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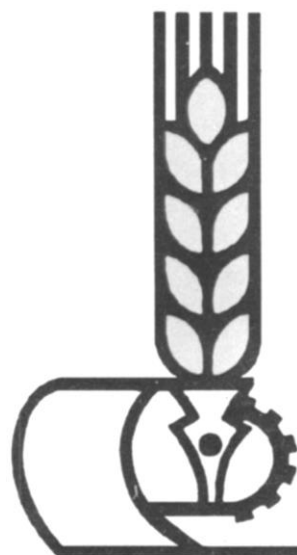
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A Well-Being Perspective on Drone Adoption by Iranian Potato Farmers

Mojtaba Shekarbaygi¹, Shahpar Geravandi^{1*}, and Farahnaz Rostami¹

ABSTRACT

Traditional technology acceptance models primarily focus on behavioral factors, with limited exploration of well-being perspectives. This study examines the role of technology well-being, based on the PERMA framework, in shaping the intention and adoption of drone technology among potato farmers in western Iran. Using a descriptive-correlational survey design, path analysis of a systematic sample of 234 farmers revealed that intention, engagement, social relationships, meaning, and accomplishments significantly influence drone adoption, with path coefficients of 0.85 for intention and 0.38 for acceptance. Positive emotions, however, showed no significant effect. These findings highlight the critical role of well-being in technology acceptance, offering novel insights for precision agriculture. The results suggest that policymakers should prioritize persuasive strategies to enhance farmers' intentions, beyond merely promoting technology use. This research lays a foundation for future investigations, emphasizing technology well-being as a key driver of agricultural innovation.

Keywords: Drone technology, PERMA framework, Precision agriculture, Technology well-being.

INTRODUCTION

Agricultural drones have transformed modern farming by enhancing productivity, operational efficiency, and sustainability (Rachmawati *et al.*, 2021). Through precise field monitoring and targeted operations, drones can improve clean crop production and bolster food security (Shouji *et al.*, 2021). In the potato trade, particularly the commercialization of sweet potatoes, these advancements play a pivotal role in improving farmers' livelihoods and fostering rural economic development (Oyebamiji *et al.*, 2024). Factors such as farm size, access to credit, market proximity, and information availability significantly influence farmers' engagement in this trade. Given the heavy reliance on pesticides in potato cultivation, drone-based spraying offers a sustainable alternative, reducing pesticide use by 30–

65%, while maintaining or enhancing pest control efficacy (Patil *et al.*, 2024). Consequently, drones represent a transformative tool for sustainable potato production and increased market competitiveness.

Potatoes, recognized globally for their high yield per unit area, are a critical food source, producing more dry matter and protein per hectare than many major crops, and serving as a nutrient-rich alternative to grains (Wijesinha-Bettoni and Mouillé, 2019; Devaux *et al.*, 2014; Gustavsen, 2021). Unlike grains, their limited trade volume makes them a reliable option for food security, particularly in politically sensitive markets (Devaux *et al.*, 2014). In Iran, the potato industry has seen significant growth from 1978 to 2023, with harvested areas expanding from 57,000 to 143,000 hectares (3% annual growth) and production rising from 735,000 to 5.5 million tons (5.5%

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annual growth), increasing yields from 8.12 to over 43 tons per hectare. In Kermanshah Province, Iran's fourth-largest potato producer, 6.3% of irrigated land is dedicated to potato farming, significantly contributing to local agricultural revenues. However, challenges such as excessive agrochemical use, climate change, outdated equipment, and declining water resources hinder productivity.

To meet the rising demand and ensure food security, increasing potato production through innovative solutions like drone technology is essential. Drones enhance weed and pest management, boosting yields while promoting economic and environmental sustainability. Despite available infrastructure, drone adoption remains low in Kermanshah County, a key production hub (Figure 1). Existing research has not sufficiently explored the reasons for this low adoption, particularly through a psychological lens. Traditional technology adoption models, such as the Technology Acceptance Model (TAM) and the Unified Theory of Acceptance and Use of Technology (UTAUT), focus on factors like perceived usefulness and ease of use, but often overlook psychological and well-being dimensions critical to rural agricultural contexts (Dissanayake *et al.*, 2022; Blut *et al.*, 2022). Factors such as trust in technology, prior experience, and risk attitudes, which significantly influence adoption, are

underrepresented in these models (Dai and Cheng, 2022).

This study addresses a critical gap in the literature by examining drone technology adoption among potato farmers in Kermanshah Province through the lens of well-being theory, specifically the PERMA model (Seligman, 2018). Unlike behavioral frameworks such as the Technology Acceptance Model (TAM) and the Theory of Planned Behavior (TPB), which focus primarily on utilitarian factors and have been widely applied in agricultural contexts, the PERMA model emphasizes psychological well-being, an under-explored dimension in agricultural behavioral economics. By employing PERMA independently, this research provides a novel theoretical framework that captures farmers' psychological and professional motivations, offering a distinct perspective beyond traditional models. Practically, the findings deliver actionable insights for policymakers and agricultural stakeholders to promote drone adoption, enhancing farm productivity and sustainability while fostering farmers' well-being.

The PERMA Model and Technology Well-Being

Traditional technology adoption models, such as the Technology Acceptance Model (TAM) and the Unified Theory of



Figure 1. Drone technology in potato farming (Kermanshah, Iran).

Acceptance and Use of Technology (UTAUT), emphasize technical factors like perceived usefulness, ease of use, and performance expectations (Rouidi *et al.*, 2022). However, these models often neglect psychological and well-being dimensions, limiting their applicability in complex settings like rural agricultural communities. Psychological and behavioral barriers significantly hinder technology adoption, particularly for innovations like drones (Lee *et al.*, 2025). Integrating psychological frameworks with traditional models is thus essential for a comprehensive understanding of adoption dynamics. To address this gap, this study adopts the PERMA model (Seligman, 2018), a foundational framework in positive psychology, as its primary lens to explore farmers' acceptance of drone technology in potato field spraying in Kermanshah Province, Iran. PERMA comprises five dimensions, namely, Positive Emotion, Engagement, Relationships, Meaning, and Accomplishment, which collectively offer a holistic perspective on how well-being shapes technology adoption (Ascenso *et al.*, 2018). Unlike TAM and UTAUT, which prioritize utilitarian factors, PERMA captures farmers' psychological and professional motivations, making it particularly suited for rural agricultural contexts (Lenzenweger, 2004).

Positive emotions, such as happiness and hope, are central to well-being and can foster technology adoption by creating favorable user experiences (Chisale and Phiri, 2022). Research shows that positive experiences with technology evoke emotions that enhance adoption intentions (Müller *et al.*, 2016; Şahin *et al.*, 2022). Engagement, defined as deep involvement in meaningful activities, is influenced by user experience quality, including challenge and perceived control (Lubis *et al.*, 2019). Higher engagement is linked to stronger adoption intentions (Hussain *et al.*, 2019). Relationships, a key PERMA component, emphasize social connections that facilitate adoption through collaboration, learning, and teaching (Taylor, 2011). This means that reflecting purpose and

life satisfaction encourages positive attitudes toward technology use (El Barachi *et al.*, 2022). Accomplishment, tied to goal achievement, enhances motivation and adoption intent by fostering a sense of mastery (Umucu *et al.*, 2022).

While PERMA is the primary framework, this study draws on insights from other behavioral models applied in Iran's agricultural sector to provide context. The Theory of Planned Behavior (TPB) highlights attitudes, norms, and control as adoption predictors (Valizadeh *et al.*, 2018). The Value-Belief-Norm (VBN) theory underscores environmental values (Zobeidi *et al.*, 2022), while Social-Cognitive Theory (SCT) emphasizes self-efficacy (Zola *et al.*, 2022). By employing PERMA independently while acknowledging these frameworks, this study offers a novel theoretical contribution, integrating well-being into technology adoption research (Ryan *et al.*, 2018). This approach not only enriches the theoretical discourse but also provides practical insights for promoting drone adoption in agriculture.

Based on this framework, the following hypotheses are proposed (Figure 2):

H1: Positive emotions toward drone technology significantly enhance farmers' intention to adopt it.

H2: Engagement with drone technology positively influences farmers' intention to adopt it.

H3: Social relationships in the context of drone technology positively impact farmers' intention to adopt it.

H4: Meaning of "derived from drone technology" is positively linked to farmers' intention to adopt it.

H5: Accomplishments in using drone technology strengthen farmers' intention to adopt it.

H6: The intention to use drone technology directly influences its actual adoption.

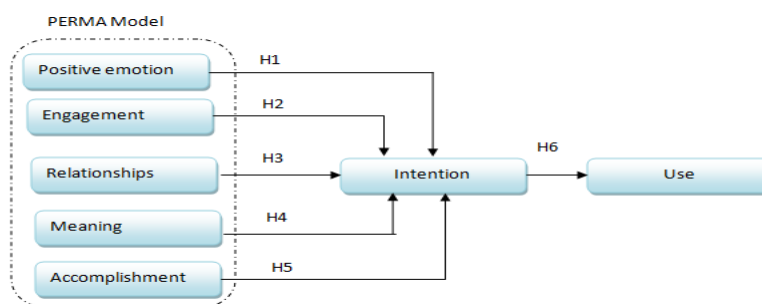


Figure 2. PERMA-based model of drone technology adoption.



Figure 3. Locations of Kermanshah Province, Iran.

MATERIALS AND METHODS

Research Design

Study Area

This study was conducted in Kermanshah Province, western Iran (Figure 3), a key agricultural region and Iran's fourth-largest potato producer. The province features a diverse climate, ranging from humid to semi-arid, with an average annual rainfall of 320 mm (1992–2014). In 2023, potato cultivation spanned 6,000–7,000 hectares, yielding approximately 350,000 tons, underscoring its critical role in food security and the rural economy, which relies heavily on agriculture and livestock. Predominant potato varieties include Agria, Marfona, Banba, and Burren. With an average yield of 50 t ha⁻¹, the adoption of drone technology for precision spraying could enhance yields to 65–70 t ha⁻¹, improving productivity and sustainability.

This quantitative study employed a descriptive survey design to examine the model of drone technology acceptance in potato field spraying. Although technology acceptance has conventionally been explored through behavioral frameworks, there is a paucity of research examining its adoption through the lens of well-being theory.

Sampling Method

A systematic sampling approach was employed to select potato farmers in Kermanshah Province. This method proves particularly effective when the population is organized physically or in a listed format, and simple random sampling is impractical or labeling all units is challenging (Hankin *et al.*, 2019). The study targeted the entire population of 596 potato farmers, as documented by the Statistical Center of Iran

(2019). Based on Krejcie and Morgan's (1970), a sample of 234 farmers was determined ($n=234$). Notably, the study focused exclusively on farmers without prior experience using drones on their farms, positioning the research within an ex-ante analytical framework (Thurow *et al.*, 1997).

Data Analysis Method

Data were collected through a self-administered questionnaire comprising two sections: (1) Demographics and Professional Characteristics (14 items) and (2) PERMA Model-Based Assessment (21 items), adapted from Ascenso *et al.* (2018), to evaluate farmers' perceptions of drone technology. The instrument's validity was established through review by a panel of experts. A pilot study involving 30 farmers from villages of Miandarband, Kuzaran, and Sarabniloofar—excluded from the final sample—was conducted to assess reliability. Study variables and Composite Reliability (CR) coefficients are presented in Table 1.

Data analysis was performed using SPSS 16 (IBM Corp., 2007) and SmartPLS 3 (Ringle *et al.*, 2015). Preliminary checks for normality, outliers, and multicollinearity were conducted to ensure data validity, with all metrics meeting acceptable thresholds,

consistent with Subhaktiyasa (2024). Partial Least Squares Structural Equation Modeling (PLS-SEM) was employed due to its robustness in analyzing complex models and suitability for smaller sample sizes (Hair and Alamer, 2022). The conceptual model incorporated latent variables (reflective and/or formative constructs), with relationships examined using SmartPLS 3.

The measurement model's validity and reliability were evaluated through Composite Reliability (CR), convergent validity (Average Variance Extracted, AVE), and discriminant validity (Fornell-Larcker criterion), all of which met established benchmarks (Hair and Alamer, 2022). The structural model was assessed by examining path coefficients, R^2 values, and the significance of relationships via a bootstrapping procedure.

RESULTS

Demographic and Socioeconomic Characteristics

The respondents in this study had a mean age of 46.2 years ($SD=15.3$), ranging from 24 to 70 years, with 96.6% male and 3.4% female. Most respondents (89.7%) were

Table 1. Survey measures and the corresponding questionnaire items.

Measure	Description	Number of items	CR ^a
Positive emotion (Pe)	Feelings of happiness, hope, and enjoyment in using drone technology (1= Very low, 5= Very high)	3	0.87
Engagement (En)	Deep involvement in meaningful and challenging activities (1= Very low, 5= Very high)	5	0.87
Relationships (Re)	User's understanding and long-term commitment to drone technology (1= Very low, 5= Very high)	4	0.95
Meaning (Me)	Sense of purpose and career significance derived from drone technology use (1 = Strongly disagree, 5= Strongly agree)	3	0.91
Accomplishment (Ac)	Farmers' sense of success in achieving professional goals through drone technology (1= Strongly disagree, 5= Strongly agree)	3	0.84
Intention (In)	Farmer's decision and willingness to adopt drone technology (1= Strongly disagree, 5= Strongly agree)	3	0.94
Use (U)	Practical application of drones in farm management (1= Strongly disagree, 5= Strongly agree)	4	0.81

^a Composite Reliability.



married, with an average household size of four members. Educationally, 50.2% held a bachelor's degree or higher, 30.9% had a diploma, and 19.9% had education below the diploma level. Occupationally, 90.6% were farmers, with 93.6% self-employed and 6.4% employed. The mean agricultural experience was 22.5 years (SD = 11.24), while potato cultivation experience averaged 2.5 years (SD= 2.52).

The average income from potato cultivation was 652.4 million Iranian Rials (approximately USD 1,305 ha⁻¹ based on the 2023 average exchange rate), with average production costs of 216.0 million Iranian Rials (approximately USD 432 ha⁻¹). Most farmers (93.6%) were native to Kermanshah Province, while only a negligible proportion were non-native. Respondents owned an average of 8.28 hectares of total farmland, including 4.37 hectares of dryland, 4.17 hectares of irrigated land, and 1.43 hectares allocated to potato cultivation. More than 90% of the farmland was personally owned, with a small proportion under lease or joint ownership arrangements. The average expenditure on pesticides for pest and weed management was 17.45 million Iranian Rials (approximately USD 35 ha⁻¹).

Evaluation of the Drone Technology Acceptance Model

Confirmatory Factor Analysis (CFA) was conducted to evaluate the fit, validity, and reliability of the PERMA-based model for drone technology acceptance in potato field spraying. The model assessed dimensions including need, positive emotions, engagement, social relationships, meaning, accomplishments, and frequency of drone

use. After removing one indicator from Social Relationships (Re5), the model demonstrated a good fit. Goodness-of-fit indices, correlation coefficients, and summary results are presented in Tables 2, 3, and 4.

All factor loadings were statistically significant ($P < 0.05$), confirming unidimensionality. Composite Reliability (CR > 0.80) demonstrated strong internal consistency, while convergent validity (AVE > 0.50) indicated that the indicators effectively captured variance in their respective constructs. Discriminant validity was also confirmed, as the square root of AVE for each construct exceeded its correlations with other constructs, ensuring construct distinctiveness (Table 3).

Based on the results presented above, the proposed measurement model for drone technology acceptance in potato field spraying, comprising seven primary latent constructs, is a suitable framework for conducting analyses in this study.

Following validation of the measurement model through confirmatory factor analysis, path analysis was employed to test the research hypotheses within the proposed conceptual framework for drone technology acceptance in potato field spraying. The path model, including standardized factor loadings (Figure 4), significant path coefficients (Figure 5), and a summary of results (Table 5), is presented to illustrate the structural relationships in the drone technology acceptance model.

The path analysis (Table 5) revealed that intention, engagement, social relationships, meaning, and accomplishments significantly influenced drone technology acceptance in potato field spraying ($P < 0.01$), while

Table 2. Goodness-of-Fit Indices for the PERMA-based drone technology acceptance model.^a

Fit index	SRMR	D_ULS	D_G	NFI	RMS_Theta
Recommended value	< 0.10	> 0.05	> 0.05	> 0.80	≤ 0.12
Estimated value	0.097	3.07	2.114	0.85	0.11

^a SRMR, NFI, and RMS_theta values met the recommended thresholds (Hu and Bentler, 1999; Henseler *et al.*, 2014), indicating acceptable overall model fit. d_ULS and d_G are reported as supplementary exact-fit indices.

Table 3. Factor loadings and reliability metrics for the PERMA-Based Drone Acceptance Model.

Latent variables (Measures)	Observed variables (Items)	Factor loadings (β)	t	CR	AVE
Pe	Pe1	0.60	**5.11	0.87	0.70
	Pe2	0.92	**69.56		
	Pe3	0.94	**114.69		
En	En1	0.85	**64.64	0.87	0.59
	En2	0.84	**39.58		
	En3	0.81	**20.67		
	En4	0.80	**21.27		
	En5	0.43	**4.09		
Me	Me1	0.94	**153.35	0.95	0.87
	Me2	0.93	**76.26		
	Me3	0.91	**98.60		
Re	Re1	0.91	**80.30	0.91	0.78
	Re2	0.89	**49.40		
	Re3	0.87	**44.02		
	Re4	0.22	*2.02		
Ac	Ac1	0.96	**138.48	0.84	0.61
	Ac2	0.90	**65.32		
	Ac3	0.89	**39.17		
In	In1	0.92	**76.15	0.94	0.85
	In2	0.92	**82.12		
	In3	0.93	**86.76		
U	U1	0.89	**76.26	0.81	0.65
	U2	0.87	**98.60		
	U3	0.74	**44.02		
	U4	0.71	**69.56		

** Significantly at 1% error level and * significantly at 5% error level.

Table 4. Discriminant validity of the PERMA-Based Drone Acceptance Model (Fornell–Larcker Criterion).^a

	Ac	En	In	Me	Pe	Re	U
Ac	0.91						
En	0.76	0.86					
In	0.87	0.82	0.92				
Me	0.87	0.76	0.89	0.93			
Pe	0.54	0.47	0.52	0.51	0.83		
Re	0.72	0.71	0.78	0.77	0.68	0.78	
U	0.51	0.57	0.62	0.61	0.43	0.65	1

^a The numbers of the table diameter are root AVE and the numbers below diameter are the correlation coefficients between the variables.

positive emotions showed no significant effect. The coefficient of determination (R^2) indicated that these factors strongly predicted acceptance ($R^2 = 0.38$) and intention to use drones ($R^2 = 0.85$). Effect size analysis (f^2) highlighted a strong effect of intention, moderate effects of engagement, social relationships, meaning, and accomplishments, and a weak effect of positive emotions. The high predictive relevance (Q^2) confirmed the model's

effectiveness in forecasting both intention and acceptance, supporting its applicability for policy development to enhance drone adoption.

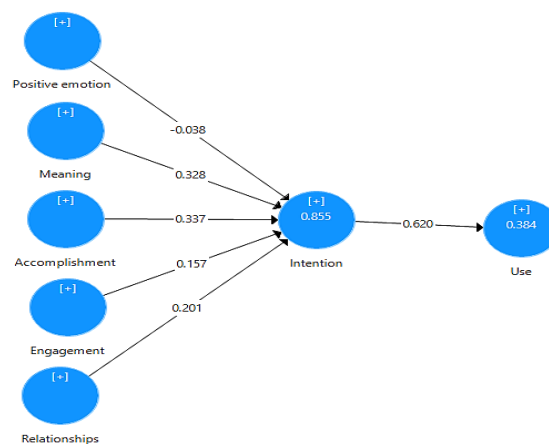
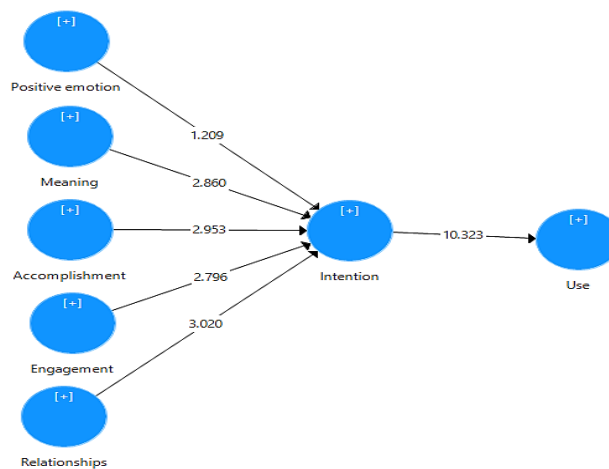
DISCUSSION

This study demonstrates that intention, engagement, social relationships, meaning, and accomplishments significantly influence

**Table 5.** Path Analysis results for the PERMA-based drone acceptance model.

Latent variables			Direct effect		Indirect effect		Total effect		2f	R2	Q2
			t	β	t	β	t	β			
Use of drone technology	Intention		0.62	**10.32	-	-	-	-	0.62		
	Positive emotion		-	-	-	1.21	-	1.21	-		
					0.02		0.02				
	Engagement		-	-	0.09	**2.84	0.09	**2.84	-	0.38	0.37
	Relationship		-	-	0.12	**2.74	0.12	**2.74	-		
	Meaning		-	-	0.20	**2.71	0.20	**2.71	-		
	Accomplishment		-	-	0.20	**2.89	0.20	**2.89	-		
Intention	Positive emotion		-	1.20	-	-	-	1.20	0.01		
			0.03				0.03				
	Engagement		0.15	**2.79	-	-	0.15	**2.79	0.04	0.85	0.72
	Relationship		0.20	**3.02	-	-	0.20	**3.02	0.08		
	Meaning		0.32	**2.86	-	-	0.32	**2.86	0.09		
	Accomplishment		0.33	**2.95	-	-	0.33	**2.95	0.17		

** Significantly at 1% error level.

**Figure 4.** PERMA-Based Drone acceptance model with standardized factor loadings.**Figure 5.** PERMA-Based Drone acceptance model with significant path coefficients.

drone adoption for pesticide spraying in potato farming, while positive emotion have no significant effect. These findings position technology well-being, as conceptualized through the PERMA framework, as a pivotal construct in technology adoption, extending beyond traditional models like Technology Acceptance Model (TAM), which focus primarily on perceived usefulness and ease of use. By employing the PERMA framework independently, this research offers a novel theoretical contribution, extending the application of technology well-being to emerging technologies like drones.

Engagement is a key driver of drone adoption, with farmers more likely to adopt drones when perceived as useful, efficient, and beneficial to their agricultural practices. This aligns with prior studies (Lee *et al.*, 2025; Taoufik, 2020), which emphasize perceived value and expectancy as core determinants of adoption intentions. In traditional farming systems, repetitive and physically demanding tasks often lead to fatigue and reduced motivation among farmers, including potato growers. The introduction of drone technology can transform agriculture into a more engaging and meaningful activity, enhancing productivity and sustainability (Nguyen *et al.*, 2024). These findings are globally relevant, as engagement-driven strategies can promote drone adoption in precision agriculture across diverse regions.

Social relationships significantly shape adoption, both directly and indirectly, through supportive networks and peer influence. Research (Irzan *et al.*, 2021; Koelle *et al.*, 2018) highlights that social connections foster technology adoption by creating enabling environments. This is particularly relevant in collectivistic agricultural societies, such as those in South Asia, Latin America, and rural Europe, where peer adoption and knowledge sharing reduce perceived risks (Antolini *et al.*, 2015). Moreover, social networks and brand identification (Thai *et al.*, 2022; Confente and Kucharska, 2021) reinforce positive attitudes toward technology across diverse cultural

contexts, enhancing the global applicability of these findings.

The role of meaning suggests that farmers who perceive drone technology as consistent with their professional values, goals, and aspirations are more likely to develop a stronger intention to adopt it. This is consistent with prior research (Rao, 1996; Yu-Hsin *et al.*, 2020), which highlights goal alignment as a key driver of technology acceptance. Drones, by providing precise data and enabling rapid decision-making, enhance farmers' knowledge and control over their fields, thereby fostering a sense of purpose and professional advancement (McCarthy *et al.*, 2023). This pattern is applicable to farming communities worldwide, where technologies that align with professional goals can drive adoption.

Accomplishments, particularly economic benefits, strongly predict drone adoption, as farmers prioritize technologies that increase yields, reduce costs, and enhance profitability. This is supported by some studies (Sunding, D. and Zilberman, D. 2001; Suvittawat, 2024), which emphasize economic incentives as universal drivers of technology adoption. Drones facilitate precise pesticide application, improving productivity and product quality, which in turn enhances job satisfaction and a sense of achievement among farmers (Olson and Anderson, 2021). These findings are relevant to both smallholder and large-scale farming systems globally.

Contrary to studies emphasizing the role of positive emotions in technology adoption (Mansoor *et al.*, 2020; Suur-Inkeroinen *et al.*, 2011; Tsaur *et al.*, 2015), this research found no significant emotional influence. Farmers without direct drone experience may struggle to associate positive emotions like joy or hope with the technology, as supported by recent evidence (Suvittawat, 2024). Instead, perceived usefulness, economic benefits, and social influence are prioritized, particularly in resource-limited contexts (Acibuca, 2024; Waris *et al.*, 2022; Zhang and Li, 2005). In wealthier agricultural systems, such as those in Europe or North America, where financial



risks are lower, positive emotions may play a more prominent role (Djamasbi *et al.*, 2010).

Despite the strong path coefficient (0.85) between intention and adoption, practical barriers such as cost, training, and access—though not assessed in this study—may hinder adoption. These barriers are prevalent globally, particularly in developing nations with limited infrastructure. Future research should explore solutions like public-private partnerships or subsidies to enhance drone adoption in regions such as South Asia, Africa, and Latin America. By contextualizing these findings within a global framework, this study advances the literature on agricultural technology adoption. The integration of well-being constructs through the PERMA framework provides a novel perspective, informing policies and practices for both smallholder and large-scale farming systems worldwide.

Theoretical Implications

The findings provide empirical validation for drone technology adoption in potato farming, confirming the relevance of psychological well-being in adoption models. By employing the PERMA framework independently, this study offers a novel theoretical contribution, extending the application of technology well-being to emerging technologies like drones. The limited use of the PERMA model in prior technology adoption research underscores the study's role in expanding the literature by incorporating diverse perspectives on acceptance and influential factors. Incorporating technology of well-being into adoption frameworks broadens the theoretical scope and highlights its critical role in shaping farmers' intentions and behaviors.

Practical and Policy Implications

Policymakers often prioritize psychological factors in designing

technology adoption strategies. This study highlights the equal importance of technology well-being. To enhance drone acceptance, planners should incorporate well-being principles into adoption programs, ensuring both psychological and well-being factors being addressed in the policy frameworks. This approach can strengthen strategies for promoting sustainable agricultural innovations in rural settings.

Limitations

This study has several limitations. First, the limited literature on technology well-being restricted comparative analyses, constraining the depth of discussion despite contributing to the study's originality. Secondly, reliance on quantitative methods may not fully capture the complexities of technology well-being. Future research should employ qualitative approaches to explore nuanced dimensions and refine the PERMA framework. Thirdly, as many potato growers lacked direct drone experience, the study focused on the prospective role of technology well-being. Retrospective studies comparing early adopters and new users could enhance model validity. Fourthly, the focus on farmers without prior drone experience may limit the generalizability of findings. Future studies should adopt ex-post or mixed methods to compare perceptions and behaviors of farmers with and without drone experience. Fifthly, the potential mediating role of financial concerns in the relationship between positive emotions and adoption behavior was not empirically tested. Future research could address this gap through mediation analysis. Finally, due to time and budgetary constraints, this study examined only the PERMA framework independently, without integration with established models like TAM or UTAUT. Future research should combine PERMA with these frameworks to provide a more comprehensive understanding of technology adoption.

CONCLUSIONS

Technology well-being, though a novel concept, emerges as a critical factor in drone technology adoption. While prior agricultural studies have largely overlooked this aspect, this research underscores its pivotal role in shaping acceptance behaviors. Incorporating technology well-being into the existing adoption models could enhance their relevance and applicability. Financial barriers remain a significant obstacle to drone adoption, particularly in developing countries. Government interventions, such as financial support and policy incentives, are essential to promote adoption. Addressing these challenges will strengthen the technology well-being model and lay a foundation for future research in this evolving field.

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نگاهی از منظر بهزیستی به پذیرش پهیاد توسط کشاورزان سیبزمینی‌کار ایرانی

مجتبی شکرپیگی، شهپر گراوندی، و فرحناز رستمی

چکیده

مدل‌های سنتی پذیرش فناوری عمدتاً بر عوامل رفتاری تمرکز داشته‌اند و توجه محدودی به ابعاد مرتبط با بهزیستی در فرایند پذیرش فناوری نشان داده‌اند. این پژوهش با هدف بررسی نقش بهزیستی فناوری، بر پایه چارچوب PERMA، در شکل‌دهی قصد پذیرش و پذیرش فناوری پهیاد در میان کشاورزان سیبزمینی‌کار غرب ایران انجام شد. پژوهش حاضر با استفاده از طرح پیمایش توصیفی-همبستگی انجام گرفت و داده‌های حاصل از نمونه‌ای نظام‌مند شامل ۲۳۴ کشاورز با استفاده از مدل‌یابی معادلات ساختاری مبتنی بر تحلیل مسیر مورد تجزیه و تحلیل قرار گرفت. یافته‌ها نشان داد که قصد پذیرش، درگیری، روابط اجتماعی، معنا و دستاوردهای حاصل از فناوری، تأثیر مثبت و معناداری بر پذیرش پهیاد دارند؛ به‌طوری‌که ضریب مسیر قصد پذیرش برابر با ۰/۸۵ و ضریب مسیر پذیرش فناوری برابر با ۰/۳۸ به دست آمد. با این حال، هیجان‌های مثبت تأثیر معناداری بر پذیرش پهیاد نداشتند. یافته‌ها بر نقش محوری بهزیستی فناوری در فرایند پذیرش فناوری تأکید کرده و بینش‌های نوینی برای توسعه کشاورزی دقیق ارائه می‌کنند. نتایج همچنین نشان می‌دهد که سیاست‌گذاران و برنامه‌ریزان باید راهبردهای مؤثری را برای تقویت قصد کشاورزان به پذیرش فناوری در اولویت قرار دهند و صرفاً بر ترویج استفاده از فناوری تمرکز نکنند. این پژوهش زمینه را برای مطالعات آینده فراهم می‌کند و بر اهمیت بهزیستی فناوری به‌عنوان یکی از محرک‌های کلیدی پذیرش نوآوری‌های کشاورزی تأکید دارد.

Assessing the Effects of Economic Structural Changes and Trade Openness on Ecological Sustainability: The Case of the MENA Region

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ABSTRACT

This study examines whether the structural transformations in the economy and trade exert significant spatial direct and spillover effects on the ecological footprint across MENA countries from 2001 to 2021. For this purpose, we used Moran's "I test" and applied fixed-effect Spatial Durbin Model (SDM). To ensure the findings, we checked the robustness of our results across alternative spatial models and other matrices. The results showed that there was significant positive spatial dependence in ecological footprints, indicating that a country's ecological footprint was influenced by those of its neighbors. We find economic structural changes played an important role in reducing the ecological footprints, both directly and through spillover effects. Similarly, trade openness had a significantly negative impact on the ecological footprint; however, the spillover effect was not significant. The results of robustness checks confirmed the environmental impact of structural economic changes, and trade openness were robust and not driven by model-specific assumptions. These findings underscore the need for MENA governments to prioritize economic restructuring and promote the adoption of environmentally friendly technologies through strategic trade policies and reduced trade barriers.

Keywords: Ecological footprint, Spatial dependence, Spatial Durbin Model.

INTRODUCTION

Environmental degradation—manifested through biodiversity loss, pollution, and land-use change—has become a critical threat to human societies (Keck *et al.*, 2025). Environmental degradation is not merely an ecological concern but a global and multidimensional threat to human well-being and long-term sustainability that directly undermines health, security, and human rights, intensifying the existing social

inequalities (Banik and Uddin, 2025). Current evidence suggests that the continuation of these trends severely weakens ecosystems' capacity to provide essential services—ranging from climate regulation to food security (Telila *et al.*, 2025).

A key factor behind this trend is extensive economic activities and the continued reliance on conventional energy sources—primarily fossil fuels—to support national economic growth (Zhou *et al.*, 2024). Such growth-oriented strategies have markedly

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increased ecological footprints across nations and become a primary threat to environmental sustainability (International Energy Agency, 2018). These impacts pose significant challenges to sustainable development, particularly in developing countries (Danish and Khan, 2020; Razzaq *et al.*, 2024).

In response to mounting environmental pressures, countries are increasingly adopting comprehensive strategies aimed at balancing economic growth with ecological sustainability. These strategies encompass adjustments across various dimensions, including economic scale, technological innovation, and structural transformation (Li *et al.*, 2021). Among these, scale and technological adjustments are often emphasized due to their immediate and visible impacts. Although slowing economic growth can effectively reduce ecological footprints, such an approach is generally undesirable from a policy perspective (Ahmad *et al.*, 2017). Technological advancements—particularly in energy efficiency—have historically contributed to curbing ecological degradation. However, the potential for further efficiency gains is diminishing, and future technological breakthroughs may yield only marginal improvements (Mi *et al.*, 2015).

As a result, structural changes in both the economy and trade have gained prominence as sustainable and strategic avenues for reducing ecological footprints. These transformations have attracted growing interest from policymakers, especially in developing regions. Notably, the expansion of the tertiary sector and increased trade openness have driven substantial structural shifts in many economies, offering new opportunities to decouple economic growth from environmental harm (Li *et al.*, 2021).

The Middle East and North Africa (MENA) region holds a strategic geographic position at the crossroads of Asia, Africa, and Europe, providing it with significant geopolitical and economic advantages. The region is also endowed with abundant natural resources, including crude oil, natural gas, and various

minerals, which have played a vital role in fostering economic development (Doğan *et al.*, 2023; El Khoury *et al.*, 2025). However, following the colonial period, many MENA countries experienced setbacks in their socioeconomic development trajectories (Mrabet *et al.*, 2021). In an effort to accelerate growth and catch up economically, these nations have heavily exploited their natural resources, often at the expense of environmental sustainability (Sun *et al.*, 2022). As illustrated in Figure 1, the ecological footprint across MENA countries has exhibited a consistent upward trend over the two decades leading up to 2021. Notably, six countries in the region—the United Arab Emirates, Qatar, Kuwait, Bahrain, Iran, and Saudi Arabia—rank among the top 20 countries with the highest ecological footprints globally (Global Footprint Network, 2024). In addition to their heavy environmental impact, most MENA nations also contend with severe environmental challenges, including water scarcity, air pollution, and deforestation (Hachaichi and Baouni, 2020). Given the growing severity of environmental degradation, this issue has emerged as a primary policy concern across the region. Consequently, a growing body of environmental economic research (e.g., Al-Mulali and Ozturk, 2015; Guliyev 2024; El Khoury *et al.*, 2025) has focused on investigating the environmental dimensions of economic development. These studies employ various theoretical frameworks and empirical approaches to assess how sustainable development goals can be achieved in the face of persistent environmental challenges.

Given the above context, the economic growth of most MENA countries remains heavily reliant on the production and export of oil and gas. However, in light of the global push to reduce environmental pollution and promote sustainability, governments in the region have begun implementing structural reforms in both the economy and trade. These efforts aim to foster economic development while simultaneously improving environmental quality. In this context,

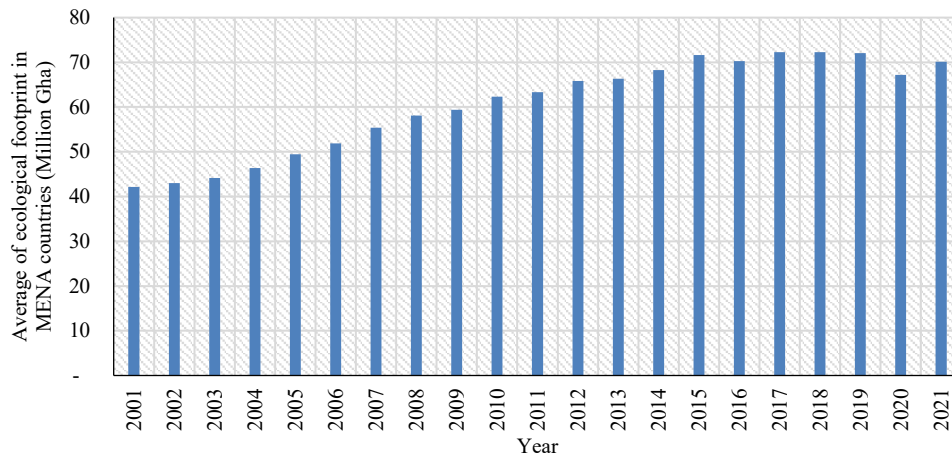


Figure 1. Average of the ecological footprint in the MENA countries (2001-2021) (Global Footprint Network, 2024).

understanding the environmental implications of such structural changes is both timely and essential.

Accordingly, this study seeks to answer two main questions. First, what is the effects of economic structural changes and trade openness on the ecological footprint in MENA countries. Second, whether structural changes in neighboring countries exert significant spatial spillover effects on a given country's environmental quality? Our findings not only will make an important contribution to the existing literature by estimating the spatial direct and spillover effects of economic structural changes and trade openness on the ecological footprint, but also, refine the main axes to enhance the environmental quality without delimiting or stopping economic growth. By doing so, it offers novel insights into the environmental implications of structural transformations in the MENA region—an area that has been underexplored in prior research. Additionally, the findings have important policy relevance, providing evidence-based guidance for designing effective strategies to enhance ecological sustainability.

A review of the existing literature reveals a significant gap concerning our research objectives. While numerous studies have explored the environmental impacts of various economic indicators—such as economic growth, economic complexity, and

financial development (Nathaniel *et al.*, 2020; Saud *et al.*, 2023; Alofaysan, 2024; El Khoury *et al.*, 2025), none, to the best of our knowledge, have specifically examined the effects of structural changes in the economy, particularly through the expansion of the services sector, on ecological sustainability in the MENA region. Moreover, prior studies have consistently shown that industrialization significantly contributes to the rise in ecological footprints (Ragmoun, 2023; El Khoury *et al.*, 2025). However, the potential mitigating role of structural shifts away from industry toward services remains underexplored, highlighting a critical gap that this study aims to address. Recent advances in Artificial Intelligence (AI) and digital technologies further reinforce this gap, as emerging evidence shows that AI-driven innovation substantially improves environmental efficiency across countries (Zhang *et al.*, 2026) and plays a growing role in shaping sustainability outcomes. Therefore, investigating the effects of economic structural changes is essential for informed policymaking aimed at environmental sustainability. Among the three major sectors (Primary sector: Agriculture sector, Secondary sector: Industry sector, Tertiary sector: Service sector), the tertiary industry is generally associated with cleaner production processes and lower levels of environmental pollution



compared to the primary and secondary sectors (Touati and Ben-Salha, 2024). Moreover, studies have shown that AI adoption is more prevalent within service-oriented sectors, and this technological diffusion contributes to enhanced environmental sustainability, partly through improved energy efficiency and alignment with Sustainable Development Goals (SDGs) related to clean energy and climate action (Wang *et al.*, 2026a). In this context, Li *et al.* (2021) demonstrated that economic structural changes—measured by the share of the tertiary sector in total employment—play a significant role in mitigating environmental pollution. Complementing this view, Wang *et al.* (2026b) showed that artificial intelligence significantly enhances corporate sustainability performance, particularly within service-dominated and transitioning economies, suggesting that technology-driven structural transformation can reinforce environmental gains. This suggests the continued development of the tertiary industry can contribute positively to environmental sustainability. Despite this, no existing studies have examined the spatial direct and spillover effects of economic structural changes on the ecological footprint within the MENA region. This spatial dimension is particularly important in regions with close economic and geographic linkages, such as MENA, where policies and economic transformations in one country may have cross-border environmental implications. Although Ramezani *et al.* (2022) applied a Spatial Durbin Model (SDM) and incorporated variables such as per capita income to analyze environmental pressures in MENA countries, their study did not account for structural economic changes. A dedicated discussion of prior spatial studies is, therefore, essential. Ramezani *et al.* (2022) demonstrated significant spatial dependence in ecological footprint patterns across MENA countries, providing a strong justification for our use of the SDM framework. Our analysis extends their work by incorporating structural economic variables—especially the shift toward the tertiary sector—that were

not included in their model, thereby adding an important new dimension to understanding spatial environmental dynamics in the region. Therefore, for a more comprehensive spatial analysis, we need that explicitly considers how economic restructuring may influence both direct and spillover ecological impacts across the region. This need is further emphasized by recent findings showing that financial development and AI together facilitate cleaner energy transitions (Wang *et al.*, 2026d), reinforcing the importance of technology-linked structural change in shaping environmental outcomes.

Furthermore, international trade plays a pivotal role in enhancing environmental quality through the diffusion of technology. Countries with more open trade structures are better positioned to import environmentally friendly and cleaner technologies from developed economies, thus reducing their ecological footprint. However, emerging research also warns that global sustainability dynamics are increasingly shaped by deglobalization pressures, which can influence the ecological footprint through changes in human development pathways and international integration (Wang *et al.*, 2026c). However, trade openness does not universally lead to improved ecological sustainability. Through the scale and composition effects, increased production and exports—often pursued for economic gain—can intensify pressure on natural and ecological resources (Aminizadeh *et al.*, 2024). Additionally, developed countries with stringent environmental regulations may relocate pollution-intensive industries to developing nations with weaker standards, a phenomenon often referred to as the “pollution haven” hypothesis (Schneider and Mellon-Bedi, 2025). The existing literature on trade in the MENA region remains limited and lacks consensus. For example, Al-Mulali and Ozturk (2015) found that trade openness significantly increases the ecological footprint in MENA countries, suggesting a negative environmental impact. In contrast, Mrabet *et al.* (2021) reported that trade

openness contributes to reducing the ecological footprint, thus enhancing ecological quality. To situate the MENA region in the global context, it is important to note that Zambrano-Monserrate *et al.* (2020) identified strong global spatial dependence in ecological footprints and highlighted biocapacity and trade as key interconnected drivers of spatial environmental dynamics. Engaging with their global findings strengthens the methodological justification for our use of a spatial econometric approach and provides an essential benchmark against which the MENA-specific patterns can be interpreted. Their results also indicate that trade-related spillovers can be substantial at the international level, raising questions about whether the absence of spillover effects reported by Ramezani *et al.* (2022) reflects regional particularities or model limitations—an issue our study directly revisits. Regarding spatial effects, Ramezani *et al.* (2022) showed that while trade openness exhibits significant spatial direct effects on the ecological footprint, its spatial indirect effects are statistically insignificant, implying that trade liberalization in neighboring countries do not significantly influence a given country's environmental outcomes. Nevertheless, findings from other regional studies like Zambrano-Monserrate *et al.* (2020) have highlighted significant spatial indirect effects of trade openness via the SDM, casting doubt on the conclusions of Ramezani *et al.* (2022). Given these mixed results, a comprehensive investigation into both the spatial direct and spillover effects of trade openness on ecological sustainability in the MENA region is both timely and necessary.

In summary, to strengthen the conceptual clarity of the study, it is important to explicitly outline the proposed relationships among the main independent variables and ecological footprint. According to the literature gaps in the MENA region, this study examines the following two main hypothesis:

H1: Economic structural changes have a negative and significant direct spatial and

spillover effects on the ecological footprint. This implies that a shift toward more efficient and service-oriented economic structures reduces environmental pressure both within a country and across its neighboring countries.

H2: Trade openness has a negative and significant direct spatial and spillover effects on the ecological footprint. In other words, higher levels of trade openness lower a country's ecological footprint while, simultaneously, reducing environmental pressure in adjacent countries through spatial transmission channels.

MATERIALS AND METHODS

Research Model

This study aimed to analyze the spatial effects of economic structural changes and trade openness on the ecological footprint in the MENA countries. Our empirical model to achieve the research purpose is as follows:

$$\begin{aligned} \text{LnEFC}_{it} = & \beta_0 + \beta_1 \text{LnGDP}_{it} + \beta_2 \text{LnGDP}_{it}^2 + \\ & \beta_3 \text{LnBIO}_{it} + \beta_4 \text{LnECS}_{it} + \beta_5 \text{LnTRA}_{it} + \\ & \beta_6 \text{LnREN}_{it} + \beta_7 \text{LnURB}_{it} + u_{it} \end{aligned} \quad (1)$$

Where, i denotes country and t shows year. EFC denotes the Ecological Footprint. Gross Domestic Product (GDP) and GDP^2 show GDP per capita (in US\$ at constant 2015 prices), and the squared GDP per capita, respectively. Most studies support the Environmental Kuznets Curve (EKC) hypothesis for the MENA countries (Nathaniel *et al.*, 2020; Touati and Ben-Salha, 2024). However, the study by El Khoury *et al.* (2025) did not confirm the EKC hypothesis. Therefore, this study anticipates the ecological footprint will be positively influenced by income ($\frac{\partial \text{EFC}_{it}}{\partial \text{GDP}_{it}} > 0$), and negatively influenced by squared income ($\frac{\partial \text{EFC}_{it}}{\partial \text{GDP}_{it}^2} < 0$), showing that the ecological footprint begins to decrease after a certain level of income. BIO denotes biocapacity. Relevant studies implied that biocapacity increases the ecological footprint (Zambrano-Monserrate *et al.*, 2020). By contrast, Jabeen *et al.* (2023) revealed the negative



relationship between biocapacity and the ecological footprint for Belt and Road Initiative (BRI) economies. Therefore, biocapacity can either increase or decrease the ecological footprint ($\frac{\partial EFC_{it}}{\partial BIO_{it}} > 0$ or < 0).

ECS denotes the Economic Structure. Following Li *et al.* (2021), the ratio of employment in service sector is defined as a proxy of economic structure. Considering that tertiary industry causes less environmental damage compared to primary and secondary industries (Abdulmagid Basheer Agila *et al.*, 2022; Li *et al.*, 2024), this study theorizes the negative effects of economic structure on the ecological footprint ($\frac{\partial EFC_{it}}{\partial ECS_{it}} < 0$). TRA reveals the Trade Openness. Previous studies determined the influence of trade openness on the ecological footprint and showed the mixed findings, like negative influence (Liu *et al.*, 2022; Mrabet *et al.*, 2021), and positive influence (Al-Mulali and Ozturk, 2015; Ramezani *et al.*, 2022). Therefore, trade openness is expected to decrease or increase the ecological footprint ($\frac{\partial EFC_{it}}{\partial TRA_{it}} < 0$ or > 0).

REN denotes Renewable Energy consumption. Most empirical research (e.g., Li *et al.*, 2023; Alofaysan, 2024; El Khoury *et al.*, 2025) showed that renewable energy consumption reduces the environmental degradation. Thus, renewable energy consumption is expected to have a negative effect on the ecological footprint ($\frac{\partial EFC_{it}}{\partial ENS_{it}} < 0$). URB reveals urbanization. Although most research showed the positive effects of urban population on the ecological footprint (Ragmoun, 2023; El Khoury *et al.*, 2025), the study by Khalfaoui *et al.* (2023) found a negative effect. Therefore, urbanization is expected to have a positive or negative effect on the ecological footprint ($\frac{\partial EFC_{it}}{\partial URB_{it}} > 0$ or < 0).

Econometrics Method

This research applies spatial econometric methods to determine the spatial direct and

spillover effects of economic structural changes and trade openness on the ecological footprint for 17 MENA countries in 2001-2022. Spatial econometric modelling is applied to capture the environmental interlinkages that arise when ecological pressures or policy actions in neighbouring countries affect local country. These models mitigate omitted-variable bias arising from ignoring geographic interlinkages and provide richer policy-relevant insights compared with standard panel techniques (LeSage and Pace, 2009; Elhorst, 2014). For these advantages, most environmental economic studies were extensively used the spatial econometrics method (Abban *et al.*, 2025; Fan *et al.*, 2026; Sunge and Espoir, 2026). However, a key methodological consideration is the specification of the spatial weight matrix, as different matrix structures may alter the detected intensity and direction of environmental spillovers. For this reason, most empirical studies (e.g., Aminizadeh *et al.*, 2024; Sunge and Espoir, 2026) were used test and error method for identifying best spatial weight matrix and conducted robustness check according to other matrices.

