared to the control group. Prohemocyte number decreased under cold too stress. The total hemocyte count in the larvae exposed to 35°C (421 $\pm$ 25 cells mm^{-3}) and 30°C (377 $\pm$ 28.1 cells mm^{-3}) was higher than that of the control larvae (340 $\pm$ 11.5 cells mm^{-3}). Moreover, cold stress at 5°C significantly decreased the number of hemocytes in larvae, reducing it to 262 $\pm$ 15 cell mm^{-3} hemolymph (Figure 3).

Furthermore, the granulocyte count was higher in larvae placed at 35°C (177 $\pm$ 14 cell mm^{-3}) cell in mm^3 and 30°C (145 $\pm$ 15.5 cell mm^{-3}) than in control larvae (107 $\pm$ 11.3 cell mm^{-3}). Similar to the previous case, the number of granulocytes significantly decreased under cold stress, being reduced to about half the number of hemocytes in the control larvae (45 $\pm$ 6.5 cell mm^{-3} hemolymph) (Figure 4). The changes observed in plasmatocytes under the influence of high and low temperatures were similar to those in granulocytes. In other words, the increase of these cells in heat stress and the decrease of plasmatocytes in cold were significant compared to the control. At 30 and 35°C, the rate of increase

of plasmatocytes, like granulocytes, fell into a statistical group (Figure 5).

Increases in oenocytoids differed at high temperatures, with 35°C caused a greater increase in the number of these cells. On the other hand, cold stress did not have a significant effect on the reduction of oenocytoids. Larvae that experienced cold for 24 hours showed no significant differences in the number of oenocytoids compared to the control group (Figure 6).

Under the influence of cold stress, prohemocytes exhibited a significant decrease compared to the control group, and their numbers reduced to about half. Based on the observations, the number of prohemocytes increased in higher temperatures but showed no significant difference with the control (Figure 7).

Morphological Changes of Hemocytes in *D. ciliatus* Affected by Thermal Stress

Heat and cold stress significantly affected the shape of hemocytes. Nonetheless, cells underwent greater changes in appearance under heat stress compared to cold. Granulocytes (55 $\pm$ 2.3%) and plasmatocytes (42 $\pm$ 7%) were deformed more than the other cells by temperatures of 30 and 35°C (Figure 9). At 30°C, the walls of the granulocytes were wrinkled; at the temperature of 35°C, after the cell wall was torn, the cell contents gradually came out of the cell (Figure 8). In some cases, cells were seen to disintegrate under the influence of 35°C. The cell walls of plasmatocytes and oenocytoids were also wrinkled by heat stress. Cold had the greatest effect on the morphology of prohemocytes (Figure 9). These cells were severely shrunk at 5°C, and the nuclei were compressed.

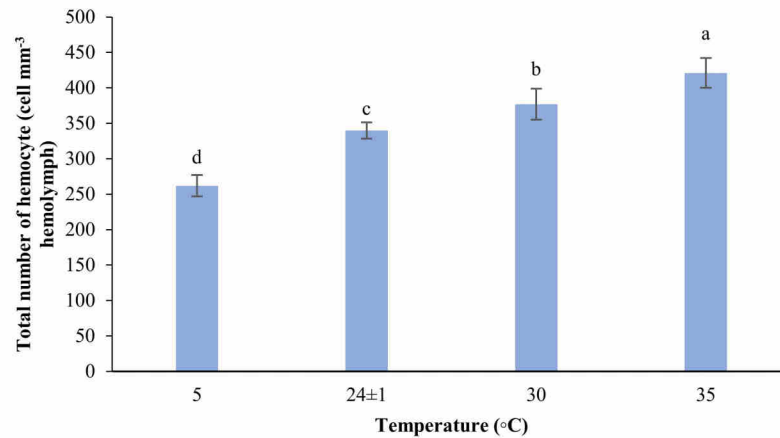


Figure 3. Effect of thermal stress on total hemocyte count in the third instar larvae of *Dacus ciliates*.

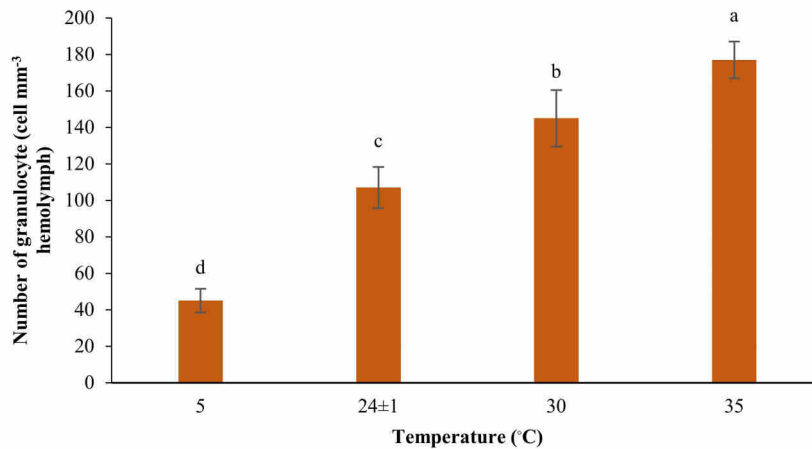


Figure 4. Effect of thermal stress on granulocyte number in the third instar larvae of *Dacus ciliates*.

Effect of Thermal Stress on Pupae

The pupal period was observed to be shorter in larvae that experienced heat stress than in the control group ($F=76.5$, $df_{t,e}=3, 10$, $P\leq 0.002$). The temperature of 35°C decreased the pupal period (6.4 ± 0.5 days) more than the temperature of 30°C (8.5 ± 0.2 days). The percentage of adult emergence in larvae subjected to both heat and cold stress was lower than in the control, although cold stress had a greater effect on this parameter ($F=98.3$, $df_{t,e}=3, 10$, $P\leq 0.0001$). The

percentage of adult emergence in the larvae exposed to cold ($58\pm1.5\%$) was reduced by half compared to the control ($98\pm5.5\%$) (Table 5).

DISCUSSION

Hemocytes play a crucial role in the cellular immunity of insects, responding to various stresses and infections by altering their number, type, size, and shape (Lavine and Strand, 2002; Ebrahimi and Ajamhassani, 2020; Duarte et al, 2020). In *Dacus ciliatus*, five types of hemocytes were identified in the hemolymph: prohemocytes,

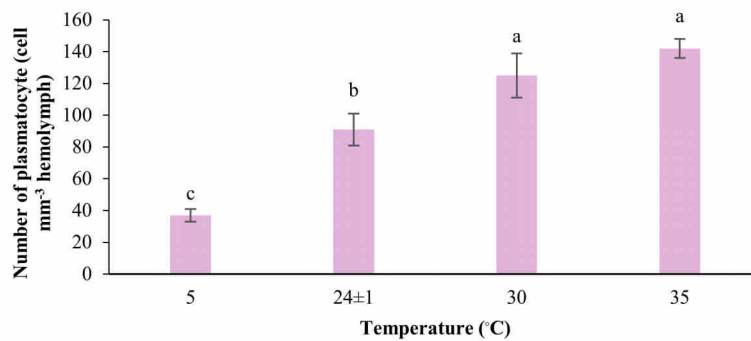


Figure 5. Effect of thermal stress on plasmatocyte number in the third instar larvae of *Dacus ciliatus*.

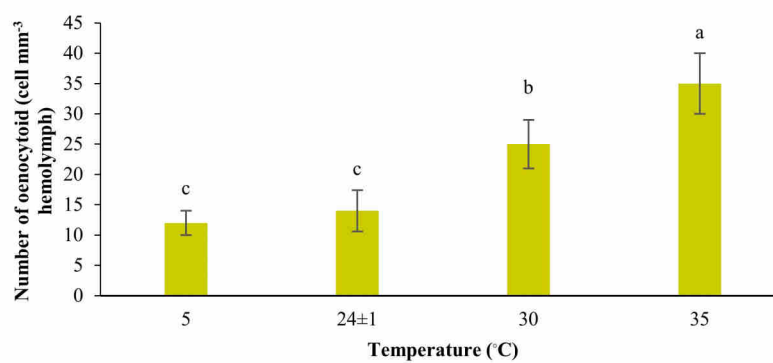


Figure 6. Effect of thermal stress on oenocytoid number in the third instar larvae of *Dacus ciliatus*.

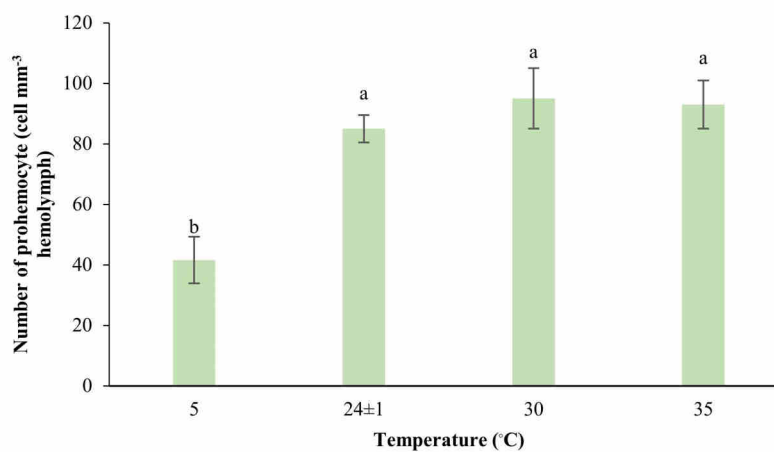


Figure 7. Effect of thermal stress on prohemocyte number in the third instar larvae of *Dacus ciliatus*.

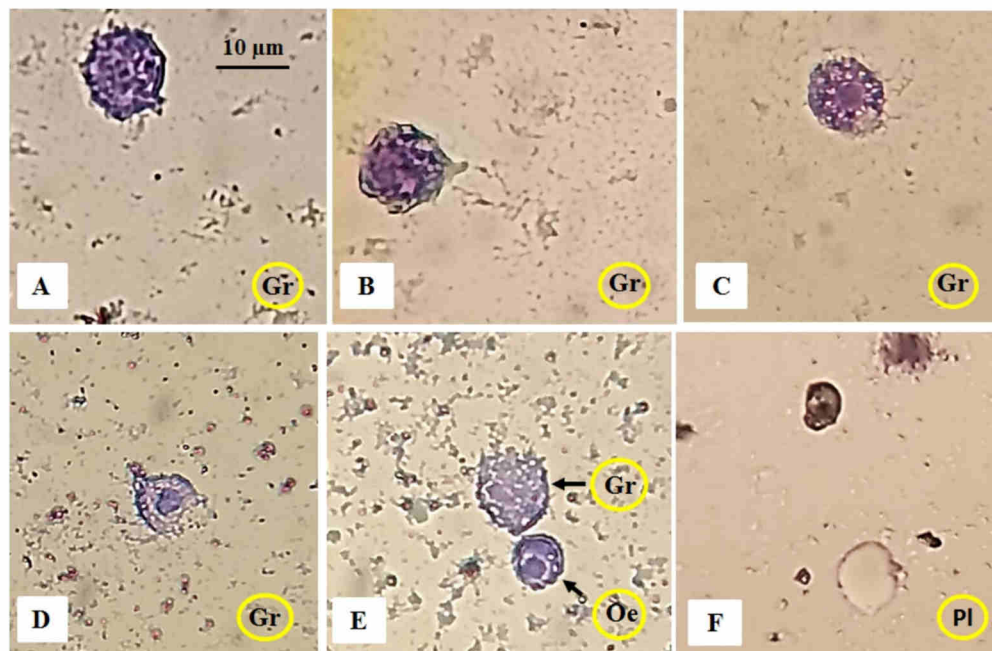


Figure 8. A, and B show the deformation of granulocytes; C shows the tearing of the cell wall in granulocytes affected by thermal stress at 30°C; D shows the exit of cellular contents from the granulocyte at 35°C; E shows the complete removal of cellular contents of the granulocyte and destruction of the nucleus as well as deformation of the oenocytoid, and F shows the shrinkage of the cell wall in plasmatocytes affected by cold stress.

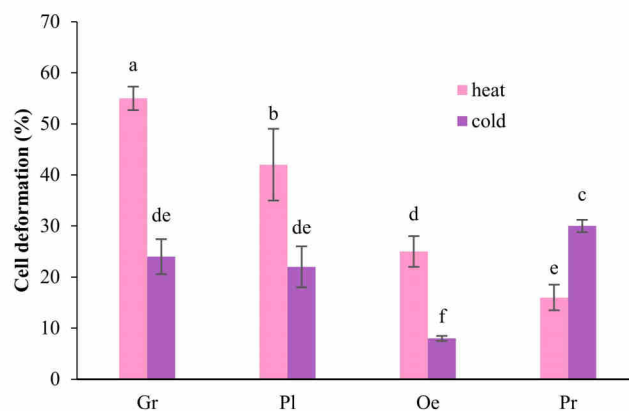


Figure 9. Cell deformation percentage of *Dacus ciliatus* affected by heat and cold stress.