Spatial Autocorrelation Test

The Moran's I index is widely used to determine the spatial dependence in empirical studies (Zambrano-Monserrate *et al.*, 2020). The Moran's I statistic (I) is calculated using the number of spatial units (n), the spatial weight between unit i and unit j (w_{ij}), the value of the variable in unit i (x_i) and unit j (x_j), and the overall mean of the variable across all units ($\bar{x}$) as follows:

$$\text{Moran's } I = \frac{n}{\sum_{i=1}^n (x_i - \bar{x})} \frac{\sum_{i=1}^n \sum_{j=1}^n w_{ij} (x_i - \bar{x})}{\sum_{i=1}^n \sum_{j=1}^n w_{ij}} \quad (2)$$

Zero score shows no spatial dependence. The positive Moran's I score shows positive spatial correlation, whereas, the negative Moran's I score indicates negative spatial correlation.

Spatial Econometrics Models

By adding the geographical information of the MENA countries to ordinary models generates spatial econometrics models, which can explain the spatial dependence of the ecological footprint between countries. The main models to determine the spatial effects are the Spatial Durbin Model (SDM), the Spatial Autocorrelation Model (SAC), the Spatial Lag Model (SLM), and the Spatial Error Model (SEM). Since SDM contains both the spatially lagged dependent and independent variables, this model is extensively used in empirical studies as a comprehensive spatial model (Ragmoun, 2023; Abban *et al.*, 2025). Considering the significant effect of spatially lagged independent variables on the dependent variable, omitting these effects can lead to bias in estimation (Elhorst, 2014). SDM is as follows (LeSage and Pace, 2009):

$$Y = \rho WY + \beta X + \theta WX + \varepsilon \quad (3)$$

Where, Y is dependent variables and X denotes independent variables. WY is spatially lagged dependent variable, and W is the non-negative spatial weight matrix. This study chooses the three nearest neighbors spatial weights matrix (W_k3) based on the test and error method. “ ρ ” is spatial autoregressive parameter. If it is statistically significant, it shows the ecological footprint in a country is significantly affected by the ecological footprint in neighboring countries.” β ” indicates the effects of the independent variables on the dependent variable. WX is spatially lagged independent variables, and “ θ ” demonstrates the effects of the spatially lagged independent variables on the dependent variable. “ ε ” is the error term.

According to the LeSage and Pace (2009), we use the decomposition method to estimate the direct and indirect effects to increase the credibility of the research results, because the interpretation of the estimated coefficients leads to erroneous conclusions. The SDM model is rewritten in vector form, which is shown in Equation (4):

$$Y_{it} = (I - \rho W)^{-1}(\beta X + \theta WX) + (I - \rho W)^{-1}(\varepsilon) \quad (4)$$

Where, “I” shows an Identify Matrix. Other variables and parameters are defined in Equation (3). The direct effect is the average of matrix diagonal elements, and the spillover effect is the average of row sums of matrix non-diagonal elements. The total effect is the sum of the direct and spillover effects (LeSage and Pace, 2009).

Data

This study determines the spatial direct and indirect effects of economic structure and trade openness on the ecological footprint using balanced panel data for the selected MENA countries during 2001-2021. The choice of time series length and sample countries is determined by data availability on the ecological footprint and independent variables. Data for the ecological footprint and biocapacity are obtained from the Global Footprint Network database (Global Footprint Network, 2024). GDP per capita is measured as constant 2015 US\$. Economic structure is the employment ratio in service sector. Trade openness is the sum of exports and imports of goods and services measured as a share of GDP. Renewable energy consumption is the share of renewable energy in total final energy consumption. Urbanization is the share of people living in urban areas in total population. Data for GDP per capita, economic structure, trade openness, renewable energy consumption, and urban population are collected from the World Development Indicators database (World Bank, 2024). The study variables are converted to logarithmic form. In panel-data econometric modelling, log transformations are widely employed because they reduce heteroscedasticity and allow coefficients to be interpreted in elasticity form. These advantages contribute to more robust estimation and more meaningful comparisons across units and periods (Greene, 2003; Wooldridge, 2010). Table 1 shows the

**Table 1.** Descriptive statistics of the variables and source of data.

Variable	Mean	Std. dev.	Min	Max	Expected Sign	Source
LnEFC	1.269	0.741	0.065	2.771		Global Footprint Network (GFN) (2024)
LnGDP	9.129	1.136	6.613	11.205	+	World Bank (2024)
LnBIO	-0.449	0.596	-1.529	1.309	+/-	Global Footprint Network (GFN)
LnECS	4.095	0.203	3.547	4.422	-	World Bank
LnTRA	4.374	0.376	3.396	5.257	+/-	World Bank
LnREN	-1.246	3.512	-6.908	3.109	-	World Bank
LnURB	4.319	0.221	3.754	4.605	+/-	World Bank

descriptive statistics of the study variables and source of data.

RESULTS AND DISCUSSION

In the first step, we tested the cross-sectional dependence and the results are presented in Table 2. According to the results of Pesaran (2004) test, cross-sectional dependence exists between countries. However, the results of Pesaran and Xie (2021) and Juodis and Reese (2022) tests revealed that there was weak cross-sectional dependence. Therefore, spatial econometrics models is an appropriate method to consider the dependence between countries (Algeri *et al.*, 2022). The results of multicollinearity test using Variance Inflation Factors (VIF) and first- and second-generation unit root tests are presented in Table 3. There is no issue of multicollinearity, because the obtained values are less than 5. The unit root tests results showed the null hypothesis was rejected at 5% level and, hence, all study variables are stationary.

The Moran's I results are shown in Table 4. Moran's I values for the ecological footprint are positive and statistically significant at 1% level, revealing that there exists spatial dependence between countries in the MENA region. Our findings agree with the results of previous studies (Ramezani *et al.*, 2022; Ragmoun, 2023), which showed the ecological footprint in local country in the MENA region is influenced by the ecological footprint in other countries.

Table 5 presents the findings of SLM, SEM, SAC and SDM models. We used the

suggested procedures by Belotti *et al.* (2017) to identify the best spatial model. The SDM model would not be simplified to the SLM and SEM, according to the results of Likelihood Ratio (LR)-test. Additionally, according to the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) results for the SDM and SAC models, we found SDM was the best model to examine the research purpose. Similarly, literature review (e.g., Ali *et al.*, 2024; Sunge and Espoir, 2026) confirms SDM is efficient compared to the other spatial models. The results demonstrate that the spatial coefficient (ρ) is positive and significant. This finding shows that ignoring the spatial effect would lead to inconsistent and biased results (Aminizadeh *et al.*, 2024). Table 6 shows the results of cross-sectional dependence for SDM residual. The Cross-Section Dependence (CD) test findings reveal cross-sectional independence and confirm the SDM results are unbiased.

Table 7 shows the results of direct, indirect, and total effects of SDM model. This research shows the expected positive spatial direct and indirect effects of GDP per capita on the ecological footprint in the MENA countries, which means that economic growth in a country leads to environmental degradation in that country and the neighboring countries. This finding is similar with the results of previous studies (Nathaniel *et al.*, 2020; Touati and Ben-Salha, 2024). However, the spatial effect of GDP per capita squared on the ecological footprint is negative and significant, confirming the EKC hypothesis for the MENA countries. This indicates that nations, after the initial level of

Table 2. The results of Croos-Sectional Dependence (CSD) tests.

Test	Pesaran (2004)	Pesaran and Xie (2021)	Juodis and Reese (2022)
CSD (P-value)	4.35 (0.000)	0.40 (0.623)	-0.49 (0.692)

Source: Research findings.

Table 3. The results of multicollinearity and first- and second-generation unit root tests using Fisher-ADF and CADF tests^a.

Variable ^b	Multicollinearity test	First-generation unit root test	Second-generation unit root test
	VIF	Fisher-ADF	CADF
LnEFC		-6.761***	-1.686**
LnGDP	3.33	-6.041***	-1.664**
LnBIO	2.22	-7.986***	-2.479***
LnECS	2.37	-5.771***	-3.237***
LnTRA	1.43	-6.868***	-2.979***
LnREN	2.24	-4.825***	-2.804***
LnURB	3.59	-9.491***	-1.937***
Mean VIF	2.53		

** P -value < 0.05, *** P -value < 0.01. Source: Research findings.

^a Fisher-ADF is the first- generation unit root test. CADF is the second-generation unit root test.

^b EFC: Ecological Footprint, GDP: Gross Domestic Product, BIO: Biocapacity; ECS: Economic Structural Changes, TRA: Trade Openness, REN: Renewable Energy Consumption, URB: Urbanization.

Table 4. The results of Moran's I index.

Year	Moran's I	P-value	Year	Moran's I	P-value
2001	0.367	0.000	2012	0.395	0.000
2002	0.411	0.000	2013	0.321	0.000
2003	0.473	0.000	2014	0.508	0.000
2004	0.426	0.000	2015	0.504	0.000
2005	0.467	0.000	2016	0.477	0.000
2006	0.460	0.000	2017	0.483	0.000
2007	0.510	0.000	2018	0.468	0.000
2008	0.533	0.000	2019	0.500	0.000
2009	0.513	0.000	2020	0.447	0.000
2010	0.545	0.000	2021	0.343	0.000
2011	0.518	0.000	mean	0.489	0.000

Source: Research findings.

economic growth, realize the importance of the environment in human well-being and begin to care about the ecological sustainability. This is consistent with the results of previous studies (Saud *et al.*, 2023; Touati and Ben-Salha, 2024), which showed the inverted U-shaped EKC between income and ecological footprint in the MENA region. Conversely, some research, like El Khoury *et al.* (2025) revealed that the inverted U-shaped EKC is not validated. In addition, we find that there is EKC relationship between the neighboring countries' income and local countries' ecological footprint, meaning that income growth in neighboring economies

improves a nation's environmental quality through channels such as technology diffusion, shared regulatory practices, and cross-border environmental externalities (Abban *et al.*, 2025). These positive spillovers enlarge the total marginal effect of income on environmental outcomes and imply that regional economic upgrading accelerates the improving ecological sustainability. These findings align with recent spatial-EKC research emphasizing the importance of regional interdependencies in shaping environmental performance

**Table 5.** The results of spatial econometrics models.^a

Variable	SLM		SEM		SAC		SDM	
	Coef.	P> z	Coef.	P> z	Coef.	P> z	Coef.	P> z
lnGDP	0.924	0.001	0.870	0.002	0.987	0.000	0.818	0.003
lnGDP2	-0.032	0.039	-0.031	0.045	-0.034	0.020	-0.023	0.132
lnBIO	0.140	0.000	0.163	0.000	0.132	0.000	0.195	0.000
lnECS	-0.648	0.000	-0.578	0.000	-0.656	0.000	-0.550	0.000
lnTRA	-0.090	0.005	-0.068	0.048	-0.098	0.001	-0.133	0.000
lnREN	-0.017	0.008	-0.018	0.006	-0.014	0.026	-0.016	0.011
lnURB	0.800	0.000	0.778	0.000	0.796	0.000	0.931	0.000
WlnGDP							1.433	0.001
WlnGDP2							-0.068	0.005
WlnBIO							0.005	0.944
WlnECS							-0.397	0.051
WlnTRA							0.006	0.907
WlnREN							0.000	0.988
WlnURB							0.017	0.973
<i>Rho</i>	0.284	0.000			0.432	0.000	0.128	0.052
<i>Lambda</i>			0.249	0.000	-0.345	0.005		
Sigma2_e	0.011	0.000	0.011	0.000	0.010	0.000	0.010	0.000
SDM-SLM							26.620	0.000
SDM-SEM							44.060	0.000
Hausman test							262.80	0.000
AIC	-578.286		-562.445		-583.259		-590.604	
BIC	-543.386		-527.545		-544.481		-528.560	
R-squared	0.870		0.870		0.846		0.865	

Source: Research findings.

^a SLM: Spatial Lag Model, SEM: Spatial Error Model, SAC: Spatial Autocorrelation, SDM: Spatial Durbin Model, EFC: Ecological Footprint, GDP: Gross Domestic Product, BIO: Biocapacity, ECS: Economic Structural Changes, TRA: Trade Openness, REN: Renewable Rnergy Consumption, URB: Urbanization.

Table 6. The results of cross-sectional dependence for SDM residual.

Test	Pesaran CD (2004)
CD	-1.45 (0.148)

Source: Research findings.

Table 7. The results of direct, indirect and total effects of SDM.

Variable	Direct effect		Indirect effect		Total effect	
	Coef.	P> z	Coef.	P> z	Coef.	P> z
lnGDP	0.874	0.002	1.687	0.000	2.561	0.000
lnGDP2	-0.026	0.104	-0.078	0.002	-0.104	0.001
lnBIO	0.201	0.000	0.031	0.651	0.232	0.001
lnECS	-0.565	0.000	-0.527	0.013	-1.092	0.000
lnTRA	-0.134	0.000	-0.009	0.874	-0.143	0.025
lnREN	-0.017	0.009	-0.001	0.922	-0.018	0.195
lnURB	0.943	0.000	0.151	0.753	1.095	0.043

Source: Research findings.

(Aminizadeh *et al.*, 2024; Schneiter and Mellon-Bedi, 2025).

It is worth nothing that the estimated “Turning Point” of the EKC—approximately 222,000 USD of per-capita income—lies far beyond the current and foreseeable income

levels of economies in the region. This shows that relying solely on economic growth to eventually reverse environmental degradation can be unrealistic under the existing development trajectories. Therefore, the EKC relationship should be interpreted

with caution, as the income-driven turning predicted by the model is unlikely to materialize in practice. Policymakers in the region, therefore, need to prioritize direct environmental interventions and economic structural reforms, rather than assuming that rising income alone will naturally lead to improved environmental outcomes. Recent empirical research shows that the EKC Turning Point often lies at unrealistically high-income levels, challenging the idea that growth alone will reduce pollution. Therefore, most developing economies cannot reach the income threshold needed for EKC-based environmental improvement (Durmaz and Thompson, 2024).

Our findings show that there is significant and positive relationship between bio-capacity and ecological footprint. This means that economies with higher bio-capacity are tempted to consume more environmental resources to achieve economic goals, leading to loss of biodiversity and ecological degradation. MENA countries, as resource-dependent economies, often prioritize economic growth over environmental sustainability. In the last two decades, there has been a lot of competition between oil-exporting countries because of rising oil prices, resulting in excessive use of bio-capacity without considering their destructive effects on the environment. For this reason, a number of MENA countries, like Saudi Arabia and United Arab Emirates, are among the top 10 polluters. Weak governance is a main reason to exacerbate degradation due to weak implementation and enforcement of environmental laws and policies and limited public accountability (Akpan and Kama, 2024; Saba *et al.*, 2025). Bilgili *et al.* (2023) stated that the reliance on resource rents and fossil fuels weakens institutional incentives to prioritize environmental sustainability. In this regard, Akpan and Kama (2023) showed that strong governance significantly increases environmental sustainability, particularly by limiting fossil fuel use. This result is consistent with the previous research (Zambrano-Monserrate *et al.*, 2020; Kassouri and Altıntaş, 2020), which found a positive

and significant relationship between the biological capacity and the ecological footprint. However, Jabeen *et al.* (2023) showed a significant negative effect of bio-capacity on the ecological footprint in Belt and Road Initiative countries.

Our results show that the economic structure has negative and significant spatial direct, spillover and total effects on ecological footprint in the MENA countries. This means that increase in proportion of service sector leads to reduce the environmental degradation. A 1% increase in the proportion of service sector decreases 1.092% ecological footprint. The economic structure dominated by the tertiary industry gradually forms by increasing the proportion of employed people in the service industry and the economic structure dominated by the secondary industry begins to weaken. Therefore, since the tertiary industry is generally associated with cleaner production processes and lower levels of environmental pollution, the demand for natural and environmental resources will be reduced. This finding shows ecological sustainability can be achieved by optimally allocating the resources and adjusting industrial structure. In terms of the spatial spillover effects, ecological footprint in a country is negatively impacted by economic structure in neighboring countries, highlighting that service-oriented transformation in neighboring economies contributes to local country's environmental improvement, likely through channels such as reduced resource intensity and diffusion of cleaner technologies. This finding implies that regional structural upgrading can mitigate cross-border environmental pressures and confirms the importance of aligning national environmental policies with broader regional development strategies (Opoku-Mensah, 2023; Abban *et al.*, 2025).

The study by Li *et al.* (2021) showed that development of service sector plays an important role in reducing environmental pollution at global level. Moreover, Abdulmagid Basheer Agila *et al.* (2022) found that improvements in the economic



structure could contribute to improve the environmental quality. In line with this, the studies by Al-Mulali and Ozturk (2015) and Ragmoun (2023) found the significant effect of industrialization in increasing MENA countries' ecological footprint. However, El Khoury *et al.* (2025) did not show the significantly negative effect of service sector on the ecological footprint. Moreover, Destek (2021) revealed that, although industrialization and reindustrialization increase the environmental degradation, reindustrialization has lower adverse impact on the environmental and ecological sustainability because of technological advancement.

Trade openness significantly reduces the ecological footprint in the MENA countries. A 1% increase in trade openness leads to 0.143% decrease in the environmental degradation. Importing new clean technologies and replacing them with old technologies is a solution to environmental problems. Therefore, international trade can improve environmental sustainability by transferring new clean and environmentally friendly technologies. In this regard, Saghaian *et al.* (2020) found that trade openness has significantly positive effect on climate-smart goods trade in the MENA region. Mrabet *et al.* (2021) revealed that trade liberalization significantly reduces MENA region's ecological footprint. By contrast, Jabeen *et al.* (2023) stated that greater trade puts more pressures on the environment. Zambrano-Monserrate *et al.* (2020) found significant positive effect of openness on the ecological footprint. We found that the spatial spillover effect of trade openness is not statistically significant, meaning that trade openness in neighboring countries has no significant effects on the ecological footprint in local country. This finding shows the structural and institutional characteristics of MENA economies. Most countries continue to rely on oil rents and exhibit limited industrial diversification, the conditions that restrict the cross-border diffusion of cleaner technologies even when trade openness expands (Beck and Richter,

2021). Weak intra-regional economic integration, shallow participation in shared value chains, and fragmented technology-transfer channels reduce the likelihood that trade reforms in one country generate significant environmental improvements in neighboring countries (Ahmed *et al.*, 2025). In addition, domestic factors such as dependence on fossil-fuel rents and constrained regulatory capacity dominate environmental outcomes, thereby overshadowing potential spatial spillovers from trade openness (Al-Saffar *et al.*, 2025). This result is consistent with the results of Ramezani *et al.* (2022), which showed that trade openness in neighboring countries has no significant effect on local country's ecological quality in the MENA region.

Renewable energy consumption has a significantly negative spatial direct effect on ecological footprint, meaning that reduction in environmental degradation in a local country is significantly affected by its increasing consumption of renewable energy. This finding is similar to the previous studies (Alofaysan, 2024; El Khoury *et al.*, 2025), which showed the significant effect of the renewable energy consumption on the ecological footprint in the MENA region. However, the spatial spillover effect of renewable energy consumption is not significant, meaning that there is no relationship between renewable energy consumption in neighboring countries and local country's ecological footprint. This may reflect the limited share of renewable energies in regional energy systems, which reduces cross-border influence (Abban *et al.*, 2025). In addition, weak grid interconnections and low levels of energy-market integration in many MENA economies restrict the physical and economic transmission of renewable-energy gains across borders. This effect is further muted by policy and technological misalignment among economies, as well as the dominance of more powerful structural drivers such as institutional quality and fossil-fuel dependence that overshadow the relatively small potential spillovers from renewable

energies (Abban *et al.*, 2025; Stiewe *et al.*, 2025). Our finding contradicts the result by Rasmoun (2023), who found that the ecological footprint in local country was negatively influenced by the consumption of renewable energy in other countries.

Urbanization has significantly positive spatial direct and total effects on ecological footprint. This shows the development of urban population increases the environmental degradation. Similar findings were observed in the MENA countries. For instance, Al-Mulali and Ozturk (2015) showed that increase in urbanization led to increase in the ecological footprint in the MENA region. Touati and Ben-Salha (2024) stated that the rapid urbanization in the MENA countries had a harmful effect on the ecological sustainability. The insignificant spatial spillover effect of urbanization reflects the fact that urbanization largely affects local country's environmental quality. Because urban systems in the region also lack spatial and institutional integration, growth in urban population in neighboring nations does not directly influence resource use or pollution levels in local nation. Consequently, stronger structural determinants such as economic structure and economic growth tend to overshadow any limited spillover effects associated with social factors like urbanization.

Robustness Check

To ensure the robustness of the empirical findings, several complementary spatial specifications were estimated in addition to the baseline static SDM. First, alternative spatial models including SLM and SAC were employed, and the results consistently confirmed the significance and direction of the main independent variables, particularly the role of structural economic changes (Table 8). Secondly, spatial weight matrices of four nearest neighbors (W_k_4) and spatial weight matrices of inverse-distance (W_i) were tested to assess sensitivity to spatial dependence assumptions, and the core

coefficients remained stable across these specifications (Table 9). Thirdly, a dynamic SDM was estimated to account for temporal persistence in environmental indicators, yielding results broadly consistent with the static framework (Table 10). Finally, the dependent variable was replaced by CO₂ emissions instead of ecological footprint, and most of the results again remained unchanged (Table 11). Consequently, these robustness checks demonstrate that the main empirical relationships are not sensitive to alternative modelling choices. The central conclusion that structural economic transformation toward services contributes to environmental improvement remains stable across other models, dynamic SDM, different spatial weight matrices, and alternative dependent variable. This convergence across specifications confirms that the environmental implications of target variables, particularly those arising from structural economic changes, are robust and not dependent on model-specific assumptions.

CONCLUSIONS

This study investigated the spatial direct, indirect, and total effects of determinants on the ecological footprint in 17 MENA countries during 2001-2021 using the SDM model. The local country's ecological footprint is significantly affected by that of other countries. Economic structural changes and trade openness have a significantly negative effect on the footprint, indicating that development of tertiary industry and increased trade improve ecological sustainability. Renewable energy consumption reduces the footprint, while biocapacity and urbanization exacerbate degradation. Policy implications include accelerating diversification from resource-intensive industries toward knowledge-based services (SDG 8, 12, 13), expanding solar and wind capacities and reforming fossil-fuel subsidies (SDG 7, 13), adopting sustainable urban-development models with green-

**Table 8.** The results of direct, indirect and total effects across alternative models.

Variable	Direct effect		Indirect effect		Total effect	
	Coef.	P> z	Coef.	P> z	Coef.	P> z
Fixed-effect SLM model						
lnGDP	1.029	0.000	0.446	0.017	1.474	0.000
lnGDP2	-0.038	0.018	-0.017	0.059	-0.055	0.019
lnBIO	0.179	0.000	0.078	0.006	0.257	0.000
lnECS	-0.606	0.000	-0.268	0.011	-0.875	0.000
lnTRA	-0.077	0.027	-0.035	0.111	-0.112	0.040
lnREN	-0.019	0.005	-0.008	0.049	-0.027	0.008
lnURB	0.874	0.000	0.389	0.023	1.263	0.000
Fixed-effect SAC model						
lnGDP	1.059	0.000	0.694	0.002	1.753	0.000
lnGDP2	-0.037	0.021	-0.024	0.035	-0.061	0.023
lnBIO	0.144	0.000	0.094	0.000	0.237	0.000
lnECS	-0.701	0.000	-0.464	0.000	-1.165	0.000
lnTRA	-0.104	0.001	-0.070	0.015	-0.174	0.003
lnREN	-0.015	0.020	-0.010	0.034	-0.025	0.022
lnURB	0.864	0.000	0.571	0.001	1.435	0.000

Source: Research findings.

Table 9. The results of direct, indirect and total effects under alternative spatial weights matrices.

Variable	Direct effect		Indirect effect		Total effect	
	Coef.	P> z	Coef.	P> z	Coef.	P> z
Fixed-effect SDM model under four nearest neighbors spatial weights matrix (W _{k4})						
lnGDP	1.204	0.000	2.745	0.000	3.949	0.000
lnGDP2	-0.054	0.001	-0.164	0.000	-0.218	0.000
lnBIO	0.268	0.000	0.089	0.453	0.357	0.003
lnECS	-0.565	0.000	-0.442	0.097	-1.007	0.001
lnTRA	-0.151	0.000	-0.048	0.475	-0.199	0.011
lnREN	-0.014	0.039	0.006	0.693	-0.008	0.615
lnURB	0.709	0.003	1.045	0.214	1.753	0.061
Fixed-effect SDM model under inverse-distance spatial weights matrix (W _i)						
lnGDP	1.323	0.000	3.881	0.004	5.204	0.001
lnGDP2	-0.062	0.000	-0.256	0.001	-0.318	0.000
lnBIO	0.300	0.000	0.744	0.001	1.044	0.000
lnECS	-0.591	0.000	-1.196	0.007	-1.787	0.000
lnTRA	-0.095	0.010	0.283	0.101	0.188	0.296
lnREN	-0.013	0.048	0.023	0.294	0.010	0.668
lnURB	0.553	0.021	-0.334	0.800	0.219	0.877

Source: Research findings.

Table 10. The results of direct, indirect, and total effects of dynamic SDM.

Variable	Short-term effect						Long-term effect					
	Direct effect		Indirect effect		Total effect		Direct effect		Indirect effect		Total effect	
	Coef.	P> z	Coef.	P> z	Coef.	P> z	Coef.	P> z	Coef.	P> z	Coef.	P> z
lnGDP	1.156	0.000	2.113	0.000	3.269	0.000	1.232	0.000	2.529	0.000	3.761	0.000
lnGDP2	-0.046	0.004	-0.118	0.000	-0.164	0.000	-0.051	0.002	-0.138	0.000	-0.189	0.000
lnBIO	0.246	0.000	0.323	0.001	0.570	0.000	0.258	0.000	0.397	0.000	0.655	0.000
lnECS	-0.652	0.000	-0.602	0.013	-1.254	0.000	-0.675	0.000	-0.768	0.005	-1.442	0.000
lnTRA	-0.123	0.000	-0.080	0.272	-0.202	0.009	-0.126	0.000	-0.107	0.189	-0.233	0.010
lnREN	-0.011	0.106	0.006	0.619	-0.005	0.772	-0.011	0.122	0.005	0.710	-0.005	0.773
lnURB	0.912	0.000	-1.152	0.065	-0.240	0.724	0.875	0.001	-1.153	0.100	-0.277	0.722

Source: Research findings.

Table 11. The results of direct, indirect and total effects of SDM (Dependent variable: LnCO2).

Variable	Direct effect		Indirect effect		Total effect	
	Coef.	P> z	Coef.	P> z	Coef.	P> z
lnGDP	3.326	0.000	0.309	0.083	3.635	0.000
lnGDP2	-0.166	0.000	-0.015	0.085	-0.181	0.000
lnBIO	0.292	0.000	0.027	0.071	0.318	0.000
lnECS	-0.261	0.001	-0.024	0.138	-0.285	0.001
lnTRA	0.047	0.129	0.004	0.317	0.051	0.130
lnREN	-0.027	0.000	-0.002	0.111	-0.029	0.000
lnURB	1.090	0.000	0.102	0.094	1.192	0.000

Source: Research findings.

building codes in expanding cities (SDG 11, 12, 13), and strengthening regional cooperation through shared renewable-energy markets and joint carbon-reduction initiatives, particularly a regional clean-energy alliance involving Saudi Arabia, the UAE, Iran, Morocco, and Egypt (SDG 7, 12, 13). Future research can investigate other determinants such as artificial intelligence, energy poverty, governance, and globalization.

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ارزیابی اثرات تغییرات ساختاری اقتصادی و باز بودن تجاری بر پایداری اکولوژیکی: مطالعه موردی منطقه منا (خاورمیانه و شمال آفریقا)

سحر عرفانیان، الهام مهرپرور حسینی، امیرحسین چیذری، حسین نوروزی، و میلاد امینی زاده

چکیده

این مطالعه با بررسی اینکه آیا تحولات ساختاری در اقتصاد و تجارت، اثرات مستقیم و سرریز فضایی قابل توجهی بر ردپای زیست‌محیطی در کشورهای MENA از سال ۲۰۰۱ تا ۲۰۲۱ اعمال می‌کنند، به این شکاف می‌پردازد. برای این منظور، از آزمون موران I استفاده کردیم و مدل SDM با اثر ثابت را به کار بردیم. برای اطمینان از یافته‌ها، استحکام نتایج خود را در مدل‌های فضایی جایگزین و سایر ماتریس‌ها بررسی کردیم. نتایج نشان داد که وابستگی فضایی مثبت قابل توجهی در ردپای زیست‌محیطی وجود دارد که نشان می‌دهد ردپای زیست‌محیطی یک کشور تحت تأثیر همسایگانش است. ما دریافتیم که تغییرات ساختاری اقتصادی نقش مهمی در کاهش ردپای زیست‌محیطی، چه به طور مستقیم و چه از طریق اثرات سرریز، ایفا می‌کنند. به طور مشابه، باز بودن تجارت تأثیر منفی قابل توجهی بر ردپای زیست‌محیطی داشت. با این حال، اثر سرریز قابل توجه نبود. نتایج بررسی‌های پایداری، تأثیر زیست‌محیطی تغییرات ساختاری اقتصادی و باز بودن تجارت را قوی و تحت تأثیر فرضیات خاص مدل قرار نداده است. این یافته‌ها بر نیاز دولت‌های منطقه خاورمیانه و شمال آفریقا به اولویت‌بندی بازسازی اقتصادی و ترویج پذیرش فناوری‌های سازگار با محیط زیست از طریق سیاست‌های تجاری استراتژیک و کاهش موانع تجاری تأکید می‌کند.

Estimation of Price and Substitution Elasticities of Cotton Production Inputs: Empirical Evidence from Baghlan Province, Afghanistan

Hafizullah Radmand^{1, 2}, Ali Keramatzadeh^{1*}, Ramtin Joolaei¹, and Farshid Eshraghi³

ABSTRACT

This study aimed to estimate the price and substitution elasticities among the production inputs of cotton in Baghlan Province, Afghanistan. Data were collected through 132 questionnaires, using stratified random sampling from cotton growers in the province. The relationships among production inputs were examined using a translog cost function in conjunction with the Seemingly Unrelated Regression (SUR) approach. The results showed that the price elasticities of demand for land, animal manure, phosphate fertilizer, urea fertilizer, seeds, labor, water, and machinery were -0.036, -0.815, -0.056, -0.050, -0.056, -0.092, -0.074, and -0.198, respectively. All price elasticities of input demand were less than one, indicating inelastic demand, that is, input use is not highly sensitive to price changes. The cross elasticities of demand for inputs were also less than one, confirming inelastic demand across all inputs. The small values of substitution elasticities further indicated that policies targeting a single input would have little impact on the allocation of other inputs. Therefore, it is recommended that policymakers avoid focusing on individual inputs and, instead, consider all inputs as an integrated system when designing agricultural policies.

Keywords: Allen and Morishima elasticities of substitution, Cost function, Demand for inputs, Seemingly Unrelated Regressions (SUR).

INTRODUCTION

Cotton is a vital global crop and a significant economic driver, especially in developing nations. Often referred to as “white gold,” it is the world’s most consumed natural fiber and a cornerstone of the textile industry (Khan *et al.*, 2020). Globally, cotton is cultivated on more than 32.5 Mha, with a total production of 24.08 million tons in 2021 (Tokel *et al.*, 2022). The industry provides livelihoods for approximately 31.5 million farmers and employs around 150 million people

worldwide (FAO, 2021).

In Afghanistan, cotton has been an important crop since 1921. Before the 1979 revolution, the country maintained a strong cotton industry, cultivating 128,000 hectares and producing 150,000 tons annually. However, political instability led to a sharp decline in cultivation. Since 2002, the Afghan Ministry of Agriculture has prioritized reviving the sector, contributing to a resurgence in agricultural production (ILO, 2017). By 2019, the area under cotton cultivation had reached 36,000 ha, with a total production of 14,000 tons across

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various provinces, including Baghlan, where farmers produced 4,620 tons from 1,650 ha (Radmand *et al.*, 2021).

Understanding the economics of production is fundamental to ensuring the long-term sustainability of any industry. According to Surya *et al.* (2021), comprehensive knowledge of production costs and factor contributions is vital for improving efficiency, enhancing competitiveness, and fostering sustainable growth. In agriculture, effective resource management remains a cornerstone of development, especially given the limited availability of key production inputs in many regions (Adom and Adams, 2020).

Analyzing the demand for production inputs requires assessing their sensitivity to price changes and understanding the relationships between them (Ray *et al.*, 2023). This analysis is essential to inform policy decisions. If inputs are complementary, reducing costs necessitates proportionally reducing all inputs. However, if inputs are substitutes, producers can lower overall production costs and increase profits by replacing more expensive inputs with cheaper alternatives (Ren *et al.*, 2021). The extent to which inputs can substitute for one another plays a key role in determining the economic impacts of policies targeting input demand. (Feng *et al.*, 2025).

Furthermore, analyzing input costs provides valuable policy insights for improving resource allocation efficiency and enhancing farmers' productivity (Du *et al.*, 2019). Elasticities are key parameters in this analysis, as they reveal how the relative cost share of production factors changes with price fluctuations (Bellocchi and Travaglini, 2023). According to Binswanger (1974), studying the elasticity of substitution is fundamental to understanding how easily, or with difficulty, one production factor can be replaced by another. A higher elasticity indicates greater ease of substitution. In this context, the present study estimates the price and substitution elasticities of cotton production inputs in Afghanistan's Baghlan

Province, offering important insights into the interrelationships among these factors.

In Afghanistan, the absence of provincial-level studies has significant implications for agricultural policy. National averages conceal substantial regional variation in resource endowments, production systems, and market access. As a result, policies designed from aggregated national data often fail to address the distinct challenges and opportunities within specific provinces. For instance, a uniform fertilizer subsidy or mechanization program may be effective in some areas but economically infeasible, or environmentally unsustainable, in others. Provincial-level analyses, such as this study in Baghlan are, therefore, essential to develop locally adapted policies that enhance productivity, ensure equitable resource distribution, and improve farmers' livelihoods. Accordingly, this study seeks to answer the following research question: What are the price and substitution elasticities among the production inputs used in cotton farming in Afghanistan's Baghlan Province, and what are the implications of these relationships for regional agricultural policy and resource allocation?

Numerous studies have utilized the Seemingly Unrelated Regression (SUR) method and the translog cost function to analyze input demand and factor relationships. For example, Deininger *et al.* (2018) investigated substitution effects within Swiss manufacturing firms and identified a complementary relationship between capital and energy, particularly in energy-intensive sectors. In the agricultural context, Du *et al.* (2019) estimated the price and substitution elasticities for peach and cherry production in Beijing, China, and found that labor serves as a substitute for land, fertilizers, and pesticides. Similarly, Pippa Rochelle and Ferreira Filho (1999) investigated the cotton input market in São Paulo, Brazil, and revealed that labor and machinery were complementary inputs, while other factor pairs showed substitution relationships.

The substitutability and complementarity of inputs often vary by context and crop. For instance, Pang *et al.* (2021) observed a complementary relationship between fertilizer and labor in corn production in China, whereas a substitutive relationship was identified in cabbage cultivation. Their findings further indicated that the price elasticity of fertilizer demand was inelastic in both production systems. Similar results have been reported in other studies, highlighting the generally inelastic nature of demand for agricultural inputs. Ansari Roshandeh *et al.* (2022) found that water demand for rice production in Iran's Gorgan County was inelastic, indicating that significant price increases would not substantially reduce water consumption. Koç and Karayığit (2022) analyzed the Australian water market and the impact of electricity price liberalization, finding that electricity demand was relatively price-sensitive and that electricity complemented both labor and capital. Forgenie *et al.* (2023) reported that, in Indonesia, the demand for animal feed exhibited greater responsiveness to variations in income than to fluctuations in price. Landolsi and Miled (2024) reached a similar conclusion regarding energy demand in Tunisia, noting that its sensitivity to income was greater than to price fluctuations. Wang and Wu (2025) examined modernization and substitution elasticities in China's grain production using a Translog production function. Their results reveal complementary relationships among several key factor pairs, including capital–fertilizer, capital–land, fertilizer–land, pesticide–land, and fertilizer–labor. In contrast, substitutive relationships were identified for capital–pesticide, fertilizer–pesticide, pesticide–labor, and land–labor. Similarly, Gu *et al.* (2025) investigated how rising rural labor prices influence land use patterns and crop allocation by applying the Translog function. Their findings indicate that higher labor costs have reduced the planted area of rice and maize, while significantly increasing the area devoted to wheat. This shift is primarily driven by

factor substitution, specifically, the replacement of labor with fertilizer and machinery, which has been instrumental in reshaping cropping patterns. These results underscore the importance of developing and promoting labor-saving technologies, such as more efficient fertilizers and small-scale agricultural machinery.

Collectively, these studies highlight the importance of analyzing input demand and factor relationships to inform effective resource management and policy. However, despite the global significance of cotton and the well-established methodologies for this type of analysis, a notable research gap exists in Afghanistan. To the best of the current knowledge, no comparable research has been undertaken at the provincial level, particularly in Baghlan, a major agricultural region. Hence, this study seeks to fill this gap by estimating the price and substitution elasticities of cotton production inputs in Baghlan Province, thereby generating valuable insights to support local agricultural development.

MATERIALS AND METHODS

Study Area

Afghanistan, with an area of 652,864 square kilometers, is divided into 34 provinces and 398 districts, based on its administrative divisions (Figure 1). As of 2020, its population was approximately 38.8 million. Approximately, 61.6% of the population relies on agriculture and animal husbandry, and the agricultural sector accounts for 23% of the GDP (Radmand *et al.*, 2021). Afghanistan possesses an estimated 75 B m³ of total annual freshwater resources, comprising roughly 57 B m³ (76%) of surface water and 18 B m³ (24%) of groundwater (Mahmoodi, 2008). Baghlan Province is one of the major industrial and agricultural regions located in northeastern Afghanistan (Figure 1). The province covers 21,118 km² and has an estimated population

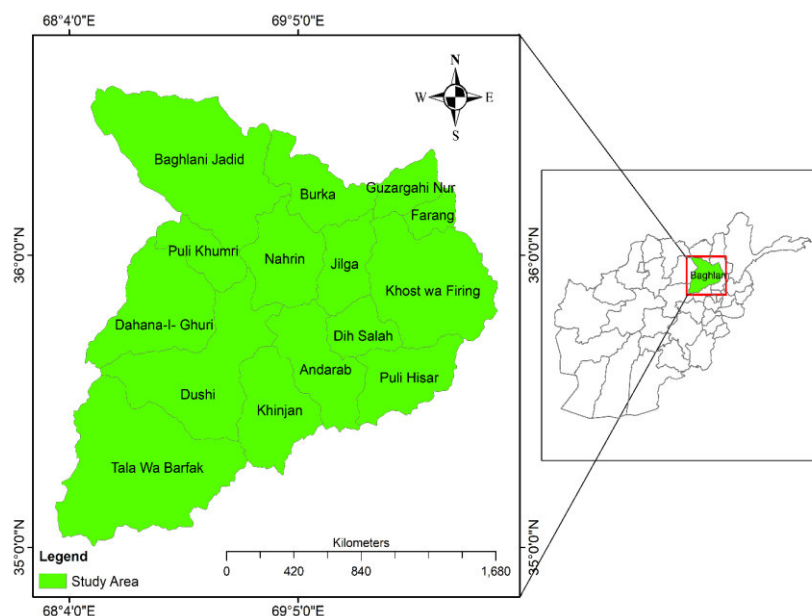


Figure 1. The geographic location of Baghlan Province in Afghanistan.

of about 1.77 million people (Radmand *et al.*, 2021).

This study estimated the input demand function to determine the price elasticity of demand and the elasticities of substitution among production inputs. Consistent with previous research (Du *et al.*, 2019; Miljkovic *et al.*, 2016; Mufutau Opeyemi, 2021; Ray *et al.*, 2023; Ren *et al.*, 2021; Ranjbar *et al.*, 2023), the Seemingly Unrelated Regression (SUR) approach was employed, as it is well-suited to cross-sectional data and effectively accounts for the interdependence among input demand equations. By considering the contemporaneous correlation among the error terms across equations within the system, the SUR method yields more efficient parameter estimates compared to the single-equation Ordinary Least Squares (OLS) approach. Since changes in prices or external shocks simultaneously affect the demand for all inputs, SUR enables the modeling of such interdependence. Consequently, SUR is a powerful tool for accurately estimating price and substitution elasticities within a system of equations (Griffin *et al.*, 1987; Du *et al.*, 2019). The general form of the SUR model is as follows (Griffin *et al.*, 1987):

$$Y_{1t} = \alpha_1 + \beta_1 X_{1t} + u_{1t} ; t = 1, \dots, T \quad (1)$$

$$Y_{2t} = \alpha_2 + \beta_2 X_{2t} + u_{2t} ; t = 1, \dots, T \quad (2)$$

Here, Y represents the level of production, and X denotes the production factor. While Equations (1) and (2) may appear independent, certain underlying factors can cause them to vary simultaneously, affecting production outcomes through the disturbance terms u_{1t} and u_{2t} . Consequently, this study utilized the SUR method to estimate both the translog cost function and the input share demand functions.

Translog Production Function

The translog production function was proposed by Christensen *et al.* (1971). This function is a logarithmic, flexible, functional form that allows substitution elasticities and partial production elasticities to vary with input levels (Debertin, 2012). The translog production function encompasses all three production regions, permitting marginal products to be increasing, decreasing, or even negative. Beyond the primary variable coefficients, the translog specification also estimates the interaction terms between variables. The general form of the translog production function is presented in Equation (3) (Griffin *et al.*, 1987).

$$\ln(Y) = \alpha_0 + \sum_{i=1}^n \alpha_i \ln(\alpha_i) + \frac{1}{2} \sum_{i=1}^n \sum_{j=1}^n b_{ij} \ln(x_i) \ln(x_j) \quad (3)$$

Where, Y represents the production amount, and x_i and x_j are the production inputs.

Translog Cost Function

The cost function describes the relationship between total cost, input prices, and the level of output. Deriving the cost function involves either minimizing total cost for a specified level of production or maximizing output within a given budget constraint. As the dual of the production function, each production function corresponds to a unique cost function, allowing the production function to be inferred from the cost function. Equation 4 shows the general form of the cost function.

$$C = f(P_1, P_2, P_3, \dots, P_n, Q) \quad (4)$$

Where, C represents the Total Cost, Q represents the Amount of Production, and P_1 to P_n represent the Prices of Production Inputs.

This study utilized the translog cost function to estimate the cost structure of production. A key advantage of this functional form is that the substitution elasticities among production factors are not constant but vary depending on both the production level and the combination of inputs used (Griffin *et al.*, 1987). This functional form has been widely used in economic literature (Christensen and Ramanantoandro, 1971; Koç and Karayığit, 2022). One key reason for its widespread application is the ease of interpreting results and deriving marginal costs (Johansen, 1972). The general form of the translog cost function is given by Equation (5):

$$\begin{aligned} \ln(C) = & \alpha_0 + \sum_{i=1}^n \alpha_i \ln Y_i + \sum_{i=1}^n \beta_i \ln P_i + \\ & \frac{1}{2} \sum_{i=1}^n \sum_{j=1}^n \alpha_{ij} \ln Y_i \ln Y_j + \\ & \frac{1}{2} \sum_{i=1}^n \sum_{j=1}^n \beta_{ij} \ln P_i \ln P_j + \\ & \frac{1}{2} \sum_{i=1}^n \sum_{j=1}^n \gamma_{ij} \ln Y_i \ln P_j + e_i \end{aligned} \quad (5)$$

Where, C represents the Total Cost, Y_i represents the Level of Production, P_i and P_j

represent the Prices of Inputs, and α_i , β_i , α_{ij} , β_{ij} , and γ_{ij} denote the estimated parameters. In general, the translog cost function possesses the properties of positivity, symmetry, and homogeneity with respect to input prices. The dependent variable of the translog function is expressed in logarithmic form, ensuring the positivity condition. The restriction $\beta_{ij} = \beta_{ji}$ must be imposed to ensure symmetry. The assumption of homogeneity with respect to input prices implies that, if all input prices double while output remains constant, the total cost will also double. To satisfy the homogeneity condition of the cost function, the following constraints must be imposed when estimating the SUR model (Garcia and Randall, 1994; Rigi and Shahraki, 2017):

$$\sum_{i=1}^n \beta_i = 1 \quad \sum_{i=1}^n \beta_{ij} = \sum_{j=1}^n \beta_{ji} = 0$$

Demand Functions of Inputs

There are two primary approaches to deriving the demand function for input. When the production function displays Increasing Returns to Scale (IRS), the input demand function is obtained by minimizing the cost function. In this scenario, differentiating the cost function with respect to input prices (Shephard's Lemma) produces an indirect (conditional) input demand function. Conversely, if the production function exhibits Decreasing Returns to Scale (DRS), the input demand function can be derived through profit maximization using Hotelling's Lemma (Varian, 1992). The general form of the conditional demand function of production inputs, according to Shephard's theorem, which represents the equations of the cost share of inputs, is given by Equation (6):

$$S_i = \frac{P_i X_i}{\sum P_i X_i} = \frac{P_i}{C} \cdot X_i = \frac{P_i}{C} \cdot \frac{\partial C}{\partial P_i} = \frac{\partial \ln C}{\partial \ln P_i} = \alpha_i + \sum_{j=1}^n \beta_{ij} \ln P_j + \gamma_{iy} \ln Y \quad (6)$$

Here, S_i denotes the cost share of the i_{th} input, C represents the total production cost, P_i and P_j denote the prices of the i_{th} and j_{th} inputs, respectively, and Y is the output level.



Because the cost shares sum to unity ($\sum S_i = 1$), the disturbance covariance matrix is singular, making only $(n-1)$ cost share equations linearly independent. Consequently, the standard procedure in Seemingly Unrelated Regression (SUR) estimation involves omitting one cost share equation from the system to overcome singularity, while expressing all input prices in relative terms (normalized by the price of the omitted input) to automatically enforce the price homogeneity condition of degree one. The parameters of the omitted equation are subsequently derived using the linear adding-up and symmetry restrictions. The standard procedure for estimating input demand functions involves omitting one cost share equation from the system of simultaneous equations and estimating the parameters for the remaining $n - 1$ equations. The parameters of the omitted equation are subsequently derived from the estimated parameters of the included equations (McGeehan, 1993; Pippa Rochelle and Ferreira Filho, 1999; Shahraki and Ali Ahmadi, 2014; Wijetunga, 2023; Rigi and Shahraki, 2016; Ning *et al.*, 2020, and Abbasian *et al.*, 2022). Therefore, one cost share equation, typically corresponding to the input with the lowest cost share, is omitted from the model. In this study, the cost of pesticides was excluded from the analysis because it represented the smallest share of the total production cost. Therefore, the cost function of each production input was estimated as follows:

$$SS = \alpha S + \beta_{SS} \log \left(\frac{PS}{PP} \right) + \beta_{SL} \log \left(\frac{PL}{PP} \right) + \beta_{SR} \log \left(\frac{PR}{PP} \right) + \beta_{SN} \log \left(\frac{PN}{PP} \right) + \beta_{SPH} \log \left(\frac{PPH}{PP} \right) + \beta_{SA} \log \left(\frac{PA}{PP} \right) + \beta_{SW} \log \left(\frac{PW}{PP} \right) + \beta_{SM} \log \left(\frac{PM}{PP} \right) + \gamma_{SY} \log Y \quad (7)$$

$$SL = \alpha L + \beta_{LS} \log \left(\frac{PS}{PP} \right) + \beta_{LL} \log \left(\frac{PL}{PP} \right) + \beta_{LR} \log \left(\frac{PR}{PP} \right) + \beta_{LN} \log \left(\frac{PN}{PP} \right) + \beta_{LPH} \log \left(\frac{PPH}{PP} \right) +$$

$$\beta_{LA} \log \left(\frac{PA}{PP} \right) + \beta_{LW} \log \left(\frac{PW}{PP} \right) + \beta_{LM} \log \left(\frac{PM}{PP} \right) + \gamma_{LY} \log Y \quad (8)$$

$$SN = \alpha N + \beta_{NS} \log \left(\frac{PS}{PP} \right) + \beta_{NL} \log \left(\frac{PL}{PP} \right) + \beta_{NR} \log \left(\frac{PR}{PP} \right) + \beta_{NN} \log \left(\frac{PN}{PP} \right) + \beta_{NPH} \log \left(\frac{PPH}{PP} \right) + \beta_{NA} \log \left(\frac{PA}{PP} \right) + \beta_{NW} \log \left(\frac{PW}{PP} \right) + \beta_{NM} \log \left(\frac{PM}{PP} \right) + \gamma_{NY} \log Y \quad (9)$$

$$SPH = \alpha PH + \beta_{PHS} \log \left(\frac{PS}{PP} \right) + \beta_{PHL} \log \left(\frac{PL}{PP} \right) + \beta_{PHR} \log \left(\frac{PR}{PP} \right) + \beta_{PHN} \log \left(\frac{PN}{PP} \right) + \beta_{PHPH} \log \left(\frac{PPH}{PP} \right) + \beta_{PHA} \log \left(\frac{PA}{PP} \right) + \beta_{PHW} \log \left(\frac{PW}{PP} \right) + \beta_{PHM} \log \left(\frac{PM}{PP} \right) + \gamma_{PHY} \log Y \quad (10)$$

$$SR = \alpha R + \beta_{RS} \log \left(\frac{PS}{PP} \right) + \beta_{RL} \log \left(\frac{PL}{PP} \right) + \beta_{RR} \log \left(\frac{PR}{PP} \right) + \beta_{RN} \log \left(\frac{PN}{PP} \right) + \beta_{RPH} \log \left(\frac{PPH}{PP} \right) + \beta_{RA} \log \left(\frac{PA}{PP} \right) + \beta_{RW} \log \left(\frac{PW}{PP} \right) + \beta_{RM} \log \left(\frac{PM}{PP} \right) + \gamma_{RY} \log Y \quad (11)$$

$$SW = \alpha W + \beta_{WS} \log \left(\frac{PS}{PP} \right) + \beta_{WL} \log \left(\frac{PL}{PP} \right) + \beta_{WR} \log \left(\frac{PR}{PP} \right) + \beta_{WN} \log \left(\frac{PN}{PP} \right) + \beta_{WPH} \log \left(\frac{PPH}{PP} \right) + \beta_{WA} \log \left(\frac{PA}{PP} \right) + \beta_{WW} \log \left(\frac{PW}{PP} \right) + \beta_{WM} \log \left(\frac{PM}{PP} \right) + \gamma_{WY} \log Y \quad (12)$$

$$SA = \alpha S + \beta_{AS} \log \left(\frac{PS}{PP} \right) + \beta_{AL} \log \left(\frac{PL}{PP} \right) + \beta_{AR} \log \left(\frac{PR}{PP} \right) + \beta_{AN} \log \left(\frac{PN}{PP} \right) + \beta_{APH} \log \left(\frac{PPH}{PP} \right) + \beta_{AA} \log \left(\frac{PA}{PP} \right) + \beta_{AW} \log \left(\frac{PW}{PP} \right) + \beta_{AM} \log \left(\frac{PM}{PP} \right) + \gamma_{AY} \log Y \quad (13)$$

$$SM = \alpha M + \beta_{MS} \log \left(\frac{PS}{PP} \right) + \beta_{ML} \log \left(\frac{PL}{PP} \right) + \beta_{MR} \log \left(\frac{PR}{PP} \right) + \beta_{MN} \log \left(\frac{PN}{PP} \right) + \beta_{MPH} \log \left(\frac{PPH}{PP} \right) + \beta_{MA} \log \left(\frac{PA}{PP} \right) + \beta_{MW} \log \left(\frac{PW}{PP} \right) + \beta_{MM} \log \left(\frac{PM}{PP} \right) + \gamma_{MY} \log Y \quad (14)$$

In these equations, S_a , S_s , S_l , S_n , S_{ph} , S_p , S_w , S_r , and S_m represent the Cost Shares of Manure, Seed, Labor, Nitrogen, Phosphate, Pesticide, Water, Land, and Machinery, respectively. Similarly, P_a , P_s , P_l , P_n , P_{ph} , P_p ,

P_w , P_r , and P_m denote their corresponding Input Prices.

Allen-Uzawa Partial Elasticities of Substitution (AES)

In empirical research, the most commonly employed measures of input substitution are the Allen–Uzawa Elasticities of Substitution (AES) (Zha and Ding, 2014), which were initially proposed by Hicks and Allen (1934) and, subsequently, refined by Allen (1938) and Uzawa (1962). Partial elasticities are a useful method for measuring the substitutability between factors of production and play an essential role in determining the optimal composition and efficient utilization of production inputs (Stern, 1997). These elasticities measure the percentage change in the ratio of two production inputs in response to a 1% change in their relative prices. A positive Allen AES signifies that the inputs function as substitutes, whereas a negative AES indicates a complementary relationship between them (Knoblach and Stöckl, 2020). Equations (15) and (16) specify the Allen cross- and own-partial elasticities of substitution.

$$AES_{ij} = \frac{\beta_{ij} + s_i s_j}{s_i s_j} \quad (15)$$

$$AES_{ii} = \frac{\beta_{ii} + s_i(s_i - 1)}{s_i^2} \quad (16)$$

Here, AES_{ij} represents Allen's Cross-Partial Elasticity of Substitution, AES_{ii} denotes Allen's Own-Partial Elasticity of Substitution, β_{ij} is the coefficient of the j^{th} input in the equation for the i^{th} input, and s_i and s_j are the cost shares of the i^{th} and j^{th} inputs, respectively. The signs of these elasticities are typically expected to be negative, reflecting the general inverse relationship between the demand for a product and its price, except in the case of Giffen goods (A Giffen good is an inferior good whose demand increases as its price increases because the income effect exceeds the substitution effect) (Varian, 1992; Henderson and Quandt, 1980). Allen's cross-partial elasticities are symmetric, if

$AES_{ij} > 0$, this indicates a substitutive relationship between the two inputs, whereas $AES_{ij} < 0$ indicates a complementary relationship (Kuroda, 1987).

Own and Cross-Price Elasticity of Input Demand

The own-price elasticity of demand for a production input reflects the percentage change in the quantity demanded of that input resulting from a 1% change in its own price. Likewise, the cross-price elasticity of demand measures the percentage change in demand for one input resulting from a 1% change in the price of another input (Zha and Ding, 2014). In the present study, cross-price elasticities of input demand are employed to analyze the substitutive or complementary relationships among production inputs. These elasticities were computed using Equations (17) and (18).

$$\varepsilon_{ii} = AES_{ii} \cdot s_i \quad \text{for } i = j \quad (17)$$

$$\varepsilon_{ij} = AES_{ij} \cdot s_j \cdot \varepsilon_{ji} = AES_{ji} \cdot s_i \quad \text{for } i \neq j \quad (18)$$

Here, ε_{ii} denotes the own-price elasticity of demand, ε_{ij} represents the cross-price elasticity of demand for the i^{th} input with respect to the price of the j^{th} input, AES_{ij} indicates Allen's Cross-Partial Elasticity of Substitution, and AES_{ii} denotes Allen's Own-Partial Elasticity of Substitution. When the price elasticity of input demand is greater than zero ($\varepsilon_{ii} > 0$), the demand for that input is considered elastic; when it is less than zero ($\varepsilon_{ii} < 0$), the demand is inelastic; and when it equals unity ($\varepsilon_{ii} = 0$), the demand demonstrates unitary elasticity. Similarly, when the cross-price elasticity of input demand is positive ($\varepsilon_{ij} > 0$), the inputs are considered substitutes, whereas a negative value ($\varepsilon_{ij} < 0$) indicates that the inputs are complements. It is important to note that, unlike the Allen elasticity of substitution, these elasticities are asymmetric—meaning that the elasticity of demand for the i^{th} input with respect to the price of the j^{th} input is not necessarily equal to that of the j^{th} input



with respect to the price of the i^{th} input ($\varepsilon_{ij} \neq \varepsilon_{ji}$).

The cross-price elasticity of input demand measures the absolute degree of substitution between inputs, but does not capture the relative substitution among production factors (Zha and Ding, 2014). In contrast, Allen's Cross-Partial Elasticity of Substitution (AES), compared with the cross-price elasticity of input demand, reflects the relative substitutability between two factors while accounting for the substitution effects of other inputs (Stern, 1997).

The Morishima Elasticity of Substitution (MES)

The MES is defined as the logarithmic derivative of the ratio of input quantities with respect to the ratio of their prices (Morishima, 1967). Introduced by Morishima in 1967, this measure serves as an alternative approach to assessing the degree of substitutability among production inputs. This measure provides a superior framework for evaluating input substitutability under relative price shifts.

The Allen–Uzawa Elasticity of Substitution (AES) fails to retain several essential characteristics of the original Hicksian concept—as follows:

(i) It does not accurately measure the ease of substitution or the curvature of the isoquant, (ii) It offers no insight into relative factor shares,

(iii) It cannot be formulated as the logarithmic derivative of an input quantity ratio with respect to a price ratio.

However, Morishima introduced the Morishima Elasticity of Substitution (MES) to overcome these limitations (Blackorby, C. and Russell, R. R. 1981). The Morishima elasticity of substitution is defined as follows:

$$MES_{ij} = \varepsilon_{ij} - \varepsilon_{jj} \quad (19)$$

Where ε_{ij} represents the cross-price elasticity of the i th input demand with respect to the j th input price, ε_{jj} represents

the own-price elasticity of demand for the j th input, and MES denotes the Morishima elasticity of substitution. A positive MES value indicates a substitution relationship between two inputs, and the magnitude of the value determines the strength of this relationship. Values greater than one ($MES > 1$) denote strong substitution, whereas values between zero and one ($0 < MES \leq 1$) indicate weak substitution. Conversely, a negative MES value signifies a complementary relationship, where the absolute magnitude reflects the degree of complementarity—values greater than one ($|MES| > 1$) suggest strong complementarity, and values between zero and one ($0 < |MES| \leq 1$) indicate weak complementarity. Overall, the MES results are consistent with the Allen–Uzawa partial elasticity estimates, reinforcing the robustness and coherence of the empirical findings.

RESULTS AND DISCUSSION

The results of this study include a descriptive analysis of cotton farmers' data (Table 1), the estimation of the translog cost function and input share demand functions, and the calculation of the Allen, Morishima, own-price, and cross-price elasticities of demand.

Based on the descriptive statistics, the average cotton farmer in Baghlan Province is a seasoned practitioner, with an average of 15.3 years of experience, despite a relatively low average education level of 6.6 years. The data highlight a high reliance on family labor, with a large average family size of 10.9 and a substantial labor input of 164.9 person-days per hectare. This contrasts sharply with the limited use of machinery (23.5 hours ha⁻¹), confirming the labor-intensive nature of cotton cultivation. Furthermore, the results reveal a strong dependency on chemical fertilizers, with high average application rates for both phosphate and urea. In contrast, the use of animal manure is exceptionally low and highly variable, indicating that it is not a

Table 1. Results of descriptive statistics for cotton farmers in Baghlan Province, Afghanistan.

Variable	Unit	Mean	Min	Maxi	Standard Deviation
Age	Years	44.9	20	80	12.97
Education	Years	6.6	0	16	6.39
Family size	Persons	10.9	5	26	3.97
Experience	Years	15.3	2	42	5.93
Animal	kg ha ⁻¹	0.9	0	5	1.45
Phosphate	kg ha ⁻¹	190	100	290	58.08
Urea	kg ha ⁻¹	178	100	250	28.30
Seed	kg ha ⁻¹	76.5	62	98	8.70
Herbicide	kg ha ⁻¹	0.9	0	3	0.95
Insecticide	kg ha ⁻¹	0.6	0	3	0.88
Fungicide	kg ha ⁻¹	0.3	0	3	0.71
Labor	Person-	164.9	133	192	12.77
Water	m ³ ha ⁻¹	4386.1	2433	6272	867
Machinery	Hours	23.5	17	30	3.18

primary input source for most farmers. Overall, these findings provide a crucial foundation for understanding the economic behavior and production constraints of farmers in the region.

Estimation Results of the Cost Function

Table 2 presents the estimation results of the translog cost function for cotton production in Baghlan Province, Afghanistan. As shown, the total cost of cotton production depends on the prices of land, animal manure, nitrogen fertilizer, phosphate fertilizer, pesticides, labor, water, and machinery. While individual coefficient interpretations in Translog models are complex due to the presence of numerous interaction terms, they are primarily used here to derive elasticity estimates, which offer more interpretable insights into the relationships among production inputs.

In this study, we employed the Variance Inflation Factor (VIF) test to assess multicollinearity (O'Brien, 2007), the Jarque-Bera test to examine the normality of

disturbance terms (Jarque and Bera, 1980 and Bera et al., 1984), the White test to diagnose heteroscedasticity (White, 1980), and the LM test to detect autocorrelation (Breusch, 1978; Godfrey, 1978). The results are presented in Tables 2 and 3. The VIF test indicates that there was no serious multicollinearity in the model, as all values were well below the standard threshold of 5. Furthermore, the results of the Jarque-Bera test indicate that the null hypothesis of normally distributed residuals cannot be rejected (Prob> 0.05). Similarly, the results of the White and LM tests confirm that the null hypotheses of homoscedasticity and no autocorrelation in the disturbances cannot be rejected (Prob.> 0.05).

Estimation Results of Input Demand Functions Using Seemingly Unrelated Regression Equations (SURE)

Table 4 presents the estimated demand functions for cotton inputs in Baghlan Province. These functions were systematically estimated using the

**Table 2.** Results of the translog cost function of cotton in Baghlan Province, Afghanistan.

Variables	Description	Coefficients	Prob.	VIF
C	Intercept	6.4317	0.0000	
Log(PA)	Logarithm of the Animal Manure Price	0.0032	0.0172	3.14
Log(PS)	Logarithm of Seed Price	0.0207	0.0000	1.69
Log(PA) ²	Square Logarithm of Animal Manure Price	0.0009	0.0000	4.46
Log(PL) ²	Square Logarithm of Labor Wage	0.1176	0.0000	3.55
Log(PN) ²	Square Logarithm of Nitrogen Price	0.0158	0.0111	1.81
Log(PP) ²	Square Logarithm of Pesticide Price	0.0003	0.0000	2.53
Log(PPH) ²	Square Logarithm of Phosphate Price	0.0293	0.0000	4.07
Log(PR) ²	Square Logarithms of the Land Price	0.1043	0.0000	1.07
Log(PW) ²	Square logarithms of water price	0.0009	0.0000	1.71
Log(Y)*Log(PL)	Interaction of Production and Labor Price	-0.0570	0.0000	4.87
Log(Y)*Log(PM)	Interaction of Production and Machinery Price	0.0662	0.0000	4.96
Log(PA)*	Interaction of Animal Manure Price and Phosphate	-0.0003	0.0049	4.28
Log(PA)*	Interaction of Animal Manure Price and Water	0.0003	0.0209	4.49
Log(PL)*Log(PN)	Interaction of Labor Wage and Nitrogen Price	-0.0212	0.0254	3.90
Log(PL)*Log(PR)	The interaction of Labor Wage and Land Price	-0.1396	0.0000	4.25
Log(PM)*Log(PP)	Interaction of Machinery and Pesticide Price	0.0001	0.0000	1.70
Log(PM)*	Interaction of Machinery Price and Phosphate Price	-0.0265	0.0000	4.03
Log(PM)*Log(PR)	The interaction of Machinery Price and Land Price	-0.0196	0.0096	3.85
Log(PPH)*	Interaction of Phosphate Price and Land Price	-0.0198	0.0000	2.98
R ²	0.9992			
Adjusted R ²	0.9991			
F-Statistics	8088.11			
Prob. (F-statistics) ^a	0.0000			

^a "Prob." (P-values of t-statistics for parameter significance) and "VIF" (Variance Inflation Factor, for multicollinearity). "Prob." denotes the *P*-value of the respective statistical test.

Table 3. Test results of normality, heteroscedasticity, and autocorrelation in the translog cost function of cotton.^a

Test	Normality (Jarque-Bera statistics)	Heteroskedasticity (White test)	Autocorrelation (LM-test)
T-statistics	4.3222	0.4267	1.3205
Prob.	0.1151	0.9821	0.2712

^a "Prob." denotes the *P*-value of the respective statistical test. *P*-values (*P*-value > 0.05) indicate that the null hypotheses of normally distributed residuals (Jarque-Bera test), homoscedasticity (White test), and no autocorrelation (LM test) cannot be rejected at the 5% significance level.

Seemingly Unrelated Regression Equations (SURE) method, and the results were subsequently used to calculate the elasticities of substitution, as described previously.

The statistical significance of the coefficients was assessed at the 5% level, with *p*-values less than 0.05 considered statistically significant. Table 4 shows that all the coefficients of the demand function for the land cost share are significant at this

Table 4. Results of estimating the demand functions of the share of inputs in cotton production in the Baghlan Province of Afghanistan.^a

Inputs	α	$\beta_{PR PR}$	$\beta_{PR PA}$	$\beta_{PR PH}$	$\beta_{PR PN}$	$\beta_{PR PS}$	$\beta_{PR PL}$	$\beta_{PR PW}$	$\beta_{PR PM}$	γ_{PRY}
Land	0.288 (6.75)*	0.208 (15.1)	-0.0004 (-10.1)	-0.024 (-19.5)	-0.0097 (-5.51)	-0.004 (-2.19)	-0.131 (-33.7)	-0.004 (-2.91)	-0.033 (-11.1)	-0.007 (-1.42)
Animal manure	α 0.0671 (1.20)	$\beta_{PA PR}$ -0.003 (-1.89)	$\beta_{PA PA}$ -0.001 (21.79)	$\beta_{PA PH}$ -0.001 (-0.53)	$\beta_{PA PN}$ 0.004 (-1.51)	$\beta_{PA PS}$ 0.0005 (0.182)	$\beta_{PA PL}$ -0.003 (-0.60)	$\beta_{PA PW}$ 0.001 (0.83)	$\beta_{PA PM}$ -0.0001 (-0.03)	γ_{PAY} -0.003 (-0.49)
Phosphate	α 0.185 (5.89)	$\beta_{PPH PR}$ -0.023 (-17.0)	$\beta_{PPH PA}$ -0.0001 (-5.82)	$\beta_{PPH PH}$ 0.070 (76.14)	$\beta_{PPH PN}$ -0.004 (-2.96)	$\beta_{PPH PS}$ -0.002 (-1.31)	$\beta_{PPH PL}$ -0.034 (-12.0)	$\beta_{PPH PW}$ 0.0004 (0.40)	$\beta_{PPH PM}$ -0.006 (-2.84)	γ_{PPHY} -0.002 (-0.62)
Nitrogen	α 0.096 (12.36)	$\beta_{PN PR}$ -0.009 (-43.5)	$\beta_{PN PA}$ -0.000 (-4.89)	$\beta_{PN PH}$ -0.002 (-10.6)	$\beta_{PN PN}$ 0.030 (88.8)	$\beta_{PN PS}$ -0.0006 (-1.75)	$\beta_{PN PL}$ -0.013 (-18.2)	$\beta_{PN PW}$ -0.0002 (-0.97)	$\beta_{PN PM}$ -0.004 (-8.02)	γ_{PNY} -0.0003 (-0.37)
Seed	α 0.064 (12.36)	$\beta_{PS PR}$ -0.007 (-48.7)	$\beta_{PS PA}$ -0.000 (-5.95)	$\beta_{PS PH}$ -0.001 (-10.9)	$\beta_{PS PN}$ -0.001 (-5.66)	$\beta_{PS PS}$ 0.020 (80.2)	$\beta_{PS PL}$ -0.008 (-18.0)	$\beta_{PS PW}$ -0.0002 (-1.29)	$\beta_{PS PM}$ -0.005 (-4.30)	γ_{PSY} 0.0007 (1.30)
Labor	α 0.079 (1.90)	$\beta_{PL PR}$ -0.1295 (-11.1)	$\beta_{PL PA}$ -0.0005 (-13.3)	$\beta_{PL PH}$ -0.032 (-26.5)	$\beta_{PL PN}$ -0.015 (-8.54)	$\beta_{PL PS}$ -0.013 (-6.80)	$\beta_{PL PL}$ 0.243 (64.0)	$\beta_{PL PW}$ -0.005 (-3.72)	$\beta_{PL PM}$ -0.046 (15.8)	γ_{PLY} 0.008 (1.78)
Water	α 0.036 (8.46)	$\beta_{PW PR}$ -0.002 (-20.4)	$\beta_{PW PA}$ -0.000 (-3.26)	$\beta_{PW PH}$ -0.0005 (-4.33)	$\beta_{PW PN}$ -0.0003 (-1.68)	$\beta_{PW PS}$ -0.000 (-0.22)	$\beta_{PW PL}$ -0.0042 (-10.7)	$\beta_{PW PW}$ 0.008 (58.39)	$\beta_{PW PM}$ -0.0008 (-2.94)	γ_{PWY} 0.0001 (0.31)
Machinery	α 0.160 (11.15)	$\beta_{PM PR}$ -0.031 (-78.5)	$\beta_{PM PA}$ -0.0001 (-7.38)	$\beta_{PM PH}$ -0.007 (-17.8)	$\beta_{PM PN}$ -0.002 (-4.36)	$\beta_{PM PS}$ -0.002 (-3.56)	$\beta_{PM PL}$ -0.042 (-32.4)	$\beta_{PM PW}$ -0.0008 (-1.81)	$\beta_{PM PM}$ 0.087 (87.71)	γ_{PMY} 0.002 (1.75)

^a Numbers in parentheses are *t*-statistics.

level, except for the production variable. The demand for land share is positively related to the price of land; that is, as the price of land increases, the share of land in the total cost of cotton production also rises. In contrast, there is a significant negative relationship between the prices of animal manure, phosphate fertilizer, nitrogen fertilizer, seed, labor, water, and machinery, indicating that as the prices of these inputs increase, the share of land in total cost decreases. The demand functions for other

input shares can be interpreted similarly.

Allen-Uzawa Partial Elasticities of Substitution (AES)

Table 5 presents the results of Allen-Uzawa's own and cross partial elasticities of substitution, calculated from the estimated coefficients of the demand functions. All the Allen-Uzawa partial elasticities of substitution (i.e., the diagonal elements of

Table 5. Partial elasticity of the Allen-Uzawa substitution for cotton production inputs in Baghlan Province, Afghanistan.

Inputs	Land	Manure	Phosphate	Nitrogen	Seed	Labor	Water	Machinery
Land	-0.109	0.831	0.090	0.122	0.078	0.033	0.057	-0.031
Manure	--	-99.080	0.725	0.858	0.831	0.811	0.764	0.885
Phosphate	--	--	-0.692	0.067	0.283	0.073	0.431	-0.249
Nitrogen	--	--	--	-1.490	0.197	0.057	0.868	-0.492
Seed	--	--	--	--	-2.508	-0.267	3.567	0.719
Labor	--	--	--	--	--	-0.221	-0.923	0.751
Water	--	--	--	--	--	--	-9.302	1.358
Machinery	--	--	--	--	--	--	--	-1.659



the elasticity matrix) have the expected negative sign, reflecting the inverse relationship between the price of an input and its quantity demanded, consistent with economic theory. The results indicate a mix of elastic and inelastic relationships. The absolute magnitude of the substitution elasticity is elastic (greater than one) for animal manure (-99.08), water (-9.302), seeds (-2.508), machinery (-1.659), and nitrogen fertilizer (-1.49), suggesting that changes in the relative prices of these inputs would lead to more than proportional changes in their usage. In contrast, the elasticity is inelastic (less than one) for phosphate fertilizer (-0.692), labor (-0.221), and land (-0.109), indicating that the demand for these inputs is relatively insensitive to price changes. The high elasticity of animal manure suggests that its consumption is highly responsive to price fluctuations, making it a viable input for rapid adjustments in production. Conversely, the low elasticity of land implies that its use is relatively stable and not significantly affected by price changes, reflecting its fundamental role as a fixed factor of production.

The cross-elasticity results provide deeper insights into the relationships among inputs. Land exhibits a complementary relationship with machinery (AES= -0.031), indicating that expanding cultivated area requires additional machinery. This finding partially aligns with Bui *et al.* (2018), who observed similar factor substitutions in rice production in Vietnam. The results also reveal a weak substitution relationship between land and most other inputs, including manure, fertilizers, seeds, and labor. These findings support the theory of agricultural intensification in developing economies, as confirmed by Hussain *et al.* (2020), where labor and other inputs are utilized to increase yield per unit of land.

Animal manure serves as a substitute for all other inputs, including phosphate, nitrogen, seeds, labor, water, and machinery. This notable finding supports sustainable agricultural practices. The capacity to

replace chemical inputs, such as phosphate and nitrogen fertilizers, with animal manure presents an opportunity to mitigate environmental externalities and reduce chemical pollution.

Both phosphate and nitrogen fertilizers exhibit a substitutable relationship with seeds, labor, and water, suggesting that increased use of labor, for instance, could reduce the need for fertilizers, with positive environmental implications. In contrast, both fertilizers show a complementary relationship with machinery, likely because the application of fertilizers depends on machinery, such that greater use of one input requires greater use of the other.

Labor is a complement to seeds (AES= -0.267) and water (AES= -0.923). The complementarity with seeds indicates that cotton cultivation in this region is labor-intensive, particularly during the manual sowing process. The complementarity with water suggests that traditional irrigation methods require a significant amount of manual labor. Labor is also a substitute for machinery (AES= 0.751). This finding is consistent with economic theory and aligns with studies by Zhan and Zhou (2019), but contrasts with Pippa Rochelle and Ferreira Filho (1999), who found a complementary relationship between these inputs in Brazil. The positive elasticity indicates that an increase in machinery prices would lead to increased labor usage, and vice versa, confirming the potential for substituting manual labor for mechanical power.

Water is a complement to labor but a substitute for all other inputs. The complementarity with labor reflects the reliance on traditional irrigation methods, which require substantial manual effort. The substitution relationship with other inputs suggests that increased use of these inputs, such as higher-quality seeds or additional fertilizers, can enhance productivity and lead to more efficient water use.

Seeds exhibit a substitution relationship with water and machinery, indicating that the use of higher-quality seeds can reduce the need for these inputs. However, they are

complementary to labor, emphasizing the traditional, labor-intensive sowing practices in the region.

Morishima Elasticity of Substitution (MES)

Table 6 reports MES among the primary cotton production inputs in Baghlan Province. Each cell in the table displays the MES value for the input listed in the row in response to a price change in the input listed in the corresponding column. For instance, the value in cell row 1, column 2, represents the elasticity of substitution of land with respect to manure following a change in the price of manure. The results indicate that most input pairs exhibit positive MES values, implying varying degrees of substitution between them. The strongest substitution occurs between manure and other inputs, particularly labor (MES= 1.146), land (MES= 1.081), and phosphate fertilizer (MES= 0.870). These values suggest that animal manure can effectively replace part of the chemical fertilizer demand, and increased labor use can complement organic input management, reflecting both economic and environmental advantages. Similarly, machinery shows moderate substitution relationships with labor (MES= 0.510), seed (MES= 0.214), and water (MES= 0.209), indicating that mechanization can partly substitute for manual operations and input handling. In contrast, the negative MES values between water and other inputs—especially labor

(MES= -0.457), manure (MES= -0.068), and land (MES= -0.055)—indicate complementarity. This pattern reflects traditional irrigation systems that rely heavily on manual labor and land expansion. Overall, the MES estimates reveal that substitution is generally stronger among organic and chemical inputs and weaker between resource-intensive factors like water and labor. These findings are broadly consistent with the Allen–Uzawa elasticity results, confirming similar substitution and complementarity patterns across key input pairs.

The results of the MES in this study are broadly consistent with findings from previous research. Similar to Du *et al.* (2019), who reported limited substitution possibilities among major agricultural inputs in fruit production, our results indicate weak substitution between labor and machinery and strong substitution between organic and chemical fertilizers. Likewise, Pang *et al.* (2021) found that substitution patterns vary by crop type, with fertilizer and labor being substitutes in some contexts and complements in others, a pattern also observed in our study. The complementary relationship between water and labor in Baghlan Province aligns with the findings of Ansari Roshandeh *et al.* (2022) for irrigated rice production in Iran, highlighting the persistence of labor-intensive irrigation practices in developing agricultural systems. In contrast, the strong substitution between animal manure and chemical fertilizers found in our analysis extends previous

Table 6. MES for cotton production inputs in Baghlan Province, Afghanistan.

Inputs	Land	Manure	Phosphate	Nitrogen	Seed	Labor	Water	Machinery
Land	--	0.042	0.043	0.040	0.038	0.050	0.036	0.033
Manure	1.081	--	0.870	0.839	0.830	1.146	0.817	0.900
Phosphate	0.086	0.062	--	0.059	0.063	0.087	0.060	0.032
Nitrogen	0.081	0.050	0.048	--	0.047	0.072	0.050	-0.007
Seed	0.083	0.063	0.080	0.063	--	-0.054	0.085	0.129
Labor	0.103	0.098	0.098	0.094	0.086	--	0.085	0.168
Water	-0.055	-0.068	-0.039	-0.045	0.006	-0.457	--	0.063
Machinery	0.188	0.204	0.178	0.181	0.214	0.510	0.209	--

**Table 7.** Domestic and intersecting price elasticities of demand for cotton production inputs in Baghlan Province of Afghanistan.

Inputs Demand function	Land	Manure	Phosphate	Urea	Seed	Labor	Water	Machinery
Land	-0.036	0.007	0.007	0.004	0.002	0.014	0.000	-0.003
Manure	0.271	-0.815	0.059	0.029	0.019	0.337	0.006	0.089
Phosphate	0.029	0.006	-0.056	0.002	0.006	0.030	0.003	-0.025
Urea	0.040	0.007	0.005	-0.050	0.004	0.031	0.007	-0.050
Seed	0.025	0.007	0.023	0.007	-0.056	-0.111	0.029	0.072
Labor	0.011	0.007	0.006	0.002	-0.006	-0.092	-0.007	0.076
Water	0.019	0.006	0.035	0.029	0.080	0.383	-0.074	0.137
Machinery	-0.010	0.007	-0.020	-0.016	0.016	0.312	0.011	-0.198

results, suggesting that organic inputs could play a larger role in sustainable cotton production. Overall, these comparisons confirm the reliability of the MES estimates and demonstrate that the substitution and complementarity relationships identified in this study are consistent with international evidence.

Own- and Cross-Price Elasticities of Input Demand

Table 7 presents the own- and cross-price elasticities of demand for cotton production inputs in Baghlan Province. All price elasticities of demand for land, animal manure, phosphate fertilizer, nitrogen fertilizer, seeds, labor, water, and machinery exhibit the expected negative sign, in line with economic theory. The absolute values of the own-price elasticities are less than 1 for all inputs, indicating that demand is inelastic. These results are partially consistent with Wijetunga (2023), who analyzed rice production in Sri Lanka. In practical terms, a 1% increase in input prices leads to a less than 1% decrease in input demand. Among the inputs, animal manure has the highest own-price elasticity, while land has the lowest.

Table 7 presents the own- and cross-price elasticities of demand for production inputs. These elasticities indicate how the demand for each input responds to a 1% change in the price of other inputs. In terms of

complementarity and substitution, the results are consistent with the Allen–Uzawa partial elasticities of substitution.

CONCLUSIONS

This study evaluated input demand and substitution elasticities of cotton production in Baghlan Province, Afghanistan, using a Translog cost function estimated via Seemingly Unrelated Regression (SUR). Results show that input demand is generally inelastic, with animal manure displaying the highest own-price elasticity and land the lowest. Substitution analyses revealed strong substitution between manure and chemical fertilizers, whereas labor and machinery exhibited weak substitution. Additionally, labor and water showed a complementary relationship due to traditional, labor-intensive irrigation practices. Policy efforts should promote organic manure to reduce fertilizer costs and environmental impacts, while mechanization strategies must balance productivity with rural employment. Future research should integrate dynamic welfare simulation models to assess policy impacts on farm incomes.

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تخمین کشش‌های قیمتی و جانشینی نهاده‌های تولید پنبه: شواهد تجربی از استان بغلان،
افغانستان

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چکیده

هدف این مطالعه تخمین کشش‌های قیمتی و جانشینی بین نهاده‌های تولید پنبه در استان بغلان افغانستان است. داده‌ها از طریق ۱۳۲ پرسشنامه و با استفاده از نمونه‌گیری تصادفی طبقه‌بندی‌شده از کشاورزان پنبه در این استان جمع‌آوری شد. روابط بین نهاده‌های تولید با استفاده از تابع هزینه ترانسلوگ و با رویکرد رگرسیون به ظاهر نامرتبط (SUR) بررسی شد. نتایج نشان داد که کشش‌های قیمتی تقاضا برای زمین، کود حیوانی، کود فسفات، کود اوره، پذر، نیروی کار، آب و ماشین‌آلات به ترتیب به $-۰/۰۳۶$ ، $-۰/۸۱۵$ ، $-۰/۰۵۶$ ، $-۰/۰۵$ ، $-۰/۰۵۶$ و $-۰/۰۹۲$ و $-۰/۱۹۸$ برآورد گردید. بر اساس نتایج همه کشش‌های قیمتی تقاضای نهاده‌ها کمتر از یک بود که نشان‌دهنده تقاضای نهاده بی‌کشش است، یعنی استفاده از نهاده‌ها به



تغییرات قیمت حساسیت زیادی ندارد. کشش‌های متقاطع تقاضای نهاده‌ها نیز کمتر از یک بود که نشان‌دهنده تقاضای بی‌کشش در بین همه نهاده‌ها است. مقادیر کوچک کشش‌های جانشینی همچنین نشان داد که سیاست‌هایی که یک نهاده واحد را هدف قرار می‌دهند، تأثیر کمی بر تخصیص سایر نهاده‌ها خواهند داشت. بنابراین، توصیه می‌شود که سیاست‌گذاران از تمرکز بر نهاده‌های به صورت انفرادی اجتناب نموده و در عوض، هنگام طراحی سیاست‌های کشاورزی، همه نهاده‌ها را به عنوان یک سیستم یکپارچه در نظر بگیرند.

Greenhouse Farmers' Behavioural Intentions to Reduce Pesticide Use in Antalya-Turkiye

Mustafa Kan^{1*}, Hasan Yilmaz², Hasan Gökhan Doğan³, Merve Mürüvvet Dağ², and Tidiani Diallo²

ABSTRACT

This study investigates farmers' intentions to reduce the use of pesticides in greenhouse cultivation in Antalya Province, Turkey. Using an extended version of the Theory of Planned Behaviour (TPB), we investigated the socio-psychological factors that influence farmers' intentions to adopt more sustainable practices. Interviews with 297 farmers growing tomatoes, peppers, and eggplants revealed that, while farmers generally have a positive attitude towards reducing pesticide use, this attitude does not have a significant impact on their actual intentions. These intentions are chiefly driven by moral norms, subjective norms, and perceived behavioural control. Improving social support, creating an enabling environment, and implementing educational programmes can significantly help farmers to adopt agroecological practices. This study emphasizes the importance of understanding the social and psychological factors that influence farmers' decisions, which is essential for developing effective strategies for environmentally friendly agriculture.

Keywords: Agroecology, Farmers' intentions, Pesticide reduction, Theory of Planned Behavior.

INTRODUCTION

Agriculture has long been a cornerstone of civilization, meeting essential human needs and supplying raw materials for various industries. However, it faces a major transformation to achieve sustainability in harmony with ecosystems. While scientific and technological advances have boosted productivity, they have also introduced complex economic, environmental, and social challenges. To address these issues and ensure a sustainable legacy, there is a growing shift away from traditional approaches toward more sustainable practices—most notably, agroecological methods.

Agroecology integrates ecological and

social principles holistically to build sustainable food systems. It promotes socially just systems where individuals decide what, how, and where to consume, while optimizing interactions between plants, animals, humans, and the environment (FAO, 2025). Agroecology aims to minimize the use of agrochemicals and energy through intentional agroecosystem design (Altieri and Nicholls, 2017), while preserving biodiversity and sustaining ecosystem services (Wezel *et al.*, 2020). Recognized by FAO as key to sustainable agriculture (FAO, 2021), agroecology highlights agricultural control strategies as a core component.

Pest-related losses pose a major threat to global food security. The Green Revolution

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relied heavily on chemical pesticides, which initially increased yields (FAO, 1984). However, concerns about health, biodiversity, and environmental harm have raised doubts regarding the sustainability of these methods (WHO/UNEP, 1989; WRI, 1992; Pimentel, 1996; Aktar *et al.*, 2009). In this context, Deguine *et al.* (2023) outline five core challenges for 21st century agricultural control: maintaining productivity, ensuring healthy food, reducing environmental harm, securing farmer incomes, and adapting to climate change. Here, Agroecological Plant Protection (APP) has emerged as a viable solution (Deguine *et al.*, 2020).

APP applies two agroecological principles: leveraging biodiversity and improving soil health. It favors preventive approaches, such as disease-resistant varieties and integrated practices, over sole reliance on pesticides (INRAE, 2023). There is an ongoing global shift towards rethinking pesticide application, with several countries working to limit chemical dependence. For instance, the EU's "Farm to Fork" strategy aims to halve pesticide use by 2030, promoting agroecology as a solution (European Commission, 2020; OECD, 2023). The FAO also advocates for widespread adoption of APP (FAO, 2021).

In Turkey, agroecological practices such as polyculture, biological control, and organic farming are recognized as effective in reducing pesticide reliance (Öztemiz, 2008; Azizoğlu *et al.*, 2012; Şişman, 2023). With 23.5 million hectares of arable land and 30 agroecological regions, Turkey has a diverse agricultural landscape (Kaymak *et al.*, 2015). Despite having 2.2 million farms and 6 million agricultural workers, which produces around 140 million tons annually (Birişik *et al.*, 2015), Turkey faces serious pest issues. Interestingly, it is among the lowest pesticide users globally, with 2.22 kg ha⁻¹, less than countries like Israel (14.71), Japan (11.63) or the USA (3.02) (FAOSTAT, 2025). However, pesticide usage is often unregulated, with over 60% concentrated in the Mediterranean, Aegean,

and Marmara Regions (Delen *et al.*, 2015). Antalya, known for its strong fresh vegetable and greenhouse production, faces significant risks from pesticide residues, driving interest in alternative practices. Studies emphasize the urgency of transitioning from chemical-intensive methods to knowledge-based, ecosystem-focused strategies (Altieri *et al.*, 2024). In Turkey, agroecological practices such as polyculture, biological control, and organic farming are seen as effective in reducing pesticide reliance (Öztemiz, 2008; Azizoğlu *et al.*, 2012; Şişman, 2023).

To understand the decision-making processes of farmers, recent research employs the Theory of Planned Behavior (TPB), developed by Ajzen (1991). TPB posits that behavioral intentions are influenced by three key components: Attitude, which reflects personal evaluations of a behavior; subjective norm, encompassing social pressures and expectations from peers; and perceived behavioral control, indicating beliefs about one's ability to perform the behavior effectively. By examining these interconnected factors, TPB elucidates the psychological drivers behind farmers' choices. This understanding offers valuable insights for designing targeted interventions that promote sustainable agricultural practices and encourage a reduction in pesticide use. Recognizing these dynamics is essential for policymakers and agricultural educators, as it helps create strategies that align with farmers' motivations and social influences, ultimately leading to more environmentally friendly farming methods.

Pesticides play a significant role in modern agriculture but have well-documented adverse impacts on ecosystems and human health (Aktar, *et al.*, 2009). These impacts have spurred research into farmers' pesticide use behaviors and the challenges involved in promoting sustainable and reduced-use practices (Pretty and Bharucha, 2015). Studies show that these TPB components significantly affect farmers' pesticide use behavior (de Bon *et*

al., 2014; Fan *et al.*, 2015; Jallow *et al.*, 2017; Wang *et al.*, 2018; Sarma, 2022; Pirmoghni *et al.*, 2024). Although attitudes and subjective norms are important variables within TPB, perceived behavioral control has emerged as a key factor in studies on pesticide use behavior (Abtey *et al.*, 2016; Schreinemachers *et al.*, 2017; Vidogbena *et al.*, 2016). Expanded versions of TPB also consider additional factors, such as moral norms and government policy awareness (Monfared *et al.*, 2015; Bagheri *et al.*, 2019; Batbay and Kahramanoğlu, 2024; Damalas, 2021).

Reducing pesticide use is critical for sustainable agriculture and human health. Understanding farmer behavior through TPB is essential, particularly in high-use systems like greenhouses. Using TPB, this study analyzes greenhouse farmers in Antalya, to assess how attitudes, norms, perceived behavioral control, and moral norms affect their behavioral intentions. The findings are key to shaping strategies that support agroecological practices in greenhouse farming.

the highest pesticide use in agriculture due to its diverse crop pattern (Özercan and Taşçı, 2022). Erdoğan (2024) attributes this to factors such as crop diversity, widespread pest and disease damage, multiple harvests per season, favorable climate and soil, irrigation availability, extensive greenhouse production, and the region's role in agricultural exports. Within this region, Antalya province has the highest pesticide use, accounting for 7.72% of the total national usage (MoAF, 2023). The prevalence of greenhouse farming and high pesticide application makes Antalya a suitable study area.

The study focused on agroecological sub-regions in Antalya where greenhouse cultivation of tomatoes, peppers, and eggplants is concentrated. These sub-regions were selected using the Purposive Sampling Method based on climate, soil, and vegetation to ensure representation of areas where all three crops are grown. Sub-regions I and II (Kumluca, Demre, Kaş, and Aksu) represent tomato and pepper production, while Sub-region III (Alanya, Gazipaşa) represents eggplant production, as shown in Figure 1.

MATERIALS AND METHODS

Research Area

In Turkey, the Mediterranean region has

Data Collection Tool and Sample

The main material of the study consisted



Figure 1. Research area map.

of survey data collected from 297 farmers who grew tomatoes, peppers, and eggplants in greenhouses in Aksu, Alanya, Demre, Gazipaşa, Kaş and Kumluca districts of Antalya province. The sampling, from the finite population, was estimated using formula in Equation (1), employing the Simple Random Sampling Method with population proportions (Güneş and Arıkan, 1988). In the study, P (0.50) and $(1-P)$ (0.50) were taken to reach the maximum sample size. In the study, the 95% confidence interval $\sigma_{(px)}$ was calculated as 0.0026 for a 10% margin of error. The sample size was calculated for each product group, and data on the population was obtained from the Ministry of Agriculture and Forestry. According to these data, there were 8,448 tomato producers, 5,433 pepper producers, and 1,156 eggplant producers forming the population in the greenhouse within the research area.

$$n = \frac{Np(1-p)}{(N-1)\sigma_{px}^2 + p(1-p)} \quad (1)$$

Where, n = Sample size; N = Population size, p = Estimation ratio (0.05 for maximum sample size), and σ_{px}^2 = Denotes the population variance.

The data come from personal surveys conducted with the farmers between August and September 2023. In this context, a survey was conducted with 103 bell pepper, 105 tomato, and 89 eggplant farmers.

Research Model and Hypotheses

The study employed the TPB to examine factors influencing greenhouse tomato, bell pepper, and eggplant farmers' intentions to reduce pesticide use. Ajzen's (1991) TPB serves as a prominent model for examining Behavioral Intentions (INT), emphasizing Attitude (ATT), Subjective Norm (SN), and Perceived Behavioral Control (PBC) as its three fundamental constructs. ATT involves an individual's evaluations and emotional responses toward a behavior; SN reflects the expectations of important individuals or groups; and PBC concerns one's perception of control over performing the behavior (Fishbein and Ajzen, 2010) (Figure 2).

In this study, the Moral Norms (MN) variable was included in the model in addition to the ATT, SN, and PBC variables. Scientific literature has documented the integration of MN into the Theory of Planned Behavior (TPB), demonstrating that this variable significantly influences behavioral intention and enhances the explanatory power of models incorporating it (Kaiser and Scheuthle, 2003; Monfared *et al.*, 2015; Bagheri *et al.*, 2019). Given the critical environmental and health implications associated with pesticide use, it is particularly relevant to consider moral norms as a key factor shaping farmers' intentions to reduce pesticide application. Therefore, the TPB framework was

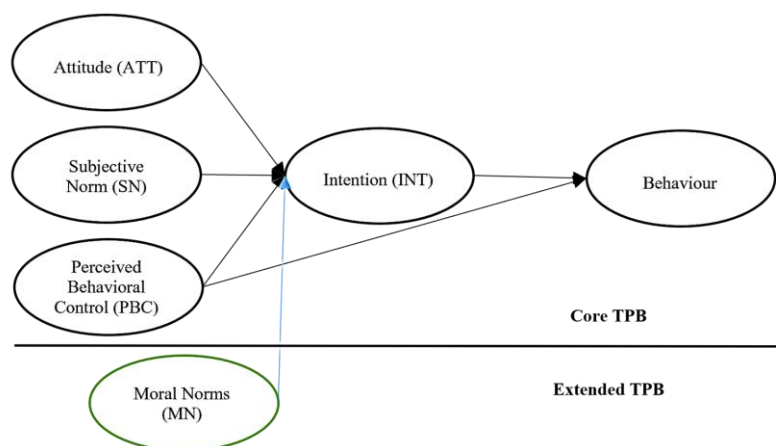


Figure 2. Theory of planned behaviour (modified from Ajzen, 1991).

extended to include moral norms to better capture these ethical considerations in producers' decision-making processes. In the study, the model was developed within the scope of Ajzen's TPB. The following hypotheses were made regarding the intention of farmers to reduce the use of pesticides in greenhouse agriculture.

H₁: There is a positive correlation between farmers' attitudes towards pesticide use and their intention to reduce pesticide use.

H₂: The variable of farmers' moral norms has a positive correlation on the intention to reduce pesticide use.

H₃: The variable of farmers' perceived behavioral control over pesticide use has a positive correlation on the intention to reduce pesticide use.

H₄: Farmers' subjective norms regarding pesticide use have a positive correlation with the behavioral intention to reduce pesticide use.

The measurement instrument used in the study consisted of scales that measure five different constructs. Three statements (ATT1, ATT2, ATT3) were used to measure Attitude (ATT); three statements (SN1, SN2, SN3) for Subjective Norms (SN); three statements (MN1, MN2, MN3) for Moral Norms (MN); three statements (PBC1, PBC2, PBC3) for Perceived Behavioral Control (PBC); and six statements (INT1, INT2, INT3, INT4, INT5, INT6) were used to measure Behavioral Intention (INT). The scales and their statements are listed in the table. The study employed a 5-point Likert scale (strongly disagree/strongly agree) for its measurements. When developing the scales used in the study, the literature review was taken into account (Table 1).

Confirmatory Factor Analysis (CFA) and Structural Equation Modeling (SEM) were among the multivariate statistical approaches utilized in the study. Cronbach's alpha was calculated to assess the internal consistency of the scales. Reliability refers to the consistency of data collection tools in yielding the same results under similar conditions (Aziz, 2014) and is defined as the extent to which an instrument accurately

measures the true value of a variable (Özdamar, 2016). Measured between 0 and 1, Cronbach's alpha assesses how well the scale reflects the construct being measured (Haytko and Matulich, 2008; Özdamar, 2016). A value above 0.70 indicates acceptable reliability. The minimum acceptable KMO sample adequacy value is 0.50 (Sipahi *et al.*, 2010; Özdamar, 2016).

Construct and convergent validity were tested using CFA, which is a statistical technique used to assess the construct validity of a measurement model by testing whether the data fit a hypothesized factor structure that is based on theory or prior empirical evidence. It is widely used to examine both construct validity and convergent validity (Brown, 2015). Validity assesses whether the instrument accurately and appropriately measures the intended construct without error (Aziz, 2014). Relationships among the study's hypotheses were analyzed using Structural Equation Modeling (SEM) with latent variables to test model accuracy. Model fit was evaluated using SmartPLS 4 software (Ringle *et al.*, 2024) based on several goodness-of-fit indicators: the Standardized Root Mean Square Residual (SRMR), which measures the average magnitude of correlation discrepancies, the Normed Fit Index (NFI), which evaluates relative fit against a null model, the Unweighted Least Squares discrepancy (dULS) and Geodesic Discrepancy (dG), which serve as exact fit criteria evaluating model-implied matrix discrepancies via bootstrap confidence intervals and the Chi-square to degrees of freedom ratio (χ^2/df). Ideal thresholds are SRMR < 0.08 (Hu and Bentler, 1999; Schermelleh-Engel *et al.*, 2003), NFI > 0.90 (Bentler and Bonett, 1980; Schermelleh-Engel *et al.*, 2003), Chi-square/df < 5 for an acceptable fit, and Chi-square/df < 3 for a good fit (Wheaton *et al.*, 1977; Schermelleh-Engel *et al.*, 2003; Kline, 2016). Since chi-square is sensitive to sample size, additional fit indices like d_ULS and d_G were used. Henseler *et al.* (2016) suggest d_ULS and d_G < 0.95 indicate good fit.