Table 5. Survival status of *Dacus ciliatus* affected by thermal stress.^a

Temperature (°C)	Days until pupation	Pupal weight (mg)	Pupal period (Day)	Percentage of adult emergence (%)	Adult longevity (Day)
24±1	2±0.3a	14±0.4a	10±1a	98±5.5a	18.5±1.7a
5	2±0.6a	13.5±0.6a	11.7±0.6a	58±1.5c	20±2a
30	1±0.2a	12±0.2a	8.5±0.2b	84±5b	16.5±1.8a
35	0.5±0.1b	11.2±0.3a	6.4±0.5c	80±2.4b	17±2.2a

^a Different letters in each column show statistical differences among biological stages (Tukey's test, $P \leq 0.05$).

plasmatocytes, granulocytes, oenocytoids, and spherulocytes. It is important to note that, in some insects, these cell types can be reduced or transformed into other forms. For instance, different types of hemocytes have been observed in flies and mosquitoes. In *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), prohemocytes, granulocytes, plasmatocytes, adipohaemocytes, oenocytoids, and spherulocytes were identified in the hemolymph (Silva *et al.*, 2002). Similarly, in *Musca domestica* Linnaeus, *Chrysomya megacephala* (Fabricius), and *Chironomus ramosus* (Fabricius), prohemocytes, plasmatocytes, granulocytes, oenocytoids, and spherulocytes were observed (Pal and Kumar, 2014; Gaikwad *et al.*, 2024). This variability suggests that there is no universal hemocyte pattern within this order (Bruno *et al.*, 2022). The types and functions of hemocytes can vary not only between different insect orders but also among families, genera, and species within the same order (Gábor *et al.*, 2020).

The Differential Hemocyte Count (DHC) in *D. ciliatus* revealed significant variations in the population of different hemocyte types across its life stages. Prohemocytes were the most abundant hemocytes in the hemolymph of the first instar larvae, but their density decreased as the larvae aged. Prohemocytes differentiate into plasmatocytes before being released from hematopoietic organs, undergoing mitosis to become plasmatocytes and granulocytes during cellular defense or wound healing processes (Yamashita and Iwabuki, 2001). Plasmatocytes and granulocytes were the most abundant hemocytes in the third instar larvae and pupae, with a higher concentration in the third instar larvae. These cells are critical for cellular defense processes. Previous research has shown that, in Lepidoptera, plasmatocytes and granulocytes account for approximately 80-90% of the total hemocyte population (Strand, 2008). It has been demonstrated that older larvae exhibit greater resistance to foreign factors compared to the younger

larvae, likely due to the higher abundance of key immune cells like plasmatocytes and granulocytes (Valadez-Lyra, 2011). In *D. ciliatus*, the number of plasmatocytes and granulocytes peaked in the third instar larvae but decreased in the pupal and adult stages. In contrast, the numbers of oenocytoids and spherulocytes were low across all developmental stages, comprising approximately 6-11% of the total hemocyte population.

The hemogram of *D. ciliatus* revealed a direct correlation between the insect's weight and both the hemolymph volume and Total Hemocyte Count (THC). The third instar larvae, which are larger and consume more food, cause more damage to crops and have greater body size and weight compared to other stages. Consequently, their blood volume and THC were significantly higher. In contrast, the first instar larvae, which were smaller with less feeding activity, exhibited lower hemocyte counts and hemolymph volume. It has been established that nutrition and food type significantly influence hemolymph volume and hemocyte density (Manjula *et al.*, 2020). Furthermore, the increased nutritional demands of older larvae and the heightened concentration of antimicrobial protein compounds may contribute to changes in the hemocyte population (Gupta, 1985; Mason *et al.*, 2014). In adult flies, the reduced nutritional intake is associated with a significant decrease in circulating hemocytes compared to the third instar larvae.

Many insects are ectotherms, i.e. their primary source of heat comes from the environment. Consequently, drastic temperature changes can significantly affect their homeostasis and survival. The ability to tolerate environmental stress is, therefore, crucial for their fitness, activity, reproduction, survival, and immunological potential (Boher *et al.*, 2016; Herren *et al.*, 2023). In our study, we observed physiological changes in *Dacus ciliatus* hemocytes under short-term temperature stress (24 hours), specifically in their number and morphology.



The effects of heat and cold stress on hemocyte count and morphology were evident. As the temperature increased to 35°C, there was a significant rise in the number of hemocytes. This increase was attributed to cell division, particularly among prohemocytes, which differentiate into immunocytes in response to stress or the presence of foreign agents (Pandey *et al.*, 2010). Additionally, granulocytes and plasmatocytes were observed to undergo cell division as a result of elevated temperatures (Amaral *et al.*, 2010). However, at these higher temperatures, the cell walls of granulocytes and plasmatocytes were compromised, leading to the rupture of cell membranes and the release of cell contents (Ghasemi *et al.*, 2013). Similar observations were made in *Scrobipalpa ocellatella* (Boyd) (Lep: Gelechiidae), where granulocytes were severely deformed, and oenocytoids, despite their thick cell walls, were torn under high-temperature stress. Prolonged heat stress in larvae led to the complete disintegration of cells as their contents were fully expelled (Ajamhassani, 2021).

Conversely, cold stress resulted in a decrease in hemocyte numbers and caused compression and shrinkage of prohemocytes. Under unfavorable weather conditions or temperature drops, insects tend to reduce their vital activities, such as feeding and mobility (Ajamhassani *et al.*, 2023). As a result, some hemocytes are removed from circulation and attach to the body walls

(Rowley and Ratcliffe, 1978). In cold-exposed cockroaches, the hemocyte area was significantly smaller compared to the control larvae. Specifically, at 4°C, the hemocyte size in *Gromphadorhina coquereliana* was markedly reduced (Lubawy and Stocinska, 2020). These cells were no longer part of the circulating hemocyte population. Similar effects have been reported in other insect species, such as *Nicrophorus vespilloides* Herbst (Coleoptera: Silphidae) (Urbanski *et al.*, 2017), *Antheraea myllita* (Drury) (Pandey *et*

al., 2010), and *Yponomeuta mallinellus* Zeller (Ajamhassani and Mahmoodzadeh, 2020). In our study, *D. ciliatus* exhibited comparable response to the thermal stress, where hemocyte size and morphology were notably altered by thermal stress. Granulocytes and plasmatocytes showed significant deformation under heat stress, while cold stress led to a marked reduction in prohemocyte size. These findings further support the sensitivity of hemocyte morphology to temperature extremes, consistent with the observations in other insect species.

We observed a shortened pupal period in *Dacus ciliatus* individuals subjected to short-term heat stress. Additionally, the percentage of adult emergence was significantly lower in those exposed to both heat and cold, with cold stress having a more pronounced impact compared to the control group. Our findings suggest that the duration of heat stress plays a crucial role in influencing survival and the population dynamics of subsequent generations, which can ultimately affect damage levels, as highlighted by Herren *et al.* (2023). In their study, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) larvae were subjected to either short (2 hours) or long (14 hours) heat stress at 38°C, and the effects on larval survival and immune response were assessed. They found that brief exposure improved survival rates and enhanced antibacterial activity, whereas prolonged or delayed heat stress had less favorable outcomes, underscoring the importance of stress duration and timing.

In contrast, Zheng *et al.* (2017) investigated the effects of a 2-hour heat stress at 35°C on *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) pupae. Their results indicated a significant increase in adult longevity and heat resistance, though fecundity was negatively impacted. This study demonstrates how, even short-term, mild heat stress can enhance certain aspects of fitness, such as longevity, which contrasts with the lack of significant changes in pupal weight and adult longevity

observed in our study. Ouda *et al.* (2022) examined *D. ciliatus* at constant temperatures of 15, 20, 25, and 30°C, maintaining infested squash fruits at these temperatures to measure developmental rates from egg to adult. Their findings showed that higher temperatures accelerated the development of immature stages, consistent with our observation of accelerated development at elevated temperatures. Similarly, Mahmoud (2016) reported a reduction in the larval period of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) with increasing temperatures from 15 to 30°C. This observation aligns with our findings on the accelerated development of *D. ciliatus*, highlighting the broader impact of temperature on developmental rates across different species.

Furthermore, high temperatures have been documented to reduce the developmental stages of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (de Campos *et al.*, 2021), and *Athetis lepigone* (Möschler) (Lepidoptera: Noctuidae) when temperatures were increased to 30°C during their development from egg to adult. (Li *et al.*, 2013). Despite insects having behavioral, morphological, and physiological adaptations to tolerate adverse environmental conditions, even slight deviations from the optimal temperature range for their growth can affect their survival and development (Mutamisva *et al.*, 2023).

In our study, we found that temperature treatments did not significantly affect pupal weight or adult lifespan. This may be due to the larvae inside the fruits experiencing only a brief 24-hour temperature stress after nearly completing their feeding, suggesting that under these conditions, pupal weight and adult longevity remained largely unchanged. However, it is crucial to consider the physiological characteristics and sensitivity of each species to environmental changes.

Our findings revealed a positive effect of thermal stress on certain biological and physiological aspects of *D. ciliatus*. While

our study primarily focuses on the effects of thermal stress, understanding the broader implications, including the insect's immune response, can provide additional context. Specifically, future research should explore how thermal stress affects the insect's immune system and its ability to cope with other stressors, such as microbial agents and toxic substances. Investigating the interaction between thermal stress and immune parameters, like antimicrobial peptides and detoxifying enzymes, could offer valuable insights into the pest's overall resilience. Such studies will be essential for developing comprehensive pest management strategies that account for both environmental stress and biological factors.

CONCLUSIONS

In the present study, we investigated the impact of thermal stress on certain developmental characteristics and the hemocyte density of *D. ciliatus*. Our findings suggest that this insect exhibits sensitivity to thermal stress, which affects its development and physiological parameters. Given these results, it is crucial to conduct further research on the effects of thermal stress in greenhouse and field settings over extended periods. Understanding how thermal stress influences the survival and immune system of the insect, in both short and long terms, could provide valuable insights for managing its population and mitigating the damage it causes.

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بررسی هموگرام و تأثیر استرس حرارتی بر هموسیت ها و رشد در *Dacus ciliatus* (Diptera: Tephritidae)

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چکیده

این پژوهش تأثیر تنشهای دمایی را بر سامانه ایمنی مگس جالیز (*Dacus ciliatus* Loew (Diptera: Tephritidae) با بررسی مرفولوژی و تراکم سلولهای خونی به عنوان اجزای اصلی ایمنی حشره نشان می دهد. فاکتورهای مختلفی مانند تنش دما، تغییرات رژیم غذایی و ورود عفونتها و آلودگیها به همولنف، با تغییر پروفایل سلولهای خونی سبب پاسخ ایمنی حشره می شوند. این تحقیق متمرکز بر پروفایل هموسیتها، هموگرام همه مراحل زیستی و تغییرات مرفولوژیکی و فراوانی هموسیتهای لاروهای سن سوم مگس جالیز در مقابل استرسهای دمایی بود. میوه های خیار آلوده به لارو، جمع آوری و به آزمایشگاه منتقل شدند. لاروهای سن سوم از بافتهای میوه خارج شدند. پس از استخراج همولنف از لاروها و رنگ آمیزی با محلول گیمسا، هموسیتها با استفاده از میکروسکوپ نوری، شناسایی شدند. در مطالعه هموگرام، پارامترهای DHC، THC، و حجم همولنف در همه مراحل زیستی اندازه گیری شد. در لاروهای سن سوم، گرانولوسیتها و پلاسماتوسیتها در مجموع شامل ۵۶ درصد فراوانی، بیشترین جمعیت را بین هموسیتها داشتند. در مقابل، پروهموسیتها در حدود ۳۷٪، بیشترین فراوانی را در لاروهای سن اول به خود اختصاص دادند. بالاترین THC در لاروهای سن سوم مشاهده شد که نشان دهنده ارتباط مستقیم بین حجم همولنف و تعداد کل سلولها بود. تنشهای دما تأثیر معنی داری بر تعداد هموسیتها نشان داد. در تنش گرما، افزایش دما تا ۳۰ و ۳۵ درجه سلسیوس، منجر به افزایش بارز تعداد کل سلولها، گرانولوسیتها و پلاسماتوسیتها شد. در مقابل، تنش سرما، سبب کاهش پروهموسیتها، گرانولوسیتها، پلاسماتوسیتها و تعداد کل سلولها در مقایسه با شاهد شد. به علاوه، تنش دما سبب تغییر شکل هموسیتها شد. پلاسماتوسیت ها و گرانولوسیت ها بارزترین تغییرات را تحت تنش گرمایی نشان می دهند، از جمله پارگی دیواره سلولی و از بین رفتن محتویات سلولی در دمای ۳۵ درجه سلسیوس. استرس سرما، تأثیر بیشتری بر انقباض پروهموسیت ها در مقایسه با سایر سلولها داشت. تنش دما همچنین به طور قابل توجهی بر ویژگی های رشدی مگس میوه تأثیر گذاشت. استرس گرمایی طول دوره شفیره و نرخ ظهور حشرات کامل را کاهش داد، در حالی که استرس سرما به طور برجسته تری بر نرخ تولد تأثیر داشت. این مطالعه نشان دهنده شناسایی اولیه هموسیت ها و تجزیه و تحلیل پاسخ ایمنی *D. ciliatus* به تغییرات دما است که پایه ای برای تحقیقات بیشتر در مورد مکانیسم های دفاع فیزیولوژیکی این آفت فراهم می کند.