**Table 1.** Measurement scale employed in the study based on the Extended Theory of Planned Behavior (TPB).

Attitudes (ATT)	
ATT1	Production without the use of pesticides is important for human health.
ATT2	Production without the use of pesticides is important for the production of safe food.
ATT3	Production without the use of pesticides is important for the environment.
Subjective Norms (SN)	
SN1	My close friends think I should use less/no pesticides on my produce.
SN2	My Like-minded farmers support pesticide-free farming.
SN3	My family urges me to limit the use of pesticides in my produce.
Moral Norms (MN)	
MN1	I feel like a better person when I don't use pesticides or use as little as possible in the right dose
MN2	I feel like I am personally contributing to something good when I use less pesticides
MN3	I believe it is morally wrong to prioritize profit over reducing pesticide use
Perceived Behavioral Control (PBC)	
PBC1	I believe that I have the means to grow my produce without pesticides.
PBC2	I would have no problems achieving non-chemical pest control.
PBC3	It would be possible for me to switch to non-chemical pest control.
Behavioral Intention (INT)	
INT1	I plan to use fewer pesticides in my production in the near future.
INT2	I plan to replace pesticides with non-chemical pest control methods.
INT3	I plan to start using biological pest control in the near future.
INT4	I am looking at non-chemical pest control options in the future.
INT5	I see myself in the future as someone who is known for selling healthy food.
INT6	I see myself in the future as someone who conducts more research into alternatives to pesticides and attends training to learn about them if needed.

Sources: Bagheri *et al.*, 2019; Yazdanpanah *et al.*, 2019; Savari and Gharechae 2020; Ataei *et al.*, 2021.

The study was analyzed using SmartPLS, which, as a data analysis method, is evaluated in two stages, the measurement model and the structural model (Hair *et al.*, 2014). The model's reliability and validity were further assessed using Composite Reliability (CR) and Cronbach's Alpha (Fornell and Larcker, 1981). Validity was evaluated using Average Variance Extracted (AVE), along with convergent and discriminant validity analyses. The Fornell-Larcker criterion (Fornell and Larcker, 1981) and the HTMT test (Heterotrait-Monotrait Ratio) (Henseler *et al.*, 2015) are used to determine discriminant validity.

RESULTS

In order to understand the intentions of greenhouse producers regarding the reduction of pesticide use and to establish a robust measurement framework for this purpose, the original model, comprising five variables and 24 indicators, was first

evaluated for reliability. During the analysis, six indicators that did not meet the reliability criteria were removed, resulting in a revised model consisting of five variables and 18 indicators. Confirmatory factor analysis demonstrated that all the remaining scales exhibited satisfactory reliability, thereby confirming the adequacy of the revised measurement model (Table 2). This step ensured that subsequent structural analyses would be grounded on a sound and reliable measurement foundation.

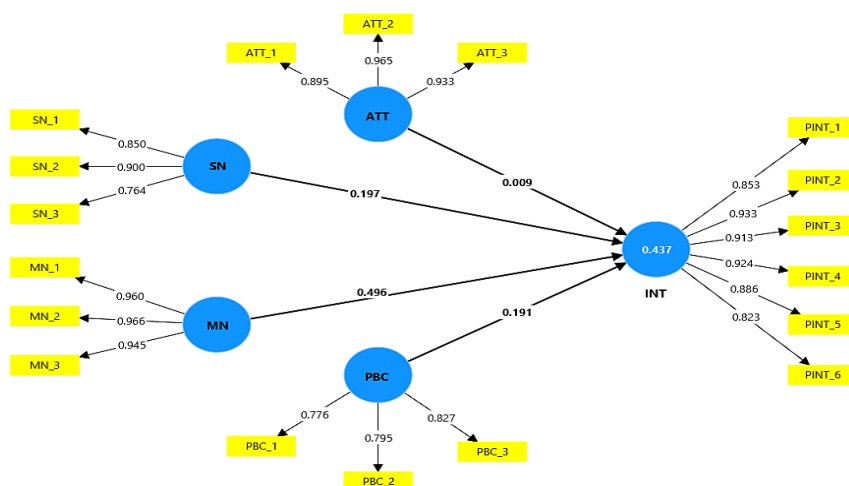
The study presents the factor loadings, the Cronbach's alpha values, the composite reliability values (rho_a and rho_c) and the AVE values of the indicators for each variable (Table 3 and Figure 3). An examination of the factor loadings of the indicators shows that they are above the acceptable criterion of 0.70. Similarly, Cronbach's alpha, rho_a, and rho_c values above 0.7, and the AVE above 0.5 indicate that the scale validity is strong (Hair *et al.*, 2022; Hair *et al.*, 2024).

Table 2. Reliability assessment of research scales.

Variables	Cronbach's α coefficient	Composite reliability		Average Variance Extracted (AVE)
		rho a	rho c	
ATT	0.923	0.946	0.951	0.867
INT	0.947	0.948	0.958	0.791
MN	0.954	0.957	0.970	0.916
PBC	0.719	0.727	0.842	0.640
SN	0.790	0.808	0.877	0.706

Table 3. Loading factor, cronbach alpha and composite reliability.

Variables	Indicators	Loading factors	Cronbach's α	rho_a	rho_c	AVE
ATT	ATT_1	0.895	0.923	0.946	0.951	0.867
	ATT_2	0.965				
	ATT_3	0.933				
MN	MN_1	0.960	0.954	0.957	0.970	0.916
	MN_2	0.966				
	MN_3	0.945				
PBC	PBC_1	0.776	0.719	0.727	0.842	0.640
	PBC_2	0.795				
	PBC_3	0.827				
SN	SN_1	0.850	0.790	0.808	0.877	0.706
	SN_2	0.900				
	SN_3	0.764				
INT	INT_1	0.853	0.947	0.948	0.958	0.791
	INT_2	0.933				
	INT_3	0.913				
	INT_4	0.924				
	INT_5	0.886				
	INT_6	0.823				

**Figure 3.** Validity and reliability of research indicator.

To ensure the distinctiveness of the constructs used in this study, discriminant

validity was assessed using the method proposed by Fornell and Larcker (1981).



Discriminant validity examines whether each construct is truly unique by comparing it with other constructs through the square root of the AVE. In the present study, the square roots of the AVE values (highlighted in bold in Table 4) were higher than the correlations with other variables, thereby confirming the discriminant validity of the scales. In addition, the Heterotrait-Monotrait (HTMT) ratio was employed as a complementary and more contemporary criterion for discriminant validity (Henseler *et al.*, 2016). HTMT values below 1 indicate adequate discriminant validity (Henseler *et al.*, 2016), and all values in this study met this requirement. These findings demonstrate that the measurement instruments effectively differentiate between constructs, providing a reliable foundation for subsequent analyses (Table 4).

SEM was employed to test the study's hypotheses. After confirming the measurement model's fit, the structural model was evaluated against the data. The model fit indices are presented in Table 5. The results indicate Chi-square= 496.562,

Degrees of freedom= 296 (χ^2/df = 1.678), NFI= 0.891, and SRMR= 0.049. Although NFI ideally exceeds 0.90, it is sensitive to sample size and the observed value is considered acceptable (Bentler and Bonett, 1980; Dash and Paul, 2021; Lin *et al.*, 2017; Yusif *et al.*, 2020; Cahigas *et al.*, 2022). Exact fit was further assessed using Squared Euclidean (d_{ULS}) and Geodesic Distances (d_G), requiring non-significant differences at 95% and 99% confidence levels (Jöreskog and Sörbom, 1996; Wah *et al.*, 2023). As shown in Table 5, no significant differences were observed in either the estimated or saturated model, confirming satisfactory model fit.

To assess the structural model, VIF, R^2 , f^2 , and path coefficient analyses were conducted. VIF analysis checks for multicollinearity between model variables (O'Brien, 2007), with acceptable values below 3 (Diamantopoulos and Siguaw, 2006). The results showed that all VIF values met this criterion (Table 6).

Next, the coefficient of determination (R^2) was examined to assess how well

Table 4. Discriminant validity assessment results.

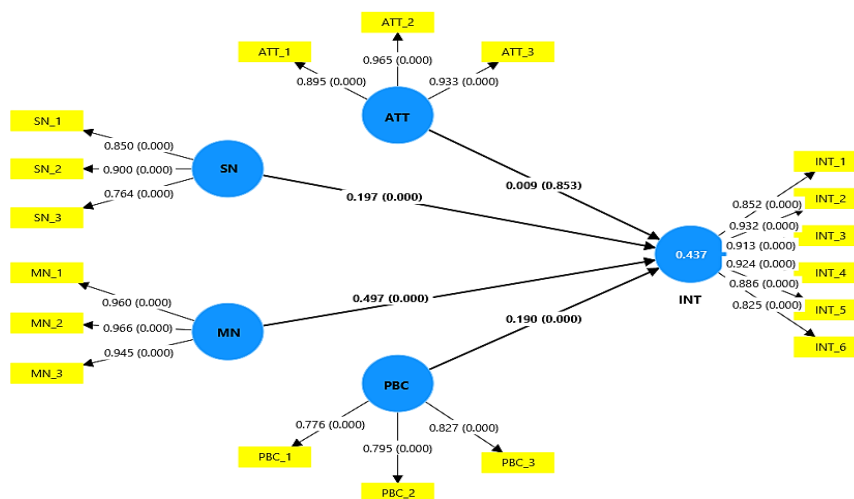
Fornell Larcker Criteria					
	ATT	INT	MN	PBC	SN
ATT	0.931				
INT	0.160	0.890			
MN	0.253	0.598	0.957		
PBC	0.025	0.339	0.239	0.800	
SN	0.117	0.368	0.280	0.155	0.840
HTMT					
ATT					
INT	0.171				
MN	0.269	0.630			
PBC	0.055	0.410	0.288		
SN	0.136	0.426	0.319	0.212	

Table 5. Summary of model fit statistics.

	Saturated model	Estimated model	P values (0.95)	P values (0.99)
SRMR	0.049	0.049	0.041	0.044
d_{ULS}	0.405	0.405	0.285	0.333
d_G	0.257	0.257	0.257	0.292
Chi-square	496.562	496.562		
NFI	0.891	0.891		

Table 6. Structural equation modelling results.

Hypothesis	Model	β	St. Dev.	T Value	P Value	VIF	f2
H ₁	ATT → INT	0.011	0.049	0.186	0.853	1.074	0.000
H ₂	MN → INT	0.495	0.045	11.116	0.000	1.201	0.365
H ₃	PBC → INT	0.194	0.040	4.747	0.000	1.074	0.060
H ₄	SN → INT	0.199	0.044	4.438	0.000	1.102	0.063

**Figure 4.** Path analyse diagram.

independent variables explained the dependent variable. The model explained 43.70% of the variance in behavioral intention. The f^2 analysis, indicating effect size, measured each independent variable's contribution to the dependent variable. Effect sizes are classified as weak ($0.02 < f^2 < 0.14$), medium ($0.15 < f^2 < 0.34$), or strong ($f^2 > 0.34$) (Cohen, 1988). The attitude variable had no significant effect on intention, while other variables generally showed a medium effect (Table 6). Path analysis, the final stage of the structural model, is detailed in Table 6 and Figure 4.

Table 6 and Figure 4 present the SEM results, showing the effects of ATT, MN, PBC, and SN on INT. The relationship between ATT and INT is positive but small ($\beta = 0.011$), suggesting a minimal influence of attitude on intention within this context. In contrast, MN exerts the strongest effect on intention ($\beta = 0.495$), indicating that higher moral standards among farmers substantially increase their intention to adopt the behavior. Both PBC ($\beta = 0.194$) and SN

($\beta = 0.199$) also positively influence intention, demonstrating that farmers' perceived control over the behavior and social pressures from peers and family meaningfully contribute to their behavioral intentions.

The f^2 values further confirm the relative importance of each predictor, with MN showing a moderate effect, while PBC and SN have small but meaningful impacts. These findings align with the Theory of Planned Behavior, i.e.: highlighting that intention is shaped not only by ATT but also by MN, PBC, and SN, providing a comprehensive understanding of the factors influencing farmers' decision-making processes.

DISCUSSION

Data collected from interviews with greenhouse farmers in Antalya were analyzed using the Theory of Planned Behavior (TPB) to examine pesticide use



behavior. Four hypotheses assessed the effects of attitude, subjective norm, moral norm, and perceived behavioral control on farmers' intentions to reduce pesticide use.

The results indicated that MN, SN, and PBC significantly influenced farmers' behavioral intentions, while ATT did not. In this context, PBC control reflects farmers' assessment of the ease or difficulty of not using pesticides, SN capture the influence of others, typically family and friends, and MN represent farmers' ethical evaluation of pesticide use. ATT, referring to the degree to which farmers believe they should reduce pesticide use, was not a significant predictor. Although previous studies often identify attitude as a key determinant of behavioral intention (Bond *et al.*, 2009; de Bon *et al.*, 2014; Fan *et al.*, 2015; Jallow *et al.*, 2017; Wang *et al.*, 2018; Sarma, 2022; Pirmoghni *et al.*, 2024), findings can be inconsistent. For example, Frey *et al.* (1993) suggest that strong social pressures can limit attitude formation, reducing its predictive power for behavioral intention. Jallow *et al.* (2017) also reported that even well-informed farmers may fail to take adequate precautions or reduce pesticide use.

In the Antalya Region, greenhouse farming is an important source of income, and high economic stakes encourage risk-averse behavior. Farmers prioritize pest control to prevent yield losses and often rely on familiar, effective, and widely used pesticides. Studies indicate that farmers generally prefer proven methods over unfamiliar alternatives (Damalas and Koutroubas, 2014; Stadlinger *et al.*, 2013). These factors may explain why attitude was not a significant predictor of behavioral intention in this study.

Aligned with the TPB, moral norm, subjective norm, and perceived behavioral control positively influenced farmers' intentions to reduce pesticide use. National and international studies report similar patterns, although regional and production system differences may affect results (Bond *et al.*, 2009; Yin *et al.*, 2010; Liu *et al.*, 2013; Abteu *et al.*, 2016; Vidogbena *et al.*,

2016; Schreinemachers *et al.*, 2017; Wang *et al.*, 2018; Sarma, 2022; Pirmoghni *et al.*, 2024). Ajzen (1991) highlighted the central role of these constructs in shaping intentions, and moral norms, in particular, have been shown to enhance environmental responsibility and promote sustainable practices (Bamberg and Möser, 2007).

Further evidence from other studies supports these findings. Batbay and Kahramanoğlu (2024) found that, in Northern Cyprus, higher perceived behavioral control, attitude, and pesticide knowledge were associated with reduced pesticide use, though moral norms were not significant. They recommended that future research consider moral norms in various contexts. In Turkey, Günay and Niyaz Altınok (2024) reported that, while attitude and subjective norm strongly influenced intentions, perceived behavioral control was not significant. Additionally, intentions did not always translate into actual behavior. Similarly, Zhang *et al.* (2024) identified attitude, followed by perceived behavioral control and subjective norm, as the strongest predictors of farmers' intentions to adopt nature-based practices. Monfared *et al.* (2015) reported that even in the face of notable environmental, economic, and health impacts, farmers in developing countries continued to apply pesticides. The study adopted an extended TPB model by including moral norm and self-identity to better predict pesticide use intentions. Findings indicated that this enhanced model explained 63% of the variation in intentions, surpassing the predictive power of the standard TPB, with attitude, subjective norm, perceived behavioral control, and self-identity as significant contributors. In the extended model, attitude, perceived behavioral control, and self-identity had positive effects, while subjective norm had a negative effect, and moral norms were not statistically significant.

Overall, these findings indicate that while attitude may not always directly predict behavioral intention, social and moral influences, along with perceived control,

play a critical role in shaping farmers' intentions to reduce pesticide use, highlighting the relevance of the extended TPB in explaining behavioral intentions in greenhouse farming contexts.

5. CONCLUSIONS

Controlling plant diseases and pests is a central challenge in greenhouse horticulture, and while chemical methods remain effective, alternative crop protection strategies are increasingly important. This study applied the Theory of Planned Behavior (TPB) to examine how ATT, SN, MN, and PBC influence tomato, bell pepper, and eggplant farmers in Antalya in their behavioral intention to reduce pesticide use. SEM showed that MN, SN, and PBC significantly shaped INT, whereas ATT had a positive but non-significant effect. In Turkey, strict pesticide residue regulations, particularly for exports, along with growing support for biological alternatives, highlight the need for effective training and policy interventions. The findings suggest that policies should strengthen moral and subjective norms and enhance perceived behavioral control to promote agroecological practices. Reducing pesticide use supports sustainability, food safety, and ecosystem health. Additionally, cost-effective, eco-friendly crop protection aligns with greenhouse production. Overall, the study underscores the role of behavioral factors in farmers' decision-making and the importance of targeted public policies to encourage sustainable horticultural practices.

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قصد رفتاری کشاورزان گلخانه‌ای برای کاهش مصرف آفتکش‌ها در آنتالیا-ترکیه

مصطفی کان، حسن ییلماز، حسن گوخان دوغان، مروه مورووت داق، و تیدیاتی دیالو

چکیده

این پژوهش قصد کشاورزان برای کاهش مصرف آفتکش‌ها در کشت گلخانه‌ای در استان آنتالیا، ترکیه را بررسی می‌کند. با استفاده از نسخه‌ای توسعه‌یافته از «نظریه رفتار برنامه‌ریزی‌شده» (TPB)، ما عوامل اجتماعی-روانشناختی مؤثر بر قصد کشاورزان برای



به‌کارگیری شیوه‌های پایدارتر را بررسی کردیم. مصاحبه با ۲۹۷ کشاورز پرورش‌دهنده گوجه‌فرنگی، فلفل و بادمجان نشان داد که اگرچه کشاورزان به‌طور کلی نگرش مثبتی نسبت به کاهش مصرف آفت‌کش‌ها دارند، اما این نگرش تأثیر معناداری بر قصد و نیت واقعی آن‌ها ندارد. این نیت‌ها عمدتاً توسط هنجارهای اخلاقی، هنجارهای ذهنی و کنترل رفتاری ادراک‌شده هدایت می‌شوند. بهبود حمایت اجتماعی، ایجاد یک محیط توانمندساز و اجرای برنامه‌های آموزشی می‌تواند به‌طور قابل توجهی به کشاورزان در اتخاذ شیوه‌های کشاورزی-اکولوژیکی کمک کند. این مطالعه بر اهمیت درک عوامل اجتماعی و روان‌شناختی مؤثر بر تصمیم‌گیری‌های کشاورزان تأکید می‌ورزد؛ امری که برای تدوین راهکارهای مؤثر در زمینه کشاورزی سازگار با محیط زیست، حیاتی است.

Grounded Theory Model for Social Media Integration in Agricultural Extension

Maryam Baharestani¹, Alireza Poursaeed^{1*}, and Marjan Vahedi¹

ABSTRACT

This study aimed to develop a grounded theoretical model that explains the drivers, barriers, and strategies for enabling social media adoption in agricultural extension. Eleven semi-structured interviews were conducted with subject-matter experts from extension institutions and digital communication units in Iran, and data were analyzed using open, axial, and selective coding procedures. The coding process generated 57 concepts and 23 categories, which were synthesized into a paradigmatic model encompassing causal, contextual, and intervening conditions, as well as strategies and outcomes. Results showed that policy coordination, institutional collaboration, infrastructure investment, and digital capacity-building are essential conditions for effective adoption. Key barriers identified include weak rural internet coverage, limited digital literacy among farmers, insufficient financial resources, and fragmented policy frameworks. The study also revealed that social media supports faster knowledge dissemination, real-time farmer engagement, and adoption of sustainable agricultural practices. The theoretical model contributes to extension scholarship by linking institutional and contextual enablers to practical outcomes, thereby complementing existing technology-adoption frameworks. Practically, the findings emphasize the need for evidence-based policy frameworks, public-private partnerships, and investments in localized content and farmer training. Strategic use of emerging tools such as big data, IoT, and artificial intelligence can further enhance the effectiveness of social media-based extension. The model provides actionable guidance for policymakers and practitioners to strengthen rural innovation and sustainable agricultural development.

Keywords: Digital capacity-building, Emerging digital technologies, Farmer decision-making, Institutional collaboration, Policy framework.

INTRODUCTION

In an era defined by rapid technological advancement, social media has become a transformative force in modern communication, influencing daily life, professional activities, and knowledge dissemination (Kipkurgat *et al.*, 2016). Beyond connectivity, these platforms have emerged as tools for networking, collaboration, and interactive engagement across diverse domains, including education, business, and agriculture (Isiaka *et al.*, 2024). Scholars emphasize the importance of

expanding virtual communication platforms and fostering innovation through specialized digital networks to address societal needs in a knowledge-driven economy (Jandaghi *et al.*, 2021).

The agricultural sector, vital for food security and rural livelihoods, is also undergoing this digital transformation. Agricultural extension services, which traditionally focused on face-to-face training, peer exchange, and mass media channels such as radio and television, have faced challenges of scalability, inclusivity, and limited interactivity (Ankita *et al.*, 2023; Ebrahimivand *et al.*, 2023; Mianabadi *et al.*,

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2023; Ndege *et al.*, 2024). Constraints such as geographic isolation, limited personnel, and dependency on physical resources highlight the need for more flexible and participatory communication approaches (Daigle, 2020; Kapoor and Singh, 2023). Social media platforms provide new opportunities to complement these traditional systems, offering real-time, accessible, and user-driven channels for information sharing and learning.

Globally, social media tools—including blogs, microblogs (e.g., platform X), podcasts, wikis, and media-sharing platforms—enable bidirectional communication, allowing users to serve simultaneously as content producers and consumers (Jebali, 2024). For agricultural extension, this participatory environment supports problem-solving, strengthens networks, and accelerates the spread of innovations (Khosravipour *et al.*, 2024). Evidence from earlier studies confirms that social media can enhance job performance (Bathaiy *et al.*, 2021), facilitate professional networking (Jafari, 2021), and improve access to scientific resources (Mirzaei *et al.*, 2016), though barriers such as infrastructural limitations and inconsistent information remain (Mohammadi *et al.*, 2019).

Despite these benefits, challenges persist. Limited digital literacy among farmers, inadequate rural internet coverage, and fragmented policy frameworks hinder the systematic integration of social media in agricultural extension (Rezvani *et al.*, 2022; Masoumi, 2025). Addressing these barriers requires coordinated policies, capacity-building initiatives, and investments in digital infrastructure (Kolahi *et al.*, 2025a; Kolahi *et al.*, 2024a; b).

While previous studies have examined the opportunities and constraints of digital platforms, there is still limited theoretical understanding of how technical and institutional conditions interact to shape social media adoption in agricultural extension systems. This research addresses that gap by applying grounded theory to develop a conceptual model that explains

causal, contextual, and intervening conditions, along with strategies and outcomes. The study provides a systematic framework for integrating social media into agricultural extension, offering both theoretical contributions and practical guidance for policymakers and practitioners.

MATERIALS AND METHODS

Methodology

This study adopts a qualitative exploratory approach using grounded theory, guided by Strauss and Corbin's (1998) systematic methodology. Grounded theory was selected because it allows for the generation of theoretical models where limited or fragmented conceptual frameworks exist. In this study, prior research had largely described the opportunities and barriers of social media in agricultural extension but lacked an integrated model to explain how contextual, causal, and institutional conditions interact to shape adoption. Grounded theory provided an inductive yet structured framework for systematically transforming data into concepts, categories, and ultimately a paradigmatic model (Kolahi and Boroumand, 2024).

The research sought to construct a theoretical framework explaining the implementation of technical and specialized social media support in agricultural extension. Following Strauss and Corbin (1998), the analytical process included three coding phases—open, axial, and selective coding—leading to the identification of six interconnected dimensions: causal conditions, contextual conditions, intervening conditions, strategies, outcomes, and the core category.

Study Population and Sampling

The study population consisted of subject-matter experts with professional roles in agricultural extension and digital

communication. Participants were drawn from the Agricultural Education and Extension Institute, the Network of Knowledge and Extension Media Office, and the Virtual Networks and Technology Group in Kermanshah Province, Iran. A purposive sampling strategy, complemented by snowball sampling, was used to identify experts with extensive knowledge and experience in agricultural extension and the use of digital technologies.

A total of 11 experts were interviewed until theoretical saturation was achieved. Of these, five were senior extension officers with more than 10 years of experience, three were ICT specialists working in agricultural communication, and three were university faculty members specializing in agricultural extension and rural development. Participants were selected on the basis of their expertise, professional role, and direct involvement with digital communication initiatives in agriculture.

Data Collection and Analysis

Data were collected through semi-structured, in-depth interviews. An interview guide was developed based on a preliminary review of the literature and expert input, and was pilot tested prior to use. Interviews were recorded with participant consent, transcribed verbatim, and analyzed immediately after each session to refine subsequent data collection (Figure 1).

Analysis followed the three stages of grounded theory coding:

Open coding: Breaking down raw data into discrete concepts, yielding 57 initial codes.

Axial coding: Grouping codes into six overarching dimensions: causal conditions, contextual conditions, intervening conditions, strategies, outcomes, and the core category.

Selective coding: Integrating and refining the categories into a paradigmatic model representing relationships among the dimensions.

Reliability and Validity

Several measures were taken to enhance rigor. To ensure the study's rigor, multiple measures of reliability and validity were employed as follows:

Confirmability, transferability, and credibility: Achieved through triangulation of data sources, thick description of processes, and participant validation. Interview summaries and emergent themes were returned to participants for feedback, ensuring accurate representation of their perspectives.

Test-retest reliability: To confirm coding stability, three interviews were re-coded after 20 days. Agreement rates were calculated using the following Kvale's (1996) formula:

$$\text{Percentage of Retest Reliability Coefficient} = \frac{\text{Number of Agreements} \times 2}{\text{Total Codes}} \times 100$$

The reliability score of 85%, well above the 60% threshold, confirmed the robustness of the coding (Table 1).

Expert Validation

The final paradigmatic model developed through selective coding was shared with five independent experts in agricultural extension and social media research. Their feedback confirmed the accuracy and practical relevance of the categories. Minor suggestions, such as clarifying the scope of contextual conditions and refining

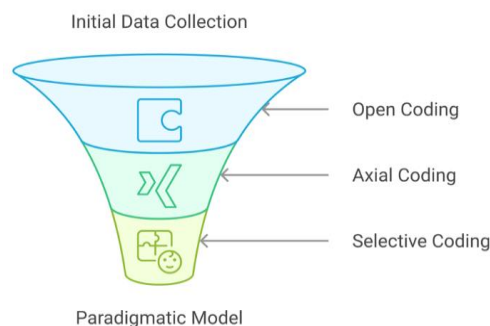


Figure 1. Transforming data into a Paradigmatic Model.

**Table 1.** Percentage of reliability through re-coding.

Interview number	Total codes	Number of agreements	Number of disagreements	Test-retest reliability (%)
1	29	12	5	83
2	21	9	3	85
3	23	10	3	87
Overall	73	31	11	85

terminology, were incorporated. Continuous consultation with methodological experts ensured that analytical procedures were transparent and consistent with grounded theory standards.

RESULTS

The findings of the grounded theory analysis are organized into six key dimensions as follows:

Causal Conditions

Causal conditions represent the fundamental drivers that influence the adoption and implementation of social media in agricultural extension. From the interviews, 17 concepts were identified and grouped into seven overarching themes (Table 2; Figure 2). These conditions provide the foundation for developing effective support mechanisms for social media integration.

One major driver is the development of farmers' technical knowledge, which motivates decision-makers to invest in social media initiatives. As an interviewee explained:

"Agricultural extension within the ecosystem requires raising public awareness in various agricultural fields."

While productivity improvements were frequently mentioned by respondents, these are considered outcomes rather than direct causal conditions. Therefore, such elements are reported in the Outcomes and Impacts section rather than listed here as drivers.

Another key causal factor is farmers' familiarity with media-related institutions (e.g., the Telecommunications Company, Ministry of Industry, Mine, and Trade) and the importance of cross-sectoral collaboration. Respondents emphasized that sustainable integration requires both hardware and software development.

Access to up-to-date global agricultural information was also identified as a crucial enabler, reflecting the role of social media in bridging knowledge gaps. One participant noted:

"Providing accurate and timely information about modern agricultural technologies through social media can enhance their adoption."

Finally, sustainable agricultural and environmental goals were consistently cited as motivating factors, aligning with the broader responsibility of extension systems to promote eco-friendly practices.

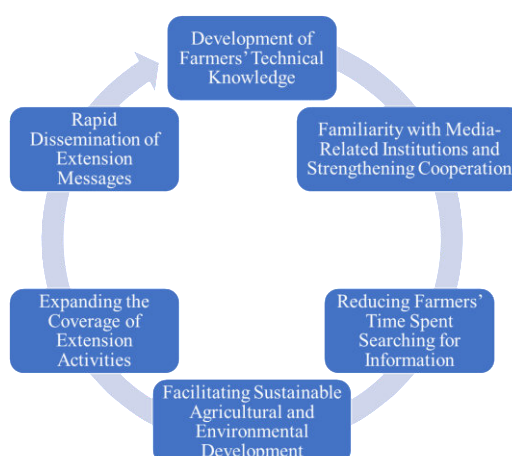
Core Category (Central Phenomenon)

The core category represents the central phenomenon around which the model is structured: the acceptance and use of social media in agricultural extension, supported by technical and specialized mechanisms.

Analysis revealed nine core concepts, grouped into three major themes (Table 3; Figure 3). These themes reflect the multidimensional nature of adoption, including technological modernization, local empowerment, and cultural adaptation.

Table 2. Identified concepts and categories related to causal conditions.

Coding dimension	Categories	Concepts
Causal conditions	Development of Farmers' Technical Knowledge	Enhancing public awareness; increasing technical expertise; expanding professional agricultural knowledge
	Familiarity with Media-Related Institutions and Strengthening Cooperation	Collaboration between institutions and farmers; improving awareness of agricultural media organizations
	Reducing Farmers' Time Spent Searching for Information	Minimizing search time; using modern technologies for access
	Facilitating Sustainable Agricultural and Environmental Development	Promoting environmental conservation; advancing sustainable agriculture
	Expanding the Coverage of Extension Activities	Linking to global networks; extending outreach of extension services
	Rapid Dissemination of Extension Messages	Exchanging knowledge; delivering diverse, relevant messages; storing and reusing messages; ensuring swift communication

**Figure 2.** Cycle of “causal conditions” for “social media integration” in Agriculture.

Contextual Conditions

Contextual conditions represent the institutional and environmental factors shaping adoption, such as supportive policies, infrastructure, training, and financing (Table 4; Figure 4).

Intervening Conditions

Intervening conditions are external dynamics—economic, technological, and political—that either enable or hinder adoption (Table 5; Figure 5). These include government promotional policies, social capital and trust, economic and market conditions, and limitations caused by international sanctions.

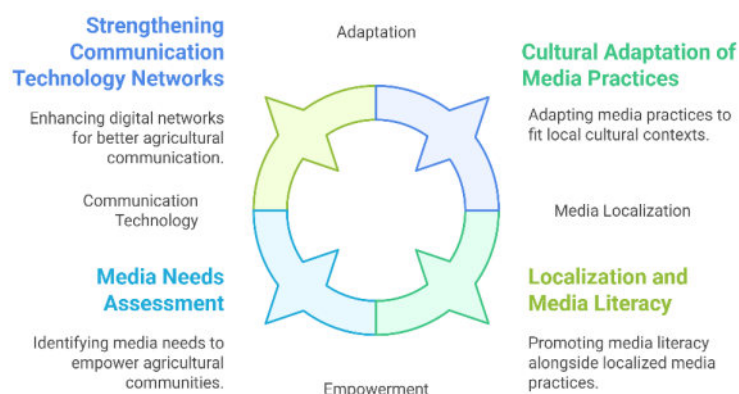


Figure 3. Core Category of social media adoption in agricultural extension.

Table 3. Identified concepts and categories related to the Core Category.

Coding dimension	Categories	Concepts
Core category	Strengthening and Modernizing Communication Technology Networks	Establishing and improving communication networks Leveraging modern communication technologies
	Media Needs Assessment and Local Empowerment	Identifying regional and local needs related to social media development Enhancing local and individual capacities
	Localization and Cultural Adaptation of Media Practices	Customizing social media platforms for local agricultural use Promoting media literacy and awareness
	Establishing Social Media Platforms and Groups	Creating appropriate social media platforms Forming discussion groups and channels on messaging apps

Strategies and Actions

Strategies highlight practical measures for implementation, including creating platforms, promoting participatory extension, and building institutional collaborations (Table 6; Figure 6).

Outcomes and Impacts

Social media integration results in transformative outcomes, including

improved agricultural performance, accelerated learning, and enhanced farmer participation (Table 7; Figure 7).

These outcomes illustrate how social media fosters innovation, expands access to global knowledge, and contributes to sustainable farming. For instance, one interviewee explained:

“Using social media in agricultural extension can significantly enhance public knowledge in various agricultural domains.”

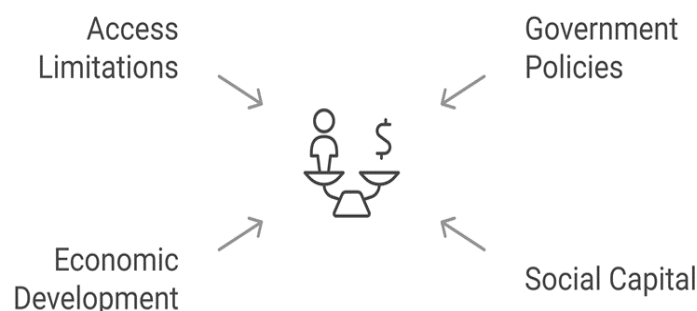
Table 4. Identified concepts and categories related to Contextual Conditions.

Coding dimensions	Categories	Concepts
Contextual conditions	Provision of Equipment and Infrastructure for Social Media	Quantitative and qualitative development of internet services Establishment of hardware infrastructure (electronic and specialized equipment) for the use of social media by the government
	Policy-Making and Inter-Institutional Cooperation Related to Social Media	Agricultural policymakers' belief in the effectiveness of social media in extension services Formulation of supportive policies and regulations for social media in agricultural extension Cooperation and interaction with agencies involved in social media operations
	Education and Research on Social Media	Research on improving the effectiveness of social media in agricultural extension Training extension agents in the use of social media for agricultural outreach
	Financial Resource Allocation for Social Media Development	Allocation of government funding and financial resources for integrating social media in agricultural extension Attracting financial sponsors for social media development Government support in the form of loans and financial incentives to help farmers acquire smartphones Providing farmers with access to smartphones to facilitate social media usage

**Figure 4.** Components of Contextual Conditions for social media integration in agriculture.

**Table 5.** Identified concepts and categories related to Intervening Conditions.

Coding dimensions	Categories	Concepts
Intervening conditions	Government Promotional Policies on Social Media	Government policies and strategies regarding social media Institutions related to agricultural extension
	Social Capital and Public Trust	Farmers' social capital Farmers' trust in social media as an agricultural extension tool
	Economic Development of Communication Technology	Development of communication technologies Economic and market conditions affecting technology access
	Limitations in Access to Advanced Technical Knowledge Due to Sanctions	Comprehensive international sanctions Lack of access to cutting-edge global technical knowledge in satellite and communication technologies Restrictions on securing internet and web-based infrastructure

**Figure 5.** Intervening Conditions impacting social media support in agriculture.

Theoretical Model and Analytical Approach

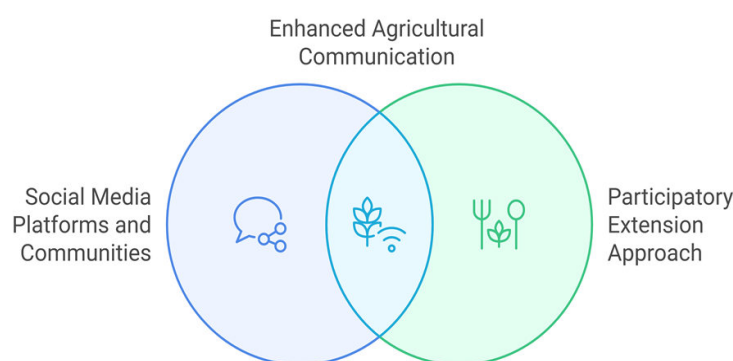
The integration of all six dimensions led to the development of a theoretical model of social media adoption in agricultural extension (Figure 8). Unlike narrow adoption models, this framework emphasizes both institutional drivers (e.g., policies, infrastructure) and external constraints (e.g., sanctions, digital divides).

DISCUSSION

This study sought to generate a grounded theoretical model explaining how technical and professional support for social media can be implemented within agricultural extension systems. The model integrates six empirically derived dimensions—causal conditions, core category (adoption/use), contextual conditions, intervening conditions, strategies, and outcomes—based on 11 in-depth expert

Table 6. Identified concepts and categories related to Strategies and Actions.

Coding dimensions	Categories		Concepts
Strategies and actions	Establishment of Social Media Platforms and Communities		Development of suitable platforms for social media engagement in agriculture
			Formation of groups and channels on social media and messaging applications
	Participatory Approach	Extension	Enhancing the attractiveness of educational content for dissemination through social media
			Strengthening connections between extension services and influential figures in the agricultural sector to facilitate content creation in digital spaces
			Expanding participatory extension efforts by leveraging the capabilities of social media
			Promoting the adoption of advanced technologies related to social media in agricultural outreach

**Figure 6.** Strategies for adopting social media in agricultural extension.

interviews and inductive coding (57 concepts → 23 categories).

Our findings show that the causal conditions (e.g., enhancement of farmers' technical knowledge; access to up-to-date information; local needs assessment) principally enable the acceptance and use of social media by shaping demand and perceived usefulness. These drivers align with the problem described in the introduction: traditional extension approaches struggle with scale and interactivity, and social media can address these gaps if enablers are present. Empirically, a plurality of interviewees emphasized knowledge-access as the primary motivation for adoption. This is consistent

with Aker (2011) and Mittal and Mehar (2016), who identify information timeliness as central to improved decision-making. In our data, the pathway from causal conditions to outcomes (for example, improved farm practices and accelerated learning) was repeatedly reported: Respondents tied knowledge access (causal) through platform use (core category) to measurable practice changes (outcomes). Consequently, the model helps explain how social media adoption contributes to agricultural sustainability — via accelerated diffusion of environment-friendly practices, faster troubleshooting (pest/disease), and improved adoption of resource-efficient techniques —

**Table 7.** Identified concepts and categories related to Outcomes and Impacts.

Coding dimensions	Categories	Concepts
Outcomes and impacts	Enhancing Farmers' Access to Global Agricultural Knowledge	Increased access to up-to-date agricultural information and knowledge Improvement of farmers' awareness and expertise through social media utilization
	Boosting Farmers' Confidence Through Accelerated Learning	Strengthening farmers' motivation and self-confidence Accelerating the learning process for farmers
	Promoting a Culture of Innovation and Entrepreneurship	Encouraging creativity and innovation in the agricultural sector Promoting entrepreneurial practices within agriculture Enhancing efficiency in agricultural education and extension activities Enriching the content of extension training programs

**Figure 7.** Enhancing Agricultural outcomes through social media integration.

all of which were cited by participants as examples.

Role of Social Media in Agricultural Extension

The qualitative evidence indicates that extension-oriented use in the respondents' context is dominated by messaging apps and locally available platforms (messaging channels, groups and channels on national apps such as Eita and Baleh), as well as broad platforms used for content dissemination

(e.g., Telegram historically, messaging and video platforms). The interview sample (experts from extension offices, ICT units and academia) reported high institutional usage of messaging channels for announcements and advisories, while direct farmer adoption was described as variable: experts noted widespread use among younger or better-connected farmers, and limited adoption among remote smallholders. These observations are consistent with Mohammadi *et al.* (2019), who documented limited effective use among farmers, and Mirzaei *et al.* (2016), who showed heavy use of social

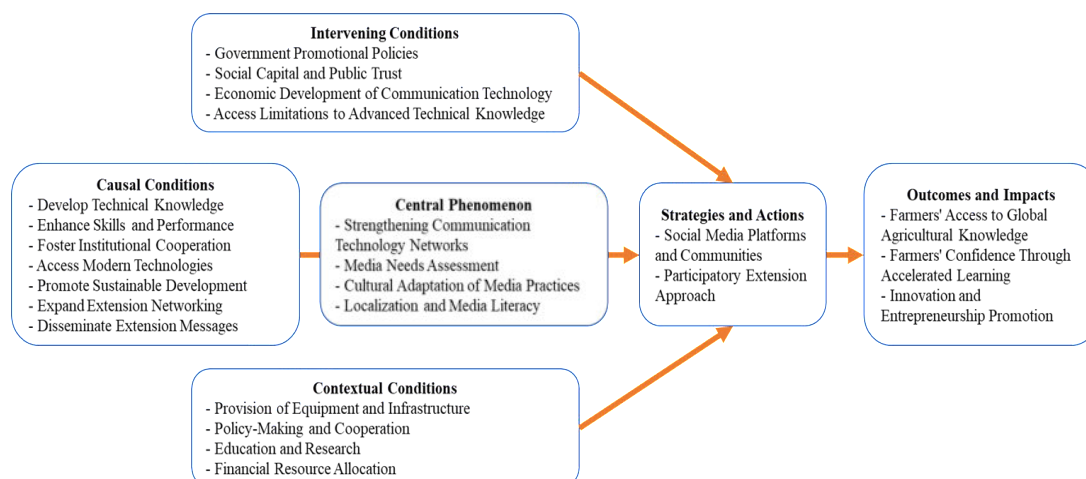


Figure 8. Theoretical model of Social Media Adoption in agricultural extension.

networks among educated students—indicating that uptake is uneven and often correlated with connectivity and digital literacy. Furthermore, our interview data substantiates patterns (Institutional use> Partial farmer uptake) and highlights the need for quantitative surveys to measure adoption rates among farmers.

Institutional Support: Forms and Mechanisms

Respondents described multiple, concrete forms of institutional support and policy instruments that enable social media integration. These included the followings:

- **Regulatory and Strategic Measures:** Formal national/regional digital extension strategies, ministerial directives, and standard operating procedures for digital advisories (e.g., endorsed content protocols and liability guidelines).
- **Budgetary/Financial Measures:** Designated budget lines for digital extension, subsidies or loan programs for farmer smartphones, and public–private financing for platform development.
- **Infrastructure Investments:** Public investment in rural broadband and targeted ICT infrastructure such as Base

Transceiver Stations (BTS) sites and fiber backhaul to improve coverage.

- **Organizational Mechanisms:** Inter-agency coordination platforms [Memoranda Of Understanding (MOUs) between agriculture and communications ministries], dedicated extension ICT units, and partnerships with telecommunication companies to prioritize agricultural messaging.

These concrete forms correspond directly to the contextual and intervening dimensions in our model and are supported by respondents' accounts. Literature (for example, Barau and Afrad, 2017) also stresses institutional backing and infrastructure as core enablers. Our study extends these insights by mapping how different institutional instruments align with model components (e.g., Budgets → Contextual condition; MOUs → Enabling policies for strategies).

Barriers and Locally Grounded Evidence

Respondents repeatedly cited limited rural internet infrastructure, digital illiteracy among certain farming cohorts, distrust in platform data quality, and financial constraints as barriers — themes that occur in the broader literature (Musa *et al.*, 2014; Van



Zoonen *et al.*, 2016). Importantly, our dataset provided local-scale nuances: experts pointed to fragmentation (multiple platforms, some requiring VPNs in the national context), lack of local language and culturally suitable content, and uneven device access. These local barriers explain why adoption is patchy and why institutional instruments should be targeted (e.g., smartphone subsidies combined with tailored local content and trust-building).

Model Novelty, Theoretical Contribution, and Relationship to Existing Frameworks

Several diffusion and technology adoption models [Technology Acceptance Model (TAM)/ Unified Theory of Acceptance and Use of Technology (UTAUT), diffusion of innovations] emphasize perceived usefulness, ease of use, and social influence. Our grounded model differs by offering an institutionally and operationally explicit account: rather than focusing only on individual perceptions. It situates adoption within a system of technical support mechanisms (policy, infrastructure, capacity building) and identifies mediating and contextual factors specific to agricultural extension. In other words, the study's novelty lies in linking institutional levers (policy, budgets, inter-agency cooperation) with micro-level outcomes (farmer learning and behavior) via operational strategies (platforms, participatory extension), which the existing individual-level adoption models do not fully specify. This contribution advances extension theory by demonstrating that achieving beneficial outcomes from social media requires simultaneous alignment across multiple institutional and technical dimensions.

Policy and Practice Implications

The policy recommendations presented in the section on "Results" and earlier (in brief: infrastructure investment, capacity building, institutional coordination, localized content, financing mechanisms, and trust-building) are not speculative; they directly follow from

empirical statements by interviewees about what they perceive as gaps and enablers. For example, the recommendation to improve farmers' access to smartphones and increase budget allocations reflects multiple respondents' concrete suggestions. Similarly, recommendations for localized content and extension agent training are grounded in participants' reports of low digital literacy and the lack of regionally relevant educational materials. Thus, each recommendation is traceable to specific categories in the model (Contextual conditions → Policy and Finance; Strategies → Platform creation and participatory extension; Outcomes → Improved learning and performance).

Limitations, Unsettled Points and Future Work

Consistent with the grounded theory tradition and the qualitative design of this study, the findings provide a contextually grounded theoretical explanation that is intended to support analytical understanding and transferability to similar contexts, rather than statistical generalization to a broader population. Notable unsettled points that merit future empirical testing include: (1) Quantifying farmer adoption rates across demographic groups and regions, (2) Testing causal linkages in the model (e.g., the mediating role of institutional coordination between infrastructure investment and outcomes), and (3) Empirically integrating technology characteristics (complexity, usability) with the institutional model (TAM/UTAUT variables). The latter was beyond the present study's scope but flagged by respondents as relevant for future mixed-methods research.

CONCLUSIONS

This grounded theory study produced an empirically-based model explaining how technical and specialized support can enable social media adoption in agricultural

extension. It highlights that coordinated policy action, targeted infrastructure investment, institutional collaboration, and context-sensitive capacity-building are necessary preconditions for realizing the knowledge-sharing and sustainability benefits of digital platforms.

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مدل نظریه‌پردازی داده‌بنیاد برای یکپارچه‌سازی رسانه‌های اجتماعی در ترویج کشاورزی

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چکیده

هدف این پژوهش، ارائه یک مدل نظری مبتنی بر نظریه داده‌بنیاد به منظور تبیین عوامل محرک، موانع و راهبردهای مؤثر بر به‌کارگیری رسانه‌های اجتماعی در نظام ترویج کشاورزی بود. بدین منظور، یازده مصاحبه نیمه‌ساختاریافته با خبرگان حوزه ترویج کشاورزی و واحدهای ارتباطات دیجیتال در ایران انجام شد و داده‌ها با استفاده از فرایندهای کدگذاری باز، محوری و انتخابی مورد تجزیه و تحلیل قرار گرفتند. در نتیجه فرایند کدگذاری، ۵۷ مفهوم و ۲۳ مقوله استخراج و در قالب یک الگوی پارادایمی مشتمل بر شرایط علی، زمینه‌ای و مداخله‌گر، راهبردها و پیامدها سازمان‌دهی شدند. یافته‌ها نشان داد که هماهنگی سیاستی، همکاری‌های بین‌نهادهی، سرمایه‌گذاری در زیرساخت‌ها و توسعه ظرفیت‌های دیجیتال، از مهمترین پیش‌نیازهای استقرار اثربخش رسانه‌های اجتماعی در نظام ترویج کشاورزی به‌شمار می‌روند. همچنین، ضعف پوشش اینترنت در مناطق روستایی، پایین بودن سواد دیجیتال کشاورزان، محدودیت منابع مالی و پراکندگی چارچوب‌های سیاستی، از مهمترین موانع شناسایی شده بودند. نتایج پژوهش همچنین نشان داد که رسانه‌های اجتماعی با تسهیل انتشار سریع دانش، افزایش تعامل برخط با کشاورزان و حمایت از پذیرش شیوه‌های کشاورزی پایدار، می‌توانند نقش مؤثری در ارتقای عملکرد نظام ترویج ایفا کنند. مدل نظری ارائه‌شده با تبیین پیوند میان ظرفیت‌های نهادی و زمینه‌ای با پیامدهای عملی، دانش موجود در حوزه ترویج کشاورزی را غنا بخشیده و مکمل چارچوب‌های رایج پذیرش فناوری است. از منظر کاربردی، یافته‌ها بر ضرورت تدوین سیاست‌های مبتنی بر شواهد، تقویت مشارکت‌های بخش دولتی و خصوصی، سرمایه‌گذاری در تولید محتوای بومی و ارتقای آموزش

کشاورزان تأکید دارند. همچنین، بهره‌گیری راهبردی از فناوری‌های نوظهور نظیر کلان‌داده، اینترنت اشیا و هوش مصنوعی می‌تواند اثربخشی نظام‌های ترویج مبتنی بر رسانه‌های اجتماعی را بیش از پیش افزایش دهد. مدل پیشنهادی، راهنمایی عملی برای سیاست‌گذاران و دست‌اندرکاران فراهم می‌آورد تا از طریق تقویت نوآوری‌های روستایی، توسعه پایدار بخش کشاورزی را تسهیل کنند.