Temperature-Dependent Development and Temperature Thresholds of total immature stage of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) in Iran

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ABSTRACT

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), is one of the most important pests of agricultural crops in tropical and subtropical regions of the world. In this study, the developmental rate of *C. ceratitidis* was studied at 10, 12, 15, 17, 20, 25, 27, 30, 32, and 35°C. The results showed a nonlinear relationship between temperatures and developmental rate. The best nonlinear models were Performance-1 and Performance-2 in Mazandaran and Fars Provinces, respectively. These models simulated the developmental rate of Mediterranean fruit fly accurately at temperatures ranging from 15 to 35 and 20 to 30°C, in Mazandaran and Fars Provinces, respectively, and the estimated optimal temperature of total immature stages was 31.9 and 31.8°C, respectively. The lower and upper temperature thresholds of the total immature stage in Mazandaran and Fars Provinces were estimated at 11.23 and 13.15°C, and 38.1 and 37.7°C, respectively. Between the two linear models, the Ikemoto linear model showed better-fit data compared with the ordinary model.

Keywords: Developmental rate, Fars Province, Linear models, Mazandaran Province, Nonlinear models.

INTRODUCTION

Fruit flies belong to the family Tephritidae, one of the largest and most economically important groups in the order Diptera (White and Elson-Harris, 1992; Li *et al.*, 2013). The larvae of most Tephritid species develop in the seed and cause severe damage to fruit and vegetable crops in most tropical and subtropical countries. The Mediterranean fruit fly is one of the most damaging agricultural pests in the world. It is a severe pest of more than 350 species of fruits and vegetables (Thomas *et al.*, 2001; Morales *et al.*, 2007; White and Elson-Harris, 1992). In the last decade, the consequences of climate change on the distribution, abundance, and phenology of insect species have been widely studied.

With an estimated further increase in mean global temperatures of 0.8°C over the next 100 years, the biosphere can be expected to experience broad climate-related changes. The occurrence of insect pests could be impacted by these changes. Insects may respond to climate change in a variety of ways (Chandrakumara *et al.*, 2024; Kambreakar *et al.*, 2015; Yamamura and Kiritani, 1998). Various factors, particularly temperature, is a critical abiotic factor affecting the development, survival, and reproduction of insect species, fitness, or performance-related traits of insects (Azrag *et al.*, 2018; Yadav *et al.*, 2014). Insect distribution and abundance are highly affected by temperature and, generally, an increase in temperature within the limits tolerated by the insect results in a rapid

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population increase (Campbell *et al.*, 1974; Bale *et al.*, 2002; Mujica *et al.*, 2017).

The developmental rate of insects and other poikilothermic invertebrates is linearly dependent on Temperature from a lower developmental threshold (T_{min}) to the optimum Temperature (T_{opt}). This is because temperature affects many physiological processes and the activity of enzymes (Trudgill *et al.*, 2005). Phenological models, using physiological time data, have been developed for Mediterranean fruit fly to predict the emergence of adults from the overwintering generation, egg hatching, larval, and pupal development, as well as generation time. These models, all based on a linear relationship between temperature and developmental rate, have been used to time pesticide application for Mediterranean fruit fly (Duyck *et al.*, 2002; Grout and Stoltz, 2007; Duyck and Quilici, 2002). Linear approximation enables the estimation of lower temperature thresholds and thermal constants within a limited temperature range (Campbell *et al.*, 1974; Honek 1999; Howell and Neven, 2000; Jarosik *et al.*, 2002). The curvilinear models have not been routinely used of their complexity (Howell and Neven, 2000). Temperature is the single most important environmental factor determining development and survival of Tephritid fruit flies (Fletcher, 1989). Temperature effects on the development and stage-specific survival have been shown to influence both the quantity and quality of Tephritid fruit flies produced (Vargas *et al.*, 1996; Vargas *et al.*, 1997; Brévault and Quilici, 2000; Vargas *et al.*, 2000; Duyck and Quilici, 2002; Trudgill *et al.*, 2005; Grout and Stoltz, 2007; Rwomushana *et al.*, 2008; Vayssières *et al.*, 2008; Liu and Ye, 2009; Salum *et al.*, 2013). Various Tephritid species have specific optimal temperature ranges for development that are limited by the lower and upper thresholds. Development does not occur below and above these temperature limits, and this can vary both with developmental stage and geographical origin (Honék and Kocourek, 1990). Information on the thermal

requirements of insect groups forms an essential basis for understanding and predicting the geographical distribution of the different insect groups.

This is the first study in which two linear and 26 nonlinear models have been used to model the effect of temperature on the development of this important fruit fly. The results will contribute to improve Integrated Pest Management (IPM) programs.

MATERIALS AND METHODS

Rearing Methods

To establish and maintain the insect colony and conduct the experiment, fruits infected with Mediterranean fruit fly were collected from citrus orchards in Mazandaran and Fars Provinces. Infected citrus fruits were transferred to the growth chamber (in plastic boxes on sterilized sand) and phytotron (rearing of colony) at a temperature of $25\pm 1^{\circ}\text{C}$ and $60\pm 10\%$ RH and 16:8 (L:D). Larvae and adults were reared for two generations on artificial food (bran, yeast, water, sugar, sodium benzoate and citric acid) and hydrolyzed protein in Petri dishes and large cylindrical containers (Mafi Pashaklai, 2013).

Experimental Conditions

Rearing was conducted at (10, 12, 15, 17, 20, 25, 27, 30, 32, and $35\pm 1^{\circ}\text{C}$), $60\pm 5\%$ RH, and a photoperiod of 16:8 (L:D) h in growth chambers. The environmental conditions of each phytotron were monitored with a temperature and relative humidity data logger.

Egg, Larval and Pupal Development

Three hundred to 1,000 eggs in groups of 100, less than one day old, were incubated on filter paper at 10, 12, 15, 17, 20, 25, 27, 30, 32, and $35\pm 1^{\circ}\text{C}$. All eggs were checked

daily for hatching. The daily growth and development on artificial food and sterilized sand for larvae and pupae was monitored and recorded until the emergence of adult flies.

Developmental Rate and Mathematical Models

Developmental rate is the reciprocal of developmental time in days. These rates are used in linear and nonlinear models where data are added daily (Arbab *et al.*, 2006). Development is completed when the sum of daily developmental rate values equals 1 (Curry *et al.*, 1978). Therefore, the integral of the developmental rate function over time can be used to simulate the development of an organism exposed to different temperatures (Arbab *et al.*, 2006). The ordinary and Ikemoto linear and 26 nonlinear descriptive models were used to determine the relationship between temperature and Mediterranean fruit fly developmental rate. The parameters of interest are the lower and upper temperature thresholds ($T_{\min}$ and $T_{\max}$, respectively), the optimal Temperature (T_{opt}), and the thermal constant (K). Most models can estimate two or more parameters. In addition to the ordinary model, the Ikemoto linear model was used to obtain more reliable estimates of the lower temperature threshold and thermal constant (Ikemoto and Takai, 2000).

Three criteria including the Sum of Squared Error (SSE), adjusted coefficient of determination (R^2_{adj}), and Akaike Information Criterion (AIC) were used to evaluate the nonlinear models. All nonlinear models in each stage were ranked using AIC, as the best statistical criterion (Akaike 1974), and the model with the smallest value of AIC was considered to be the best model for describing the temperature-dependent development of *C. capitata*. According to Burnham *et al.* (2011), models with $\Delta > 7$ were dismissed where Δ is the difference between AIC of the best model and the i^{th} model. T_{fast} , the temperature that the

maximum development rate occur was calculated directly from some of the nonlinear models (Arbabtafti *et al.*, 2023). In addition to statistical criteria accuracy (Kontodimas *et al.*, 2004), biological significance (Briere *et al.*, 1999) were considered to select the best nonlinear model. The observed total development times of *C. capitata* in Mazandaran and Fars Provinces were compared with those estimated using the selected nonlinear models.

Statistical Analysis of Developmental Rate

To determine the effect of different temperatures on the developmental time of the Mediterranean fruit fly, data were checked for normality. Then, one-way Analysis Of Variance (ANOVA) was used to determine the significant differences in developmental time of total immature stages (from egg to pupal stage) at constant temperatures (Minitab, 2000). The differences among the treatments were compared using Tukey's test ($\alpha = 0.05$). Comparison of development time of the two provinces was done by the Student's t-test. Minitab (ver. 19.2) software was used for all analyses. Excel 2016 was used for graph construction. Evaluation of the two linear and 26 nonlinear models was done by using Arthro Thermo Model (ATM) software (Mirhosseini *et al.*, 2017) to describe the development rate (the reciprocal of development time) of *C. capitata* as a function of temperature. The ATM software calculates criteria and parameters for all models.

RESULTS

Developmental Time

No development occurred at 10 and 12°C (in Fars Province) and 10, 12, 15 and 17°C (in Mazandaran Province). The mean developmental time of total immature stages (from egg to pupal stage) at ten constant temperatures in two provinces, is shown in Table 1. One-way ANOVA showed a

**Table 1.** Developmental time of *Ceratitis capitata* total immature stages at ten constant temperatures.^a

Geographical population	Temperature (°C)	Total (Day) Mean±SE
Mazandaran	10	-
	12	-
	15	52.50±0.5 ^{aA} N=2
	17	38.2±0.87 ^{bA} N=20
	20	20.11±0.28 ^{cB} N=70
	25	15.09±0.22 ^{dA} N=94
	27	13.30±0.12 ^{eA} N=121
	30	11.24±0.21 ^{defA} N=66
	32	-
	35	13±0.57 ^{defA} N=3
	<i>F</i>	632.27
	<i>df</i>	6,369
	<i>P</i>	0.000
Fars	10	-
	12	-
	15	-
	17	-
	20	26.01±0.17 ^{aA} N=121
	25	14.52±0.16 ^{bA} N=129
	27	13.39±0.10 ^{cA} N=133
	30	11.22±0.14 ^{dA} N=68
	32	-
	35	-
	<i>F</i>	1832.81
	<i>df</i>	3,447
	<i>P</i>	0.000

^a Means followed by different lowercase letters in the columns are significantly different between different temperatures in each population (Tukey's test, $P < 0.05$) and the means followed with by capital letters were significantly different between two populations at each temperature (T-test, $P < 0.05$).

significant effect of temperature on development time for total immature stages of Mediterranean fruit fly in Mazandaran and Fars Provinces ($P < 0.05$). Total developmental time was extended at 15°C (52.50 days) and 20°C (26.01 days) in the population of Mazandaran and Fars Provinces, respectively.

Model Evaluation

Linear Models

Both linear models showed an acceptable fitness for total immature stages. The linear regression equation, the lower temperature threshold, and the thermal constant of the total immature stages of *C. capitata* are shown in Table 2. The Ikemoto linear model

Table 2. Linear regressions, lower Temperature threshold (T_{min}), and thermal constant (degree days) of *Ceratitis capitata* immature stages using two linear models.

Geographical population	Model	Stage	Linear equation	T_{min} (°C)	K (DD)	R^2	R^2_{adj}	P
Mazandaran	Ordinary	Egg-pupa	$R= 0.024385+0.0033905T$	7.1921	294.9416	0.84082	0.80898	0.003
	Ikemoto	Egg-pupa	$DT= 228.8627+10.8028D$	10.8028	228.8627	0.94398	0.93278	0.00007
Fars	Ordinary	Egg-pupa	$R= 0.060777+0.0050415T$	12.0553	168.3525	0.98692	0.98039	0.006
	Ikemoto	Egg-pupa	$DT= 188.5943+12.695D$	12.695	188.5943	0.99341	0.99011	0.0002

had a higher value of R^2 and R^2_{adj} than the ordinary model, indicating a slight degree of confidence in parameter estimates provided by the Ikemoto linear model. In addition to the ordinary model, the Ikemoto and Takai linear models were used to obtain more reliable estimates of the lower temperature threshold and thermal constant (Ikemoto and Takai, 2000). The Ikemoto linear model estimated lower temperature thresholds for total developmental of *C. capitata*, were 10.80 and 12.69°C, while the thermal constants of the total immature stages were 228.86- and 188.59-Degree Days (DD) in Mazandaran and Fars Provinces, respectively.

Nonlinear Models

The curves of the influence of temperature on the developmental rate of the total immature stages (from egg to pupae stage) fitted by 18 models in Mazandaran Province (Figure 1) and five models in Fars Province are shown in Figure 2. The values of R^2 , RSS (SSE), AIC (Akaike Information Criterion), and R^2_{adj} used to determine the goodness-of-fit, the models of the nonlinear models of the Mazandaran Province are shown in Table 3. Considering the AIC and biological criteria (T_0 , T_U , and T_{opt}), the Logan 6 model had the poorest and the Briere-1 model had the best fitness to the data for total immature development in Mazandaran (Table 5). The values of measurable parameters of the nonlinear developmental rate models in Fars are presented in Table 4. Among the non-linear

models obtained from the Fars, only the polynomial model was accepted for total immature development based on AIC, however, biological criteria (T_0 , T_U , and T_{opt}) had the poorest ability to provide the growth and development model. Performance-2, and Briere-1 models provided more accurate estimates for T_0 , T_U , and T_{opt} in Fars Province (Table 6).