Knowledge-Behavior Gap in Soil Conservation: An Interventional Study with Small-Scale Farmers in Chile

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ABSTRACT

The purpose of the study was to analyze the impact of a soil management course on the farmers' soil science knowledge and their soil conservation behaviors. The research involved 33 small-scale farmers in the Arica and Parinacota Region, Chile. This study employed both qualitative and quantitative methods, with data collected through surveys (pre- and post- course, and a subsequent retention test) and interviews following the retention test. The training course significantly enhanced farmers' understanding of soil science, as demonstrated by their performance on the post-course survey and retention test. However, no statistically significant improvement was observed in farmers' soil conservation behavior. Interview results confirmed these quantitative findings, and provided insights into the underlying mechanisms, which are consistent with the theories of the stage model of self-regulated behavioral change. Our study offers current evidence of the knowledge-behavior gap in agricultural decision-making. This study challenges the typical focus of agricultural extension activities on knowledge alone, highlighting how this approach overlooks potential behavioral changes resulting from the intervention.

Keywords: Agricultural extension, Behavioral change, Land degradation, Pro-environmental behavior, Soil protection.

INTRODUCTION

Soil management practices are a critical component of human civilization's life support system (Liang *et al.*, 2025; Maruthi *et al.*, 2024). Indeed, improper soil management practices have been implicated in the collapse of several ancient societies (Hillel, 1992; Diamond, 2011). Taking this historical context into account, scholars emphasize the importance of soil conservation for advancing sustainable development (Koch *et al.*, 2013; Lal *et al.*, 2021).

Over the last decades, researchers have studied the factors that influence farmers' adoption of environmentally sustainable practices (reviews conducted by Baumgart-Getz *et al.*, 2012; Prokopy *et al.*, 2019; Ranjan *et al.*, 2019; Lu *et al.*, 2022). Research has shown that economic constraints and incentives are important drivers of farmers' decision-making (review by Bartkowski and Bartke, 2018). However, the review by Chouinard *et al.* (2008) revealed that many farmers are motivated by conservation goals, even if it means sacrificing immediate profits.

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Currently, there is no single overarching theory that accounts for farmers' decision-making in relation to conservation practices (Dessart *et al.*, 2019). For instance, Winkler-Schor *et al.* (2024) identified 29 unique theories used in land management research.

A prominent theory explaining farmers' decision-making is based on a complex relationship between farmers' soil science knowledge, their attitudes toward soil conservation, and their actual soil conservation practices (Gholami and Papzan, 2021; Burnham *et al.*, 2023; Jalilian *et al.*, 2025; Neaman, 2025b; Piran *et al.*, 2025). Farmers' soil science knowledge refers to applied understanding of soil management techniques aimed at mitigating global soil degradation (Neaman *et al.*, 2024). In turn, "attitude" can be defined as a mental position with regard to a fact or state or a feeling or emotion toward a fact or state (Merriam-Webster Dictionary, 2025). The "knowledge-deficit theory" (Schultz, 2002) posits that a lack of environmental knowledge can hinder pro-environmental actions. Equally, it is well known that pro-environmental attitudes influence farmers' decisions to engage in conservation behaviors (Farani *et al.*, 2021). Therefore, a combination of improved attitudes toward soil and enhanced soil science knowledge among farmers will likely contribute to the adoption of more sustainable soil conservation practices (Jalilian *et al.*, 2025).

This study focused on small-scale farmers in Chile. It focused primarily on soil science knowledge, attitudes toward soil conservation, and soil conservation behavior as previous studies (Burnham *et al.*, 2023; Neaman, 2024; 2025) have provided extensive evidence for the validity of scales to measure these constructs among Chilean farmers.

Scientific literature proposes that the central objective of soil science education, particularly within the context of formal soil management education, is to promote soil conservation (Muggler *et al.*, 2006). This objective of soil science education implies the need for longitudinal studies that

investigate whether interventions are effective in promoting soil conservation actions among the participating farmers.

The review conducted by Floress *et al.* (2018) emphasizes the scarcity of interventional studies in soil conservation research. Likewise, the review conducted by Lobry de Bruyn *et al.* (2017) highlights the frequent reliance on simplistic metrics, such as attendance numbers and participant satisfaction in agricultural extension activity reports. Despite the recognized importance of behavioral factors, few studies have employed longitudinal designs with validated reliable scales to evaluate behavioral change among farmers (Neaman, 2025a). Many other studies primarily evaluate knowledge gains, overlooking potential behavioral changes (Rejesus *et al.*, 2012; Pan and Zhang, 2018). This limited scope undermines our understanding of the true impact of these interventions.

This study focuses on small-scale farmers in Chile. Previous research in the area (Burnham *et al.*, 2023; Neaman *et al.*, 2024) demonstrated that the farmers' possessed limited understanding of soil management principles, despite showing favorable attitudes toward soil conservation. Accordingly, our intervention specifically targets knowledge gaps in soil science rather than attitudinal aspects. We hypothesized that a soil management training course will improve farmers' knowledge, which, in turn, will lead to observable changes in soil conservation practices.

Some researchers posit that the evaluation of training programs should also analyze the impact on agricultural productivity and farm economic rentability (Kirkpatrick and Kirkpatrick, 2006). However, the study by Roesch-McNally *et al.* (2018) demonstrated that some farmers are able to overcome the issue of an apparent trade-off between short-term productivity goals and long-term conservation goals. For these reasons, we limited our analysis to farmers' knowledge and behavior, excluding long-term impacts on agricultural productivity and farm economic rentability.

While the chosen study area is particularly significant for Chile, it is important to recognize that soil degradation is a global issue (Editorial, 2004) undermining agricultural sustainability worldwide. This emphasizes the broader importance of our study, extending its implications beyond the local context.

It should be noted that this study was designed with a specific emphasis on scrutinizing the effects of a soil management course, administered by soil professionals, not only on enhancing farmers' soil science knowledge but also on influencing their soil conservation behaviors. Crucially, this dual focus on knowledge acquisition and behavioral adjustments distinguishes our study from others, offering a more comprehensive understanding of the impact of the course on farmers' practices and knowledge in the realm of soil conservation. This emphasizes the innovative nature of this study.

MATERIALS AND METHODS

Study Area

The study area comprised the Arica and Parinacota Region, located in the

northernmost part of Chile (Figure 1), known for the world's driest desert, the Atacama Desert (Figure 2-A). However, in the Altiplano (a high Andean Plain), there exists an interesting phenomenon, which brings heavy rain during the summer months. This phenomenon allows groundwater and rivers to replenish (Kikuchi and Ferré, 2017). This, in turn facilitates agricultural activities in the coastal areas of the region. Agricultural areas are located in the plain parts of the river basins (Figure 2-B). The region's favorable climate permits the year-round open-field cultivation of crops, including the winter months. Aridisols and Entisols are the predominant soil types in the region (Casanova *et al.*, 2013).

One of the most pressing agricultural challenges in the region is the proliferation of plant diseases and pathogens (González Vallejos *et al.*, 2013; Mazuela Águila, 2013), which causes wide-spread use of synthetic pesticides. In general, farmers are uncertain of what to do with the agricultural production waste that is generated on their farm. In some cases, they dispose of it somewhere, e.g., along a road (Figure 3-A). In other instances, farmers attempt to compost their agricultural production waste, but they approach it incorrectly. Instead of forming a pile, they place the waste in a box

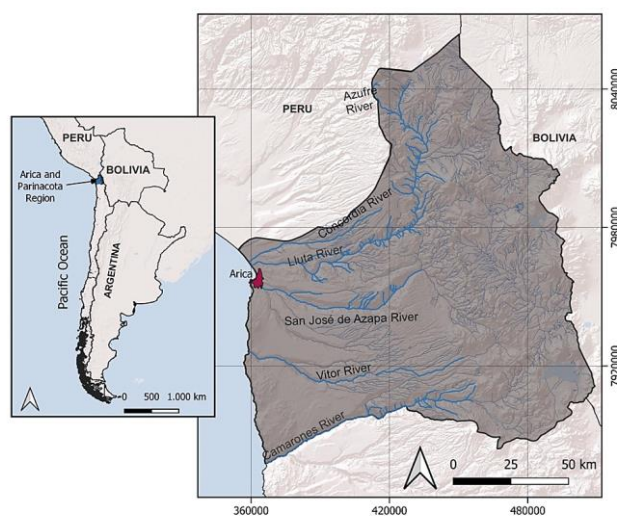


Figure 1. Geographical location of the study area.

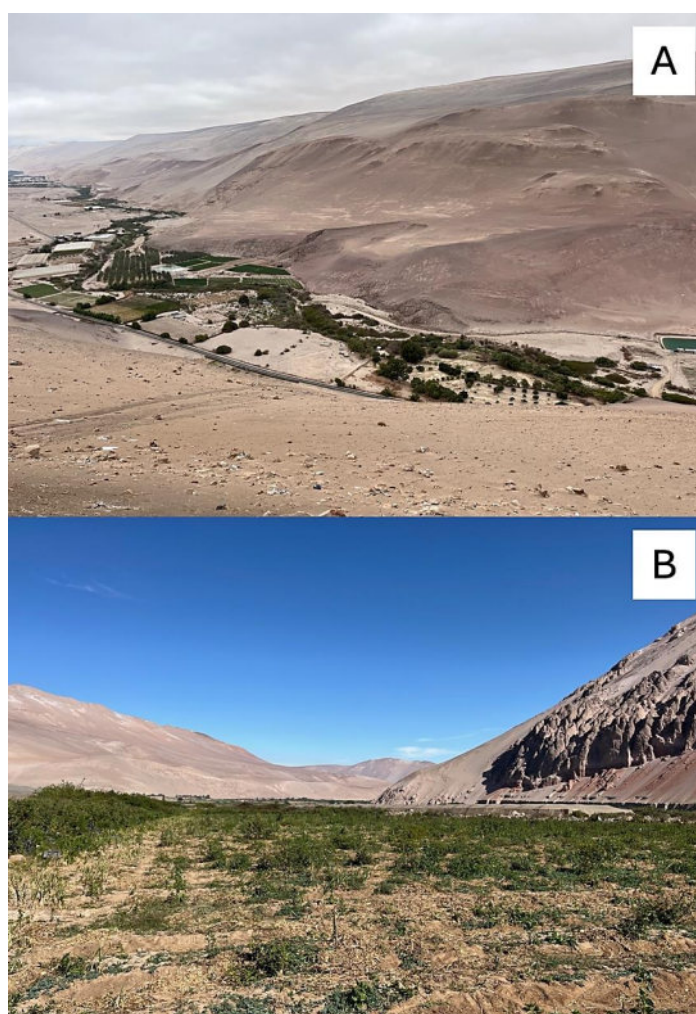


Figure 2. Typical view of the coastal area of the Arica and Parinacota Region (northern Chile), illustrating extreme aridity of the Atacama Desert (A). Agricultural areas are located in the plains of the rivers basins (B).

(Figure 3-B), which prevents them from periodically turning the pile to ensure the required aeration. Only a small fraction of the farmers conducts soil chemical analyses. This is particularly concerning given the widespread nutrient deficiencies observed in the region, as illustrated by a partially exfoliated citrus tree (Figure 3-C) and tomato plants exhibiting clear symptoms of iron chlorosis (Figure 3-D).

Study Population

The total population of the Arica and Parinacota Region is ~226,000 people,

concentrated predominantly in the city of Arica, contrastingly the rural population is only about 4,500 people (ODEPA, 2020). Given the extensive area of the region (16,873 km²) and the difficult accessibility to the Altiplano areas (located at an elevation of more than 3000 m above sea level), it was essential that we focused our research on the coastal area of the region, around the city of Arica.

The population of interest for this study were small-scale farmers because, as mentioned above, they possess a poor understanding of soil management principles and there is a need to enhance their soil conservation actions (Burnham *et al.*, 2023;



Figure 3. Examples of typical agricultural management issues in the area under study: (A) Agricultural production wastes disposed of along a road, (B) An attempt to compost agricultural production wastes by placing them in a box, instead of creating a pile, (C) A partially exfoliated citrus tree, and (D) A tomato plant with visible symptoms of iron chlorosis.

Neaman *et al.*, 2024). Around the city of Arica, such small-scale farmers are located in the Concordia and Lluta river basins (Figure 1). Accordingly, we approached the following two agricultural communities located in these river basins:

- 1) A group of 80 farmers, located in the Concordia River basin,
- 2) A group of 50 farmers, situated in the Lluta River basin.

We announced a free-of-charge training course on soil management, delivered by soil science experts. Similar training courses for farmers were held in Chile on other

topics, such as biodiversity conservation (Márquez-García *et al.*, 2018).

In response, 33 small-scale farmers enrolled in the course, i.e. the response rate was 25%. This approach corresponded to convenience sampling (also known as opportunity sampling), a common approach in research. However, it is important to note that findings derived from convenience samples are not generalizable to the broader population (APA, 2018).

Furthermore, it is important to acknowledge that this study is somewhat limited, given the relatively small sample



size. With 33 participants, we were able to detect large effects (Faul *et al.*, 7 2007). Nonetheless, rural studies notably use both qualitative and quantitative research methods (Strijker *et al.*, 2020). Therefore, we decided to mitigate the limitation of small sample size by including qualitative analysis (see “Qualitative method” below).

Socio-demographic characteristics of the participants were as follows: Age of 52±15 (range of 52–67) years old; 48% females, 67% self-identifying as Aymara (an indigenous ethnicity of northern Chile) and 33% did not belong to any indigenous ethnicity; 58% without any professional degree and 42% possessing a professional degree, 85% declared not having any previous training in soil management and 15% had such previous training. Importantly, none of the participating farmers had a professional degree related to agriculture. All participating farmers possessed relatively small land parcels (smaller than four hectares), which were used for both annual crops (e.g. tomato, Figure 3-D) or fruit trees (e.g. citrus, Figure 3-C).

All participants received comprehensive information regarding the study’s objectives, methodology, and potential implications. The informed consent procedure highlighted the voluntary participation principle, guaranteeing farmers complete autonomy to withdraw from the survey at any stage without consequence. Data collection procedures were designed to minimize participant burden while strictly maintaining confidentiality, in full compliance with established research ethics protocols.

Training

The training program combined traditional lectures with interactive discussions among participating farmers. This is a typical format used in Chile for farmers’ training programs (Márquez-García *et al.*, 2018). The interactive discussions focused on: (1) Farmers’ evaluations of their current soil

management practices, (2) The applicability of knowledge gained from the training, and (3) Challenges in adopting new practices based on their learning.

It is important to mention that the income of small-scale farmers in Chile tend to be unstable (Castillo *et al.*, 2022). Furthermore, economic constraints are the most important factors on farmers’ decision-making worldwide (review by Bartkowski and Bartke, 2018). For this reason, we decided to focus our training on soil conservation practices that require minimal (if any) economic investment. Specifically, six sessions were delivered over a six-month period, covering the following topics:

- 1) Importance of soil as a resource in agriculture,
- 2) Management of saline and calcareous soils,
- 3) Compost, fermented organic fertilizers, *Rhizobium* bacteria and other beneficial bacteria, symbiotic fungi (mycorrhiza).
- 4) Soil disinfection by solarization, fallow use, stubble burning alternatives, alternative pesticides (bio-pesticides).
- 5) Soil structure, soil erosion, use of mulch and cover crops.
- 6) Importance of the chemical analysis of the soil, irrigation water, and organic matter to be applied to the soil. Estimation of the dose of fertilizers to be applied based on the results of the analyses.

Research Method

This research employed both quantitative and qualitative approaches. Data was collected through surveys and interviews, as described in detail below.

Enrolled farmers were initially screened with the question: “Do you make decisions related to soil management on your farm?”. Among 33 participating farmers, 21 had made independent decisions regarding soil management on their farms. An additional 12 farmers had relied on decisions made by external soil management experts who

provided professional consulting services to them.

Based on this initial screening, we decided to consider the responses from all the participating farmers ($n = 33$) on soil science knowledge and attitudes toward soil. However, in the analysis of the soil conservation behavior results, we only considered farmers who made independent decisions regarding soil management on their farms ($n = 21$). Similarly, only farmers who made independent decisions regarding soil management on their farms were involved in the interviews.

Process

A pre-test was administered at the beginning of the first class, a post-test was administered at the end of the sixth class (6 months after the beginning of the course), and a retention test was administered at the follow-up class (4 months after the post-test). As previously mentioned, a confidential code was used to match the two tests completed by each respondent.

In Supplementary Tables 1-3, specific items of the scales under study are ranked based on the mean score obtained from all the participating farmers' pre-test responses. This form of presenting the results allowed us to identify items with the lowest scores, which were appropriately addressed during our intervention.

After the completion of the sixth session, there was a four-month break. Farmers were requested to implement soil management practices, according to the knowledge obtained during the training. Four months later, a follow up session was held in which participating farmers were given a retention test and had their questions answered. In parallel, an interview was conducted with each of the participating farmers who were making independent decisions regarding soil management on their farms.

Quantitative Method

The quantitative approach involved a self-administered pen-to-paper survey, which

incorporated three key scales: (1) Soil science knowledge, (2) Attitudes toward soil, and (3) Soil conservation behavior.

1) The scale of soil science knowledge (Supplementary Table 1) utilized true/false questions to assess farmers' objective understanding of soil management practices related to preventing various types of global soil degradation (Editorial, 2004). Validity criteria for this scale are presented in our previous Chilean Research (Neaman, 2024; Neaman *et al.*, 2024).

2) We used a 5-point Likert scale to measure farmers' attitudes toward soil (Supplementary Table 2). This scale included the following five options: (1) Strongly disagree; (2) Disagree; (3) Neither agree nor disagree; (4) Agree; (5) Strongly agree, allowing for nuanced responses. Validity criteria for this scale are presented in our previous Chilean Research (Neaman, 2024; Neaman *et al.*, 2024).

3) The scale of soil conservation behavior (Supplementary Table 3) evaluated actions related to various areas of soil management. Soil conservation behavior was assessed using a 5-point Likert scale with options ranging from "never" to "always". It is worth noting that the soil conservation behavior scale utilized in the present study included a "not applicable" option. This allowed farmers to skip a question if it did not apply to their specific farming situation. Validity criteria for this scale are presented in our previous Chilean research (Burnham *et al.*, 2023; Neaman *et al.*, 2025).

Reliability is a key concept in psychometrics, referring to the trustworthiness or consistency of a measure (APA, 2018). One common method for assessing reliability is Cronbach's alpha (Cronbach, 1951). The scales of soil conservation behavior and soil science knowledge used in this study demonstrated acceptable reliability (Cronbach's alpha values of 0.74 and 0.72, respectively), whereas the scale of attitudes toward soil demonstrated an excellent Cronbach's alpha value of 0.95. The reliability of the scales measuring soil science knowledge and



attitudes toward soil was assessed using pre-test responses from all participating farmers ($n=33$). However, the reliability of the scale measuring soil conservation behavior was determined based on the pre-test responses of farmers actively making decisions about soil management on their property ($n=21$).

With respect to the statistical analysis of the quantitative survey, Paired Analysis Of Variance (ANOVA), also known as repeated measures ANOVA, was used to examine the effect of the training course on farmers' soil science knowledge, attitudes toward soil, and their soil conservation behavior. A confidential code was used to match pre-, post-, and retention test scores for each farmer. A Tukey test was used for multiple comparisons.

Qualitative Method

The qualitative approach consisted of interviews. Each farmer was visited by the research team on their farm. Based on the recommendations of Berroeta *et al.* (2024), we introduced the interview as follows: "Let us go for a walk on your farm; please show us your farm". This approach allows participants to feel more relaxed during the interview (Berroeta *et al.*, 2024). It also allowed the interviewers to observe and validate the adoption, or lack of, diverse soil management practices, such as the following:

- Use of cover crops and/or mulch,
- Composting of vegetal materials or, opposingly, piling plant debris as waste,
- Production of fermented organic liquid fertilizers,
- The use of fallow land,
- Soil disinfection by solarization,
- Use of conventional synthetic agrochemicals (pesticides, herbicides, fungicides, nematicides),
- Burning of stubble and/or pruning residues and/or leaves.

During the farm visit, we asked each farmer the following questions:

How do you perceive the impact of the course on your knowledge of soil management practices?

What do you think about the current soil management practices on your farm?

Are you interested in implementing some of the soil management practices that were discussed during the course?

What practices have you been able to implement during the three-month period since the last session of the course?

What challenges are associated with implementing this/these practice(s)?

Based on the information you gained during the training sessions, what additional information do you need in order to implement soil management practices?

Through these interviews, the research team gained a nuanced understanding of each farmers' situation, as well as a deeper insight into the factors influencing the adoption of soil conservation behaviors among the participating farmers. To guarantee the validity of the qualitative analysis, members of the research team conducted an independent analysis of the interviews, and then discussed the results, reaching a consensus.

RESULTS AND DISCUSSION

The training course resulted in a statistically significant improvement in farmers' soil science knowledge (Figure 4-A). This knowledge level was sustained, as reflected by the results of the retention test. Specific "*P*" values for multiple comparisons (Tukey test) were as follows: Pre-test versus post-test: $P < 0.0001$; Pre-test versus retention test: $P = 0.003$; Post-test versus retention test: $P > 0.05$. The Cohen's (1988) *d* values were 1.8 and 1.2 for the comparisons of pre-test versus post-test and pre-test versus retention test, respectively. These effects are large according to the Cohen's (1988) classification. However, there was no statistically significant improvement in farmers' attitudes toward

soil (Figure 4-B, $P > 0.05$) nor in their soil conservation actions (Figure 4-B, $P > 0.05$).

Based on the qualitative analysis, farmers' opinions during interviews can be summarized as follows:

The delivered course greatly improved farmers' knowledge about soil management

practices,

Farmers recognized that current soil management practices on their farms could be improved.

Farmers were interested in implementing soil management practices that were discussed during the training course, such as

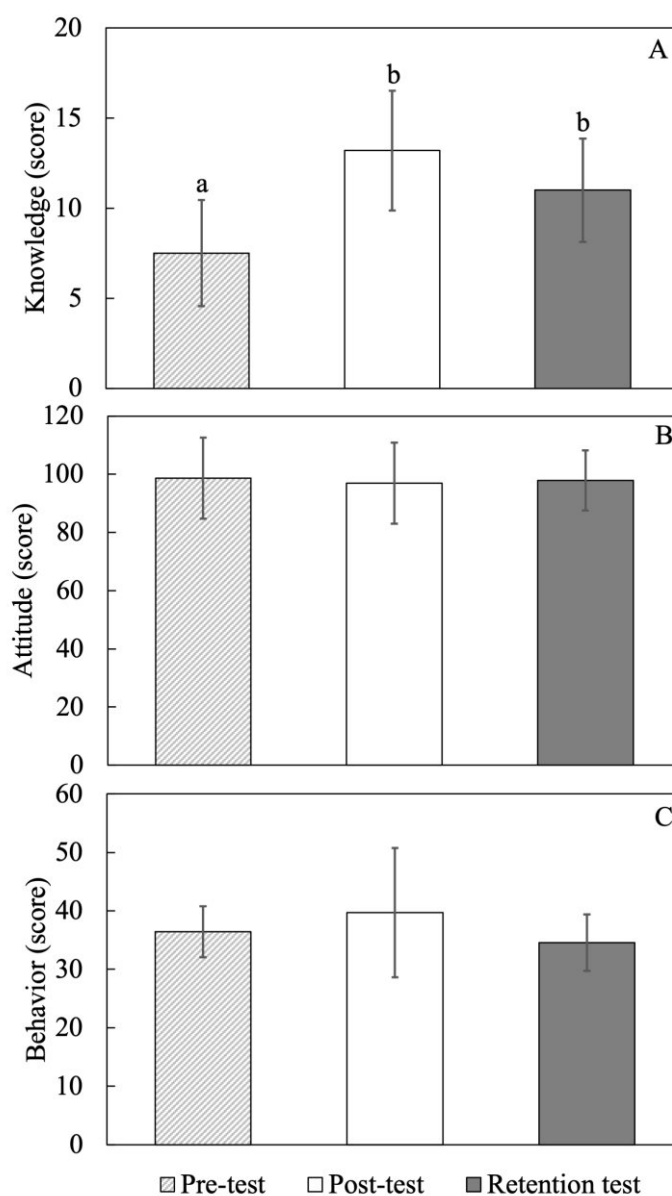


Figure 4. Quantification of the impact of farmers' training on: (A) Soil science knowledge, (B) Attitude(s) toward soil, and (C) Soil conservation behavior. The mean scores for each scale are shown; error bars show standard deviation. In the case of soil science knowledge, different letters represent statistically significant differences between the tests (ANOVA, Tukey test for multiple comparisons). Pre-test versus post-test: $P < 0.0001$; Pre-test versus retention test: $P = 0.003$; Post-test versus retention test: $P > 0.05$.



the use of compost, solarization, fermented organic fertilizers, as well as performing soil chemical analysis.

Despite the aforementioned interest, farmers were unable to implement any new practices during the three-month period since the last training session.

The primary barrier to implementing such practices was uncertainty about how to effectively put them into action, rather than economical constraints or other limitations.

Farmers requested additional practical workshops and real-scale demonstrations of the recommended practices, to better equip them to implement these actions on their farms.

In other words, the training course only resulted in a significant improvement in farmers' knowledge, while their soil conservation practices remained unaffected. These results partially confirm our original hypothesis, which stated that training course improve farmers' knowledge, which, in turn, will lead to observable changes in soil conservation practices.

As mentioned above, the format of our training program (traditional lectures and interactive discussions) is quite typical in Chile (Márquez-García *et al.*, 2018). This emphasizes the importance of having a platform to publish the results of studies that did not work out. Negative results are important and should be a vital part of the scientific community's body of knowledge. Below, we discuss in detail the implications of the results of the study.

Implications of the Study Results

The opinion that increased environmental knowledge leads to pro-environmental behavior is disputed. For example, in their seminal paper in the field of environmental education, Hungerford and Volk (1990) demonstrated that knowledge does not automatically lead to behavior change in the environmental dimension. Furthermore, the review conducted by Kollmuss and Agyeman (2002) notably highlights the

well-documented gap between possessing environmental knowledge and actually implementing pro-environmental behaviors. In the literature, this phenomenon is known as "knowledge-action gap" (Colombo *et al.*, 2023).

In the light of previous research, it is not surprising to observe this knowledge-behavior gap in the present study. The findings of this study challenge the typical focus of agricultural extension activities on knowledge alone, pointing out how this neglects potential behavioral changes resulting from the intervention (Rejesus *et al.*, 2012; Pan and Zhang, 2018). Our findings reflect the results of the interventional study of Márquez-García *et al.* (2018), which involved training Chilean winegrowers on the topic of biodiversity conservation on their farms. The latter study showed that farmers' knowledge about the importance of biodiversity was considerably improved as a result of the training. However, the biodiversity conservation behavior of participating farmers remained unchanged in the study of Márquez-García *et al.* (2018).

It is well established that behavior change is a gradual process (Prochaska and Velicer, 1997). The Stage Model of Self-regulated Behavioral Change (SSBC) offers a framework for understanding this process, outlining four distinct stages that individuals progress through (Bamberg, 2013):

- 1) Pre-decision: At this initial stage, individuals may lack awareness of the problem or its consequences.
- 2) Pre-action stage: individuals recognize the need for change but are yet to form a concrete plan of action.
- 3) Action: individuals actively implement new behavior(s).
- 4) Post-action: focus shifts to maintaining the adopted behavior(s) and preventing relapse.

Informed by the SSBC model, at the beginning of the training course, farmers participating in the present study were at the pre-decision stage. Based on the aforementioned interview results and the

quantitative results (Figure 1-A), the intervention successfully raised awareness of the issues of soil conservation, and helped foster a sense of personal responsibility among the farmers. Furthermore, the training course provided farmers with valuable information on alternative behaviors, including their pros and cons. As a result, farmers developed a willingness to achieve a soil conservation goal, although they lacked a specific plan for how to accomplish it. Consequently, as a result of the training, farmers progressed from the pre-decision stage to the pre-action stage, based on the SSBC model classification (Bamberg and Schulte, 2019).

A future interventional study is necessary to support farmers at the action and post-action stages, as outlined by the SSBC model. Specifically, a new training course could focus on practical workshops and real-scale demonstrations of recommended practices, as requested by participating farmers. During this proposed future training, farmers may require support in developing detailed implementation plans to translate intentions into actions, and maintain the changed behavior (Gollwitzer, 1999). Specifically, agricultural extension agents could help farmers to form a behavioral intention (a concrete plan to perform a specific soil conservation action), and subsequently an implementation intention (a detailed strategy for “how, when, and where” to achieve the intended soil conservation goal) (Gallo and Gollwitzer, 2007).

CONCLUSIONS

Our study provides timely and pertinent evidence of the knowledge-behavior gap in agricultural decision-making. The findings of this study challenge the common focus of agricultural extension activities on knowledge, overlooking any potential behavioral changes due to the intervention.

In our opinion, there is an urgent need to address a widespread reliance on knowledge

as an expected predictor of farmers' conservation behavior. There is also a need for future longitudinal studies to enhance our understanding of the mechanisms underlying the knowledge-behavior gap, in the context of farmers' conservation practices. The scales used in the present study will be useful in these future studies on diverse farmer populations.

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شکاف میان دانش و رفتار در حفاظت از خاک: مطالعه‌ای با کشاورزان خرده‌پا در شیلی

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چکیده

هدف این پژوهش تحلیل تأثیر یک دوره مدیریت خاک بر دانش علوم خاک و رفتارهای حفاظت از خاک کشاورزان بود. این پژوهش شامل ۳۳ کشاورز خرده‌پا در منطقه «آریکا و پاریناکوتا» (Arica and Parinacota Region) در شیلی بود. این تحقیق از روش‌های کیفی و کمی استفاده کرد و داده‌ها از طریق نظرسنجی‌ها (پیش و پس از دوره و یک آزمون ماندگاری متعاقب آن) و مصاحبه‌های پس از آزمون ماندگاری گردآوری شدند. این دوره آموزشی درک کشاورزان از علوم خاک را به‌طور قابل‌توجهی ارتقا داد، به طوری که در عملکرد آنان در نظرسنجی و آزمون سنجش ماندگاری آموخته‌ها پس از پایان دوره، مشهود بود. با این حال، هیچ‌گونه بهبود معناداری از نظر آماری در رفتار حفاظت از خاک کشاورزان مشاهده نشد. نتایج مصاحبه‌ها این یافته‌های کمی را تأیید کردند و بینش‌هایی را درباره سازوکارهای زیربنایی فراهم آوردند که با نظریه‌های «مدل مرحله‌ای تغییر رفتار خودتنظیمی» (Self-Regulated Behavioral Change) همسو هستند. بررسی ما شواهد به‌روزی از شکاف میان دانش و رفتار در تصمیم‌گیری‌های کشاورزی ارائه می‌دهد. این پژوهش، تمرکز

معمول فعالیت‌های ترویج کشاورزی "صرفاً بر مقوله دانش" را به چالش می‌کشد و نشان می‌دهد که چگونه این رویکرد، تغییرات رفتاری بالقوه ناشی از مداخلات را نادیده می‌گیرد.

Role of Characteristics and Competencies in the Business Performance of Functional Beverage SMEs

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ABSTRACT

This study examines the effects of owner characteristics, product competence, and business capital on the business performance of Small and Medium Enterprises (SMEs) in the functional beverage sector. It also investigates the mediating role of owner competence in the relationship between owner characteristics and business performance. Employing a quantitative approach, data were collected via a structured questionnaire from 105 functional beverage SMEs in Solo Raya, Indonesia. Structural Equation Modeling with Partial Least Squares was used for analysis. The results indicate that business capital and product competence significantly and positively influence business performance. Owner characteristics do not have a direct effect on business performance, but influence it indirectly through full mediation by owner competence. The findings suggest that functional beverage entrepreneurs should enhance their self-competence encompassing knowledge, skills, and business experience, and strengthen business capital to improve performance. Additionally, developing entrepreneurial characteristics is crucial for boosting business outcomes through improved owner competence.

Keywords: Business capital, Owner characteristics, Owner competence, Product competence.

INTRODUCTION

The restructuring of food systems plays a vital role in enhancing food resilience, while simultaneously safeguarding food security and nutrition. A notable trend influencing this shift is the increasing public demand for functional foods. These are products that deliver additional health advantages beyond their basic nutritional function. They contain bioactive compounds (such as vitamins, minerals, antioxidants, and phytochemicals) that help strengthen immune defences, lower the likelihood of long-term illnesses, and enhance general well-being. Given Indonesia's rich food resources and local wisdom, there is a significant opportunity to develop functional foods that align with

global trends and contribute to both public health and economic growth.

Functional beverages are a type of functional food that not only provide essential nutritional intake but also offer sensory satisfaction, such as favorable taste and texture (Setyowati, *et al.*, 2023a). These beverages can provide extra features, such as probiotics, enhanced nutrient intake, improved endurance, and reduced disease risk. Functional beverages can be derived from natural ingredients commonly found in daily life, such as plant-based materials including leaves, stems, flowers, roots, fruits, or even multiple parts of the plant (Wahyuningsih, *et al.*, 2022).

The Solo Raya region holds potential for the development of functional beverage Small and Medium Enterprises (SMEs)

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offering a diverse range of products. Despite their strengths, these enterprises face challenges such as low hygiene awareness, limited innovation, outdated management practices, and restricted marketing reach. Furthermore, they face stiff competition from other functional beverage products from outside Solo Raya and other popular beverages in the region. To succeed in this competitive environment, SMEs must differentiate their products by offering unique characteristics and benefits that resonate with consumers. This requires strong entrepreneurial skills, innovative approaches, and sufficient capital to support business growth and sustainability. By addressing these challenges and focusing on product differentiation, functional beverage SMEs in Solo Raya can carve out a successful niche in the market.

Previous studies have extensively examined the impact of entrepreneurial characteristics and competencies on business performance (Alizar and Mariyanti, 2024; Dewantoro *et al.*, 2023; Isma and Aliasyahbana, 2022; Istinganah and Widiyanto, 2020; Karimah and Meliza, 2023; Sarwoko *et al.*, 2013; Sudiarti and Juliarsa, 2020; Suindari and Juniariani, 2020). However, a comprehensive analysis encompassing entrepreneur characteristics, entrepreneur competence, product competence, and digital business competence remains lacking. This study seeks to address this gap by investigating the mediating role of owner competence in the relationship between owner characteristics and business performance in SMEs.

While research on functional beverages has predominantly focused on nutritional content, health benefits, and sensory evaluations (Dini, 2019; Harmayani *et al.*, 2019; Pamela *et al.*, 2021; Samilyk *et al.*, 2024; Widyaningsih *et al.*, 2020; Yusuf Alkandahri *et al.*, 2021), limited attention has been paid to the specific business dynamics of functional beverage SMEs, particularly in the Solo Raya region.

Therefore, this study aimed to explore the influence of product characteristics,

entrepreneur characteristics, and business capital on the business performance of functional beverage SMEs. Additionally, it examined the mediating effect of owner competence on the relationship between owner characteristics and business performance within this sector and region.

Capital is a crucial factor in starting a business. According to the Resource-Based View (RBV), organizations gain a competitive edge by efficiently managing their existing resources (Barney *et al.*, 2021). In this perspective, a company is regarded as a collection of tangible and intangible assets that can be leveraged to achieve strategic advantages (Ozdemir *et al.*, 2023). RBV emphasizes that heterogeneous business capital, such as financial capital, knowledge, experience, and human capital (Rumangkit and Prabowo, 2025). Recent research proves that venture capital, including determination, experience, and networking, significantly improves the performance of Micro, Small, and Medium Enterprises (Renaldo *et al.*, 2021). However, without sufficient financial capital, it can be challenging for a business to grow and perform well. Entrepreneurial knowledge and partner networks are critical dimensions of capital for Micro, Small, and Medium Enterprises (MSMEs). A study by Yani *et al.* (2020) reinforces these findings by showing that social capital, such as partner networks and trust, also contributes to improving MSME business performance. In addition, managerial competence, business experience, and competent human resources are also important parts of non-financial capital that contribute to the success of MSMEs. For MSMEs, business growth is often influenced by the amount of capital invested. In their study, Surya *et al.* (2021) stated that business capital and the strengthening of human resource capacity can increase MSME performance. Business capital is an essential element of MSMEs development (Isma and Aliasyahbana, 2022; Istinganah and Widiyanto, 2020; Syam *et al.*, 2023).

Characteristics refer to the traits, qualities, or attributes that define an individual and

distinguish them from others. In the context of entrepreneurship, essential traits include self-confidence, a future-oriented mindset, risk-taking ability, responsibility, and strong motivation to grow a business (Istinganah and Widiyanto, 2020). Recent findings indicate that the need to achieve, openness to risk-taking, and an internally driven sense of control are among the entrepreneurial qualities that strongly influence business success (Obschonka and Stuetzer, 2017). In addition, Self-efficacy remains a critical psychological mechanism that shapes entrepreneurial behavior and business outcomes (Newman *et al.*, 2019). According to prior studies, SME performance is shaped considerably by the attributes of their owners (Cahyati, 2023; Hidayah *et al.*, 2024).

A business's competence is determined by its owner. Stronger characteristics will increase the competence of business owners (Sarwoko *et al.*, 2013). Owner characteristics in this context are the entrepreneurial traits of the business owner, reflected in the competencies they develop; stronger personal traits often lead to higher levels of competence. Key entrepreneurial traits that contribute to competence include motivation, personality, knowledge, and skills. Essential aspects impacting owner competence include work experience, skills, confidence, responsibility, and goal-oriented priorities, which is difficult for competitors to imitate (Sarwoko *et al.*, 2013; Zainol *et al.*, 2018). This suggests a significant relationship between owner characteristics and competence.

Owner competence refers to the entrepreneur's ability to use resources efficiently and fulfill managerial roles to achieve desired business outcomes. This competence involves mastery of knowledge, skills, and relevant experience that directly influence an entrepreneur's ability to meet business objectives (Sudiarti and Juliarsa, 2020). Based on Human Capital theory and a meta-analysis by Unger *et al.* (2011), this competency is the outcome of human resource investment (such as education and training) that has been shown to significantly

affect business performance, especially when grounded in specific knowledge (task-related knowledge) and direct skills. Competence is often a predictor of performance (Suindari and Juniariani, 2020). For SMEs, enhancing business performance is challenging without strong owner competence. A study by (Unger *et al.*, 2011) also shows that competencies related to risk management and strategy making have a stronger influence than general competencies. Competence serves as a key factor in the success and growth of SMEs (Dewantoro *et al.*, 2023; Sarwoko *et al.*, 2013; Suindari and Juniariani, 2020).

Product competence refers to the extent to which a product contributes to achieving a company's goals. High product competence is a critical factor in business success (Cahyati, 2023). Products that attract consumers typically possess distinctive characteristics and strongly reflect the company's vision. Product competence can drive SMEs' growth by enhancing product appeal and competitiveness (Cahyati, 2023). Product competence can be reflected in the ability to produce innovative, high-quality products. Product and process innovation in SMEs contributes significantly to business performance, especially when supported by competitive strategies such as differentiation and focus on certain segments (Pusung *et al.*, 2023).

The characteristics of business owners are certainly the main foundation for achieving optimal business performance. However, it should be understood that the characteristics of business owners, such as daring to take risks, are not enough to develop a business; owners also need appropriate competencies, such as developing business strategies, recognizing opportunities, and understanding the market. This line of thinking is supported by theories such as Human Capital Theory, which states that investments such as education and training will only impact business performance when they are actualized into practical competencies, such as risk-taking ability (Unger *et al.*, 2011).

Owner's competence functions as a behavioral embodiment of entrepreneurial



characteristics that directly contribute to business performance (Man *et al.*, 2002). Business owners' characteristics can improve business performance through the development of competencies (Sarwoko *et al.*, 2013).

Therefore, this study aimed to explore the influence of product characteristics, entrepreneur characteristics, and business capital on the business performance of functional beverage SMEs. Additionally, it examined the mediating effect of owner competence on the relationship between owner characteristics and business performance within this sector and region. This study recommends the hypotheses as follows:

H1: Business capital has a positive and significant effect on business performance

H2: Owner characteristics have a positive and significant effect on business performance.

H3: Owner characteristics have a positive and significant effect on owner competence.

H4: Owner competence has a positive and significant effect on business performance.

H5: Product competence has a positive and significant effect on business performance.

H6: Owner characteristics have a positive and significant effect on business performance through owner competency.

MATERIALS AND METHODS

This quantitative study employs survey techniques as outlined by (Creswell and Creswell, 2018). It investigates the influence of owner characteristics, product competence, and business capital on the business performance of functional beverage SMEs. Additionally, the study examines the mediating role of owner competence in the relationship between owner characteristics and business performance (Table 1). A cross-sectional methodology was applied, with data obtained from subjects within a defined time frame (Creswell and Creswell, 2018). Data collection took place between June and August 2024. The study location was

purposively selected in the Solo Raya region, known for its significant potential in developing functional beverage businesses. Sukoharjo Regency serves as the primary hub of this potential, while other areas within Solo Raya also present promising opportunities for similar functional beverage products.

A five-point Likert scale was employed to measure the constructs, where 1 represented "strongly disagree" and 5 indicated "strongly agree". This scale was chosen because of its balance between the convenience of respondents and the adequacy of the information produced. In addition, the use of this scale also aims to minimize ambiguity in the answers given by respondents (Roselidyawaty and Rokeman, 2024). Drawing from prior research, the indicators were formulated to capture and represent the dimensions of each variable.

Data Collecting and Sampling Technique

The research utilized purposive sampling to gather data from all regencies and the city within the Solo Raya Region, namely, Boyolali, Sragen, Klaten, Wonogiri, Karanganyar, Sukoharjo, and Surakarta. The sample size was determined based on the variable-to-sample ratio of 1:20 (Hair *et al.*, 2018). This research model includes five variables; the required sample size was 100. In each regency/city, 15 functional beverage SMEs were selected, resulting in a total of 105 functional beverage SMEs as samples. Data collection was conducted through surveys (direct interviews) with the owners of these SMEs using a structured questionnaire. Moreover, in-depth interviews were conducted with seven personnel of the cooperatives and SMEs Office from the seven regencies/cities in Solo Raya to gather additional insights, particularly on government initiatives for functional beverage development in the area. The research model is presented in Figure 1.

Data Analysis

To test the research model, this study employed Structural Equation Modeling (SEM) using Partial Least Squares (PLS) as the analytical tool. PLS focuses on predictive

modeling, enabling the examination of relationships among dependent and independent variables to predict existing or new observations. This approach allows for drawing conclusions based on the significance of relationships, relative influences, explained

Table 1. Operational definition and variables measurement.

Description	Indicator	Code ^a	Source
Business Capital			
Business Capital is defined as the essential resources possessed by business owners to operate and grow their enterprises.	I have good business knowledge.	BC1	(Isma and Aliasahbana, 2022; Syam <i>et al.</i> , 2023)
	I have adequate financial resources.	BC2	
	I have a network of partners who support my business.	BC3	
	I have competent human resources.	BC4	
	I have sufficient business experience.	BC5	
	I have the availability and adequacy of business capital.	BC6	
Business Performance			
Business Performance is defined as the outcomes achieved by a business in terms of growth in sales, profits, market reach, and assets.	My product sales have increased as expected.	KB1	(Absah, Chairunisa Muchtar, and Qamariah, 2018; Augustinah <i>et al.</i> , 2022)
	My profits are steadily growing.	KB2	
	The market for my products is expanding.	KB3	
	My customers are growing.	KB4	
	The quality of my products is getting better.	KB5	
	There is an increase in production process capabilities.	KB6	
	Increase in the number of business assets owned.	KB7	
Product Competencies			
Product Competencies are defined as the distinctive attributes of a product that make it desirable, unique, high-quality, and competitive in the market.	My products are desired by consumers.	KP1	(Cahyati, 2023; Sarwoko <i>et al.</i> , 2013)
	Consumers often buy my products (repeat orders).	KP2	
	My products easily attract consumers.	KP3	
	My product has uniqueness and advantages compared to competitor products.	KP4	
	My product has the potential to be innovative.	KP5	
	My product is superior to similar products.	KP6	
	My products are guaranteed quality.	KP7	
Owner Competency			
Owner Competency is defined as the essential skills business owners possess to manage, develop, and sustain their enterprises.	I have conceptual competencies (ideas, solutions).	OC1	(Dewantoro <i>et al.</i> , 2023; Sarwoko <i>et al.</i> , 2013)
	I have the competence to read and take advantage of business opportunities.	OC2	
	I have the competence to learn.	OC3	
	I have the competence to build a network.	OC4	
	I have managerial competence.	OC5	
	I have entrepreneurial competence.	OC6	
	I have technical competence (using equipment) in running a business.	OC7	
Owner Characteristic			
Owner Characteristic is defined as the personal traits of business owners that drive motivation, confidence, responsibility, and risk-taking in achieving business goals.	I dare to take risks for the advancement of business.	OCh1	(Cahyati, 2023; Hidayah <i>et al.</i> , 2024)
	I have a strong motivation in running a business.	OCh2	
	I am oriented towards future business development.	OCh3	
	I have strong confidence.	OCh4	
	I want to achieve my business goals.	OCh5	
	I am responsible for the success of the business I run.	OCh6	

^a Business Capital (BC), Business Performance (KB), Product Competencies (KP), Owner Competency (OC), Owner Characteristic (OCh).

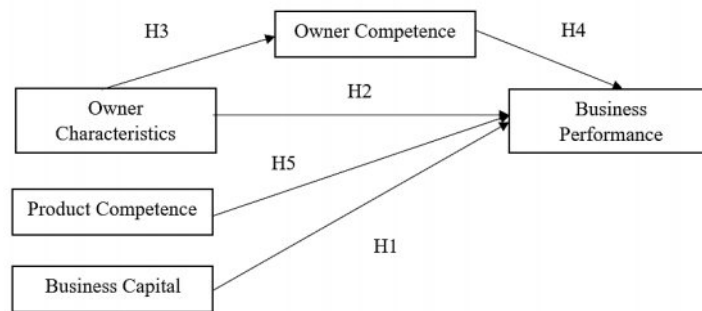


Figure 1. Proposed research model.

variance, and hypothesis testing. The choice of PLS was motivated by its nature as a variance-based SEM technique, which provides a robust analytical framework with minimal reliance on stringent assumptions. Notably, PLS does not require the data to follow a normal distribution, making it well-suited for survey data that may violate this assumption. Furthermore, PLS is effective for analyzing relatively small sample sizes while maintaining high statistical power. It also accommodates constructs measured by either reflective or formative indicators (Hair *et al.*, 2018). Data analysis was conducted using SmartPLS version 4. In PLS, two types of components are recognized within the causal model, as detailed below.

Measurement Model

The measurement model was applied directly to the indicator (manifest) variables associated with the latent variables. These indicators define the dimensions of the latent constructs. The reflective measurement model was evaluated using several criteria, including factor loadings. Convergent validity measured by Average Variance Extracted (AVE), discriminant validity, and internal consistency, assessed through composite reliability and Cronbach's alpha (Hair *et al.*, 2022).

Structural Model

The structural model comprises latent constructs that cannot be directly observed but are theorized to have relationships with one another. Testing the structural model involves estimating path coefficients, which indicate the strength and direction of relationships between dependent and independent latent variables. The significance of these path relationships was assessed using the bootstrapping technique, allowing for robust hypothesis testing. The rule of thumb for structural model testing is in Table 2.

RESULTS AND DISCUSSION

Characteristics of Respondents

Based on the survey, the owner of a functional beverage SME in Solo Raya is predominantly female, comprising 61%. Production activities require perseverance, patience, and consistency. For instance, women are more diligent in concocting herbal medicine, choosing ingredients, or selling products. The majority of functional beverage SME owners are of productive age, which is 80% aged 26-55 years. This means that SME owners have the potential to create

Table 2. Rule of thumb for structural model testing.

No	Criteria	Rule of Thumb	Reference
1	<i>R-Square</i>	Values of 0.75, 0.50, and 0.25 indicate a strong, moderate, and weak model, respectively.	(Hair <i>et al.</i> , 2019)
2	<i>Q² Predictive relevance</i>	$Q^2 > 0$ indicates that the model has predictive relevance, whereas $Q^2 < 0$ suggests the model lacks predictive relevance. Values of 0.02, 0.15, and 0.35 indicate weak, moderate, and strong predictive relevance, respectively.	(Hair <i>et al.</i> , 2019)
3	Significance (<i>two-tailed</i>)	P-value < 0.05 (5%) and a t-statistic > 1.96	(Hair <i>et al.</i> , 2019)
4	Upsilon V	Values of 0.010, 0.075, and 0.175 correspond to low, moderate, and high mediation, respectively.	(Lachowicz <i>et al.</i> , 2018) (Ogbeibu <i>et al.</i> , 2021)

and are open to innovation. A productive age provides a quick response and is able to formulate solutions more effectively. Based on the level of education, SME owners are dominated by high school graduates, which is 59%. As many as 34% have an education level up to Master's degree (S2). Adequate education is the capital of human resources that will support business development. A high level of education makes it easier to absorb knowledge and skills, plan a business well, and have good communication skills, so as to support the development of business networks. Based on the length of operation, the majority of functional beverage MSMEs are 1 to 10 years old. This shows that functional beverage MSMEs in Solo Raya are still in the development stage. At this age, MSMEs must continue to open themselves up to technological developments and continue to innovate to build a business.

Outer Model

Convergent Validity

To evaluate convergent validity in the reflective measurement model, the correlation between each indicator's score and the corresponding construct score, as computed through PLS, was examined. An indicator is regarded as having strong reflective properties when its loading factor exceeds 0.70, indicating a substantial association with the construct it represents. The loading factor results obtained in this research are presented in Table 3.

The next validity test was conducted by examining the Average Variance Extracted (AVE) value to assess the validity. The following are the AVE values from the validity test in this study.

Table 4 shows that all constructs in this study meet the validity requirement, as their AVE values exceed the threshold of 0.50. This indicates that all variables can explain the variance of their respective indicators. In the outer model, instrument reliability is determined through the analysis of CR and CA, which are both indicators of internal consistency. A construct meets the criteria when the CR score is greater than 0.6 and CA is above 0.7. The results indicate that each variable met the criteria, with CA and CR values exceeding 0.7 for all variables. This demonstrates that each variable is considered reliable, consistent and stable responses.

The loading factor for each indicator with its respective variable exceeds its cross-loading with other variables, indicating that the data meet the criteria for discriminant validity (Table 5).

Inner Model

Assessment of the structural model was carried out through the R-squared statistics of the dependent constructs. Within PLS analysis, the initial step in model evaluation involves reviewing the R² values of each endogenous latent variable. Variations in these R-squared values reflect the influence of independent construct on the dependent constructs. The coefficient of determination

**Table 3.** Factor loading.

	Business Capital (BC)	Business Performance (KB)	Owner Characteristics (OCh)	Owner Competency (OC)	Product Competence (KP)
BC2	0.803				
BC3	0.777				
BC4	0.685				
BC5	0.600				
BC6	0.769				
KB1		0.694			
KB2		0.679			
KB3		0.811			
KB4		0.834			
KB5		0.665			
KB6		0.829			
KB7		0.696			
KP1					0.600
KP4					0.730
KP5					0.788
KP6					0.795
KP7					0.773
OC1				0.668	
OC2				0.646	
OC3				0.823	
OC4				0.758	
OC5				0.729	
OC6				0.667	
OCh2			0.730		
OCh3			0.764		
OCh4			0.813		
OCh5			0.754		
OCh6			0.814		

Source: Primary Data Analysis, 2024.

Table 4. AVE, Cronbach Alpha (CA), and Composite Reliability (CR) values.

Variable	AVE	Decision	CA	CR	Decision
Business capital	0.525	Valid	0.776	0.845	Reliable
Business performance	0.558	Valid	0.868	0.898	Reliable
Owner characteristics	0.602	Valid	0.836	0.883	Reliable
Owner competency	0.515	Valid	0.810	0.864	Reliable
Product competency	0.549	Valid	0.799	0.857	Reliable

Source: Primary Data Analysis, 2024.

ranges from 0 to 1, with values of 0.67, 0.33, and 0.19 indicating strong, moderate, and weak explanatory power. The R² values are presented in Table 6. Furthermore, the relationships between latent variables were evaluated using the Q² statistic, which measures the predictive relevance of the model by indicating how well the observed values align with the values predicted by the model (Ghozali, 2014).

Hypothesis Testing (Bootstrapping)

In this research, hypotheses were evaluated using a 5% significance level (Table 7). A P-value not exceeding 0.05 led to acceptance of the hypothesis, while a value above “ α ” resulted in rejection. Figure 2 displays the research model obtained from the bootstrapping procedure.

Table 5. Cross-loading values.

	Business capital	Business performance	Owner characteristics	Owner competency	Product competence
BC2	0.803	0.335	0.470	0.568	0.482
BC3	0.777	0.448	0.327	0.503	0.461
BC4	0.685	0.459	0.232	0.332	0.217
BC5	0.560	0.191	0.371	0.456	0.293
BC6	0.769	0.393	0.352	0.457	0.484
KB1	0.283	0.694	0.140	0.354	0.214
KB2	0.282	0.679	0.222	0.357	0.144
KB3	0.504	0.811	0.339	0.560	0.464
KB4	0.435	0.834	0.288	0.353	0.415
KB5	0.384	0.665	0.336	0.465	0.466
KB6	0.398	0.829	0.241	0.364	0.395
KB7	0.420	0.696	0.224	0.375	0.244
KP1	0.324	0.150	0.367	0.461	0.599
KP4	0.451	0.394	0.259	0.254	0.730
KP5	0.400	0.347	0.438	0.473	0.788
KP6	0.437	0.274	0.331	0.378	0.795
KP7	0.362	0.454	0.282	0.387	0.773
OC1	0.379	0.232	0.408	0.668	0.345
OC2	0.326	0.383	0.387	0.646	0.172
OC3	0.493	0.435	0.442	0.823	0.379
OC4	0.466	0.427	0.470	0.758	0.401
OC5	0.513	0.394	0.420	0.729	0.347
OC6	0.479	0.473	0.428	0.667	0.488
OCh2	0.197	0.124	0.730	0.361	0.176
OCh3	0.243	0.249	0.764	0.436	0.168
OCh4	0.457	0.384	0.813	0.538	0.473
OCh5	0.413	0.288	0.754	0.470	0.404
OCh6	0.411	0.268	0.814	0.465	0.373

Source: Primary Data Analysis, 2024.

Table 6. R² and Q² values for the Functional Beverage Business Performance Model.

Variable	R ²	Q ²	Status
Business performance	0.37	0.18	Moderate- Has predictive relevance.
Owner competency	0.35	0.17	Moderate- Has predictive relevance.

Source: Primary Data Analysis, 2024.

Effect of Business Capital on Business Performance

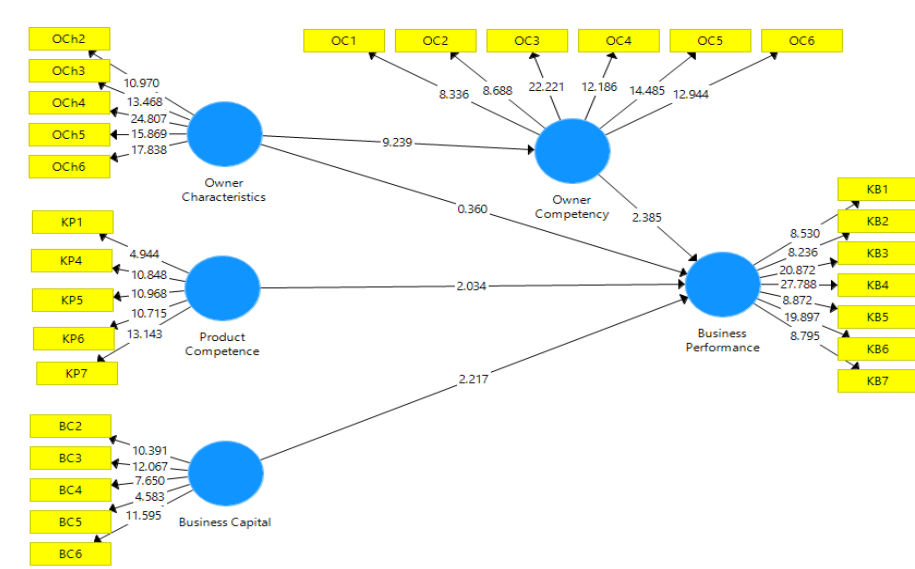
The bootstrapping analysis indicates that business capital exerts a significant and positive influence on the business performance of SMEs in the functional beverage sector, with a p-value of 0.037, thereby confirming H1. This suggests that increased business capital correlates with improved business performance in the functional beverage sector. SMEs that possess adequate financial resources, skilled labor, relevant experience, and extensive business networks are better equipped to

improve their outcomes. The finding supports prior empirical research (Augustinah *et al.*, 2022) that employees with high competencies and skills can consistently produce high-quality functional beverages. High-quality products not only attract potential consumers but also foster customer loyalty (López Rodríguez and Serrano Orellana, 2020). Furthermore, a skilled workforce adds value to the business, providing a competitive advantage and enabling superior service delivery to customers (Absah *et al.*, 2018). The capacity to build business networks also plays a crucial role in enhancing business performance (Parida *et al.*, 2017; Riptanti *et al.*, 2022). Business experience, as a

**Table 7.** Hypothesis testing results.

Hypothesis		Path coefficient	P-value	Decision	F-Square	Result
Business capital -> business performance	H1	0.239	0.037	Accepted	0.051	Low
Owner characteristics -> business performance	H2	0.036	0.707	Non-accepted	-	-
Owner characteristics -> owner competency	H3	0.595	0.000	Accepted	0.548	High
Owner competency -> business performance	H4	0.327	0.018	Accepted	0.084	Low
Product competence -> business performance	H5	0.199	0.031	Accepted	0.042	Low

Source: Primary Data Analysis, 2024.

**Figure 2.** Research model based on bootstrapping results.

component of business capital, further enhances performance by enabling the identification of business opportunities and fostering the creation of strategic networks (Othman *et al.*, 2016). Notably, 79% of entrepreneurs in this sector possess over 10 years of business experience, which supports their expertise in production, management, and network development. Based on the F-square value, the effect of business capital on functional beverage business performance is categorized as low, with an F-square value of 0.051.

Effect of Owner Characteristics on Business Performance

This study found no direct or significant relationship between owner characteristics and the performance of functional beverage SMEs, as evidenced by a p-value of 0.707, suggesting that H2 is not accepted. This implies that the strength or weakness of the owner's characteristics in functional beverage businesses does not directly influence their performance. This finding is inconsistent with the results of the previous studies (Alizar and Mariyanti, 2024; Cahyati, 2023) that strong entrepreneurial characteristics can drive business performance improvement. In the context of

this study, SMEs in the functional beverage with entrepreneurs who possess strong entrepreneurial traits do not directly lead to increased product sales, expanded market share, improved profit, or business asset growth. Characteristics such as confidence and courage to take risks need to be realized in the form of competence so that they will have an impact on business performance. According to Man *et al.* (2002), entrepreneurial competence serves as a mediator between personal characteristics and business performance. Unger *et al.* (2011) also said that human capital, such as new traits, experiences, and education, will actively contribute to performance when converted into specific competencies. Therefore, owner competence is needed as a mediator in the influence of owner characteristics on business performance.

Effect of Owner Characteristics on Owner Competency

The outcomes of the hypothesis test indicate that owner characteristics have a positive and significant effect on owner competency in the functional beverage business. This is evidenced by a P-value of 0.000, thus H3 is accepted. This suggests that stronger owner characteristics in functional beverage SMEs are associated with higher levels of competency. As entrepreneurs' motivation and self-confidence increase, so does their orientation and sense of responsibility towards achieving business goals and targets, which in turn enhances their competencies (Ode, *et al.*, 2024). This is because the achievement of business goals requires essential competencies, such as knowledge and skills. The effect size of 0.548 indicates a high influence, meaning that strengthening the characteristics of functional beverage SME entrepreneurs can improve their competencies by 54.8%. A strong character is, therefore, a crucial factor for entrepreneurs to possess.

Effect of Owner Competency on Business Performance

The findings from the hypothesis testing reveal that owner competency has a positive and significant effect on business performance. This is evidenced by a p-value of 0.018; thus, H4 is accepted. This suggests that higher competency levels in functional beverage SME entrepreneurs are associated with improved business performance. This finding is consistent with the outcomes of previous research (Alizar and Mariyanti, 2024; Karimah and Meliza, 2023) that human resource competency influences business performance. Entrepreneurs in the functional beverage industry are required to possess conceptual competency (ideas, solutions, concepts), the ability to identify business opportunities, learning competency, networking competency, entrepreneurship competency, and managerial competency. Mastery of these competencies enables entrepreneurs to achieve business performance outcomes that align with their expectations. This is especially significant given the competitive landscape in the functional beverage market, both from outside Solo Raya and the rise of beverages popular with younger generations. The effect size of 0.084 suggests that the influence of owner competency on business performance is relatively low.

Effect of Product Competence on Business Performance

In this study, product competence has been shown to have a positive and significant effect on the business performance of functional beverage SMEs. This is indicated by a P-value of 0.031; thus, H5 is accepted. This means that the higher the product competence of functional beverages, the higher the business performance of functional beverage SMEs. Product competence in functional beverages is reflected in the uniqueness, advantages, and quality of the products. These beverages attract consumer



interest through their innovation and use of natural ingredients, offering health benefits. For example, ginger drinks are known for their ability to alleviate colds, improve circulation, and reduce cholesterol levels (Setyowati *et al.*, 2023b). Functional beverages meet consumer demand for products with nutritional content that promotes health, while also offering a pleasurable taste experience. The influence of product competence on business performance is relatively weak, as indicated by the effect size of 0.042. This suggests that improvements in product competence could lead to a modest increase in the business performance of functional beverage SMEs, with a potential increase of only 4.2%.

Mediating Role of Owner Competency in the Effect of Owner Characteristics on Business Performance

The findings reveal that owner competency completely serves as a mediator in the relationship between owner characteristics and the performance of the functional beverage SMEs. Consistent with Sarwoko *et al.* (2013), owner competency functions as an intermediary linking owner characteristics to business outcomes. This is evidenced by the significant and positive effect of owner characteristics on owner competency. Likewise, the influence of owner competency on business performance is both positive and significant. However, the direct effect of owner characteristics on business performance is not significant. While identifying the significance of the mediation is important, it is equally crucial to assess the magnitude of its mediating role. This can be done using the Upsilon V parameter,

specifically the effect size (F-square) for the mediation function (Lachowicz *et al.*, 2018; Ogbeibu *et al.*, 2021). The results of the Upsilon V calculation ($a^2 \times b^2$) are presented in Table 8.

As presented in Table 8, the Upsilon V value for owner competency is 0.038. Referring to the mediation effect categorization according to Ogbeibu *et al.* (2021), a large mediation effect is indicated by Upsilon V > 0.175, a moderate effect by > 0.075, and a small effect by > 0.010. Therefore, it can be concluded that owner competency provides a small mediating effect on the relationship between owner characteristics and business performance in functional beverage SMEs. With a 95% confidence interval, owner competency can mediate the influence of owner characteristics on business performance between 0.005 and 0.337. Although the mediation effect is small, it warrants attention, as strong owner characteristics can contribute to improving business performance through the competencies possessed.

CONCLUSIONS

Efforts to enhance the business performance of functional beverage SMEs can be effectively achieved by strengthening key aspects related to the entrepreneur, the product, and business resources. The study's findings confirm that business capital, product competence, and owner competence contribute significantly to improving the business performance of functional beverage SMEs. Conversely, owner characteristics do not directly impact business performance; instead, their influence operates indirectly

Table 8. Result of the effect mediation test.

Hypothesis	P Value	95% Confidence Interval		Upsilon V (F Square)	Description
		Lower limit	Upper limit		
Owner characteristics -> Owner competency -> Business performance	0.019	0.005	0.337	0.038	The mediating effect is small.

Source: Primary Data Analysis, 2024.

through the full mediating role of owner competence. This indicates that while owner characteristics are important, their impact on business performance is primarily realized through the enhancement of owner competence. The study also has certain limitations, such as its cross-sectional design and the limited scope regarding geography and research variables.

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نقش ویژگی‌ها و شایستگی‌ها در عملکرد تجاری شرکت‌های کوچک و متوسط تولیدکننده نوشیدنی‌های فراسودمند

نونینگ ستیواتی، کوسندار، محمد هاریسودین، ارلینا ویدا ریپتانتی، ایستی خومه، و رادن
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چکیده

این پژوهش تأثیر ویژگی‌های مالک، شایستگی محصول و سرمایه کسب‌وکار را بر عملکرد بنگاه‌های کوچک و متوسط (SMEs) در حوزه نوشیدنی‌های فراسودمند بررسی می‌کند. این پژوهش همچنین نقش میانجی شایستگی مالک را در رابطه میان ویژگی‌های مالک و عملکرد کسب‌وکار بررسی می‌کند. با استفاده از یک رویکرد کمیتی، داده‌ها از طریق پرسشنامه ساختاریافته از ۱۰۵ شرکت کوچک و متوسط تولیدکننده نوشیدنی‌های کاربردی در سولو رایا (Solo Raya)، در اندونزی، جمع‌آوری شد. برای تجزیه و تحلیل از مدل‌سازی معادلات ساختاری با حداقل مربعات جزئی (Partial Least Squares) استفاده شد. نتایج نشان می‌دهد که سرمایه کسب‌وکار و شایستگی محصول، تأثیری مثبت و معنادار بر عملکرد کسب‌وکار دارند. ویژگی‌های مالک تأثیر مستقیمی بر عملکرد کسب‌وکار ندارد، بلکه از طریق میانجی‌گری کامل شایستگی مالک (Full Mediation by Owner Competence)، به‌طور غیرمستقیم بر آن تأثیر می‌گذارد. یافته‌ها نشان می‌دهد که کارآفرینان نوشیدنی‌های کاربردی باید خودکفایی خود را که شامل دانش، مهارت‌ها و تجربه تجاری است، افزایش دهند و سرمایه تجاری را برای بهبود عملکرد تقویت کنند. علاوه بر این، توسعه ویژگی‌های کارآفرینی برای افزایش نتایج تجاری از طریق بهبود شایستگی مالک بسیار مهم است.

Properties of Ethyl Cellulose/Polycaprolactone Electrospun Nano-fibers Incorporated with Gallic Acid and Natamycin for Cheese Packaging

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ABSTRACT

In this study, polymers of Ethyl Cellulose (ECL) and Polycaprolactone (PCL) were blended in ratios of 70:30, 80:20, and 90:10 (% w/w), and combined with Natamycin (NAT) at concentrations of 1 and 2% (w/w) and Gallic Acid (GAL) at 10 and 30% (w/w). Nano-fibers were produced via electro-spinning under the following conditions: voltage of 16kV, flow rate of 1 mL/hour, and a tip-to-collector distance of 15 cm. The nano-fibers were characterized for their morphology, FTIR, thermal, mechanical, antioxidant, water contact angle, and antifungal properties. The results indicated that ECL90/PCL10, ECL80/PCL20, and ECL70/PCL30 nano-fibers had average diameters of 1250.86 ± 18 , 1174 ± 21.4 , and 754.73 ± 23 nm, respectively. The ECL70/PCL30 ratio was selected and combined with NAT and GAL. The average diameter of these nano-fibers ranged from 779.32 to 924.63 nm. The addition of NAT and GAL enhanced the thermal stability, mechanical properties, and antifungal efficacy of the ECL/PCL nano-fibers. Notably, nano-fibers containing 2% NAT combined with either 10 or 30% GAL completely inhibited the growth of molds. Cheese samples packaged with nano-fibers containing NAT and GAL exhibited a significantly lower increase in mold and yeast counts during storage compared to the control samples. Furthermore, sensory evaluation revealed no significant differences in scores among the packaged samples. Therefore, ECL/PCL nano-fibers incorporated with 2% NAT and 10% GAL present an effective alternative to the direct incorporation of NAT in cheese formulations.

Keywords: Dair products, Electrospinning, Food preservation.

INTRODUCTION

Fungi are among the primary agents of food spoilage, rendering food unfit for human consumption by diminishing its nutritional value and producing harmful mycotoxins (Kumar, Mishra *et al.*, 2007). Molds belonging to the *Aspergillus* and *Penicillium* genera are among the most

frequently encountered contaminants during the production, ripening, curing, and refrigerated storage of food products prior to consumption (Gandomi *et al.*, 2009; Beikzadeh, *et al.*, 2021).

Natamycin (also known as pimaricin) is a polyene macrolide antifungal compound produced by species of *Streptomyces*, and is commonly classified within the polyene

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macrolide fungicide group (Delves-Broughton, *et al.*, 2005). It is widely used as an antimicrobial agent for surface preservation in various sausages and cheeses. Compared to other fungicides, natamycin demonstrates superior efficacy, with most fungi being inhibited or destroyed at concentrations ranging from 0.5 to 6 $\mu\text{g mL}^{-1}$ (Koontz and Marcy 2003; Delves-Broughton, *et al.*, 2005). However, the efficacy of natamycin may be diminished when directly incorporated into food matrices, due to interactions with other food components (Fajardo *et al.*, 2010). Therefore, incorporating Natamycin (NAT) into polymer matrices can enhance its stability and enable controlled release, thereby maintaining effective concentrations of the compound on the food surface.

Gallic Acid (GAL) (3,4,5-trihydroxybenzoic acid) and its derivatives, including catechin and tannin, are naturally present in a variety of plants, as well as in wine, tea, cereals, citrus fruits, and berries (Laura *et al.*, 2009). Owing to its potent antioxidant and antimicrobial properties, Gallic acid is widely utilized as an additive in the food and pharmaceutical industries. However, its application in food products is often limited by its inherent bitterness and astringency. Additionally, GAL is sensitive to environmental factors such as light, oxygen, pH, and temperature, which can affect its stability and efficacy (Fang and Bhandari 2010; Li, *et al.*, 2016). Encapsulation techniques are employed to prevent oxidation, enhance stability, and mask the undesirable taste of Gallic acid. Common encapsulation methods include lyophilization, co-crystallization, liposome entrapment, coacervation, extrusion, spray cooling or chilling, and spray drying (Fang and Bhandari, 2010).

Novel encapsulation technologies, such as electro-spinning, offer significant advantages by eliminating the need for toxic solvents and high processing temperatures. Electro-spinning is particularly suitable for encapsulating food and pharmaceutical compounds for several reasons. Notably, the

process occurs at room temperature, which helps preserve the integrity of sensitive active compounds. This technique enables the formation of nano-sized fibers, providing a high surface-area-to-volume ratio that can enhance the release of encapsulated agents. Furthermore, the polymer matrix within the nano-fibers facilitates sustained or controlled delivery of the active compounds (Mohammadi, *et al.*, 2018; Beikzadeh *et al.*, 2021; Beikzadeh, *et al.*, 2025). Moreover, a limited surface area can slow the release of active substances. By reducing fiber size from the micro-scale to the nano-scale, electro-spun fibers provide a significantly increased surface area, which facilitates more efficient and controlled release of encapsulated compounds (Neo, *et al.*, 2013; Aytac, *et al.*, 2017; Beikzadeh, *et al.*, 2021). In the electro-spinning process, both synthetic and natural polymers can be utilized to fabricate nano-fibers (Beikzadeh, *et al.*, 2021). The accumulation of packaging waste, especially from synthetic polymers that exhibit long environmental persistence, poses a major worldwide environmental challenge. To address this issue, the use of biodegradable biopolymers in packaging has emerged as a viable approach to reducing plastic waste (Pilevar, *et al.*, 2019; Beikzadeh, *et al.*, 2020; Beikzadeh, *et al.*, 2020; Javdani, *et al.*, 2025). Among biopolymers, ethyl cellulose stands out as one of the most important and widely utilized cellulose ether derivatives. It is characterized by its hydrophobic nature and resistance to salts and alkaline conditions (Beikzadeh, *et al.*, 2021). Blending different polymers is one of the most economical and practical approaches to producing materials with enhanced mechanical and thermal properties (Gregorova, *et al.*, 2012; Beikzadeh, *et al.*, 2021). Polycaprolactone (PCL) is recognized for its compatibility with various polymers. It is a six-carbon polymer featuring hydroxyl end groups. However, its relatively low melting point ($\sim 60^\circ\text{C}$) limits its thermal performance. To overcome this constraint, PCL is often blended with other polymers to enhance its

thermal and mechanical properties (Chrissafis, *et al.*, 2007).

In terms of degradability, electro-spinning enables rapid drying because the fibers produced are extremely fine, which limits the time available for crystal growth. Additionally, since the annealing process is performed below the melting point, it further contributes to reduced crystallinity. Consequently, the brief annealing of the electrospun mats appears to be a promising strategy for creating materials that degrade quickly (Salević-Jelić, *et al.*, 2024). Also, PCL demonstrates favorable biodegradability under appropriate environmental conditions. Different microorganisms, including bacteria and fungi, produce extracellular depolymerases that enable them to break down PCL. The breakdown of PCL-based films happens through the attachment of compost microorganisms to the surface, followed by enzymatic action, fragmentation of the film, and the release of degradation byproducts (Salević-Jelić, *et al.*, 2024). The incorporation of ethyl cellulose into polymer matrices has been reported to enhance biodegradability. For instance, Girija *et al.* (2010) demonstrated that blending ethyl cellulose with Poly(Ethylene-co-Vinyl Acetate) (EVA) led to improved biodegradation characteristics.

In this study, ECL/PCL [Ethyl Cellulose (ECL) and Polycaprolactone (PCL)] nano-fibers containing varying concentrations of NAT and GAL were fabricated. The thermal, mechanical, morphological, and antifungal properties of the nano-fibers were evaluated. Subsequently, cheese samples were packaged with the optimized nano-fibers, and their mold growth and overall sensory acceptance were assessed.

MATERIALS AND METHODS

Required Materials

Fungal strains were obtained from the Iranian Research Organization for Science

and Technology. Polymers, including PCL and ECL, along with NAT and GAL, were procured from Sigma-Aldrich.

Production Steps of Electrospinning Solutions

Approximately 12.5% (w/v) solutions of ECL and PCL were prepared in a 50:50 (v/v) ethanol/chloroform solvent mixture at 25 °C. Polymer blends with ECL/PCL ratios of 90:10, 80:20, and 70:30 were subsequently formulated. Ultimately, the ECL/PCL ratio of 70:30 was selected (Beikzadeh, *et al.*, 2021). NAT at 1 and 2% (wt%, weight percentage), indicating the weight of natamycin relative to the total weight of the polymer solution) and GAL at 10 and 30% (wt%) were then incorporated into the nano-fiber solutions (Amjadi, *et al.*, 2020).

Electro-spinning Operation

The electro-spinning process was conducted at an applied voltage of 16kV, a flow rate of 1 mL hr⁻¹, and a tip-to-collector distance of 15 cm. The operation was performed at a temperature of 25±2°C and relative humidity of 30±1%. The resulting nano-fibers were collected on the grounded collector (Amjadi, *et al.*, 2020; Beikzadeh, *et al.*, 2025).

Characteristics of Produced Nanofibers

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) (Philips XL 30, Holland) operated at 26kV and was employed to examine the microstructure of the nano-fibers. Fiber diameters were quantified using NIS Elements 0.3 image analysis software (Nikon Instruments Inc., Japan).



Fourier Transforms Infrared

Fourier-Transform Infrared (FTIR) spectra were recorded using a Tensor 27 spectrometer (Ettlingen, Germany). Interferograms were collected over the spectral range of 1,000–4,500 cm^{-1} (Sheikhi, *et al.*, 2020a).

Antioxidant Activity

DPPH radical scavenging method evaluates antioxidant activities (Altan, *et al.*, 2018). The antioxidant activity of the nano-fibers was evaluated using the DPPH radical scavenging assay. Briefly, 100 mg of nanofibers were suspended in 2 mL of water and stirred. Subsequently, 1 mL of the resulting solution was mixed with 0.2 mL of DPPH solution. After incubating the mixture at 25°C for 30 minutes, the absorbance was measured at 517 nm using a spectrophotometer. The scavenging activity was then calculated using the appropriate equation to determine the antioxidant capacity.

Water Contact Angle (WCA)

A drop of water (5 μL in volume) was deposited onto the nano-fiber surface, after which the contact angle was photographed and analyzed (Heidari, *et al.*, 2019; Sheikhi, *et al.*, 2020b).

Mechanical Properties

To assess the mechanical properties, the nano-fibers were cut into dumbbell-shaped specimens (0.6×9 cm) and subjected to tensile testing at a crosshead speed of 1.0 mm min^{-1} (Amjadi, *et al.*, 2019).

Thermo-Gravimetric Analysis (TGA)

For the TGA, approximately 15 mg of nano-fibers were heated under a nitrogen atmosphere at a rate of 10°C min^{-1} up to 600°C using a thermo-gravimetric analyzer (STA PT1600, Germany).

Release Kinetics of NAT and GAL

The release profiles of Gallic Acid (GAL) and Natamycin (NAT) were evaluated by immersing nano-fibers (15.1 cm^2 area) in 10 mL of distilled water at 4°C. At predetermined time intervals (0, 250, 500, 750, 1,000, and 1,250 hours), the absorbance of GAL and NAT was measured at 260 nm and 320 nm, respectively, using a UV-Visible spectrophotometer. The concentration of each compound was determined based on calibration curves constructed from standard solutions with concentrations ranging from 1 to 50 mg L^{-1} (Titone, *et al.*, 2025).

Antifungal Properties

To evaluate antifungal activity, *Aspergillus niger* (PTCC 5012) and *Penicillium notatum* (PTCC 5014) strains were cultured on Potato Dextrose Agar (PDA) media. A spore suspension (10^6 spores mL^{-1}) was prepared, and 1 mL of this suspension was homogenized with 99 mL of PDA. Nanofiber samples (1×1 cm) were sterilized under ultraviolet light for 1 hour, then placed onto the inoculated plates. The plates were incubated for 5 days, after which the diameter of the inhibition zone around the nano-fibers was measured to assess antifungal efficacy (Veras, *et al.* 2016).

UF Cheese Packaging

Based on the evaluation of the morphological, thermal, mechanical, and antifungal properties of the produced nano-fibers, those exhibiting

optimal characteristics were selected for packaging Ultra-Ultrated (UF) cheese. For this purpose, aluminum foils coated with nano-fibers (both with and without preservatives) were sterilized using Ultraviolet (UV) light for 40 minutes. Subsequently, UF cheese was produced and cut into pieces measuring 5×25×30 mm. The cheese samples were sterilized under UV light for 20 minutes before being packaged using the nano-fiber-coated aluminum foils (Beikzadeh, *et al.*, 2025).

Microbial Analysis of Produced Cheeses

Mold and yeast counts were performed using Sabouraud dextrose agar culture medium via the pour plate method, with a 0.01 dilution, under refrigerated storage conditions throughout the storage period.

Sensory Analysis of Manufactured Cheeses

The sensory evaluation was conducted by a panel of 30 trained assessors. Cheese samples were removed from refrigeration one hour prior to testing to equilibrate to room temperature and were coded using a random number table. Each panelist received the samples along with a standardized evaluation questionnaire and assessed them based on texture, taste, aroma, and overall acceptance. Evaluations were performed using a 5-point hedonic scale.

Statistical Evaluation

Data obtained from three independent replicates were expressed as mean ± standard deviation. Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) performed in SPSS software. Duncan's multiple range test was applied to determine significant differences at a confidence level of $P < 0.05$.

RESULTS AND DISCUSSION

Scanning Electron Microscopy

The SEM images and corresponding mean diameter distributions of the electrospun ECL/PCL nano-fibers are presented in Figure 1. The average fiber diameters for ECL90/PCL10, ECL80/PCL20, and ECL70/PCL30 were 1250.86 ± 18 nm, 1174 ± 21.4 nm, and 754.73 ± 23 nm, respectively. For comparison, Wang *et al.* (2020) reported an average diameter of 649 nm for pure ECL nano-fibers (Wang, *et al.*, 2020). Liu *et al.* (2018) reported nano-fibers with a spindle-like morphology and an average diameter of 413 nm (Liu, *et al.*, 2018). In contrast, PCL nano-fibers have been reported to exhibit an average diameter of approximately 419 nm. As shown in Figure 2, the average diameters of nano-fibers containing ECL70/PCL30 with varying concentrations of NAT and GAL (ECL70/PCL30/NAT1/GAL10, ECL70/PCL30/NAT2/GAL10, ECL70/PCL30/NAT1/GAL30, and ECL70/PCL30/NAT2/GAL30) were 779.32 ± 35 nm, 886.27 ± 13.5 nm, 805.45 ± 12 nm, and 924.63 ± 19 nm, respectively. The highest average diameter was observed for the ECL70/PCL30/NAT2/GAL30 sample. Overall, the incorporation of NAT and GAL led to an increase in fiber diameter. Neo *et al.* (2013) reported that the addition of GAL to zein solutions resulted in increased fiber diameters. This effect was attributed to the elevated solution viscosity caused by GAL, which enhances molecular entanglement and, consequently, leads to larger fiber diameters (Neo, *et al.*, 2013). Similarly, Veras *et al.* (2020) reported that the incorporation of NAT into the polycaprolactone electro-spinning solution led to an increase in fiber diameter. Notably, tripling the NAT concentration compared to the initial sample resulted in 64% increase in the average fiber diameter (Veras, *et al.*, 2020). These findings are consistent with the results obtained in the present study.

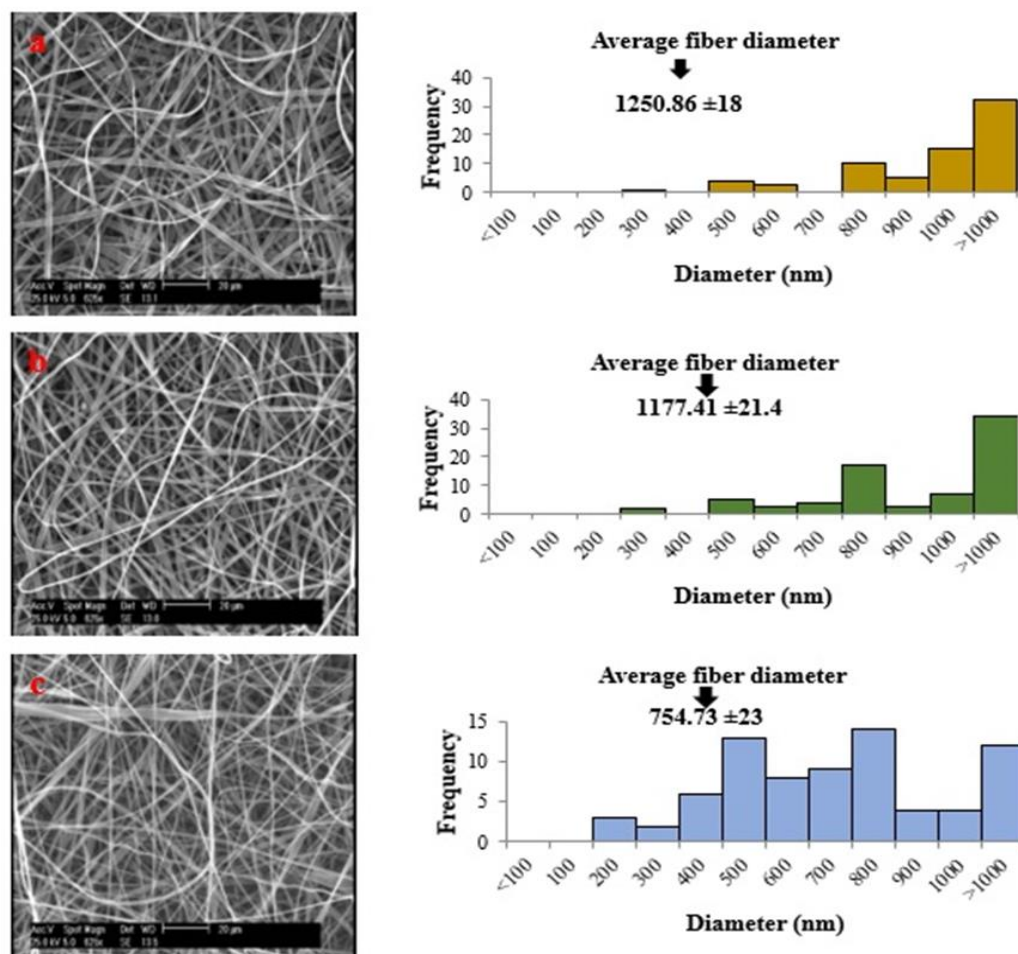


Figure 1. Fiber diameter distributions of electro-spun nano-fibers: (a) ECL90/PCL10, (b) ECL80/PCL20, and (c) ECL70/PCL30. Abbreviations: NAT: Natamycin; GAL: Gallic Acid; ECL: Ethyl Cellulose, and PCL: Polycaprolactone.

Fourier Transforms Infrared (FTIR)

Table 1 and Figure 3 present the wavenumbers of characteristic peaks observed in the nano-fibers. For the ECL nano-fiber, the peaks at 2,879, 3,450–3,741, 2,621–2,742, and 1249 cm^{-1} correspond to $-\text{CH}_3$ stretching vibration, O–H vibration, CH_2 and CH stretching, and C–O–C linkage, respectively (Beikzadeh, Hosseini et al. 2021). For PCL, the peaks observed at 2,889–3,030, 1,743, 1,375, and 1,047–1,257 cm^{-1} can be attributed to CH_2 stretching vibrations, C=O stretching, C–C stretching, and C–O–C stretching vibrations, respectively (Beikzadeh, et al., 2021, Beikzadeh, et al., 2025). For NAT, the

presence of peaks in the range of 1,100–1,750 cm^{-1} was attributed to the C=O bond. In the case of GAL, the peaks at 3490–3748 cm^{-1} corresponded to O–H stretching vibrations. The peaks observed at 1,520–1,710 cm^{-1} were related to the stretching vibrations of C–C and C–H aromatic groups. Additionally, the peaks at 1,193–1,411 cm^{-1} were assigned to C–O stretching vibrations. Changes in the ECL spectrum were observed following the addition of PCL, with the peaks at 2,870 and 3,450 cm^{-1} shifting to higher wavelengths. Furthermore, a new peak appeared at 2,504 cm^{-1} . Upon the incorporation of NAT and GAL, further modifications were detected in the ECL70/PCL30 spectrum, including the

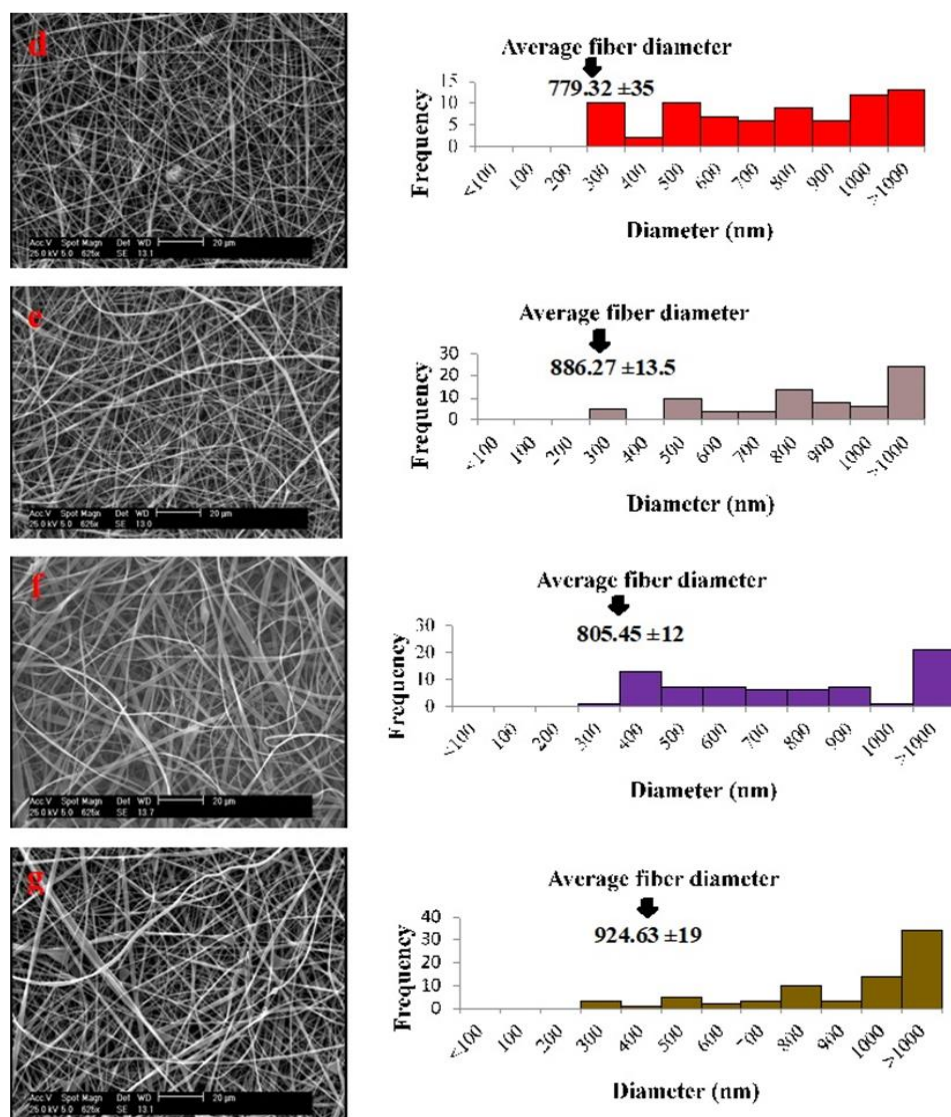


Figure 2. SEM images and average nano-fiber diameters of ECL70/PCL30/NAT1/GAL10 (d), ECL70/PCL30/NAT2/GAL10 (e), ECL70/PCL30/NAT1/GAL30 (f), and ECL70/PCL30/NAT2/GAL30 (g).

Table 1. Types of nano-fibers, connections, and wavenumber of specified peaks of nano-fibers.

Nano-fiber types	Connection	Wavenumber (cm ⁻¹)
ECL	–CH ₃ connection vibration	2879
	O–H vibrations	3450-3741
	CH ₂ and CH stretches	2621-2742
	C–O–C connection	1249
PCL	CH ₂ stretching vibration	2889-3030
	CO connection vibration	1743
	CC connection vibration	1375
	C–O–C connection vibration	1047-1257
NAT	C=O connection	1100-1750
GAL	O–H stretching vibration	3490-3748
	C–C/C–H stretching vibration	1520-1710
	C–O stretching vibration	1193-1411

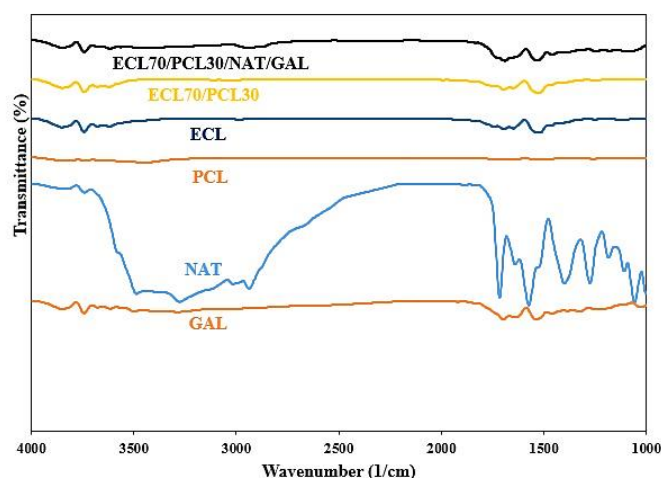


Figure 3. FTIR results of various nano-fibers. PCL: Polycaprolactone; ECL: Ethyl cellulose; NAT: Natamycin, and GAL: Gallic acid.

emergence of novel peaks at 3,380, 3,220, and 3,114 cm^{-1} , while the peak at 2804 cm^{-1} shifted to lower wavelengths. Veras et al. (2020) reported that mixing NAT with PCL did not cause significant changes in the nano-fiber spectra, likely due to the absence of chemical bonding between the polymers and antifungal compounds (Veras, et al., 2020).

Mechanical Properties

The mechanical properties of fibers containing NAT and GAL are presented in Table 2. For ECL70/PCL30 nano-fibers, thickness, tensile strength, and elongation at break were measured as 0.112 ± 0.007 mm, 8.22 ± 0.4 MPa, and $4.49 \pm 0.3\%$, respectively. ECL70/PCL30 exhibited superior mechanical properties compared to ECL90/PCL10 and ECL80/PCL20. The increase in tensile strength is attributed to

the formation of new interactions between the polymers (Liu, *et al.*, 2018), a phenomenon also supported by the FTIR results. Additionally, the incorporation of NAT and GAL further enhanced the mechanical properties of the nano-fibers.

Water Contact Angle (WCA)

WCA analysis was conducted to assess the surface wettability of the fabricated nano-fibers, with the results presented in Figure 4. The WCA values for ECL90/PCL10, ECL80/PCL20, and ECL70/PCL30 were measured as 49.03° , 54.84° , and 59.77° , respectively. An increase in PCL content corresponded with a higher WCA, indicating enhanced hydrophobicity. This behavior may be attributed to hydrogen bonding interactions between ECL and PCL polymers (Beikzadeh, *et al.*, 2021). Additionally, the incorporation of NAT and

Table 2. Mechanical properties of nano-fibers.

Sample	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
ECL90/PCL10	0.108 ± 0.005^b	5.93 ± 0.6^d	2.66 ± 0.2^c
ECL80/PCL20	0.109 ± 0.001^b	6.78 ± 0.4^c	3.12 ± 0.4^c
ECL70/PCL30	0.112 ± 0.007^b	8.22 ± 0.4^b	4.49 ± 0.3^b
ECL70/PCL30/NAT/GAL	0.124 ± 0.003^a	9.12 ± 0.5^a	6.32 ± 0.4^a

^{a-d} Mean $\pm$ Standard deviation. Values with different lowercase letters are significantly different at the level ($P < 0.05$) between the treatments.

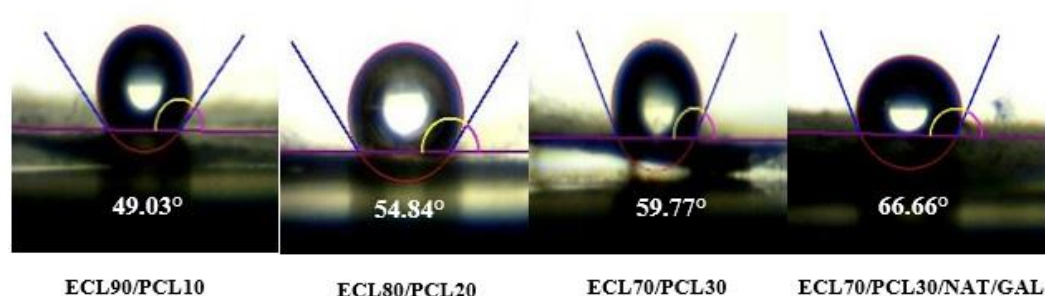


Figure 4. Water contact angles of the formed nano-fibers.

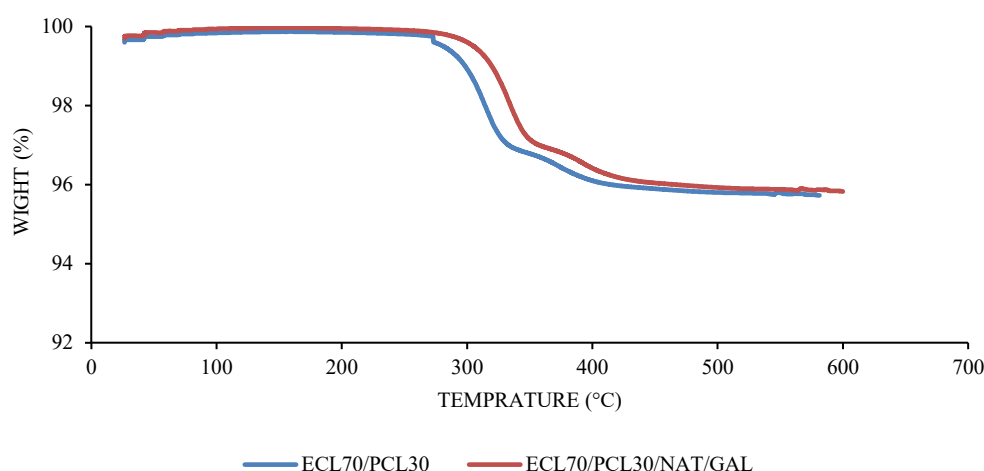


Figure 5. TGA curves of ECL70/PCL30 and ECL70/PCL30/NAT/GAL nanofibers.

GAL into the ECL70/PCL30 nano-fibers further increased the WCA values. Shen *et al.* (2021) reported that increasing the weight of encapsulated natamycin led to a rise in the WCA of gelatin/zein/polyurethane nano-fibers, attributed to the hydrophobic nature of NAT (Shen, *et al.*, 2021).