DISCUSSION

Determining the developmental time at different temperatures is necessary to calculate the developmental rate. The present findings in Mazandaran were different from the results of Ricalde *et al.* (2012) and Grout and Stoltz, 2007 at similar temperatures. Therefore, Ricalde *et al.* (2012), obtained the most extended period of developmental time of total immature stages of *C. capitata* was 71.20 days at 15°C and the shortest, 16.90 days at 30°C. Furthermore, Grout and Stoltz (2007) reported that the longest developmental time of the total immature stages of *C. capitata* at 14°C was 83.6 days and the shortest, 21.2 days at 30°C. The differences in the obtained results can be caused by regional climatic variability (e.g., temperature, humidity and rainfall), which can affect the development and survival of *C. capitata* populations (Papadogiorgou *et al.*, 2024). Linear models only estimate a lower temperature threshold, and this is proper for analysis of the phenology of insect populations due to simplifying the analysis (Ikemoto and Kiritani, 2019).



Table 3. Comparison of 26 developmental rate models based on the number of parameters, SSE, Akaike Information Criterion (AIC), and adjusted R^2 (R^2_{adj}) for predicting egg, larva, pupa and total immature development stages of *Ceratitis capitata* in Mazandaran Province.

Model	No. of parameters	Total			
		SSE	R^2_{adj}	AIC	Rank ^a
Pradhan-Taylor	3	8.000005	0.9698	-73.1459	6
Davidsons logistic	3	0.0043	-0.4999	-45.8029	24
Logan-6	4	1.00004	0.9454	-69.0103	18
Hilbert and Logan	5	9.3209e-05	0.9346	-68.5861	19
Lactin-1	3	1.00004	0.9408	-68.4299	20
Lactin-2	4	5.000005	0.9752	-74.5425	3
Logan-10	5	9.000005	0.9552	-68.3887	21
Analytis-1	5	6.000005	0.9538	-71.0189	12
Analytis-2	5	8.000005	0.9414	-69.3496	17
Analytis-1/Allahyari	5	7.000005	0.9498	-70.431	15
Analytis-3	5	6.000005	0.9530	-70.8958	14
Briere-1	3	6.000005	0.9785	-75.5218	1
Briere-2	4	6.000005	0.9716	-73.5736	5
Analytis-3/Kontodimas	3	7.000005	0.9746	-74.3637	4
Janisch/Kontodimas	4	8.000005	0.9610	-71.3618	11
Janisch/Rochat	4	1.00004	0.9509	-69.747	16
Sharpe and DeMichele	7	0.0274	NaN ^b	-26.8023	26
Sharp and DeMichele/Schoolfield	7	0.0039	NaN	-40.4897	25
Sharp and DeMichele/Kontodimas	6	0.0013	-0.7976	-48.2395	23
Polynomial (cubic)	4	7.000005	0.9662	-72.3688	9
Sharpe-Schoolfield-Ikemoto (SSI model)	7	3.000005	NaN	-71.4554	10
Performance-1	5	4.000005	0.9653	-73.0182	7
Performance-2	4	5.000005	0.9766	-74.9404	2
Wang	6	4.000005	0.9303	-70.9876	13
Ratkowsky	4	3.00004	0.9705	-61.6343	22
Beta'	4	7.000005	0.9669	-72.5221	8

^a Rank is based on the AIC criteria. ^b NAN: The number of model parameters is equal to or greater than the observations and cannot be calculated Model.

- Data could not be fitted by the model.

In the present study, lower temperature thresholds and thermal constant were estimated using both ordinary and Ikemoto linear models. A comparison of the total developmental time at different temperatures showed that the linear range was up to 30°C for the population of Mazandaran and Fars Provinces. The R^2_{adj} coefficients used to fit the regression between temperature and the developmental rate were higher for the Ikemoto linear model on the two populations tested.

The lower temperature threshold for total immature stages was estimated by the

Ikemoto linear model at 10.80 and 12.69°C, for Mazandaran and Fars Provinces, respectively. The lower temperature threshold values estimated by Ricalde *et al.* (2012) (9.10, 9.30, 9.60°C) and Grout and Stoltz (2007) (9.9°C) are closer in our result of Mazandaran Province. Based on Honek and Kocourek (1990) T_0 decreased if K increased therefore, the thermal constant of total immature stages of *C. capitata* for the two linear ordinary and Ikemoto linear models were obtained at 294.94- and 228.86-degree days in Mazandaran

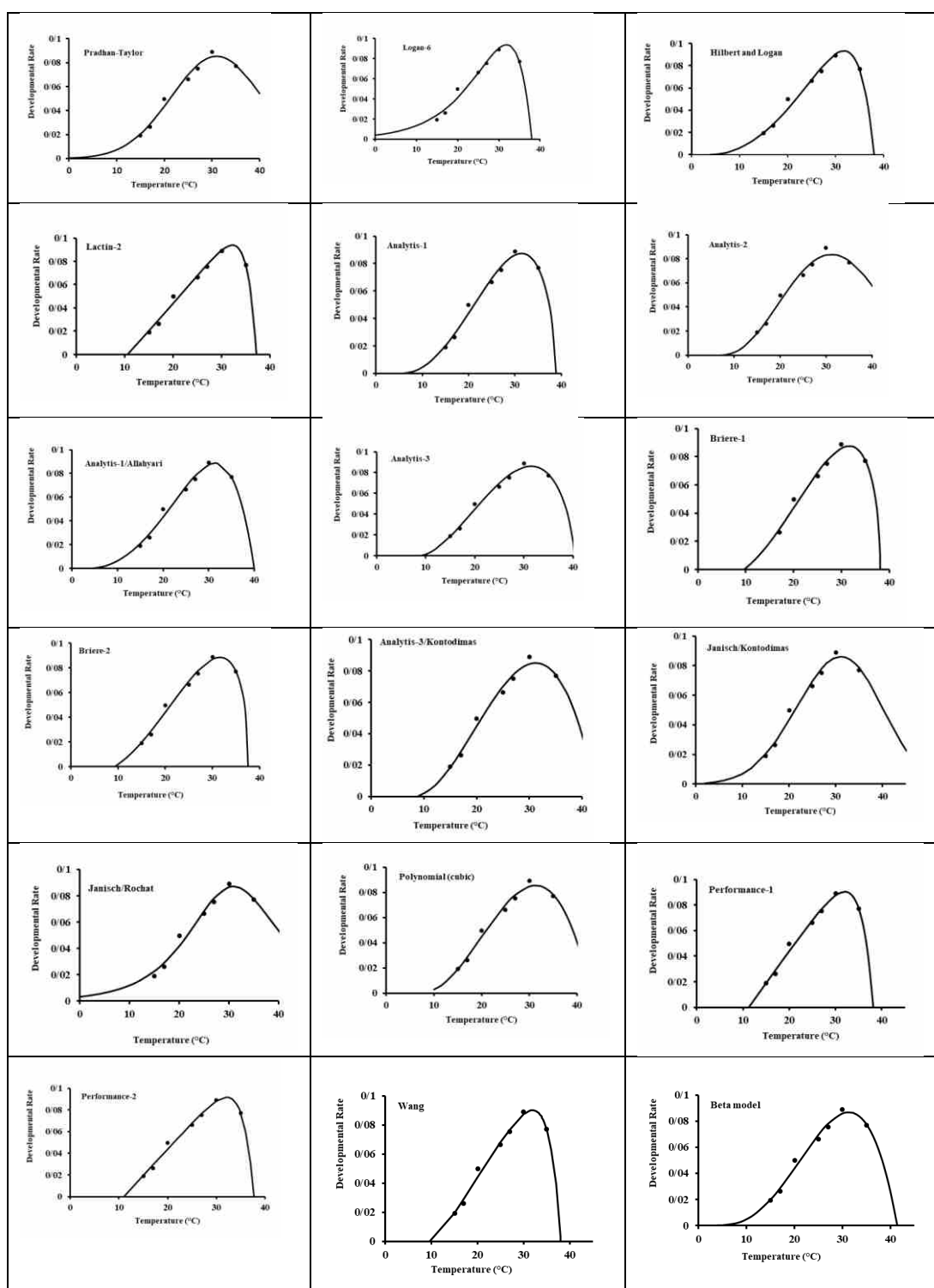


Figure 1. Observed development rate for the total immature stages of Mazandaran Province of *Ceratitis capitata* (dots) and 18 fitted nonlinear models (lines).

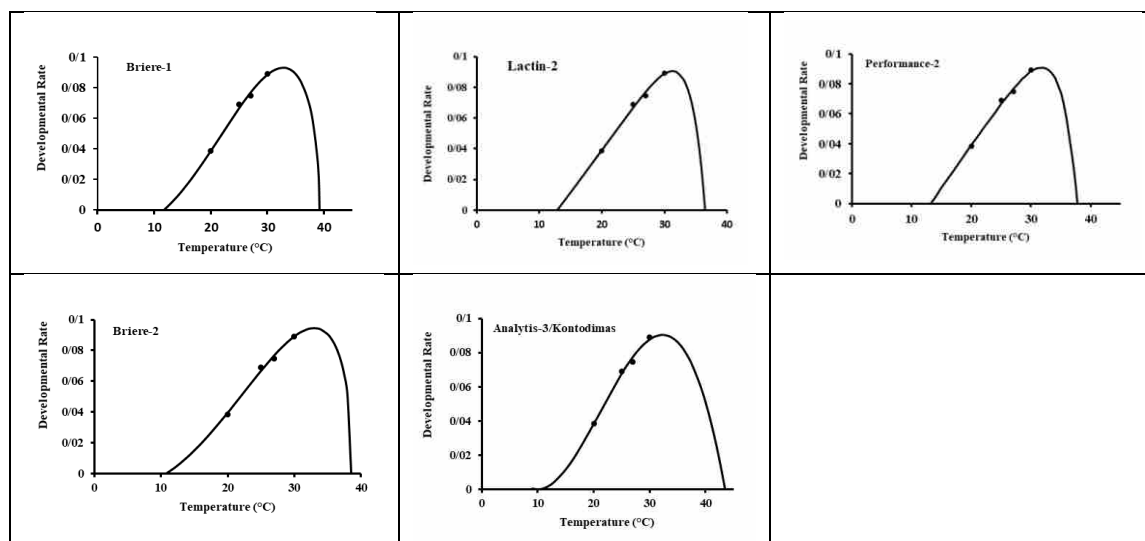


Figure 2. Observed development rate for total immature stages of Fars Province of *Ceratitis capitata* (dots) and 5 fitted nonlinear models (Lines).

Table 4. Comparison of 26 developmental rate models based on the number of parameters, SSE, Akaike Information Criterion (AIC), and adjusted R^2 (R^2_{adj}) for predicting total immature development of *Ceratitis capitata* in Fars Province.

Model	No. of parameters	Total			
		<i>SSE</i>	R^2_{adj}	<i>AIC</i>	Rank ^a
Pradhan-Taylor	3	0.00001	0.9763	-45.3017	3
Davidsons logistic	3	0.0014	-1.9999	-25.9319	13
Logan-6	4	0.00001	NaN ^b	-41.6177	10
Hilbert and Logan	5	- ^c	-	-	-
Lactin-1	3	0.00001	0.9647	-43.7057	5
Lactin-2	4	0.00001	NaN	-43.3518	8
Logan-10	5	-	-	-	-
Analytis-1	5	-	-	-	-
Analytis-2	5	-	-	-	-
Analytis-1/Allahyari	5	-	-	-	-
Analytis-3	5	-	-	-	-
Briere-1	3	0.000009	0.9791	-45.7944	2
Briere-2	4	0.00001	NaN	-43.5121	7
Analytis-3/Kontodimas	3	0.00001	0.9749	-45.0691	4
Janisch/Kontodimas	4	0.0015	NaN	-23.6038	14
Janisch/Rochat	4	0.00001	NaN	-40.8527	11
Sharpe and DeMichele	7	-	-	-	-
Sharp and DeMichele/Schoolfield	7	-	-	-	-
Sharp and DeMichele/Kontodimas	6	-	-	-	-
Polynomial (cubic)	4	0.0000	NaN	-227.7655	1
Sharpe-Schoolfield-Ikemoto (SSI model)	7	-	-	-	-
Performance-1	5	-	-	-	-
Performance-2	4	0.00001	NaN	-43.5767	6
Wang	6	-	-	-	-
Ratkowsky	4	0.00004	NaN	-37.9192	12
Beta ^d	4	0.00001	NaN	-43.2408	9

^a Rank is based on the AIC criteria. ^b NAN The number of model parameters is equal to or greater than the observations and cannot be calculated Model. ^c Data could not be fitted by the model.

Table 5. Values of the fitted coefficients and measurable parameters of 18 developmental rate models to describe immature stage development of the *Ceratitidis capitata* in Mazandaran Province.

Model	Parameter	Value
Pradhan-Taylor	r_m	0.08524 (0.07732, 0.09316)
	T_{opt} (°C)	31
	T_{σ} (°C)	9.459 (7.418, 11.5)
Logan-6	Δ_T	4.99 (-27.04, 37.02)
	ψ	0.004267 (-0.00202, 0.01055)
	ρ	0.1296 (-0.4758, 0.735)
	T_{max} (°C)	38.01 (31.58, 44.44)
	T_{opt} (°C)	31.9
Hilbert and Logan	D	56.45 (-1.123e+05, 1.124e+05)
	Δ_T	3.526 (-62.35, 69.41)
	ψ	0.5187 (-2048, 2049)
	T_{min} (°C)	3.911 (-112.4, 120.3)
	T_{max} (°C)	40 (-140.6, 220.6)
	T_{opt} (°C)	31.72
	Δ	1.929 (-4.485, 8.344)
Lactin-2	λ	-1.047 (-1.08, -1.015)
	ρ	0.00435 (0.002843, 0.005857)
	T_{min} (°C)	10.59
	T_{max} (°C)	41.55 (20.71, 62.39)
	T_{opt} (°C)	32.3
	P	0.3931 (-11.38, 12.16)
	m	0.6314 (-14.28, 15.54)
Analytis-1	n	2.2 (-29.77, 34.17)
	T_{min} (°C)	5.52 (-142.3, 153.3)
	T_{max} (°C)	38.93 (-58.59, 136.4)
	T_{opt} (°C)	31.09
	P	3.286e+05 (-4.495e+12, 4.495e+12)
	m	2.735 (-25.89, 31.35)
	n	4.132 (-3.505e+06, 3.505e+06)
Analytis-2	T_{min} (°C)	6.376 (-94.12, 106.9)
	T_{max} (°C)	55.92 (-39.66, 151.5)
	T_{opt} (°C)	31.09
	P	0.2319 (-6.458, 6.922)
	m	4 (-96.35, 104.4)
	n	2.038 (-54.85, 58.92)
	T_{min} (°C)	3.903 (-328, 335.8)
Analytis-1/Allahyari	T_{max} (°C)	39.97 (12.65, 67.29)
	T_{opt} (°C)	31.09
	a	9.417e-05 (-0.0102, 0.01039)
	m	0.6681 (-14.83, 16.17)
	n	1.72 (-17.79, 21.23)
	T_{min} (°C)	8.793 (-72.46, 90.05)
	T_{max} (°C)	40.2 (-84.11, 164.5)
Analytis-3	T_{opt} (°C)	31.09
	a	4.951e-05 (3.529e-05, 6.373e-05)
	t_{min} (°C)	9.553 (6.264, 12.84)
	T_{max} (°C)	38.07 (36.43, 39.7)
	T_{opt} (°C)	31.9
	a	5.522e-05 (-0.0001026, 0.0002131)
	n	2.192 (-3.56, 7.945)

Table 5 continued...



Continued of Table 5.

Model	Parameter	Value
Analytis-3/Kontodimas	T_{min} (°C)	9.267 (-0.188, 18.72)
	T_{max} (°C)	37.62 (25.82, 49.42)
	T_{opt} (°C)	31.64
	a	1.351e-05 (6.226e-06, 2.08e-05)
	T_{min} (°C)	7.963 (5.177, 10.75)
	T_{max} (°C)	42.87 (39.27, 46.47)
Janisch/Kontodimas	T_{opt} (°C)	31.23
	D_{min}	4.968 (0.4977, 9.439)
	k	0.0742 (-0.2308, 0.3792)
	λ	0.05016 (-0.06608, 0.1664)
Janisch/Rochat	T_{opt} (°C)	34.25 (-13.09, 81.58)
	C	0.08659 (0.05224, 0.1209)
	a	1.116 (0.7389, 1.492)
	b	1.143 (0.9574, 1.328)
	T_{max} (°C)	30.06 (10.85, 49.28)
	T_{opt} (°C)	30.9
Polynomial (cubic)	a_0	-1.372e-05 (-4.413e-05, 1.669e-05)
	a_1	0.000812 (-0.001498, 0.003122)
	a_2	-0.01055 (-0.06684, 0.04575)
	a_3	0.04067 (-0.3963, 0.4777)
	T_{min} (°C)	42.849
	T_{max} (°C)	31.3
Performance-1	T_{opt} (°C)	31.3
	C	2.162 (-474.5, 478.8)
	$K1$	0.002354 (-0.5263, 0.531)
	$K2$	0.3431 (-3.903, 4.59)
	T_{min} (°C)	11.23 (-3.707, 26.17)
	T_{max} (°C)	38.1 (14.27, 61.92)
Performance-2	T_{opt} (°C)	31.94
	$K2$	0.3956 (-0.8109, 1.602)
	m	0.004867 (0.002948, 0.006786)
	T_{min} (°C)	10.99 (7.62, 14.36)
	T_{max} (°C)	37.71 (30.48, 44.93)
	T_{opt} (°C)	32.071
Wang	C	0.2473 (-68.09, 68.59)
	$K1$	0.001469 (-14.51, 14.51)
	$K2$	0.3611 (-75.46, 76.18)
	m	3.15 (-3.069e+04, 3.07e+04)
	T_{min} (°C)	9.626 (-89.9, 109.2)
	T_{max} (°C)	38.06 (-284.2, 360.3)
Beta	T_{opt} (°C)	31.97
	r_m	0.08657 (0.07226, 0.1009)
	T_{min} (°C)	3.865 (-35.25, 42.98)
	T_{max} (°C)	41.44 (31.5, 51.38)
	T_{opt} (°C)	31.29 (29.23, 33.35)

Province, while it was 168.35- and 188.59-degree days in Fars Province, respectively. The result reported by Grout and Stoltz

(2007) 337.8 (DD), and Ricalde *et al.* (2012) 350, 341 and 328 (DD) were higher than our results.

Table 6. Values of the fitted coefficients and measurable parameters of 5 developmental rate models to describe total immature stage development of the *Ceratitis capitata* in Fars Province.

Model	Parameter	Value
Lactin-2	Δ	-
	λ	-
	ρ	-
	T_{min} (°C)	-
	T_{max} (°C)	-
	T_{opt} (°C)	-
Analytis-3	a	-
	m	-
	n	-
	T_{min} (°C)	-
	T_{max} (°C)	-
	T_{opt} (°C)	-
Briere-1	a	5.284e-05 (-0.0001954, 0.0003011)
	t_{min} (°C)	11.61 (-21.42, 44.65)
	T_{max} (°C)	39.25 (-21.12, 99.62)
	T_{opt} (°C)	32.81
Briere-2	a	6.26e-05
	n	2.383
	T_{min} (°C)	10.78
	T_{max} (°C)	38.57
Performance-2	T_{opt} (°C)	33.01
	$K2$	0.3271
	m	0.005697
	T_{min} (°C)	13.15
	T_{max} (°C)	37.74
	T_{opt} (°C)	31.8

Many abiotic factors affect the growth and development of insects. Temperature is the most significant environmental factor influencing insect development, survival, behavior, and distribution (Fletcher, 1989). Biological parameters like developmental zero and the thermal constant are supposed to be the limiting factors in the geographic distribution of fruit flies (Ye, 2001). The developmental response of insects to temperature can help to predict their occurrence and, therefore, assist in monitoring and control strategies for pests. Different species of Tephritidae have particular optimal temperature ranges for development, which are limited by low and high thresholds (Honék and Kocourek, 1990). Different temperature characteristics, may be affected by pest species (Honék, 1999), pest population (Lee and Elliott, 1998), growth, and development stages

(Honék, 1996; Kocourek and Stara, 2005) and other ecological factors such as food source (Golizadeh *et al.*, 2007), and interspecies and intraspecies competition (Duyck and Quilici, 2004) and the difference may be due to one or a set of the above factors. Model selection is critical because of the significant differences between model predictions. Rebaudo and Rabhi (2018) point out that each of the criteria for model selection has its advantages and disadvantages, therefore, a combination of different methods should be used in model selection, e. g. the AIC criteria can separate several models with the same R^2_{adj} and SSE. In most studies, the AIC index has been mentioned as the best statistical parameter to measure the validity of the models. Furthermore, model selection should be performed based on observations and biological and ecological information or



biological significance (Arbabtafti *et al.*, 2023). A standard method for evaluating the accuracy of the estimated critical temperatures is based on their comparison with experimental data (Kontodimas *et al.*, 2004).

CONCLUSIONS

The findings of this study, especially in relation to temperatures, can be used to accurately predicting *C. capitata* population development in different provinces and enable us to choose the best time for controlling this pest. Since the development rate of *C. capitata* may be influenced by factors such as host plants of *C. capitata*, further studies should be done on different host plants to obtain the best development models.

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رشد نمو وابسته به دما و آستانه‌های دمایی کل مراحل نابالغ مگس میوه مدیترانه‌ای،
Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae)
ایران

نجمه ابراهیمی

چکیده

مگس میوه مدیترانه‌ای (*Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae)) یکی از مهمترین آفات محصولات کشاورزی مناطق گرمسیری و نیمه-گرمسیری جهان است. در این پژوهش نرخ رشد و نمو مگس میوه مدیترانه‌ای در دماهای 10، 12، 15، 17، 20، 25، 27، 30، 32، 35 درجه سلسیوس بررسی شد. یافته‌های حاصل وجود رابطه غیرخطی بین دما و نرخ رشد و نمو را نشان داد. از بین مدل‌های غیر خطی، مدل پرفورمانس-1 و پرفورمانس-2 به ترتیب در دو استان مازندران و فارس بیشترین برازش را روی مقادیر مشاهده شده نرخ رشد و نمو را نشان دادند. در استان مازندران، مدل پرفورمانس-1 ضمن شبیه سازی نزدیک به واقعیت رشد و نمو مگس میوه مدیترانه‌ای در گستره دمایی 15 تا 35 درجه سلسیوس، دمای بهینه رشد و نمو کل دوره نابالغ مگس میوه مدیترانه‌ای را 31.94 درجه سلسیوس برآورد کرد و همچنین در استان فارس مدل پرفورمانس-2، در گستره دمایی 20 تا 30 درجه سلسیوس، دمای بهینه رشد و نمو کل دوره نابالغ مگس میوه مدیترانه‌ای را 31.8 درجه سلسیوس برآورد کرد. دمای آستانه پایین برای رشد و نمو کل دوره نابالغ مگس میوه مدیترانه‌ای در استان مازندران با استفاده از مدل پرفورمانس-1، 11.23 و در استان فارس با استفاده از مدل پرفورمانس-2، 13.15 درجه سلسیوس برآورد شد. دمای آستانه بالا نیز برای رشد و نمو کل مراحل نابالغ مگس میوه مدیترانه‌ای در استان مازندران با استفاده از مدل پرفورمانس-1، 38.1 درجه سلسیوس و در استان فارس مدل پرفورمانس-2، 37.74 درجه سلسیوس، تخمین زده شد. در بین مدل‌های خطی مورد بررسی، مدل خطی ایکیموتو توانست دمای آستانه پایین و نیاز دمایی مراحل مختلف نابالغ مگس میوه مدیترانه‌ای را با دقت بیشتری برآورد نماید.

Volatile Organic Compounds (VOC) Produced by *Paraconiothyrium archidendri* F10 as Biofungicidal Materials for *Ganoderma boninense*

Anisa Lutfia^{1*} and Bedah Rupaedah¹

ABSTRACT

In this study, a soil fungus isolated from a healthy, disease-free oil palm plantation was evaluated for its inhibitory activity *in vitro*, with the aim of assessing its effectiveness as a bio-inoculant. The soil fungus was sequenced for the ITS-rDNA region, and its similarity was analyzed through bioinformatics using BLASTn searches and phylogenetic tree construction. Volatile Organic Compounds (VOCs) were produced through batch fermentation on Potato Dextrose Agar (PDA). The inhibitory activity against the radial growth of *G. boninense* was evaluated using the vapor assay method. The VOC profile and other metabolites were analyzed using GC-MS. The inhibitory mechanism between VOCs and target proteins was studied through *in silico* analysis. VOCs produced by *P. archidendri* F10 were found to inhibit *G. boninense* mycelium growth by up to 55.8% in four days, with the mycelium exhibiting wavy, non-smooth, and wrinkled morphology, abnormal branching, fused, defective hyphae, and lysis through microscopy imaging. The molecular docking analysis revealed that 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione had the strongest binding affinity at -8.5 kcal mol⁻¹, forming one hydrogen bond with Tyr646 at a distance of 2.98 Å. Another notable ligand was 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate, with a binding affinity of -5.6 kcal mol⁻¹ and one hydrogen bond with His698 at 3.05 Å. The remaining ligands did not form hydrogen bonds. Thus, *P. archidendri* F10 has potential as a biofungicide for controlling *G. boninense* in the future.

Keywords: Antifungal metabolites, Basal stem rot, Biofungicide, Soil fungus, Vapour assay.