Thermogravimetric Analysis (TGA)

The TGA results of the nano-fibers containing natamycin and gallic acid are presented in Figure 5. The decomposition temperature of pure ECL was approximately 310.56°C, whereas the ECL/PCL and ECL/PCL/NAT/GAL nano-fibers exhibited decomposition temperatures around 315 and 325.3°C, respectively. These results indicate that the thermal stability of the nano-fibers

improved with the incorporation of NAT and GAL into the ECL matrix. Additionally, the observed shift in thermal decomposition profiles between the neat ECL/PCL blend and the NAT/GAL-loaded nano-fibers confirms the successful incorporation of the active compounds. Similarly, Neo *et al.* (2013) reported that blending GAL with zein enhanced the decomposition temperature of the resulting nano-fibers (Neo, *et al.*, 2013).

Antioxidant Activity Measurements

The antioxidant activity of the nano-fibers in 80% ethanol aqueous solutions is presented in Figure 6. GAL reduces free radicals by acting as a hydrogen or electron donor (Chuysinuan, *et al.*, 2009). ECL70/PCL30 electrospun nano-fibers

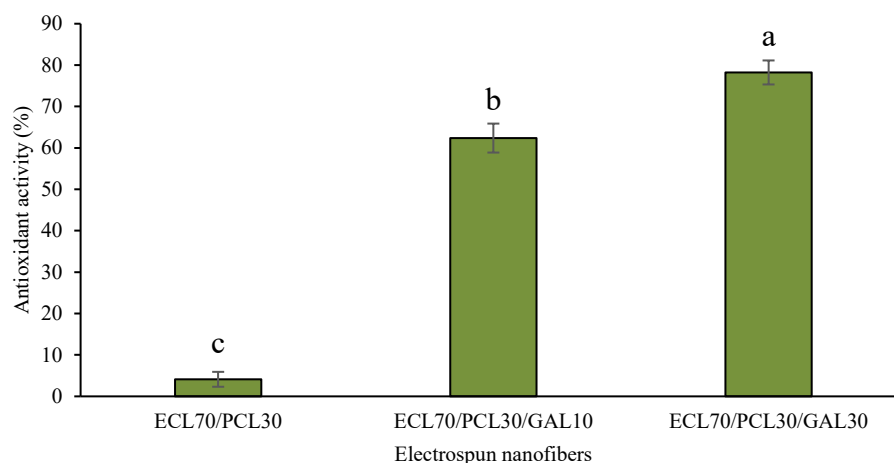


Figure 6. Antioxidant properties of electrospun nano-fibers.

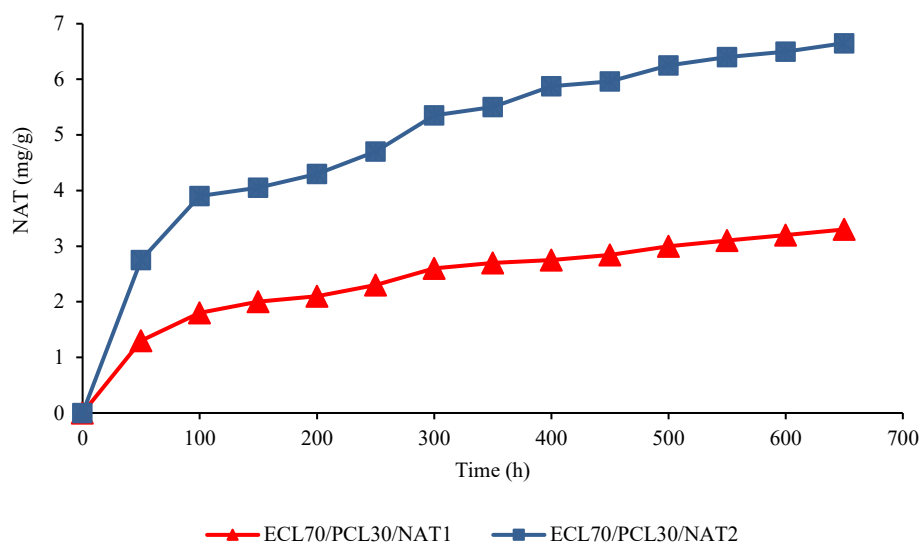
incorporated with 10% and 30% GAL exhibited antioxidant activities of approximately $62.38 \pm 3.5\%$ and $78.23 \pm 2.9\%$, respectively. In contrast, the neat ECL70/PCL30 nano-fiber showed only $4.12 \pm 1.8\%$ antioxidant activity. Neo *et al.* (2013) reported antioxidant properties ranging from 58% to 89% for zein nano-fibers containing GAL.

Release Kinetics of NAT and GAL

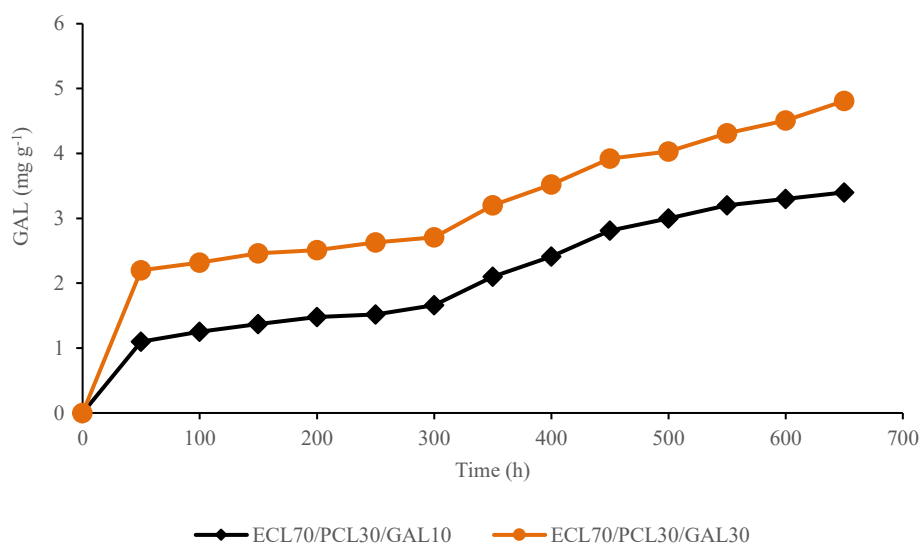
Figure 7(a) illustrates the release profiles of Natamycin (NAT) from the ECL70/PCL30/NAT1 and ECL70/PCL30/NAT2 nano-fibers. The NAT2 formulation demonstrated a higher cumulative release of approximately 6.7 mg g^{-1} , compared to around 3.3 mg g^{-1} for NAT1, over a period of nearly 700 hours. Both samples exhibited an initial burst release within the first 100 hours, which is characteristic of electrospun nano-fibers and is likely caused by the rapid desorption of NAT molecules located near the fiber surface (Sill and Von Recum, 2008). Following this phase, the release continued at a slower, sustained rate, consistent with diffusion-controlled mechanisms commonly observed in polymeric nano-fiber systems (Li and Xia, 2004). The differences in release profiles may be attributed to

variations in fiber morphology, polymer crystallinity, and drug-polymer interactions influencing the diffusion rate of NAT. The U.S. Food and Drug Administration (FDA) regulation No. 155-172 (200) authorizes the application of NAT on cheese surfaces, with the stipulation that its concentration in the final product does not exceed 20 ppm (Türe, *et al.*, 2008).

Also, the release profiles of GAL from ECL 70/PCL 30/GAL10 and ECL 70/PCL 30/GAL30 are shown in Figure 7(b). Both samples exhibited an initial burst release within the first 100 hours, followed by a sustained and gradual release over the subsequent period (up to 700 hours). The GAL30 formulation released a significantly higher amount of GAL ($\sim 5.2 \text{ mg g}^{-1}$) compared to GAL10 ($\sim 3.6 \text{ mg g}^{-1}$), indicating that increasing the GAL loading enhances cumulative release. The burst release phase is commonly attributed to the presence of GAL near the fiber surface, allowing for rapid diffusion into the release medium (Sill and Von Recum, 2008). After this stage, the release rate decreased, suggesting that the migration of GAL molecules through the polymer matrix was primarily governed by diffusion (Kenawy, *et al.*, 2002). Variations between the two formulations might result from modifications in the nano-fiber structure due to elevated GAL content. A higher



(a)



(b)

Figure 7. (a) Cumulative NAT release from ECL/PCL/NAT, and (b) Cumulative GAL release from ECL/PCL/GAL.

concentration of GAL can impact the fiber's porosity, hydrophilic properties, and degree of crystallinity, which collectively affect the release behavior (Kenawy, *et al.*, 2002). Additionally, the small molecular size and hydrophilic characteristics of GAL facilitate its prolonged release from the hydrophobic PCL/ECL mixture, as this blend restricts water penetration and slows down drug diffusion (Bhardwaj and Kundu, 2010). These controlled and sustained release characteristics are beneficial for antifungal drug delivery, as they help maintain the

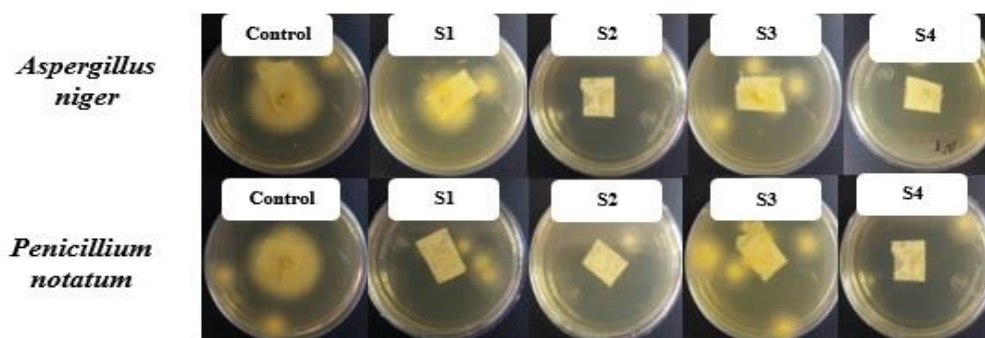
prolonged bioactivity of both NAT and GAL (Agarwal, *et al.*, 2008).

Antifungal Properties

The results of the antifungal activity of nano-fibers containing NAT and GAL are shown in Table 3 and Figure 8. Control samples (ECL70/PCL 30) and samples containing 1% NAT and 10% or 30% GAL did not exhibit antifungal activity against the growth of *Aspergillus niger* and *Penicillium*

Table 3. The colony diameter of *Aspergillus niger* and *Penicillium notatum*.

Nano-fiber samples	Colony diameter (mm)	
	<i>Aspergillus niger</i>	<i>Penicillium notatum</i>
ECL70/PCL30	38 ± 1.4	36 ± 1.6
ECL70/PCL30/NAT1/GAL10	32 ± 0.9	25 ± 1.1
ECL70/PCL30/NAT2/GAL10	0	0
ECL70/PCL30/NAT1/GAL30	18 ± 0.7	12 ± 1.3
ECL70/PCL30/NAT2/GAL30	0	0


Figure 8. Antifungal properties of nanofibers: (ECL70/PCL30 (control), ECL70/PCL30/NAT1/GAL10 (S1), ECL70/PCL30/NAT2/GAL10 (S2), ECL70/PCL30/NAT1/GAL30 (S3), and ECL70/PCL30/NAT2/GAL30 (S4) nanofibers.

notatum. However, nanofibers containing 2% NAT inhibited fungal growth. ECL/PCL/NAT/GAL nano-fibers also showed lower antifungal activity against *Aspergillus niger* compared to *Penicillium notatum*. Overall, the results indicate that the antifungal properties of the nano-fibers improve with increasing amounts of NAT. Additionally, adding 2% NAT combined with 10 or 30% GAL completely prevented the growth of both molds. Veras *et al.* (2020) reported that the inhibition zones of PCL nano-fibers containing 1 to 4% NAT ranged from approximately 6.6 to 24 mm against *Aspergillus flavus* and *Penicillium citrinum*. With increasing amounts of NAT, the antifungal properties of nano-fibers increased (Veras, *et al.*, 2016).

Mold and Yeast Analysis of Cheeses Packaged by Nano-fibers

Changes in the amount of mold and yeast in cheeses packaged with nano-fibers are shown in Table 4. In all treatments, the

levels of mold and yeast increased over time, with the control sample (cheese packed with nano-fibers without NAT and GAL) showing a higher increase compared to the other samples.

Fajardo *et al.* (2010) packaged semi-hard cheese (Saloio cheese) with chitosan and chitosan coatings containing natamycin for 37 days at 4°C. Microbial analysis of the cheese samples showed that the amount of mold and yeast ranged from 4.53 to 6.06 Log (CFU g⁻¹). The shelf life of Saloio cheese is about 50 days, but after 37 days, fungal growth on the surface of the cheese ended the microbial analysis (Fajardo, *et al.*, 2010). These findings are consistent with our results. Also, Jalilzadeh *et al.* (2020) used whey protein containing NAT to package ultra-refined cheese, and the results showed that the amount of mold and yeast in the control samples was higher than in the coated samples after 28 days (Jalilzadeh, *et al.*, 2020). The lower levels of mold in cheeses packaged with nano-fibers containing NAT and GAL, compared to the control sample, demonstrate the stability of

Table 4. Mold and yeast levels Log (CFU g⁻¹) of cheeses packaged with nano-fibers.

Packaging type	Storage period (Days)			
	1	14	28	56
Without packaging	5.16 ± 0.11 ^{aC}	6.29 ± 0.43 ^{aB}	7.55 ± 0.54 ^{aA}	-
ECL70/PCL30	5.09 ± 0.26 ^{aC}	5.33 ± 0.47 ^{bBC}	5.98 ± 0.29 ^{aAB}	6.28 ± 0.61 ^{aA}
ECL70/PCL30/NAT2/GAL10	5.12 ± 0.09 ^{aC}	5.28 ± 0.16 ^{bBC}	5.78 ± 0.24 ^{aAB}	6.03 ± 0.27 ^{aA}
ECL70/PCL30/NAT2/GAL30	5.04 ± 0.17 ^{aB}	5.15 ± 0.46 ^{bB}	5.69 ± 0.15 ^{aA}	5.97 ± 0.35 ^{aA}

^{a-b} and ^{A-C} Mean±Standard deviation. Values with different lowercase letters are significantly different according to Duncans test at the level (P< 0.05) in each day between the treatments and with different capital letters on the maintenance days.

Table 5. Sensory evaluation score of cheeses packaged with nanofibers.

Overall acceptance	Storage time (Days)		
	1	14	28
Without packaging	4.0 ± 0.18 ^{aA}	3.8 ± 0.35 ^{aA}	3.6 ± 0.16 ^{bA}
ECL70/PCL30	3.8 ± 0.14 ^{aA}	4.0 ± 0.15 ^{aA}	4.1 ± 0.37 ^{abA}
ECL70/PCL30/NAT2/GAL10	4.2 ± 0.17 ^{aA}	3.9 ± 0.39 ^{aA}	4.2 ± 0.32 ^{aA}
ECL70/PCL30/NAT2/GAL30	3.9 ± 0.36 ^{aA}	4.0 ± 0.10 ^{aA}	4.0 ± 0.29 ^{abA}

^{a-b} and ^A Mean±Standard deviation. Values with different lowercase letters are significantly different according to Duncans test at the level (P< 0.05) in each day between the treatments and with capital letter on the maintenance days.

these active compounds during storage and their antimicrobial effects.

Overall Acceptance of Cheeses Packaged by Nano-fibers

Overall acceptance of cheese samples packed with nanofibers during refrigerated storage is shown in Table 5. The results of the sensory evaluation scores for the packaged cheese samples did not show a significant difference between samples packaged with NAT-free nano-fibers, nano-fibers containing 2% NAT and 10% GAL, and nano-fibers containing 2% NAT and 30% GAL. The control sample (without packaging) had a lower score in terms of overall acceptance. Nottagh *et al.* (2020) used chitosan containing natamycin to package ultra-refined cheese for 6 weeks. The highest scores for taste, smell, and overall acceptance were observed in the packaged samples at the third week compared to the control sample (without packaging) (Nottagh, *et al.*, 2020).

CONCLUSIONS

This study investigated the antioxidant, antifungal, morphological, and thermal properties of ECL/PCL nano-fibers containing NAT and GAL. The results revealed that ECL70/PCL30 exhibited the desired morphological and mechanical properties compared to ECL90/PCL10 and ECL80/PCL20. Additionally, mixing NAT with GAL increased the average fiber diameter, mechanical characteristics, Water Contact Angle (WCA), and thermal stability. Electrospun ECL70/PCL30 nano-fibers containing 30% GAL had the highest antioxidant content. Furthermore, ECL70/PCL30/NAT2/GAL10 and ECL70/PCL30/NAT2/GAL30 nano-fibers completely prevented the growth of *Aspergillus niger* and *Penicillium notatum*.

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Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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بررسی ویژگیهای فیزیکی، مکانیکی و ضد قارچی نانوالیاف الکتروریسی شده اتیل سلولز/پلی کاپرولاکتون حاوی گالیک اسید و ناتامایسین برای بسته‌بندی پنیر

سمیرا بیگ زاده، علی احسانی، امیر محمد مرتضویان، ژیان شیخی، مرجان قربانی، و صغری رمضان

چکیده

الکتروریسی فرآیندی برای تولید نانوالیاف است که می‌تواند برای بسته‌بندی فعال مواد غذایی به کار رود. در این مطالعه، از پلیمرهای اتیل سلولز (ECL) و پلی‌کاپرولاکتون (PCL) با نسبت‌های وزنی ۷۰:۳۰، ۸۰:۲۰ و ۹۰:۱۰ درصد و ناتامایسین (NAT) (۱ و ۲ درصد وزنی/وزنی) و گالیک اسید (GAL) (۱۰ و ۳۰ درصد وزنی/وزنی) استفاده شد. نانوالیاف پس از فرآیند الکتروریسی (ولتاژ ۱۶ کیلوولت، سرعت ۱ میلی‌لیتر در ساعت، فاصله ۱۵ سانتی‌متر) تولید شدند. سپس، نانوالیاف از نظر ویژگیهای مورفولوژیکی، FTIR، حرارتی، مکانیکی، آنتی‌اکسیدانی، زاویه تماس آب و خواص ضد قارچی بررسی شدند. نتایج نشان داد که ECL70/PCL30 و ECL80/PCL20 دارای قطر متوسط $18 \pm$ نانومتر، $1174 \pm 21/4$ نانومتر، $754/73 \pm 23$ نانومتر بودند. PCL و ECL با

نسبت ۷۰ به ۳۰ انتخاب و با NAT و GAL ترکیب شدند. قطر متوسط نانوالیاف تولید شده بین ۷۷۹/۳۲ تا ۹۲۴/۶۳ نانومتر بود. همچنین با افزودن NAT و GAL، پایداری حرارتی، خواص مکانیکی و ضد قارچی ECL/PCL افزایش یافت. افزودن ۲ درصد NAT به همراه ۱۰ یا ۳۰ درصد GAL به طور کامل از رشد هر دو کپک جلوگیری کرد. نمونه‌های پنیر بسته‌بندی شده با نانوالیاف حاوی NAT و GAL در تمام تیمارها، افزایش کمتری در میزان کپک و مخمر در طول دوره نسبت به نمونه کنترل داشتند. همچنین، نتایج ارزیابی حسی تفاوت معنی‌داری بین نمونه‌های بسته‌بندی شده نشان نداد. بنابراین، نانوالیاف ECL/PCL ترکیب شده با ۲ درصد NAT و ۱۰ درصد GAL می‌توانند به عنوان جایگزینی مؤثر برای افزودن مستقیم NAT به فرمولاسیون پنیر مورد استفاده قرار گیرند.

Genetic Structure and Diversity of the Beet Armyworm *Spodoptera exigua* (Hübner) (Lepidoptera, Noctuidae) in Iran

Somayeh Ebrahimi¹, Mehdi Esfandiari^{1*}, and Lida Fekrat²

ABSTRACT

Spodoptera exigua is a significant agricultural pest in Iran. In this research, we analyzed a 700 base pair DNA fragment of *COI* to assess the genetic diversity of this harmful pest. In total, 54 specimens were sampled across six Iran's geographic areas, revealing six distinct haplotypes. The overall populations exhibited low genetic diversity ($h = 0.463 \pm 0.068$, $\pi = 0.00096 \pm 0.00017$). No distinct geographic pattern was observed in the haplotype distribution, as indicated by phylogenetic and median-joining network analyses. The median-joining network of Iranian haplotypes, combined with sequences from other global regions, demonstrated lack of geographical clustering, with Hap.1 as the most prevalent haplotype spanning Iran, Europe, Asia, and Australia, indicative of extensive gene flow and shared evolutionary history. The *Fst* values for the Iranian populations ranged from -0.12240 to 0.07189, revealing no significant differentiation among the populations. Analysis of molecular variance showed a larger proportion of the variation within rather than between them. After 1000 random permutations, the level of population differentiation was found to be non-significant. The combination of an unimodal mismatch distribution and the non-significant results of Tajima's *D* ($D = 0.02011$, $P > 0.05$) and Fu's *Fs* ($Fs = 3.33384$, $P > 0.05$) suggested the beet armyworm may have expanded recently (~10,000–50,000 years ago) but has returned to a state resembling neutrality without significant selection pressures acting on it. Insights from this population genetic study can aid in crafting targeted approaches for managing this extensively migratory pest.

Keywords: Agricultural pest, *COI*, Gene flow, Haplotype distribution, Population genetics.

INTRODUCTION

Spodoptera exigua (Hübner) is a polyphagous pest that feeds on over 130 plant species across more than 30 families (Robinson *et al.*, 2010). Originated from South Asia, *S. exigua* has a broad distribution throughout the tropical and temperate regions of Europe, Africa, North America, and Asia (Wei *et al.*, 2010). This pest's capacity for long-distance migration promotes the expansion of its populations beyond native ranges, resulting in broad dispersal (Fu *et al.*, 2017; Ma *et al.*, 2024). In Iran, this pest

causes substantial economic losses by damaging diverse host plants (Karimzadeh *et al.*, 2014). Larval feeding on leaves significantly reduces crop yields and can lead to plant death. In Iran's key beet-growing regions, encompassing approximately 160,000 hectares across 21 provinces, this pest poses a major threat to sugar beet production, especially during outbreaks. Effectively managing *S. exigua* is challenging due to the species' migratory nature and rapid insecticide resistance development (Hu *et al.*, 2021; Huang *et al.*, 2021).

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Genetic diversity is inevitable in ecological and evolutionary contexts, as it enhances individual fitness and ecosystem functionality. In pest management, elucidating the genetic composition and population structure of pest species is critical for developing effective and sustainable control strategies. Genetic studies reveal adaptive potential, dispersal patterns, and resistance mechanisms of pest populations, thereby enabling targeted interventions that reduce economic losses and environmental impact (Cao *et al.*, 2025; Peng *et al.*, 2025). Moreover, elucidating population structure informs the identification of distinct genetic units, which is fundamental for monitoring gene flow and predicting the spread of resistance alleles (Greenbaum *et al.*, 2016). Factors such as climate change, habitat variability, natural barriers, migration patterns, and anthropogenic pressures influence genetic variation and population structure, shaping evolutionary trajectories and pest success under environmental shifts (Crispo *et al.*, 2011; Martins *et al.*, 2018; Song *et al.*, 2023). Integrating genetic data into pest management frameworks enhances the precision and adaptability of control measures. DNA markers are widely recognized as primary tools for evaluating genetic diversity within and among individuals, species and populations, because of their extensive polymorphism (Behura, 2006). Various molecular markers have been employed to deduce the geographical distribution and evolutionary background of species (Urantowka *et al.*, 2017). Notably, the *Cytochrome Oxidase Subunit I* (COI) gene is particularly effective for phylogenetic analyses because of its moderate evolutionary rate and distinct evolutionary pathway (Hebert *et al.*, 2003; Wang *et al.*, 2014). Studying genetic diversity and population structure is key to gaining insights into dispersal behaviors and the causes of outbreaks in pest species. Despite the ecological and agricultural significance of *S. exigua*, genetic studies in Iran are scarce particularly using COI gene (Golikhajeh *et al.*, 2018).

This study addresses this gap by examining mtDNA COI variation across six Iranian *S. exigua* populations.

MATERIALS AND METHODS

Collection of Samples

The larvae of *S. exigua* were collected from sugar beet fields in six geographical regions of Iran (Table 1) between May and September of 2021-22. The larvae were reared under optimal conditions until adulthood (Zhang *et al.*, 2011; Jaba *et al.*, 2020). Once the insects matured, they were preserved in 96% ethanol and stored at -20°C until DNA extraction. Ten individuals from each region were chosen for this process (Endersby *et al.*, 2006; Anderson *et al.*, 2016).

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from ten randomly selected moths per population, using the Favorgen tissue genomic DNA extraction mini kit. The second and third legs of each specimen were ground in liquid nitrogen prior to extraction. The extracted DNA was stored at -20°C until PCR amplification of a COI mitochondrial gene fragment using primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). The PCR mixture contained 12.5 µL of PCR master mix (Amplicon), 0.5 µL from each primer set (10 pmol µL⁻¹), and 2.5 µL of template DNA. The PCR amplification was performed as follows: 94°C for 3 minutes; 35 cycles of 92°C for 30 seconds, 50°C for 40 seconds, and 72°C for 60 seconds; followed by a final extension at 72°C for 5 minutes. PCR products were assessed on a 1% agarose gel and subsequently sequenced by Pishgam Biotech Co.

Data Analysis

Iranian population sequences were manually modified using the BioEdit software (Hall, 1999). Bidirectional sequencing was utilized to enhance the precision of sequencing. The DNA Baser software package (version 5.15) was utilized to generate consensus sequences. The accuracy of the *COI* sequences was confirmed by verifying that they could be translated into proteins via MEGAX (Kumar *et al.*, 2018). The alignment of nucleotide sequences was carried out using the ClustalW program implemented in MEGA X. For each population, population diversity indices including haplotype (h) and Nucleotide (π) diversity as well as the Number of Haplotypes (H) Nei and Miller (1990) were computed using DnaSP v6 software presented by Rozas *et al.* (2017). The Median-Joining (MJ) network, as described by Bandelt *et al.* (1999), was generated utilizing the PopART software of Leigh and Bryant (2015) for Iranian sequences alone and in combination with additional sequences from GenBank, representing various countries (Table S₁). Genetic differentiation among populations was assessed using pairwise F_{st} (fixation index) values. To evaluate genetic variation between population groups from diverse regions (Table 1), an Analysis Of Molecular Variance (AMOVA) was conducted. Both F_{st} and AMOVA employed the Kimura 2-parameter model within the Arlequin software (Excoffier and Lischer, 2010). Visualization of AMOVA results was conducted using an integrated R script in Arlequin. The Mantel

test (Mantel, 1967) utilizing 10,000 permutations was applied to examine the correlations between genetic distance (F_{st} from Arlequin) and geographic distance (km) in IBD v1.52 (Bohonak, 2002). The estimation of the geographic distance between pairs of populations was done with the help of Google Maps Distance Calculator. Two neutrality tests were used to analyze the changes in demographic history for *S. exigua*, Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997), throughout the six geographic populations. Ultimately, Arlequin 3.0 computed the mismatch distributions between the observed and expected data (Appendix 2).

RESULTS

A 700 bp fragment of the mitochondrial *COI* gene was sequenced from 54 *S. exigua* individuals sampled across six geographic regions (Table 1). The sequences were submitted to GenBank (see Table S₁ for accession numbers) and revealed five variable sites, identifying six distinct haplotypes. The most prevalent Haplotypes (Hap.) across all populations were Hap.₁ and Hap.₂, found in 38 and 12 individuals, respectively (Appendix 1). Haplotypes Hap.₃, Hap.₄, Hap.₅ and Hap.₆ were unique, each occurring only in one population (Appendix 1). Table 2 presents the genetic diversity indices including haplotype diversity (h) and nucleotide diversity (π). The average values for h and π for all populations were 0.463 and 0.00096, respectively. Haplotype and nucleotide diversities are highest in Safiabad

Table 1. Information on the sampling locations of *Spodoptera exigua* in Iran.

Location	Code	Geographic region	Latitude/Longitude	Collection date
West Azerbaijan, Oshnavieh (Az)	Pop1	Northwest East	37°02'14.9"N/45°08'57.5"E	Aug./18/2022
Razavi Khorasan, Chenaran (Ch)	Pop2	East	36°43'27.1"N/58°59'55.2"E	Sept./07/2022
Khuzestan, Safiabad (D)	Pop3	Southwest	32°16'48.9"N/48°26'15.8"E	Jul./07/2022
North Khorasan, Garmeh (G)	Pop4	Northeast	37°21'23.8"N/56°19'44.2"E	Sept./07/2021
Markazi, Mahallat (M)	Pop5	Center	33°52'55.9"N/50°30'01.9"E	Jul./30/2022
Razavi Khorasan, Sabzevar (S)	Pop6	East	36°37'15.6"N/57°25'17.9"E	Jul./6/2022



(Pop3). This population exhibits significantly higher diversity than the others ($P > 0.05$) (Table 2), primarily because it contains divergent haplotypes (Appendix 1).

Median-Joining Network

A significant degree of gene flow in Iranian *S. exigua* may be inferred from the random distribution of mitochondrial DNA *COI* gene haplotypes. Furthermore, no clear geographical grouping of haplotypes was observed in the constructed median-joining network (Figure 1). The network's shape revealed that other haplotypes were separated from Hap.₁ or Hap.₂ by a single mutation. In addition to the haplotype network, phylogenetic analysis was utilized to investigate the links among haplotypes of the beet armyworm and to uncover any groupings associated with geographic

distribution. The phylogenetic tree's topology (Figure 2) provided further confirmation of the results from the haplotype network and no clear geographic distribution pattern was observed among the haplotypes.

Using one or two representative sequences of each haplotype from Iran, we combined these sequences with additional haplotypes sourced from GenBank representing various countries to construct a MJ network (Figure 3). The haplotype network revealed that Hap.₁ emerged as the most prevalent haplotype. Notably, sequences from the Iranian Hap.₁ were integrated within this Hap.₁ haplotype, along with sequences from Europe, Asia, and Australia. This widespread distribution of Hap.₁ suggests it may represent an ancestral or widely dispersed haplotype, potentially indicating historical migration patterns or shared evolutionary history among these geographically diverse populations. The widespread prevalence of

Table 2. Haplotype diversity (h) and nucleotide diversity (π) from six Iranian *Spodoptera exigua* populations.

Marker		H	Haplotype diversity (h)	Nucleotide diversity (π)
COI	Pop1 (Oshnavieh)	2	0.5000 ± 0.1283	0.001464 ± 0.001227
	Pop2 (Chenaran)	3	0.4167 ± 0.1907	0.000975 ± 0.000930
	Pop3 (Safiabad)	4	0.6444 ± 0.1518	0.002152 ± 0.001603
	Pop4 (Garmeh)	3	0.6071 ± 0.1640	0.001622 ± 0.001341
	Pop5 (Mahallat)	2	0.3556 ± 0.1591	0.001041 ± 0.000960
	Pop6 (Sabzevar)	2	0.4286 ± 0.1687	0.001255 ± 0.001120

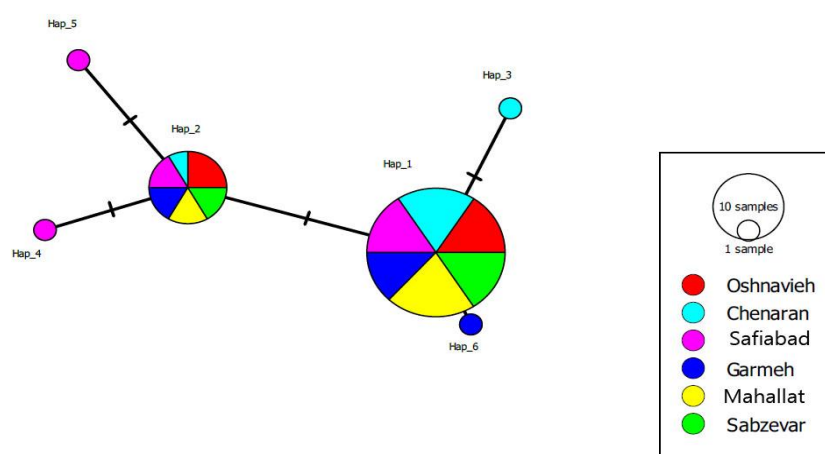


Figure 1. Haplotype network derived from partial mtDNA *COI* sequences of *Spodoptera exigua* from Iranian populations. Each circle represents a unique haplotype, identified by a number. The colors represent different populations, with the sizes of the slices and circles corresponding to the number of individuals carrying each haplotype.

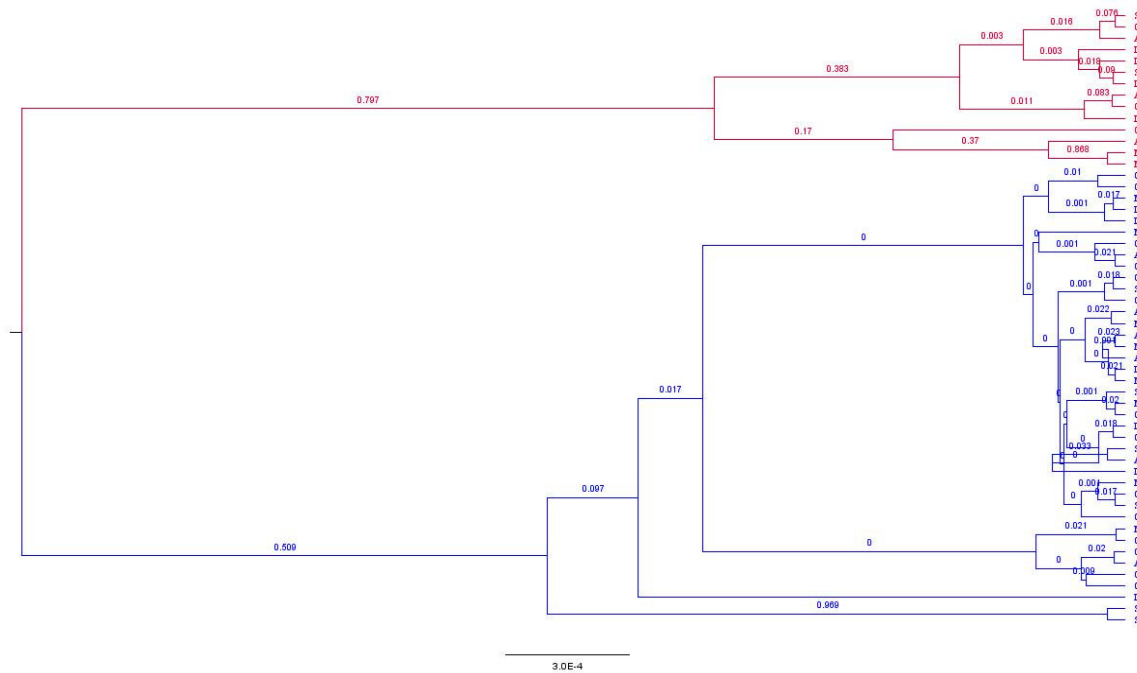


Figure 2. Phylogenetic tree based on Bayesian analysis. Numbers on nodes are posterior probabilities. Region codes are explained in Table 1.

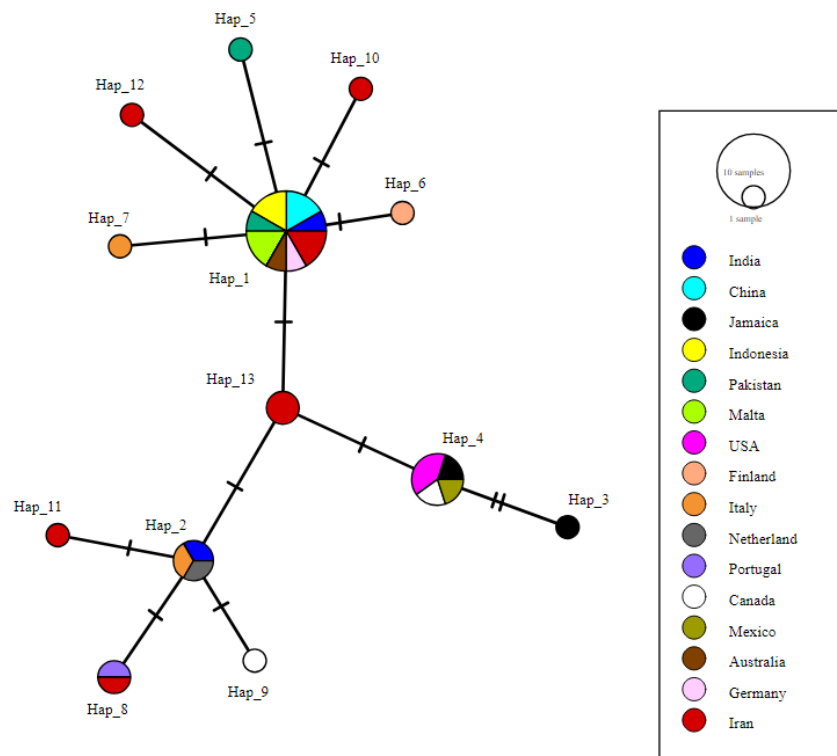


Figure 3. Haplotype network created using partial mtDNA *COI* sequences of *Spodoptera exigua*, encompassing both Iranian and global populations. Each circle represents a unique haplotype, identified by a number. The colors represent different populations, with the sizes of the slices and circles corresponding to the number of individuals carrying each haplotype.



origin and early emergence, enabling its persistence across diverse regions, as supported by Kang *et al.* (2023) and Ramesh *et al.* (2025). Furthermore, human-mediated factors, including agricultural practices and crop transport, have facilitated gene flow, promoting the dispersal of common haplotypes like Hap.1, consistent with patterns observed in highly mobile pest species (McCulloch and Waters, 2023). The second most frequent haplotype, Hap.4, was exclusively composed of sequences from North American countries. This geographical clustering of Hap.4 may indicate restricted gene flow between North American and other global populations, potentially due to geographical barriers or historical colonization events. Limited gene flow critically influences population structure by reducing migration-mediated genetic homogenization, thereby increasing differentiation among populations. Over time, such isolation can enhance the effects of evolutionary processes, such as genetic drift, which drives stochastic shifts in haplotype frequencies, and local adaptation and fosters genetic divergence in response to region-specific selective pressures. The network topology revealed a high proportion of unique haplotypes. Notably, most haplotypes were separated by only one mutation.

Genetic Distance and Genetic Diversification

Pairwise genetic distances between populations, studied via the Kimura 2-parameter technique, are summarized in Table 3, and average pairwise sequence variation among the populations was 0.1846% and varied from 0.108 to 0.279%.

The pairwise F_{st} values among the populations varied from -0.00794 to 0.07189. The analysis revealed that, on the whole, there was no notable differentiation among the populations (Table 3, Appendix 2).

Analysis of Population Genetic Structure

Grouping populations based on geographic regions through AMOVA revealed no notable differences between the groups (Table 4). No significant association was detected between genetic distances (pairwise F_{st}) and geographic distances in the Mantel's test for *COI* ($r^2=0.0017$, $P=0.519000$). This non-significant P-value indicates that geographic distance does not explain genetic differentiation among populations at the *COI* locus, thus supporting the null hypothesis of no population structure related to spatial separation. A unimodal mismatch graph was observed in the mismatch distribution analysis (Figure 4), potentially pointing to a recent population expansion. Nevertheless, our finding does not align with the mitochondrial DNA genealogy, which did not exhibit a star-like pattern. Additionally, neither the Sum-of-Squares Deviation ($SSD=0.26357$, $P=0.18486$) nor Harpending's raggedness index (0.55378, $P=0.50367$) showed significance (Figure 4), failing to indicate a population expansion. A positive SSD combined with a non-significant raggedness index usually implies that, while there is some deviation from equilibrium (indicated by SSD), these deviations are not strong enough to indicate significant demographic changes. This often points to a scenario where the population has expanded recently without severe historical bottlenecks. The findings imply that the population structure has remained stable over time. The evidence against population expansion was reinforced by Tajima's D (0.02011, $P=0.56038 > 0.1$) and Fu's ($F_s=3.33384$, $P=0.92865 > 0.1$), both of which were positive and statistically non-significant.

DISCUSSION

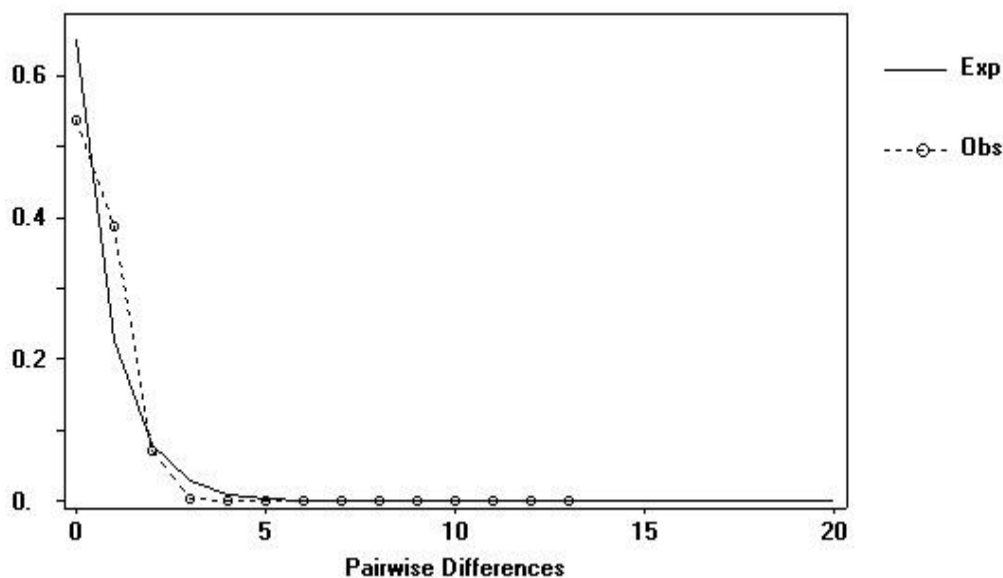
Genetic variability underpins evolutionary processes across all organisms. Assessing intraspecific genetic variation enables the

Table 3. *F_{st}* values for pairwise comparisons between six *Spodoptera exigua* populations in Iran.

Marker		Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
COI	Pop1	0.00000					
	Pop2	0.02175	0.00000				
	Pop3	-0.08972	0.07189	0.00000			
	Pop4	-0.09878	-0.04368	-0.05313	0.00000		
	Pop5	-0.06790	-0.06930	-0.00794	-0.09787	0.00000	
	Pop6	-0.11504	-0.05338	-0.06173	-0.12240	-0.11863	0.00000

Table 4. Results of the AMOVA analysis of six Iranian *Spodoptera exigua* populations, arranged by geographic areas, $P < 0.05$.

Marker	Source of variation	d.f.	SSD	Variance components	Percentage of variation	F-statistic
COI	Among groups	3	0.954	0.01354	2.90	0.02905
	Among populations within groups	2	0.380	-0.03591	-7.70	-0.07932
	Within populations	48	23.457	0.48869	104.80	-0.04797
	Total	53	24.791	0.46632		

**Figure 4.** Mismatch distribution of the 54 *COI* sequences of *Spodoptera exigua* for Iran.

identification of potential novel species and elucidates the evolutionary histories of extant populations (McEntire *et al.*, 2021). Our analysis of *S. exigua* populations from the major sugar beet cultivation regions in Iran revealed low genetic diversity, as evidenced by limited haplotype and nucleotide diversity, with only five polymorphic sites

and six haplotypes detected. This finding aligns with previously reported restricted genetic variation in *S. exigua* (Wang *et al.*, 2020). The low genetic variation observed in *S. exigua* populations in Iran may stem from a relatively recent population expansion, limiting the accumulation of genetic diversity. Additionally, the limited sample



size may influence these results. The observed low genetic variance across different geographic populations suggests substantial gene flow and high genetic connectivity, supporting the prevailing hypothesis that the flight capacity of *S. exigua* is adequate for dispersal. The current study's findings confirmed this assertion, indicating that Iranian haplotypes lack well-defined geographic distribution patterns. The presence of shared haplotypes across various regions of Iran implies that recent gene flow between these populations has blurred the original relationships among them. This low level of genetic differentiation is a hallmark of frequent migration and dynamic population turnover, typical of highly mobile pests like *S. exigua*. Such extensive movement not only facilitates the rapid spread of common haplotypes but also prevents the formation of distinct genetic clusters, thereby maintaining genetic homogeneity across wide geographic areas. Observing four unique haplotypes in pop₂, pop₃, and pop₄ might be a consequence of the small sample size. Similarly, the global haplotype network (Figure 3) revealed a lack of clear geographical structuring among haplotypes from Asian, Australian and European populations, suggesting a high degree of genetic admixture or historical gene flow among these regions. The widespread presence of a shared haplotype across continents underscores the species' dispersal capacity. The integration of Iranian Hap.₁ sequences within the global Hap.₁ haplotype suggests significant genetic connectivity between Iranian *S. exigua* populations and those from other continents. This pattern aligns with the observations of limited phylogeographic structure in *S. exigua*, as seen in other studies where haplotypes showed random distribution across large geographic areas (Wang *et al.*, 2020). The observed geographical clustering of North American haplotypes suggests a potential regional differentiation or a founder effect suggesting limited gene flow between North American and other global populations. However, the close genetic proximity,

differing by only a single mutational step from haplotypes elsewhere, implies a complex evolutionary scenario involving recent divergence, ongoing gene flow, or a mix of these two processes. The single-mutation difference could indicate that the differentiation of North American populations is a relatively recent phenomenon, or that there is still some level of genetic exchange occurring, albeit at a reduced rate. This pattern underscores the importance of considering both geographical distribution and genetic distance in interpreting population structure. The haplotype network analysis reveals two key features: most haplotypes are separated by only one mutation, and there is a high proportion of unique haplotypes. The findings shed light on the population structure and evolutionary history of *S. exigua*, suggesting recent divergence or limited genetic drift among lineages. Such shallow genetic differentiation could be attributed to high gene flow between populations. Such a pattern is often observed in species with high dispersal capabilities. The abundance of unique haplotypes may be an artifact of low sample numbers. In cases of limited sampling, rare haplotypes are more likely to appear unique simply because their counterparts in the population have not been sampled.

The detection of two distinct clusters in the Bayesian phylogenetic tree, despite the absence of clear separation among the six geographic populations of *S. exigua* and the presence of mixed population membership within each cluster, suggests that gene flow among populations is high and geographic barriers to dispersal are weak or absent. This pattern is further corroborated by the random distribution of mitochondrial *COI* haplotypes and the lack of geographic structure in the haplotype network, where most haplotypes are separated by only a single mutation. Such findings are consistent with studies in other insect species, where extensive gene flow and recent demographic expansion can obscure phylogeographic structure, leading to the formation of clusters in phylogenetic trees

that do not correspond to discrete geographic groupings (Kim and Sappington, 2013).

Generally, species that can actively disperse usually show minimal genetic variation across populations, and this is likely the case for migratory noctuid pests, including *S. exigua*. In this research, there were no evident and statistically significant genetic distances, and the pairwise sequence differences between the populations were minor. Furthermore, the analysis revealed no significant differentiation in pairwise F_{st} values among the populations. This result supports the earlier findings of several research, which provide strong evidence of low genetic divergence among the majority of individuals in this species (Wang *et al.*, 2014; 2020). The low genetic differentiation and minimal pairwise sequence differences observed in our study of *S. exigua* populations align closely with findings reported for this species on other host crops. Investigations on *S. exigua* from vegetable crops such as tomato, cotton, and pepper have similarly documented low genetic diversity and weak population structuring (Lee, 2017; Wang *et al.*, 2020).

A detailed comprehension of the genetic structure of the pest populations can reveal important biological information that is essential for their effective management. This knowledge improves our understanding of their occurrence patterns, migration trends across different areas, and the geographic differences within these populations (Zhang *et al.*, 2018). Since there was no apparent habitat loss or fragmented distribution of the hosts for this pest species in the studied regions of Iran, *S. exigua* is likely to exhibit little genetic differentiation throughout the sugar-beet areas. A non-significant result indicates that, while the observed Tajima's D is positive, it does not differ significantly from what would be anticipated according to a neutral model. This suggests that demographic factors, such as population size changes or expansions, may not have dramatically influenced genetic diversity in the studied populations. A positive non-significant Fu's F_s value indicates genetic

stability and neutrality within the population, while also suggesting that there is insufficient evidence to conclude recent demographic changes or selective pressures affecting genetic diversity.