INTRODUCTION

The pathogenic fungus, *Ganoderma boninense* Pat., is a significant issue in industrial plants, leading to basal stem rot disease and a decrease in oil palm production. *Ganoderma boninense* Pat. is a soil-borne fungal pathogen that causes Basal Stem Rot (BSR) disease in oil palm (Paterson, 2019). The pathogen is difficult to control, and the use of synthetic fungicides is not a sustainable solution, given their adverse impact on the environment and public health. Synthetic fungicides, such as dazomet and hexaconazole, offer a

temporary solution for controlling *G. boninense* (Maluin *et al.*, 2020). However, the prolonged use of synthetic antifungal agents can lead to the antifungal resistance, death of non-target microorganisms, and degradation of ecosystem function (Fang *et al.*, 2018). One approach that has gained attention in recent years is biological control, which involves the use of Microbial Biocontrol Agents (BCAs) to control plant diseases. Fungi have been shown to be effective biocontrol agents against a wide range of phytopathogens due to their diverse mechanisms, including antibiosis, host resistance induction, mycoparasitism, and niche competition for nutrients and space

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(Latz *et al.*, 2018). One promising area of research in fungal BCAs is Volatile Organic Compounds (VOCs) that exhibit strong inhibitory activity against phytopathogenic microbes. These compounds are defined as small, carbon-based molecules that have a low water solubility and a high vapour pressure, which allows them to be present in a gaseous state under normal ambient conditions, such as at a pressure of 1 atm and a temperature of 25°C. VOCs are a blend of volatile metabolites produced by both microbial and plant sources, which is referred to as "volatilome" (Farbo *et al.*, 2018). These compounds are distinguished by functional effects in the soil, greater ability to disperse, and stronger antifungal properties. *Muscodor albus* (Xylariaceae) was the first commercially and successful fungus being a BCA, known for its bioactive volatilome. This endophytic fungus, found in *Cinnamomum zeylanicum*, produces a range of volatiles, including acids, alcohols, esters, and terpenoids that exhibit antimicrobial activity against post-harvest pathogens responsible for the decay of perennial fruit trees (Saxena and Strobel, 2021). In more recent studies, some fungal species have been investigated and reported to produce antifungal VOCs such as *Aureobasidium pullulans* (Sarcotheciaceae) (Don *et al.*, 2021), *Lasiodiplodia avicenniae* (Botryosphaeriaceae) (Hartanto *et al.*, 2023), *Sarocladium brachiariae* (Sarocladiaceae) (Yang *et al.*, 2021), *Trichoderma atroviride* (Hypocreaceae) (Rao *et al.*, 2022), and *Trichoderma koningiopsis* (Kong *et al.*, 2022).

In this study, the development of effective BCAs for controlling *G. boninense* was investigated in soil inhabitants of healthy and non-infected oil palm plantations. The aim of this study was to evaluate the *in vitro* bioactivity of a soil fungus obtained from local oil palm plantations against *G. boninense*. The results will contribute to the development of sustainable and eco-friendly approaches to controlling BSR disease, ensuring the continued productivity of oil palm cultivation while reducing the impact

of synthetic biocides on the environment and public health.

MATERIALS AND METHODS

Fungal Isolate and Molecular Identification

The soil fungus, *Paraconiothyrium* isolate F10, was isolated from a healthy and uninfected oil palm plantation soils in Bogor, Indonesia. The pathogenic fungus, *G. boninense* strain SSU008 used in this study, is a collection of the Indonesian Oil Palm Research Institute (PPKS Marihat), Simalugun Regency, North Sumatra, Indonesia. This study was conducted in April 2023 at the Laboratory of Microbiology, Research Centre for Applied Microbiology, National Research and Innovation Agency (BRIN), Serpong, Indonesia. The isolates were maintained in Potato Dextrose Agar (PDA) medium. Molecular identification was performed commercially by sending the fungal specimen, isolate F10 to Macrogen, Inc. (Singapore). Raw sequences was retrieved and checked for its similarity to online database using BLASTn for ITS-rDNA region. A phylogenetic tree was constructed to assign the fungal species based on clustering analysis among accessions using MEGAXI. The confirmed species was submitted to GenBank and given with an accession code for *P. archidendri* F10 (OQ835627).

VOC Production in Submerged Fermentation

Potato Dextrose Broth (PDB) was used as a fermentation medium for VOCs production. The fungus, *P. archidendri* F10 was grown in 50 mL of PDB in a 250-mL flask at 28°C for 14 days. The Cell-Free Supernatant (CFS) was filtered using a Whatman filter paper No. 1 and centrifuged at 10,000×g for 15 minutes. The resulting

CFS was extracted thrice using a laboratory grade Ethyl Acetate (EtOAc) in a ratio of 1:1 and shaken vigorously for 3 days. The EtOAc layer was separated using a separator funnel and concentrated *in vacuo* using a Buchi Rotavapor® R-300.

Antifungal Vapor Assay

The antifungal activity of VOCs produced by *P. archidendri* F10 against *G. boninense* was assessed using a modified disc diffusion or vapor assay (Bismarck *et al.*, 2019). An active-growing colony of *G. boninense* was placed in the center of PDA medium, while a disc (Ø 6-mm) containing Ethyl Acetate (EtOAc) extract or saturated VOCs was placed in the center of the lid of the agar plate. The plates were then incubated for five days at 28°C while standing on their lids. A control plate was also prepared with only the colony of *G. boninense* in the center. The assay was performed in triplicate. The percentage of radial growth inhibition (%) was calculated as follows:

$$(\%) = [(D1 - D2)/D1] \times 100\%,$$

Where, D1 is the radial growth (mm) of the control plate and D2 is the radial growth of the treated plates. The surface morphology of the treated colony or mycelium was examined using a scanning electron microscope (JSM-6510LA JEOL SEM). The slide was fixed and coated with platinum (35 s: 30 mA) using 10kV.

GC-MS Profiling of VOC

A qualitative analysis of the sample containing VOCs was conducted using an Agilent column (Type 19091S-433: 93.92873 DB-5MS UI, 5% Phenyl Methyl Silox) with dimensions of 30 m×250µm×0.25 µm and a temperature range from 0 to 325°C (with a final hold time of 1 min) for injection. The analytical instrument used an Agilent-type 7890 (GC) and 5977A (MSD).

Molecular Docking of Antifungi as Anti-*Ganoderma boninense*

Major compounds as determined from the highest relative peak area were subjected to molecular docking studies. Chemical structure of each compound was retrieved from an online database. Protein target used in this study was chitin synthase as commonly involved in the cell wall synthesis, which serves as the factory of protective layer of *G. boninense*. The sequence of protein target with an entry code: A0A5K1JXQ5 was retrieved from UniProt database (<https://www.uniprot.org/>) and modelled utilizing the SWISS-MODEL web server (<https://swissmodel.expasy.org/>).

RESULTS AND DISCUSSION

Species Assignment of Isolate F10

Molecular identification based on the ITS-rDNA sequence analysis and BLASTn results showed that isolate F10 had the closest similarity (> 99%) with *Paraconiothyrium archidendri*. Further analysis through phylogenetic construction using a neighbor-joining tree showed that isolate F10 was clustered together and had a similar resemblance with *P. archidendri* CBS 168.77 (Figure 1), a type fungus material isolated from its host plant, *Archidendron bigeminum* (Fabaceae) in Myanmar (Verkley *et al.*, 2014). To our understanding, the discovery of *P. archidendri* F10 may be regarded as a new report as a soil inhabitant, especially from healthy oil palm plantation soils. Furthermore, it has been reported that certain related taxa belonging to the genera *Paracamarosporium* and *Pseudopithomyces* have been found to act as leaf endophytes, specifically in the petioles of *E. guineensis*. Notably, these taxa exhibit the ability to produce exopolysaccharides under laboratory conditions (Yurnaliza *et al.*, 2021). It can be implied that the presence of *Paraconiothyrium* members and other related taxa within the soils may establish

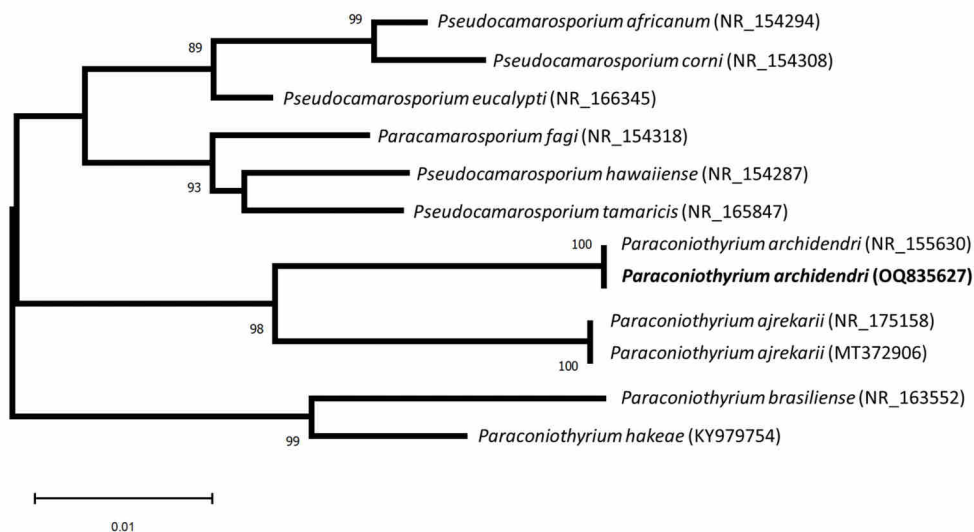


Figure 1. Phylogenetic tree of *P. archidendri* isolate F10 using ITS-rDNA for sequence homology studies. Sequence of reference strains was retrieved from GenBank accessions. Bootstrap value (%) of 1000 replications.

functional plant-microbe associations through a pathway that transitions from saprotrophic to hemi-/biotrophic modes in the living tissue of oil palm. However, the specific functional traits are yet to be fully uncovered. A more recent study has reported the occurrence of *P. archidendri* as a saprophytic fungus in the plant litters of *Magnolia* sp. in China (Tennakoon *et al.*, 2022). The presence of *P. archidendri* and other taxa within the Didymosphaeriaceae family is expected to have a crucial impact on nutrient cycling in forest ecosystems. This is because the majority of members within this family are cosmopolitan, and most of them are known to function as saprobes (Zhang *et al.*, 2012). The ability of these fungi to decompose organic matter, including plant litter, results in the release of crucial nutrients back into the soil. This process, in turn, promotes the growth and development of new vegetation, highlighting the critical role played by these fungi in the maintenance of healthy and sustainable ecosystems (Wanasinghe and Mortimer, 2022). There is still limited research on the role of *P. archidendri* as a biocontrol agent.

However, other related species, like *Paraconiothyrium brasiliense* LT161, have shown potential as bio-control agents due to their production of antifungal metabolites effective against various phytopathogens (Han *et al.*, 2012). Additionally, *Coniothyrium minitans*, reclassified as *Paraphaeosphaeria minitans*, has demonstrated mycoparasitic activity against *Sclerotinia sclerotiorum* (Verkley *et al.*, 2014; Patel *et al.*, 2021).

Inhibition of *Ganoderma boninense*

The production of VOCs by *P. archidendri* F10 was found to inhibit the growth of *G. boninense* mycelium (Figure 2). On PDA medium, the maximum growth of *G. boninense* was observed on the fifth day of incubation, reaching 9 cm. Conversely, on disc volatilization plate, the radial growth of *G. boninense* was halted at 4.81 cm. The inhibition commenced on the second day and peaked on the fourth day, with a recorded inhibition rate of 55.8%, which subsequently declined on the fifth day

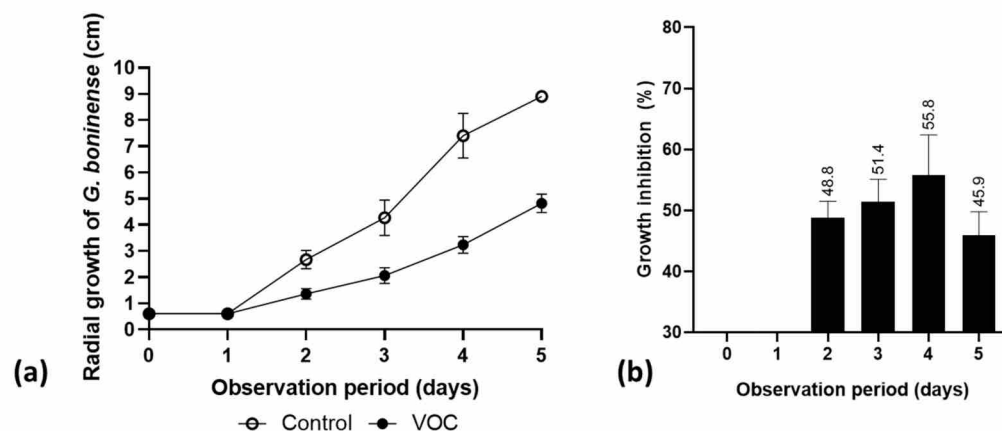


Figure 2. (a) Radial growth of *G. boninense* exposure to VOCs produced by *P. archidendri* F10, and (b) Growth inhibition (%). The error bars indicate the standard deviation that was calculated from three replicates.

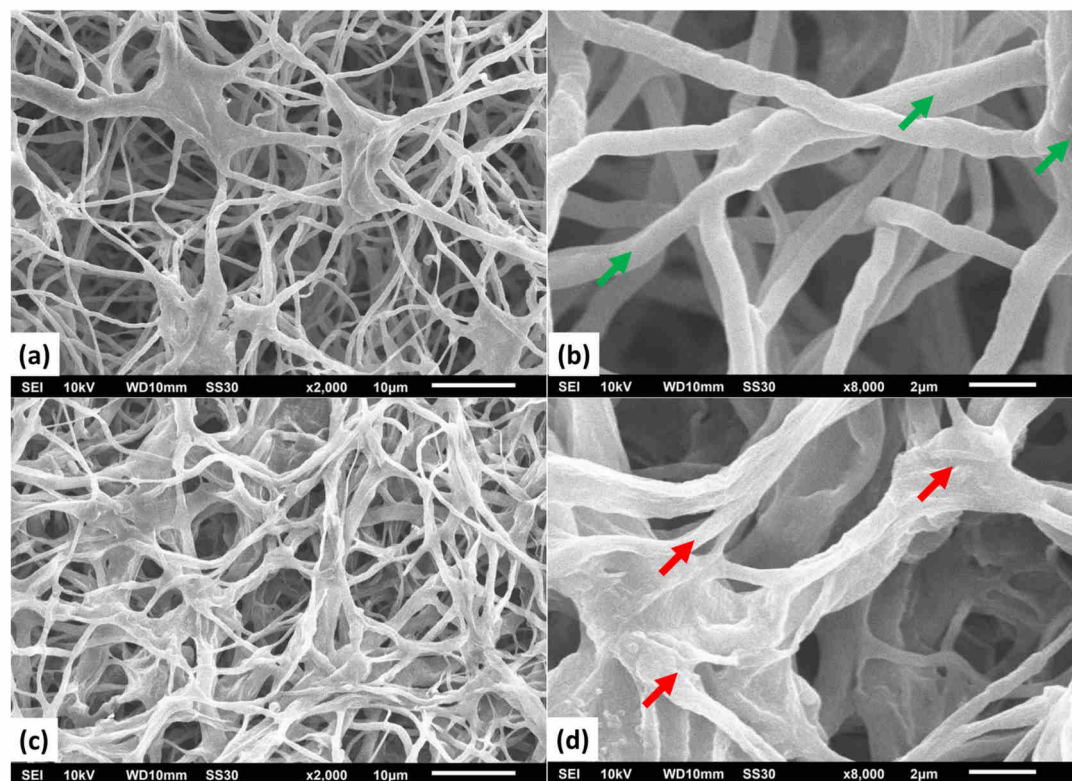


Figure 3. (a) The ultrastructure of *G. boninense* in the control plate at 2000 $\times$, and (b) 8000 $\times$ magnification. The images as pointed with green arrows showed smooth, and well-branched hyphal networks, indicative of healthy fungal growth without damage. (c) The ultrastructure of *G. boninense* after 5-d exposure to VOCs at 2000 $\times$, and (d) 8000 $\times$ magnification. The images as pointed with red arrows showed thinning, shrinkage, and reduced network density, with some sections collapsed or broken.



(45.9%). This decline may be attributed to the maximum growth of *G. boninense* colony (9 cm), which likely outcompeted the rate of inhibition induced by the VOCs. The observation of maximum inhibition of radial growth on the fourth day, followed by stagnation in inhibition thereafter, can be attributed to the assumption that the gaseous form of VOCs had diffused completely into the fungal colony and reached saturation. This may explain why there was no further inhibition of growth on the subsequent day. VOCs emission is a crucial antifungal mechanism exhibited by antagonistic microorganisms. The activity of these VOCs is observed to range from proximal interactions through water diffusion to distant interactions via air diffusion (Spadaro and Droby, 2016). Due to their volatile nature, microbial VOCs have shown great potential as biofumigants in air-tight environments (Tilocca *et al.*, 2020). These compounds possess physical properties that allow for the rapid saturation of the atmosphere with bioactive concentrations. When applied using an antagonistic isolate, the continuous exposure of VOCs may occur, leading to a potential permanent inhibition within a closed system, for example the porous soil against the soil-borne pathogen, *G. boninense*. The disc volatilization assay was initially employed to demonstrate the existence of VOCs produced by *P. archidendri* F10, which could then be subjected to profiling using Gas Chromatography-Mass Spectrometry (GC-MS). The ultrastructure of *G. boninense* mycelium following exposure to *P. archidendri* F10 VOCs after five days is presented in Figure 3. Under 2,000× magnification, the branching pattern and hyphal diameter of mycelium in the control and VOCs treatment were found to be nearly indistinguishable. However, at 8,000× magnification, differences between the two treatments were observed. Specifically, in the control plate, the mycelium displayed normal diameter, smooth surface, and proper branching. On the other hand, in the VOCs treatment, the mycelium appeared to be

wavy, non-smooth, and wrinkled, with abnormal branching and fused, defective hyphae that underwent lysis from within. This structural difference is likely responsible for the observed inhibition of maximum growth in *G. boninense* under the VOCs treatment. Similar observations of abnormal hyphal morphology and wrinkling have been reported in *G. boninense* exposed to VOCs produced by *Streptomyces* sp. GMR22 and *Nocardiosis alba* GME01 and GME22 (Islamiati *et al.*, 2022; Widada *et al.*, 2021).

Volatile Organic Compounds (VOC) as Antifungal Metabolites

GC-MS analysis was performed to determine the profile of VOCs produced by *P. archidendri* F10, which produced a total of 54 detections (Figure 4). A total of 27 VOCs was identified in the aromatic groups such as alcohols, alkanes, esters, ketones, and lipids. The major chemical components were esters while the relative abundance based on the percentage of peak area were ranked as follow: 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione (16.72%), followed by 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate (8.71%), methyl heptadecanoate (8.66%), butyl acetate (5.66%), and other minor components (<5%). The existence of 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione has been documented from various plant sources such as *Allium chinense* (Amaryllidaceae) (Rhetso *et al.*, 2021), *Garcinia cambogia* and *Garcinia indica* (Clusiaceae) (Jayakar *et al.*, 2020), *Nigella sativa* (Ranunculaceae) (Pachaiappan *et al.*, 2022), *Portulacaria afra* (Didiereaceae) (Tabassum *et al.*, 2023), and a seaweed, *Chara baltica* (Characeae) (Tatipamula *et al.*, 2019). Despite the lack of information towards its antifungal properties, this compound has been reported to exhibit several pharmacological activities, including antibacterial, antioxidant, anti-inflammatory, anti-analgesic, and cytotoxic effects (Pachaiappan *et al.*, 2022; Tabassum

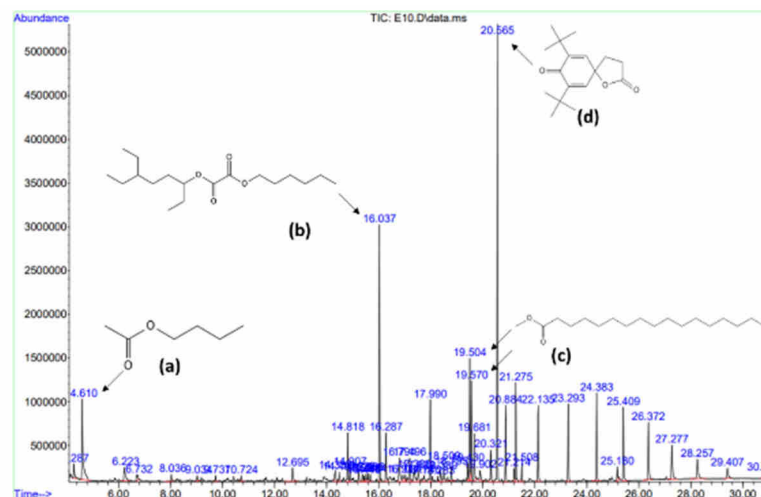


Figure 4. GC-MS spectra of EtOAc extract of *P. archidendri* F10 showing three major compounds based on the highest relative peak area (%). (a) butyl acetate, (b) 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate, (c) methyl heptadecanoate, and (d) 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione.

et al., 2023; Tatipamula *et al.*, 2019). The second most abundant compound, oxalic acid, 6-ethyloct-3-yl hexyl ester has been reported to display a broad spectrum of antifungal activity against *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium funiculosum*, and *Trichoderma reesei* in the form of phytosterols from *Anogeissus pendula*: Combretaceae (Sharma *et al.*, 2019). Methyl heptadecanoate, a member of the Fatty Acid Methyl Ester (FAME) family, shows promise as a bio-fungicide against *G. boninense*. These esters have also been designated as biomarkers to screen for resistant oil palm progenies, as they exhibit elevated expression levels during *G. boninense* interactions (Rozlianah *et al.*, 2015). Fatty acid derivatives have been identified as regulators of various responses to *G. boninense* in both non-infected and infected roots. The increased abundance of these metabolites in infected roots is attributed to their crucial role in pathogen defense mechanisms (Isha *et al.*, 2020). Therefore, the external application of *P. archidendri* F10 into oil palm seedlings may be prospective in the future. Butyl acetate is an ester form of acetic acid that is found to exhibit diverse antifungal activities. For instance, the compound can initiate

apoptotic cell death mechanisms in baker's yeast, *Saccharomyces cerevisiae* (Rego *et al.*, 2014). Additionally, a mixture of Volatile Organic Compounds (VOCs) produced by *Nocardioopsis alba*, including acetic acid and its derivatives, has been shown to be effective against *G. boninense* (Widada *et al.*, 2021). In another study, the VOCs produced by *Hanseniaspora uvarum* (Saccharomycodaceae) were found to effectively control the incidence of *Botrytis cinerea* in cherries and strawberries (Ruiz-Moyano *et al.*, 2020). The VOCs produced by *Phaeosphaeria nodorum* (Phaeosphaeriaceae) contained a significant proportion of acetic acid and its derivatives, which inhibit the growth of post-harvest phytopathogenic fungi, such as *Monilinia fruticola* (Pimenta *et al.*, 2012). Based on the SEM analysis results, it can be inferred that the inhibitory mechanism of the VOCs may involve the activation of multiple mechanisms and targets, working together synergistically to control the mycelium mass. The observed reduction in hyphal size and wrinkling of the mycelial network may be a result of signal molecules from external sources (i.e., VOCs) triggering intrinsic mechanisms that lead to delayed growth. It is also possible that these mechanisms target



less common components beyond the general aspects of fungal cell walls, such as chitin, mannoproteins, and glucans, which are usually the first structures to be inhibited (Ibe and Munro, 2021).

Docking Study of Antifungal Compounds

Chemical information of the selected compounds or VOCs produced by *P. archidendri* F10 and a standard antifungal compound in the field, dazomet, is presented in Table 1. In Table 2, the data of protein modeling was provided based on the representative criteria or descriptions given in the web server. The model was solely chosen for its high Global Model Quality Estimation (GMQE) and sequence identity based on the available model from *Ganoderma sinense* ZZ0214-1 (Figure 5). The protein model was validated using the Ramachandran plot, constituting a high score of core value, which was 80%, to represent an excellent quality of target protein (Figure 5). The binding affinity of each VOC produced by *P. archidendri* F10 produced a higher score than dazomet as a control (Table 3). The interactions between ligands or VOCs with the target protein are presented in Figures 6 and 7. Visualization of the docking results revealed distinct patterns of chemical bonding interactions for each compound, where all compounds tended to bind to the hydrophilic region of the target protein. Only methyl heptadecanoate demonstrated a tendency to interact with the hydrophobic region. These differences were due to the different types of amino acids involved in the interactions and their residues.

The obtained binding affinity values (ΔG) for the ligands in this study provide insights into their potential interactions with the target protein. The negative ΔG values indicated thermodynamically favorable binding, with more negative values representing stronger interactions. The ligand with the most negative ΔG value, 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, suggested the strongest binding affinity to the

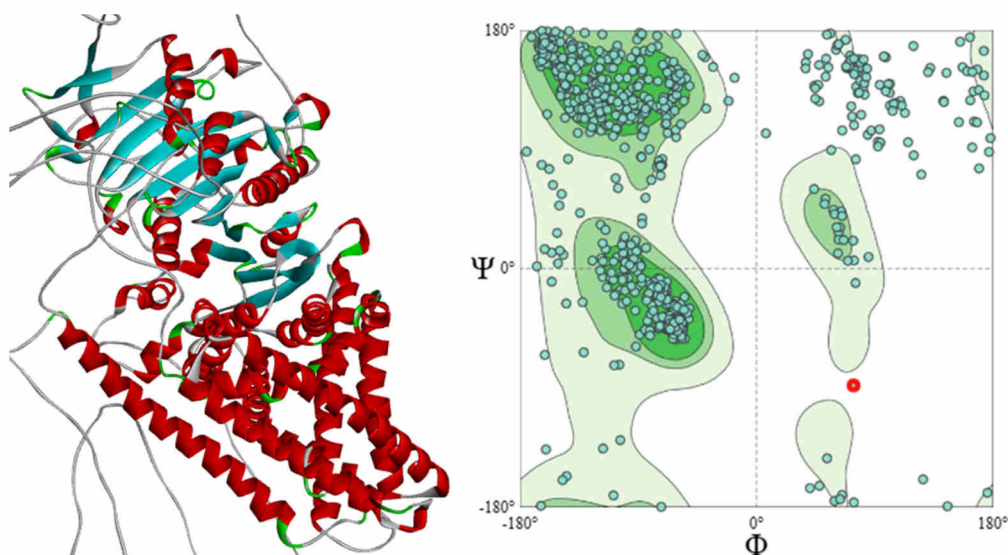
target protein. The absence of reported hydrogen bonds and interaction distances for Dazomet and Butyl acetate might suggest that their binding may be primarily driven by hydrophobic interactions or other non-covalent forces. On the other hand, Methyl heptadecanoate showed a higher binding affinity and a possible hydrophobic interaction, possibly implying that the hydrophobic region of the target protein plays a role in its binding. In contrast, 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate and 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione exhibited hydrogen bonding interactions with specific amino acid residues. This suggests the presence of potential hydrogen bond donor and acceptor sites in the ligands and the target protein, indicating specific binding pockets. The distances observed between the ligands and the interacting amino acid residues are consistent with typical hydrogen bond lengths, reinforcing the possibility of these interactions. Overall, the diversity in binding affinities and interactions observed among the ligands reflects the complexity of ligand-protein interactions and highlights the role of different forces contributing to the binding. Fungal Cell Wall (FCW) is a fundamental element of hyphae, essential for providing structural support, maintaining cellular morphology, and defending against environmental stresses. FCW is primarily composed of polysaccharides, including chitin and β -glucans, as well as proteins and lipids (Gow *et al.*, 2017). Disruption or inhibition of these cell wall components can result in significant morphological abnormalities, undermining the integrity of the hyphae and ultimately leading to cellular death (Zhang *et al.*, 2019). Molecular docking analysis indicates that the identified Volatile Organic Compounds (VOCs) may interact with specific targets within the fungal cell wall or associated proteins, potentially leading to the observed structural damage. For example, VOCs such as Dazomet and Butyl acetate exhibit significant hydrophobic interactions with the target proteins, despite the absence of specific hydrogen bond

Table 1. PubChem CID, molecular weight and formula of the tested compounds.

No.	Compound(s)	PubChem CID	Molecular weight (g mol ⁻¹)	Molecular formula
1.	Dazomet	10788	162.3	C ₅ H ₁₀ N ₂ S ₂
2.	Butyl acetate	31272	116.16	C ₆ H ₁₂ O ₂
3.	2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate	6420420	314.5	C ₁₈ H ₃₄ O ₄
4.	Methyl heptadecanoate	15609	284.5	C ₁₈ H ₃₆ O ₂
5.	7,9-Ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	545303	276.4	C ₁₇ H ₂₄ O ₃

Table 2. The information of 3D structure model of target protein.

Protein name (Sequence ID)	GMQE	Amino acid(s)	Sequence similarity	Sequence identity (%)	Description (Sequence ID)
Chitin synthase (A0A5K1JXQ5)	0.66	1102	0.60	95.17	Chitin synthase (A0A2G8SQ05)

**Figure 5.** Three dimensional structure of the protein model, chitin synthase (Left). Ramachandran plot of a model chitin synthase of *Ganoderma boninense* (Right).**Table 3.** Docking profile of the tested compounds against chitin synthase of *G. boninense*.

Ligand(s)	Binding affinity/ ΔG (kcal/mol)	Number of H-bond (Residue)	Interaction of hydrogen with amino acid residues (Distance)
Dazomet	-4.1	-	-
Butyl acetate	-4.4	-	-
Methyl heptadecanoate	-4.6	-	-
2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate	-5.6	1	His698 (3.05 Å)
7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	-8.5	1	Tyr646 (2.98 Å)

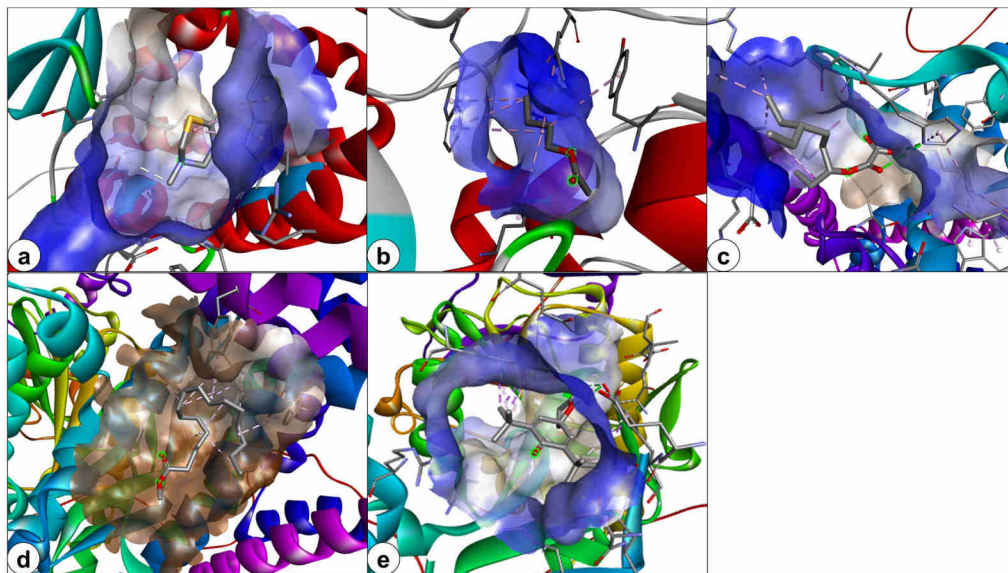


Figure 6. Three-dimensional interaction between VOC and chitin synthase from *G. boninense*. Interacting pockets represent the degree of hydrophobic region of target protein ranging from high (brown) to low (blue): (a) Dazomet, (b) Butyl acetate, (c) 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate, (d) Methyl heptadecanoate, and (e) 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione.

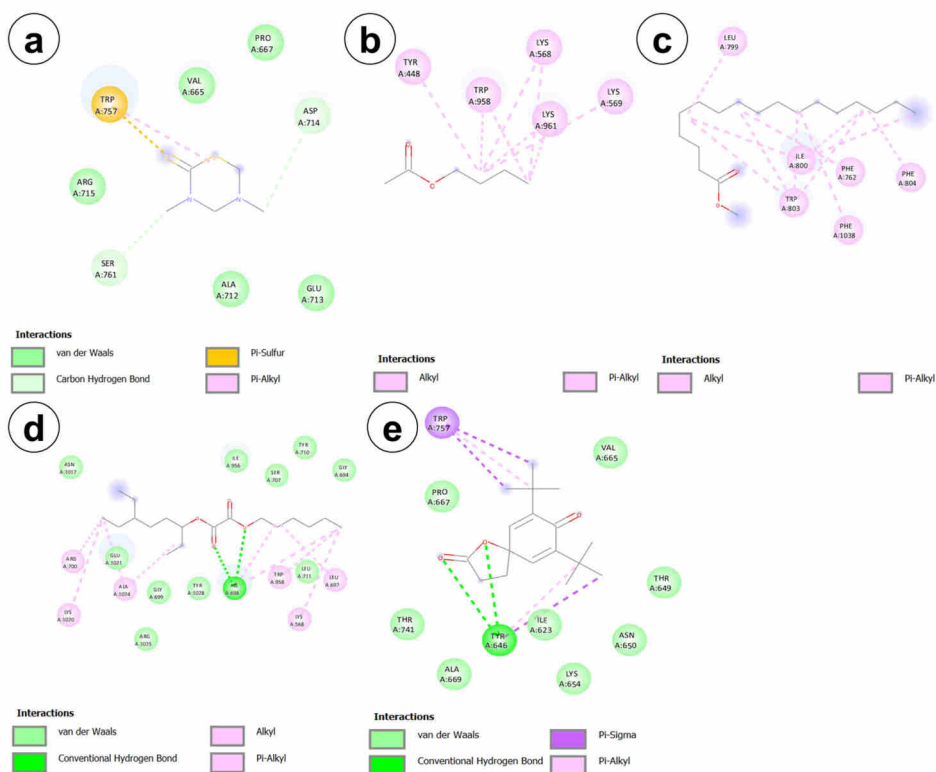


Figure 7. Two-dimensional interaction between VOC and chitin synthase from *G. boninense*. Possible bonds were visualized between receptor or amino acid residues with the ligands: (a) Dazomet, (b) Butyl acetate, (c) 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate, (d) Methyl heptadecanoate, and (e) 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione.

interactions. These hydrophobic interactions are likely to disrupt the hydrophobic domains within cell wall proteins or enzymes involved in cell wall synthesis (Dover *et al.*, 2007). The disruption may compromise the integrity of the cell wall, resulting in thinning and weakening of its structure. This weakening renders the cell wall more susceptible to environmental stress and mechanical damage, as evidenced by the alterations observed in the treated hyphae. Butyl acetate and its derivatives exhibit both antifungal and fungal-stimulating properties, which vary depending on the target species and source. In co-cultures of *Trichoderma* sp. and *Bacillus subtilis*, butyl acetate was identified as the predominant compound exerting antifungal activity against *Colletotrichum gloeosporioides*, effectively inhibiting its growth and spore formation (Emanuel *et al.*, 2020). Conversely, butyl acetate and other acetate esters derived from apple fruit were found to stimulate the adhesion and germination of *Botrytis cinerea* conidia, indicating a potential role in the fungal life cycle (Filonow, 2002). In contrast, VOCs like 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate and 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione demonstrate strong binding affinities and form specific hydrogen bonds with amino acid residues such as His698 and Tyr646. These interactions likely occur at critical sites on fungal enzymes or structural proteins. The binding of these VOCs to key residues may inhibit the function of the enzymes involved in chitin or glucan synthesis, which are crucial for cell wall construction. This inhibition can lead to defective synthesis and, consequently, a weakened cell wall structure.

CONCLUSIONS

A soil-borne fungus, *P. archidendri* F10, isolated from healthy oil palm plantation soils showed antifungal activity against the basal stem rot agent, *G. Boninense*. This was

evidenced through *in vitro* assay, surface morphology of abnormal hypha formation, and potent bioactive VOCs revealing four major components. i.e., 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate, methyl heptadecanoate, butyl acetate, and other minor components. Based on the *in silico* evaluation, four VOCs may have targeted the cell wall integrity by binding with the chitin synthase as a mode of antifungal action.

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ترکیبات آلی فرار (VOC) تولید شده توسط *Paraconiothyrium archidendri* F10 به

عنوان مواد زیستی ضد قارچ برای *Ganoderma boninense*

انیسا لطفیا، و بداح روپایده

چکیده

در این پژوهش، یک قارچ خاکری جدا شده از یک مزرعه نخل روغنی (oil palm) سالم و عاری از بیماری، به منظور بررسی فعالیت مهارکنندگی آن در شرایط آزمایشگاهی (*in vitro*) و با هدف ارزیابی اثربخشی آن به عنوان یک مایه تلقیح زیستی، ارزیابی شد. قارچ خاکری برای ناحیه ITS-rDNA توالی‌یابی شد و شباهت آن از طریق بیوانفورماتیک با استفاده از جستجوهای BLASTn و ساخت درخت فیلوژنتیکی تجزیه و تحلیل گردید. ترکیبات آلی فرار (VOCs) از طریق تخمیر دسته‌ای روی محیط کشت سیب‌زمینی دکستروز آگار (PDA) تولید شد. فعالیت بازدارندگی در برابر رشد شعاعی *G. boninense* با استفاده از روش سنجش بخار (vapor assay) ارزیابی شد. پروفیل VOC و سایر متابولیت‌ها با استفاده از GC-MS تجزیه و تحلیل گردید. مکانیزم مهار (inhibitory mechanism) بین VOCها و پروتئین‌های هدف از طریق آنالیز *in silico* بررسی شد. VOCهای تولید شده توسط *P. archidendri* F10 رشد میسلیم *G. boninense* را تا ۵۵.۸٪ در چهار روز مهار کرد، به طوری که میسلیم از طریق تصویربرداری میکروسکوپی، مورفولوژی موج‌دار، غیرصاف (non-smooth) و چروکیده (wrinkled morphology)، شاخه‌بندی غیرطبیعی، هیف‌های متصل (fused) و معیوب و لیز (lysis) را نشان داد. آنالیز داکینگ مولکولی (molecular docking analysis) نشان داد که 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione قوی‌ترین میل ترکیبی را در ۸.۵ - کیلوکالری بر مول دارد و یک پیوند هیدروژنی با Tyr646 در فاصله ۲.۹۸ آنگستروم تشکیل می‌دهد. لیگاند قابل توجه دیگر ۲-O-(۶-اتیل اکتان-۳-یل)-۱-O-هگزیل اگزالات بود که میل ترکیبی ۵.۶- کیلوکالری بر مول و یک پیوند هیدروژنی با His698 در ۳.۰۵ آنگستروم داشت. یکی دیگر از لیگاندهای قابل توجه، 2-O-(6-ethyloctan-3-yl) 1-O-hexyl oxalate بود که میل ترکیبی آن ۵.۶ - کیلوکالری بر مول و یک پیوند هیدروژنی با His698 در ۳.۰۵ آنگستروم بود. لیگاندهای باقی مانده پیوند هیدروژنی تشکیل ندادند. بنابراین، *P. archidendri* F10 پتانسیل استفاده به عنوان یک قارچ کش زیستی برای کنترل *G. boninense* در آینده را دارد.

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