The combination of an unimodal mismatch distribution and non-significant positive values for Tajima's D and Fu's F_s indicates a population that most likely underwent historical expansion in size, but is currently stable, with no significant evidence of ongoing evolutionary pressures affecting its genetic structure. Previous studies on *S. exigua* populations across Asia and neighboring regions have reported similarly low genetic differentiation and high gene flow, consistent with our findings. Wang *et al.* (2024) demonstrated that extensive migratory behavior and continuous host availability contribute to weak population structuring in *S. exigua* populations across China. This aligns with our observation of non-significant F_{st} values and lack of distinct haplotype clustering within Iranian populations, suggesting extensive gene flow and a shared evolutionary history. Moreover, a study focusing on populations in Pakistan and adjacent areas (Wang *et al.*, 2022) similarly reported low genetic differentiation in *S. exigua*, reinforcing the hypothesis that populations inhabiting contiguous agro-ecosystems and lacking significant geographic or ecological barriers tend to maintain genetic homogeneity. Although geographic distance can drive genetic divergence, its influence is often attenuated by the species' high dispersal capacity and the continuous availability of host plants, as reported by Wang *et al.* (2020). The limited sample size and geographic coverage may restrict the robustness of our conclusions. Consequently, there is a possibility that fine-scale genetic structure, or recent demographic changes, have not been fully captured in this study. Therefore, further sampling across broader spatial and temporal scales is required to fully elucidate the evolutionary dynamics and genetic structure of *S. exigua* populations in Iran.



In conclusion, while chemical treatment remains the primary method for controlling *S. exigua*, its genetic flexibility, high reproductive rate, and particularly strong selection pressure drive rapid insecticide resistance (Huang *et al.*, 2021). Significant gene flow and long-distance migration facilitate the spread and accumulation of resistance traits across populations (Bouvier *et al.*, 2001). Consequently, elucidating the mechanisms of resistance, population genetic structure, and gene flow is imperative to advance resistance monitoring and to inform the design of sustainable, effective pest management strategies.

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ساختار و تنوع ژنتیکی کرم برگخوار چغندرقد (*Spodoptera exigua* (Hübner) (Lepidoptera, Noctuidae) در ایران

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چکیده

کرم برگخوار چغندرقد (*Spodoptera exigua* (Hübner) یک آفت کشاورزی مهم در ایران است. در این تحقیق، یک قطعه ۷۰۰ جفت بازی DNA از زیر واحد I سیتوکروم اکسیداز (COI) برای ارزیابی تنوع ژنتیکی این آفت خسارتزا تجزیه و تحلیل شد. در مجموع، ۵۴ نمونه در شش منطقه جغرافیایی ایران نمونه‌برداری شد که شش هاپلوتایپ متمایز را نشان داد. جمعیت‌های کلی تنوع ژنتیکی پایینی را نشان دادند ($h = 0.463 \pm 0.068$)، ($\pi = 0.00096$)، (± 0.00017). همانطور که توسط تجزیه و تحلیل‌های فیلوژنتیکی و شبکه Median-joining نشان داده شده است، هیچ الگوی جغرافیایی مشخصی در توزیع هاپلوتایپ‌ها مشاهده نشد. شبکه Median-joining هاپلوتایپ‌های ایرانی، همراه با توالی‌هایی از سایر مناطق جهان، عدم خوشه‌بندی جغرافیایی را نشان داد، به طوری که Hap.1 به عنوان شایع‌ترین هاپلوتایپ در ایران، اروپا، آسیا و استرالیا قرار داشت که نشان‌دهنده جریان ژنی گسترده و تاریخ تکاملی مشترک است. مقادیر F_{st} برای جمعیت‌های ایرانی از -0.12240 تا 0.07189 متغیر بود که نشان‌دهنده عدم تمایز معنی‌دار بین جمعیت‌ها بود. تجزیه و تحلیل واریانس مولکولی نشان داد که تغییرات درون جمعیت‌ها نسبت به بین جمعیت‌ها بیشتر است، پس از ۱۰۰۰ جایگشت تصادفی، سطح تمایز جمعیت‌ها غیر معنی‌دار بود. ترکیب توزیع عدم تطابق تکوجهی و نتایج غیر معنی‌دار D تاجیما ($D = 0.02011$, $P > 0.05$) و ($F_s = 3/33384$, $P > 0.05$) F_u 's نشان داد که کرم برگخوار چغندر *S. exigua* ممکن است اخیراً گسترش یافته باشد، اما بدون اعمال فشارهای انتخابی قابل توجه بر آن، به حالتی شبیه به خنثی بودن بازگشته است. بینش‌های حاصل از این مطالعه ژنتیکی جمعیت می‌تواند به ایجاد رویکردهای هدفمند برای مدیریت این آفت با توانایی مهاجرت گسترده کمک کند.

Grafting Hybrid and Monoecious Cucumbers onto Cucurbit Rootstocks Enhances Growth and Yield under Net House Conditions

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ABSTRACT

This study evaluated suitable cucurbitaceous rootstocks for improving cucumber yield and quality under net house conditions. Two cucumber cultivars, namely, Green Long (GL an OPV: Open Pollinated Variety) and Namdhari Seeds (NS) 408 (a F1 hybrid), were grafted onto five rootstocks: *Cucurbita ficifolia*, *Cucurbita moschata*, *Cucurbita maxima*, *Lagenaria siceraria*, and *Luffa cylindrica*. Results showed that NS 408 outperformed GL in both grafted and non-grafted conditions. Among the rootstocks, NS 408 grafted onto *C. maxima* (winter squash) had the highest graft survival (79±1.8%), most extended vine length (700±16 cm), and maximum fruit yield (8.3±0.02 kg per plant, 118.4% increase over non-grafted NS 408). Grafting GL onto winter squash improved yield by 42.11% over non-grafted NS 408 and by 86.21% over GL control. Fruit quality parameters such as total soluble solids, palatability, ascorbic acid, and soluble protein were unaffected or improved, confirming that grafting did not negatively influence market quality. Economic analysis revealed the highest benefit-cost ratio (> 2.5) in NS 408/Winter Squash (WS), indicating that grafting is a cost-effective alternative to expensive hybrid seeds. These findings highlight the potential of grafting to optimize cucumber production, reduce reliance on pesticides, and improve profitability in protected cultivation.

Keywords: Early flowering, Fruit quality, Fruit yield and rootstock.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an economically important vegetable crop cultivated globally for fresh consumption and processing. However, its production faces challenges from biotic and abiotic stresses, including soil-borne diseases like Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*) and root-knot nematodes (*Meloidogyne incognita*), as well as drought

and salinity (Patil *et al.*, 2017; Bayoumi *et al.*, 2021). These factors reduce yields and increase production costs, particularly for farmers using hybrid seeds, which are costlier than open-pollinated varieties.

Grafting has emerged as an effective horticultural technique to enhance resilience by combining the disease resistance and stress tolerance of rootstocks with the desirable traits of scions (Davis *et al.*, 2008a; Guan *et al.*, 2012). In cucumbers,

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grafting effectively mitigates biotic and abiotic stresses while improving yield under challenging conditions (Sarwar *et al.*, 2019a, b; Davis *et al.*, 2008b).

First introduced in Japan in the 1960s to combat Fusarium wilt and improve cold tolerance, cucumber grafting is now widely practiced in cucurbit crops to enhance resistance to soil-borne pathogens and increase plant vigour (Davis *et al.*, 2008a). The success of grafting largely depends on the compatibility between the rootstock and scion. Rootstocks from cucurbit species such as *Cucurbita ficifolia*, *Cucurbita moschata*, and *Cucurbita maxima* are commonly used due to their strong root systems, improved water and nutrient uptake, and ability to confer resistance to soil-borne diseases (Heidari *et al.*, 2012; Rouphael *et al.*, 2012; Elsheery *et al.*, 2020; Shehata *et al.*, 2022). Grafting also enhances stress tolerance and crop performance under suboptimal growing conditions, leading to improved yields (Gaion *et al.*, 2018; Sallaku *et al.*, 2019; Bayoumi *et al.*, 2021).

In addition to improving plant vigour and stress tolerance, grafting offers economic benefits by reducing reliance on chemical pesticides. Enhanced natural resistance in grafted plants lowers pesticide use, cutting production costs and minimizing environmental impact, while promoting residue-free produce (Guan *et al.*, 2012; Liu *et al.*, 2015). Beyond yield improvements, grafting enhances fruit quality (Davis *et al.*, 2008b; Izaba *et al.*, 2021; Sakthivel *et al.*, 2024). In high-tunnel systems, grafted cucumbers yield higher net returns and better benefit-cost ratios than non-grafted crops (Izaba *et al.*, 2021). Its adoption is increasing in greenhouse and net house systems due to its role in sustainable, low-input farming.

Despite its advantages, research on the performance of monoecious cucumber cultivars grafted onto different *Cucurbita* rootstocks under net house conditions remains limited, particularly in the Indian subcontinent. This study addresses this gap by evaluating two cucumber cultivars—

Green Long (monoecious) and NS 408 (hybrid)—grafted onto five cucurbit rootstocks. The study aims to assess grafting effects on plant growth, yield, and fruit quality, focusing on (1) Rootstock influence on plant vigour and graft survival and (2) The impact of grafting on yield and fruit traits. These insights will contribute to optimizing cucumber production, reducing reliance on costly hybrid seeds and pesticides, and improving sustainability in net house cultivation systems.

MATERIALS AND METHODS

Experimental Site and Planting Materials

The present investigation was conducted at the College Orchard, Department of Vegetable Science, Tamil Nadu Agricultural University (TNAU), Coimbatore, India (11° N latitude, 76.9° E longitude, and 424 meters above mean sea level) from March to June 2022. Two monoecious cucumber cultivars and five cucurbit rootstocks were evaluated (Table 1). Green Long (GL) is a monoecious, open-pollinated variety (ECL Agrotech Limited, Bengaluru) with medium vine growth and uniform cylindrical fruits, while NS 408 is a vigorous F1 hybrid (Namdhari Hybrid Seeds Pvt. Ltd. Bengaluru) with high yield potential. Rootstock origins were specified as follows: *Cucurbita ficifolia* (landrace, Ooty), *C. moschata* cv. CO-2 (TNAU), *C. maxima* cv. Arka Suryamukhi (IIHR), *Lagenaria siceraria* CO-1 hybrid (TNAU), and *Luffa cylindrica* (landrace, Dehradun).

Grafting, Healing, and Acclimatization

Seeds of the rootstock and scion were sown in pro-trays filled with enriched, composted, and sterilized coir pith. The seedlings of both the rootstock and scion were ready for grafting when they developed their first true leaves, typically 9 to 15 days after sowing. Grafting was performed using the hole-insertion method, as described by

Table 1. Scion/Rootstock graft combinations used under study.

Treatment	Treatment denotation	Treatment details (Graft combination with species details)
T1	Green Long	Green Long (Non-grafted control)
T2	Green Long/FG	Green Long/FG (Green Long grafted onto <i>Cucurbita ficifolia</i> , Fig Leaf Gourd)
T3	Green Long/PM	Green Long/ PM (Green Long grafted onto <i>Cucurbita moschata</i> , Pumpkin)
T4	Green Long/WS	Green Long/WS (Green Long grafted onto <i>Cucurbita maxima</i> , Winter Squash)
T5	Green Long/BG	Green Long/BG (Green Long grafted onto <i>Lagenaria siceraria</i> , Bottle Gourd)
T6	Green Long/SG	Green Long/SG (Green Long grafted onto <i>Luffa cylindrica</i> , Sponge Gourd)
T7	NS 408	NS 408 (Non-grafted control)
T8	NS 408/FG	NS 408/FG (Green Long grafted onto <i>Cucurbita ficifolia</i> , Fig Leaf Gourd)
T9	NS 408/PM	NS 408/PM (Green Long grafted onto <i>Cucurbita moschata</i> , Pumpkin)
T10	NS 408/WS	NS 408/WS (Green Long grafted onto <i>Cucurbita maxima</i> , Winter Squash)
T11	NS 408/BG	NS 408/ BG (Green Long grafted onto <i>Lagenaria siceraria</i> , Bottle Gourd)
T12	NS 408/SG	NS 408/ SG (Green Long grafted onto <i>Luffa cylindrica</i> , Sponge Gourd)

Davis *et al.* (2008a), wherein the scion was thinner and smaller than the rootstock at the time of grafting.

The growing point of the rootstock was removed, leaving only the cotyledons, and a hole was created using a bamboo toothpick. The scion was prepared by cutting the seedling stem 3-4 cm below the cotyledons and shaping the basal end into a wedge approximately 7-8 mm in length as per the standard method (Thangamani *et al.*, 2019). The prepared scion was then carefully inserted into the hole of the rootstock.

Grafted seedlings were placed in a mist chamber at 90% relative humidity and 25–30 °C. Grafts were kept in the dark for two days to promote union healing. After this initial period, the relative humidity was gradually reduced to 70% over a period of seven days. The plants were then transferred to a shade net house for six days to

acclimate. Grafting clips were detached before transplanting.

Seedling Transplanting and Aftercare

Both grafted and non-grafted seedlings were transplanted under insect-proof net house conditions. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Blocking was done across the length of the net house to account for variations in light and temperature. Each treatment had 25 plants per replication, and observations were recorded on five representative plants per plot. Spacing was maintained at 1.5 m between rows and 1.0 m between plants. Inside temperature was 2–3°C lower than the open field, with natural ventilation and 10% shade nets; no humidifiers were used. All recommended cultural practices, including fertilizers, plant



protection measures, trailing, and pollination, were uniformly applied.

Growth, Floral Traits, and Yield Parameters

Five healthy plants from each treatment and replication were tagged for growth, floral, and yield data. The graft survival percentage was recorded 30 days after transplanting. Floral traits (days to the first female flower, node number, male: female ratio, and days to first harvest) were observed. Vine length (cm) and root length (cm) were measured with a measuring tape, stem diameter (mm) with a digital Vernier calliper, and root weight (g) with a digital balance. Primary branches were manually counted. Fruit count, mean fruit weight (g), and total yield per plant (kg) were recorded at each harvest.

Fruit Quality Parameters

At peak harvest (55 days after

transplanting), ten fruits per treatment were analyzed for physical and biochemical traits. Total soluble solids (°Brix) were measured with a digital refractometer (ATAGO, Japan). Total sugars were estimated using the Anthrone method (Hedge & Hofreiter, 1962), ascorbic acid content as per AOAC (2001), and calcium by Mani *et al.* (2007). The soluble protein was determined by the method of Lowry *et al.* (1951). An organoleptic evaluation was conducted by a semi-trained panel of 20 members using a nine-point Hedonic Scale (Kumar *et al.*, 2019a).

Statistical Analysis

Data were analyzed using a two-way ANOVA (scion, rootstock, and interaction effects). Graft survival percentage was arcsine transformed. Tukey's Honest Significant Difference (HSD) test ($P < 0.05$) was used for mean comparisons. All analyses were conducted in R (R Core Team, 2022) using the "agricolae" package (Mendiburu & Yaseen, 2020).

Table 2. Graft survival percentage and biometric characters of cucumber grafts and self-rooted control plants.^a

Graft combinations	Graft survival percentage (30 DAT)	Vine length at final harvest (cm)	Number of primary branches	Root length (cm)	Root weight (g)
GL	-	400 ± 6.1 ^g	3.6 ± 0.075 ^{cd}	51 ± 0.9 ^g	35 ± 0.46 ^f
GL / FG	78 ± 1.6 ^a	460 ± 5.2 ^{ef}	3.6 ± 0.033 ^{cd}	68 ± 1.1 ^e	60 ± 0.21 ^d
GL / PM	72 ± 0.22 ^{bc}	470 ± 10 ^{df}	3.3 ± 0.07 ^{de}	83 ± 1.9 ^d	46 ± 0.072 ^e
GL / WS	75 ± 0.12 ^{ab}	530 ± 1.4 ^c	4.1 ± 0.049 ^b	98 ± 2.5 ^b	99 ± 1 ^b
GL / BG	68 ± 0.6 ^{cd}	440 ± 9.5 ^{fg}	2.2 ± 0.037 ^f	86 ± 1.5 ^{cd}	48 ± 0.38 ^e
GL / SG	49 ± 0.31 ^f	270 ± 0.83 ^h	2.2 ± 0.048 ^f	59 ± 0.99 ^f	33 ± 0.6 ^f
NS 408	-	500 ± 4.7 ^{cd}	4.4 ± 0.041 ^{ab}	68 ± 1.2 ^e	47 ± 0.098 ^e
NS 408/ FG	71 ± 0.15 ^{bc}	600 ± 8.4 ^b	4.5 ± 0.094 ^a	92 ± 0.38 ^{bc}	74 ± 0.35 ^c
NS 408/ PM	75 ± 0.079 ^{ab}	670 ± 9.4 ^a	3.7 ± 0.065 ^c	90 ± 0.28 ^c	45 ± 0.16 ^e
NS 408/ WS	79 ± 1.8 ^a	700 ± 16 ^a	4.6 ± 0.11 ^a	110 ± 0.65 ^a	120 ± 3.1 ^a
NS 408/ BG	64 ± 0.76 ^d	480 ± 12 ^{de}	3.2 ± 0.072 ^c	92 ± 1.5 ^{bc}	46 ± 0.81 ^e
NS 408/ SG	54 ± 0.25 ^e	300 ± 7.4 ^h	2.5 ± 0.062 ^f	65 ± 1 ^{ef}	36 ± 0.45 ^f

^a Values are means±SEM. Means in a row without a common superscript letter indicate significant differences based on pair wise comparison at $P < 0.05$ as analyzed by one-way ANOVA and the TUKEY test.

Economics

Economic analysis included cost of grafting labor, nursery preparation, cultivation practices, gross returns, net returns, and Benefit-Cost (BC) ratio, calculated following Izaba *et al.* (2021).

RESULTS AND DISCUSSION

Effect of Grafting on Survival, Vine Growth, and Root Development

The graft survival percentage, vine length, primary branch number, root length, and root weight of grafted cucumber plants and non-grafted controls showed significant differences across treatments (Table 2). Graft survival ranged from $49.03 \pm 0.31\%$ [GL/Sponge Gourd (SG)] to $79 \pm 1.8\%$ (NS 408/WS), with NS 408/WS demonstrating the highest survival. The most extended vine length was observed in NS 408/WS (700 ± 16 cm), significantly outperforming the control plants and other graft combinations. The number of primary branches was highest in NS 408/WS and NS 408/FG (4.6 ± 0.11 and 4.50 ± 0.094 , respectively), with significant differences observed between treatments. Root length and root weight also varied, with NS 408/WS showing the highest values for both parameters (110 ± 0.65 cm and 120 ± 3.1 g, respectively), indicating superior root development compared to other combinations.

Although graft survival percentages were moderate compared to solanaceous crops, all combinations except GL/SG exhibited normal growth and vigour, confirming compatibility between most scion-rootstock pairs. The GL/SG combination showed reduced vigour, suggesting partial incompatibility.

In contrast, GL and NS 408 self-rooted control plants exhibited comparatively lower biometric performance in all parameters. All the measured traits, including graft survival, vine length, number of primary branches,

root length, and root weight, were significantly influenced by the graft combination with graft survival and a highly significant level of difference ($P < 0.05$) for all traits.

The significant variation in graft survival, vine growth, and root development across the treatments highlights the importance of selecting the right rootstock to enhance plant vigour. The highest graft survival and vine length in NS 408/WS suggest superior compatibility, a stronger root system, and better resource acquisition, aligning with recent reports that *C. maxima* promotes both above- and below-ground growth in cucumbers (Rouphael *et al.*, 2010; Asghar *et al.*, 2024; Sakthivel *et al.*, 2024). In contrast, the lower performance of GL/SG highlights potential incompatibility issues with certain scions, resulting in suboptimal growth.

Effect of Grafting on Earliness and Male: Female Ratio

The earliness and male: female ratio (sex ratio) of cucumber grafts and self-rooted control plants varied significantly across treatments (Table 3). The days to first female flower appearance ranged from 14 ± 0.081 days in NS 408/FG to 18 ± 0.057 days in GL/PM. Among the grafted plants, NS 408/FG and NS 408/PM showed earlier flowering with 14 ± 0.081 and 14 ± 0.16 days, respectively. The node number to first female flower appearance was lowest in GL/PM (12 ± 0.15 days) and highest in GL/BG (18 ± 0.057 days). Lowest male: female ratios observed in NS 408/FG (20:1) and NS 408/WS (21:1), indicating a favourable balance between male and female flowers.

In terms of days to first harvest, NS 408/PM exhibited the shortest period to first harvest (26 ± 0.46 days), followed closely by NS 408/FG (26 ± 0.35 days) and NS 408/BG (26 ± 0.24 days).

The significant variation in earliness, sex ratio, and time to first harvest among cucumber graft combinations highlights the

**Table 3.** Performance of cucumber grafts and self-rooted control plants for earliness and sex ratio.^a

Graft combinations	Days to first female flower appearances	Node number to first female flower appearance	Sex ratio (No. of male/female flower)	Days to first harvest
GL	16 ± 0.042 ^b	16 ± 0.25 ^b	30 ± 0.22 ^a	29 ± 0.54 ^{cd}
GL / FG	16 ± 0.037 ^b	15 ± 0.21 ^c	24 ± 0.084 ^c	27 ± 0.026 ^{de}
GL /PM	18 ± 0.057 ^a	12 ± 0.15 ^{gh}	22 ± 0.16 ^{de}	28 ± 0.55 ^{cd}
GL /WS	15 ± 0.39 ^{bd}	14 ± 0.26 ^{cde}	24 ± 0.11 ^{cd}	30 ± 0.12 ^c
GL /BG	17 ± 0.43 ^a	18 ± 0.057 ^a	27 ± 0 ^b	34 ± 0.67 ^b
GL / SG	14 ± 0.21 ^{cde}	12 ± 0.16 ^h	27 ± 0.13 ^b	42 ± 0.35 ^a
NS 408	14 ± 0.34 ^c	15 ± 0.054 ^{bc}	28 ± 0.15 ^b	26 ± 0.38 ^e
NS 408 / FG	14 ± 0.081 ^{de}	13 ± 0.19 ^{fh}	20 ± 0.36 ^f	26 ± 0.35 ^e
NS 408 /PM	14 ± 0.16 ^{de}	14 ± 0.085 ^{df}	22 ± 0.34 ^{de}	26 ± 0.46 ^e
NS 408 /WS	16 ± 0.24 ^{bc}	13 ± 0.25 ^{efg}	21 ± 0.48 ^{ef}	28 ± 0.098 ^{de}
NS 408 /BG	15 ± 0.31 ^{bc}	14 ± 0.0074 ^{cd}	23 ± 0.024 ^{cd}	26 ± 0.24 ^e
NS 408 / SG	15 ± 0.12 ^{bc}	13 ± 0.3 ^{fh}	30 ± 0.65 ^a	41 ± 0.55 ^a

^a Values are means±SEM. Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the TUKEY test.

impact of rootstock selection on reproductive traits and yield potential. Earlier flowering and favourable male:female ratios in NS 408/FG and NS 408/PM suggest that these rootstocks enhance reproductive efficiency, likely due to improved hormonal balance and nutrient uptake (Lee *et al.*, 2010; Sakthivel *et al.*, 2024). In contrast, the higher sex ratios and delayed harvest times in GL and NS 408/SG indicate suboptimal compatibility, which may limit yield potential (Rouphael *et al.*, 2010).

Yield Performance, Crop Duration

The yield parameters, crop duration, and total harvests of cucumber grafts and self-rooted control plants showed significant variation across the treatments (Table 4). The highest number of fruits per vine (21 ± 0.12) and fruit weight (400 ± 4.6 g) were recorded in NS 408/WS, resulting in a maximum fruit yield of 8.3 ± 0.02 kg per vine. This treatment also had the longest crop duration (140 ± 2.7 days) and highest number of harvests (15 ± 0.22), significantly outperforming other combinations and controls. Among the Green Long

combinations, GL/WS performed best, with 15 ± 0.14 fruits per vine, a fruit weight of 320 ± 8 g, and a yield of 5.4 ± 0.13 kg per vine, with crop duration of 120 ± 0.063 days and 12 ± 0.12 harvests.

Across all treatments, grafting significantly improved yield-related parameters compared to non-grafted controls, with significant differences ($P < 0.05$) for all traits. The superior yield and extended crop duration in NS 408/WS confirm the advantage of *C. maxima* rootstocks in promoting vigorous root systems, high water and nutrient uptake, and improved vegetative growth, aligning with reports by Davis *et al.* (2008a) and Bayoumi *et al.* (2021). Similarly, GL/WS showed strong performance among Green Long combinations. In contrast, GL/SG recorded poor yields and shorter crop duration, indicating compatibility limitations of *Luffa cylindrica*.

Fruit Characteristics and Quality

The fruit characters and quality of cucumber grafts and self-rooted controls varied significantly across treatments (Table 5). NS 408/WS had the highest fruit length

Table 4. Evaluation of cucumber grafts and self-rooted control plants for yield characters, crop duration, and total harvests.^a

Graft combinations	Number of fruits per vine	Fruit weight (g)	Fruit yield per vine (kg)	Crop duration (Days)	Total number of harvests
GL	10 ± 0.24 ^f	280 ± 0.58 ^{cd}	2.9 ± 0.034 ^g	97 ± 1.4 ^{fg}	8.4 ± 0.096 ^d
GL / FG	13 ± 0.31 ^d	270 ± 3.7 ^{de}	3.6 ± 0.031 ^{ef}	120 ± 1.5 ^c	11 ± 0.22 ^{bc}
GL /PM	8.7 ± 0.086 ^g	250 ± 2.3 ^{ef}	2.6 ± 0.062 ^h	120 ± 2.7 ^{cd}	10 ± 0.11 ^c
GL /WS	15 ± 0.14 ^c	320 ± 8 ^b	5.4 ± 0.13 ^c	120 ± 0.063 ^c	12 ± 0.12 ^b
GL /BG	12 ± 0.078 ^e	280 ± 4.7 ^{cd}	3.4 ± 0.0017 ^f	100 ± 1.5 ^{ef}	6 ± 0.063 ^f
GL / SG	7.5 ± 0.027 ^h	230 ± 4.3 ^f	2.3 ± 0.024 ⁱ	85 ± 0.044 ^h	5.9 ± 0.098 ^f
NS 408	13 ± 0.027 ^d	300 ± 7.5 ^{bc}	3.8 ± 0.002 ^e	120 ± 0.31 ^{cd}	11 ± 0.26 ^{bc}
NS 408/ FG	19 ± 0.38 ^b	380 ± 9.5 ^a	7.1 ± 0.12 ^b	140 ± 1.1 ^{ab}	15 ± 0.078 ^a
NS 408/ PM	12 ± 0.11 ^{de}	290 ± 2.5 ^{cd}	3.4 ± 0.048 ^f	130 ± 1.2 ^b	11 ± 0.13 ^b
NS 408/ WS	21 ± 0.12 ^a	400 ± 4.6 ^a	8.3 ± 0.017 ^a	140 ± 2.7 ^a	15 ± 0.22 ^a
NS 408/ BG	13 ± 0.13 ^d	300 ± 5 ^{bc}	4.2 ± 0.0088 ^d	110 ± 2.6 ^{de}	7.5 ± 0.14 ^e
NS 408/ SG	9.4 ± 0.0049 ^g	280 ± 3.6 ^{ce}	3 ± 0.019 ^g	89 ± 0.88 ^{gh}	6.5 ± 0.058 ^f

^a Values are means ± SEM. Means in a row without a common superscript letter differ (P< 0.05) as analyzed by one-way ANOVA and the TUKEY test.

(29±0.51 cm) and diameter (5.4±0.11 mm), followed by NS 408/FG (28±0.057 cm and 5.4±0.059 mm). Total Soluble Solids (TSS) were highest in NS 408/WS (4.2±0.042 °Brix), NS 408/FG, and NS 408 (4.2±0.028 °Brix). Total sugars were also highest in NS 408 (2.9±0.027 mg 100 g⁻¹), NS 408/FG (2.9±0.023 mg 100 g⁻¹), and NS 408/PM (2.8±0.04 mg 100 g⁻¹).

No negative effects were observed on critical fruit quality attributes such as TSS, visual appearance, or texture, confirming that grafting maintained or improved market quality. The larger fruit size, high TSS, and sugar content in NS 408/WS and NS 408/FG validate the positive impact of *Cucurbita* rootstocks on nutrient transport and assimilation (Rouphael *et al.*, 2010). Poor fruit quality in GL/SG further supports incompatibility issues, as this rootstock produced smaller fruits with lower sugar content.

Fruit Biochemical Composition

The fruit quality traits, including palatability, total sugars, ascorbic acid, and

soluble protein content, varied significantly across grafted and non-grafted cucumber plants (Tables 5 and 6). The highest palatability score was recorded for NS 408/PM, showing that grafting onto *Cucurbita moschata* improved taste and texture. Conversely, GL/PM (6.6±0.12) and GL/WS (6.6±0.093) had the lowest scores.

Ascorbic acid content was highest in NS 408/FG (0.82 mg 100 g⁻¹), followed by GL/WS (0.8±0.0042 mg 100 g⁻¹), while GL/SG had the lowest (0.47±0.002 mg 100 g⁻¹). For soluble proteins, GL/SG showed the highest content (13±0.17 mg g⁻¹), and GL/BG the lowest (6.3±0.052 mg g⁻¹). Calcium was highest in NS 408/PM (8.9±0.18 mg 100 g⁻¹).

These results demonstrate that grafting improved or maintained all measured biochemical traits, with *Cucurbita* rootstocks exhibiting superior effects on vitamin C, calcium, and protein content, consistent with the findings of Aslam *et al.* (2020). The enhanced biochemical values indicate that grafting improves nutrient uptake and fruit nutritional quality, reinforcing its agronomic advantage.

**Table 5.** Evaluation of cucumber grafts and self-rooted plants for fruit characters and fruit quality.^a

Graft combinations	Fruit length (cm)	Fruit diameter (mm)	TSS (°brix)	Total sugars (mg 100 g ⁻¹)
GL	20 ± 0.02 ^h	4.8 ± 0.02 ^c	3.5 ± 0.087 ^{de}	2.5 ± 0.045 ^{bc}
GL / FG	23 ± 0.22 ^{ef}	4.8 ± 0.12 ^{bc}	3.8 ± 0.021 ^{bc}	2.4 ± 0.018 ^{cd}
GL / PM	22 ± 0.023 ^{fg}	4.8 ± 0.038 ^{bc}	3.5 ± 0.055 ^{de}	2.2 ± 0.05 ^{de}
GL / WS	25 ± 0.56 ^{cd}	5.1 ± 0.032 ^{ab}	3.5 ± 0.035 ^{de}	2.3 ± 0.0071 ^{de}
GL / BG	21 ± 0.053 ^{gh}	4.6 ± 0.077 ^{cd}	3.7 ± 0.054 ^{cd}	1.9 ± 0.019 ^f
GL / SG	20 ± 0.43 ^h	3.5 ± 0.015 ^f	3.3 ± 0.012 ^e	1.6 ± 0.0065 ^g
NS 408	26 ± 0.16 ^{cd}	5.2 ± 0.033 ^a	4.2 ± 0.028 ^a	2.9 ± 0.027 ^a
NS 408/ FG	28 ± 0.057 ^{ab}	5.4 ± 0.059 ^a	4.2 ± 0.028 ^a	2.9 ± 0.023 ^a
NS 408/ PM	25 ± 0.16 ^{cd}	5.2 ± 0.057 ^a	4 ± 0.0083 ^{ab}	2.8 ± 0.04 ^a
NS 408/ WS	29 ± 0.51 ^a	5.4 ± 0.11 ^a	4.2 ± 0.042 ^a	2.6 ± 0.045 ^b
NS 408/ BG	24 ± 0.48 ^{de}	4.5 ± 0.063 ^d	4 ± 0.052 ^{ab}	2.3 ± 0.019 ^{de}
NS 408/ SG	26 ± 0.66 ^{bc}	3.9 ± 0.002 ^e	3.9 ± 0.087 ^{bc}	2.2 ± 0.054 ^c

^a Values are means±SEM. Means in a row without a common superscript letter differ (P< 0.05) as analyzed by one-way ANOVA and the TUKEY test.

Table 6. Evaluation of cucumber grafts and self-rooted plants for fruit quality.^a

Graft combinations	Reducing sugars	Ascorbic acid	Calcium	Soluble protein	Palatability score
GL	0.56 ± 0.013 ^{cd}	0.65 ± 0.013 ^b	8.2 ± 0.0042 ^{bcd}	9.5 ± 0.22 ^{de}	7.1 ± 0.14 ^{cd}
GL / FG	0.6 ± 0.013 ^{bc}	0.49 ± 0.0076 ^c	8.1 ± 0.036 ^{bcd}	13 ± 0.18 ^b	7.2 ± 0.041 ^{cd}
GL / PM	0.39 ± 0.003 ^g	0.64 ± 0.0023 ^b	7.8 ± 0.081 ^{cd}	11 ± 0.061 ^c	6.6 ± 0.12 ^d
GL / WS	0.44 ± 0.0055 ^f	0.8 ± 0.0042 ^a	8.2 ± 0.068 ^{bc}	8.8 ± 0.13 ^f	6.6 ± 0.093 ^d
GL / BG	0.53 ± 0.003 ^{de}	0.65 ± 0.016 ^b	8.2 ± 0.16 ^{bc}	6.3 ± 0.052 ^h	7.6 ± 0.024 ^{ac}
GL / SG	0.64 ± 0.013 ^{ab}	0.47 ± 0.002 ^c	8.1 ± 0.08 ^{bcd}	13 ± 0.17 ^a	6.7 ± 0.16 ^d
NS 408	0.46 ± 0.01 ^f	0.65 ± 0.012 ^b	8.4 ± 0.0087 ^{ac}	9.1 ± 0.019 ^{ef}	8 ± 0.17 ^{ab}
NS 408/ FG	0.42 ± 0.00022 ^{fg}	0.82 ± 0 ^a	8.2 ± 0.2 ^{bc}	7.6 ± 0.19 ^g	7.6 ± 0.12 ^{ac}
NS 408/ PM	0.67 ± 0.013 ^a	0.49 ± 0.0051 ^c	8.9 ± 0.18 ^a	9.3 ± 0.049 ^{ef}	8.2 ± 0.2 ^a
NS 408/ WS	0.51 ± 0.0027 ^c	0.65 ± 0.0017 ^b	8.1 ± 0.14 ^{bcd}	9.2 ± 0.1 ^{ef}	7.8 ± 0.065 ^{ac}
NS 408/ BG	0.31 ± 0.0044 ^h	0.62 ± 0.013 ^b	7.6 ± 0.099 ^d	6.3 ± 0.092 ^h	7.4 ± 0.031 ^{bc}
NS 408/ SG	0.56 ± 0.0038 ^{cd}	0.49 ± 0.0038 ^c	8.6 ± 0.14 ^{ab}	10 ± 0.12 ^{cd}	7.8 ± 0.16 ^{ab}

^a Values are means±SEM. Means in a row without a common superscript letter differ (P< 0.05) as analyzed by one-way ANOVA and the TUKEY test.

The highest palatability scores were observed in *Cucurbita moschata* grafts, indicating that this rootstock can enhance fruit sensory attributes such as taste and texture. This suggests that *Cucurbita moschata* rootstocks may facilitate better nutrient translocation and metabolism, contributing to improved flavour (Aslam et al., 2020). In contrast, the lower palatability scores recorded in GL/PM and GL/WS indicate that these rootstocks might not be as effective in enhancing the sensory appeal of cucumbers. Total Soluble Solids (TSS), a

key indicator of sweetness and fruit quality, were significantly higher in grafted plants such as NS 408/WS and NS 408/FG, highlighting the potential of *Cucurbita* rootstocks to enhance sugar accumulation in cucumber fruits. These findings align with earlier research that suggests grafting improves the plant's ability to accumulate carbohydrates, resulting in sweeter fruits (Rouphael et al., 2010; Bayoumi et al., 2021). Similarly, total sugar content was highest in NS 408 and its graft combinations, further supporting the positive

impact of *Cucurbita* rootstocks on enhancing sweetness. Soluble protein content was also significantly higher in grafted plants, particularly in GL/SG and GL/FG, compared to non-grafted controls.

The increased protein content in these grafts suggests that grafting can enhance nitrogen assimilation and protein synthesis, which are essential for overall fruit nutritional quality. These findings agree with previous reports that grafting improves protein content due to the enhanced metabolic activity in grafted plants (Kumar *et al.*, 2019b). Ascorbic acid (vitamin C) content, an important antioxidant, was highest in NS 408/FG and GL/WS, surpassing their non-grafted counterparts. The higher ascorbic acid levels in these grafted plants may be attributed to improved nutrient absorption and plant metabolism, which is commonly enhanced in grafted plants. The improved ascorbic acid content in these grafts not only enhances the nutritional value of the fruits but also contributes to longer shelf life and better post-harvest quality. Calcium content, crucial for fruit firmness and texture, was also significantly higher in grafted plants, particularly in NS 408/PM and NS 408/SG. These findings suggest that grafting onto *Cucurbita* rootstocks improves calcium uptake, which is critical for maintaining fruit structure and reducing post-harvest losses. The enhanced calcium content in these graft combinations supports the role of grafting in improving fruit firmness and overall quality (Davis *et al.*, 2008b, Aslam *et al.*, 2020; Asghar *et al.*, 2024).

Significantly higher fruit yield per plant (8.3 ± 0.02 kg) was observed in the NS 408/WS graft combination. The enhanced yield in grafted plants is primarily attributed to the vigorous root system and improved water balance provided by *Cucurbita* rootstocks, allowing more efficient resource acquisition compared to non-grafted plants. This finding aligns with Khapte *et al.* (2021), who identified interspecific *Cucurbita* hybrids as the best rootstocks for increasing cucumber yield. Figure 2

demonstrates significant differences in economic yield, gross income, cost of cultivation, net return, and BC ratio between grafted and non-grafted treatments. In this study, fruit yields ranged from 2.3 ± 0.02 to 8.3 ± 0.02 kg per plant, with the highest yields following the order: NS 408/WS > NS 408/FG > GL/WS > NS 408/BG > NS 408 > GL/FG > GL/BG > NS 408/SG > GL > GL/PM > GL/SG. Grafting increased yields by 118.42% in NS 408/WS and 86% in NS 408/FG compared to non-grafted NS 408. Similarly, GL/WS showed an 86.21% increase over GL control and a 42.11% increase over NS 408 control. Notably, the yield increase in the grafted open-pollinated variety (GL/WS) over the non-grafted hybrid (NS 408) was 42.11%, highlighting the cost-effectiveness of grafting OPVs onto resistant rootstocks over using expensive hybrid seeds. Kumar *et al.* (2019a) also reported a 30% yield increase in grafted cucumber plants (*Cucurbita ficifolia* rootstock) compared to non-grafted plants.

Correlation Analysis of Growth Traits and Yield

Pearson correlation analysis revealed strong and significant positive associations between vegetative and root traits with fruit yield in grafted cucumber plants (Figure 1). Root weight showed the highest correlation with fruit yield ($r = 0.89$, $P < 0.0001$), followed by root length ($r = 0.74$, $P < 0.0001$), vine length ($r = 0.70$, $P < 0.0001$), and number of primary branches ($r = 0.70$, $P < 0.0001$). Root weight also exhibited strong correlations with vine length ($r = 0.68$) and root length ($r = 0.78$). The strong correlations between yield and root system attributes highlight the central role of root vigour in resource acquisition and overall productivity. Enhanced root weight and length in grafted combinations such as NS 408/WS (*C. maxima* rootstock) were associated with superior shoot growth and higher fruit yields, underscoring the contribution of grafting to improved water

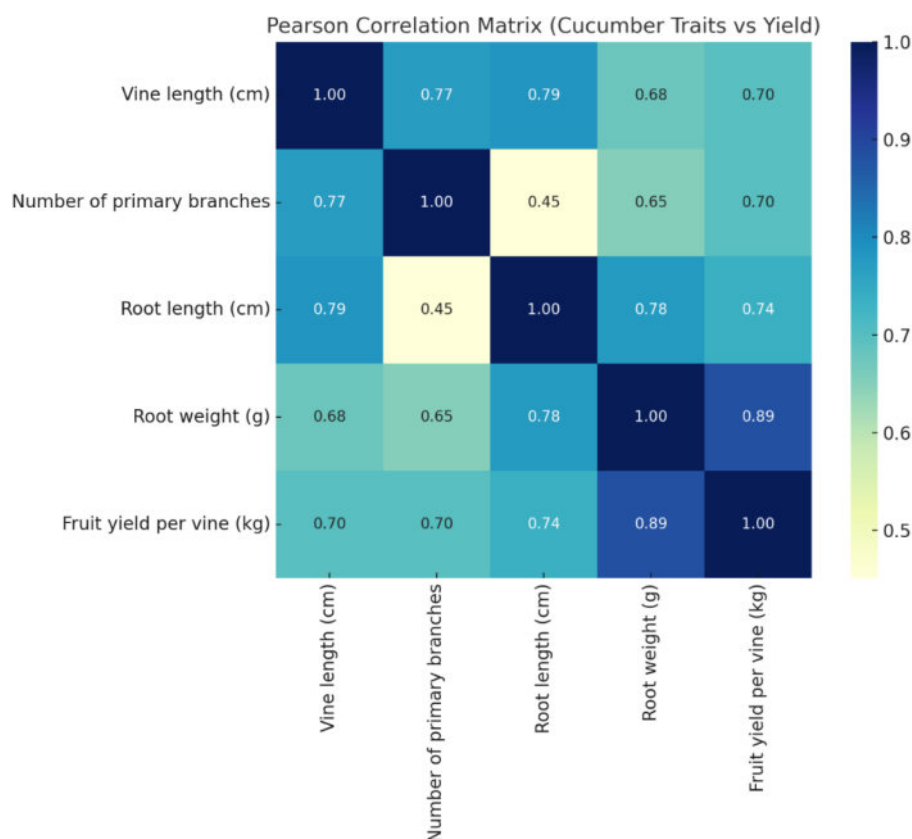


Figure 1. Pearson correlation heatmap showing relationships between key vegetative traits (vine length, number of primary branches, root length, root weight) and fruit yield in grafted cucumber plants. Darker blue-green colors represent stronger positive correlations (r -values closer to +1). Root weight and root length exhibited the strongest associations with yield ($P < 0.0001$), highlighting the importance of root vigour in productivity under net house conditions.

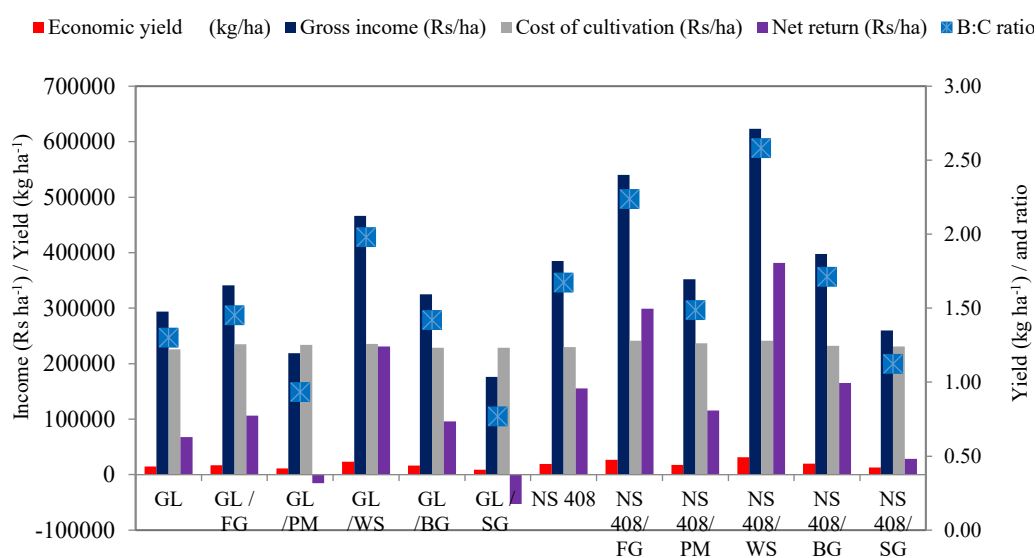


Figure 2. Yield and economics of monoecious cucumber grafted and non-grafted conditions.

and nutrient uptake. Similar findings have been reported in cucurbits, where vigorous rootstocks enhanced nutrient transport, photosynthetic activity, and fruit set, leading to greater yields (Rouphael *et al.*, 2010; Bayoumi *et al.*, 2021; Asghar *et al.*, 2024). The positive correlations between vine length, primary branches, and yield reflect the strong influence of shoot architecture on reproductive output. Plants with extended vine growth and more branches likely provided increased sites for flowering and fruiting, contributing to higher productivity. These relationships suggest that both shoot and root traits should be considered key selection criteria in grafting studies and breeding programs aimed at optimizing yield potential.

Economics

Economic analysis revealed substantial differences between grafted and non-grafted treatments (Figure 2). NS 408/WS had the highest economic yield (8.3 ± 0.02 kg/plant) and BC ratio (> 2.5), while NS 408/FG and NS 408/BG also performed well (BC ratio > 2.0). GL/WS outperformed the non-grafted hybrid, showing a 42.1% yield advantage over the NS 408 control and an 86.2% yield advantage over the GL control.

Non-grafted GL, NS 408, and GL/SG were less profitable, with BC ratios below 1.0, demonstrating that grafting significantly improves profitability in protected cultivation. The data confirm that grafting monoecious cultivars onto resistant rootstocks like *C. maxima* is a cost-effective alternative to hybrid seeds.

CONCLUSIONS

This study demonstrates the significant impact of rootstock selection on the growth, yield, and fruit quality of grafted cucumber plants. Grafting onto *Cucurbita maxima* (NS 408/WS) resulted in increased fruit yield compared to non-grafted controls, along

with improvements in fruit size, total soluble solids, and ascorbic acid content. Similarly, grafting onto *Luffa cylindrica* enhanced soluble protein and calcium content, although it did not match the overall performance of *C. maxima*. These findings highlight the importance of selecting suitable rootstocks to improve productivity, quality, and profitability in cucumber cultivation. The study is the first to demonstrate under Indian net house conditions that a grafted monoecious open-pollinated cultivar (GL/WS) can outperform a non-grafted hybrid, highlighting grafting as a practical and cost-effective alternative to expensive hybrid seeds. Results align with previous research (Rouphael *et al.*, 2010; Sarwar *et al.*, 2019a; Asghar *et al.*, 2024) showing that *Cucurbita* rootstocks enhance stress tolerance, root vigour, and nutrient acquisition, leading to improved market quality and higher returns. Overall, grafting is a promising, scalable solution for sustainable cucumber production, particularly in resource-limited settings, offering reduced pesticide dependence, lower input costs, and improved farmer profitability.

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پیوند خیارهای هیبرید و تک‌پایه روی پایه‌های خانواده کدونیان، رشد و عملکرد را در شرایط گلخانه توری بهبود می‌بخشد

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چکیده

این پژوهش، پایه‌های مناسب از تیره کدونیان را برای بهبود عملکرد و کیفیت خیار در شرایط گلخانه توری (نت‌هاوس) ارزیابی کرد. دو رقم خیار، شامل «Green Long» (یک رقم گرده‌افشانی آزاد با OPV) و «NS 408» (یک هیبرید F1)، بر روی پنج پایه پیوند زده شد: *Cucurbita ficifolia**, *Cucurbita moschata**, *Cucurbita**, *Luffa cylindrica**, *Lagenaria siceraria**, *maxima** و *C. maxima**. نتایج نشان داد که NS 408 هم در شرایط پیوندشده و هم در شرایط غیرپیوندی، عملکرد بهتری نسبت به GL داشت. در میان پایه‌های مورد بررسی، رقم NS 408 پیوندشده روی *C. maxima** (کدو زمستانه) بیشترین میزان بقای پیوند (۷۹±۱/۸٪)، بیشترین طول بوته (سانتی‌متر ۷۰۰±۱۶) و بیشترین عملکرد میوه (۸/۳±۰/۱۷ کیلوگرم در هر بوته، معادل ۱۱۸٪ افزایش نسبت به NS 408 غیرپیوندی) را نشان داد. پیوند رقم GL روی کدو زمستانه، عملکرد را در مقایسه با رقم غیرپیوندی NS 408 به میزان ۴۲/۱۱٪ و در مقایسه با شاهد GL به میزان ۸۶/۲۱٪



افزایش داد. پارامترهای کیفیت میوه مانند مواد جامد محلول، خوش خوراکی، اسید آسکوربیک و پروتئین محلول تحت تأثیر قرار نگرفتند یا بهبود یافتند، که تأیید می‌کند پیوند تأثیر منفی بر کیفیت بازارپسندی نداشته است. تحلیل اقتصادی نشان داد که بالاترین نسبت سود به هزینه (بیش از ۲/۵) مربوط به تیمار NS 408/WS است؛ این امر حاکی از آن است که پیوندزنی جایگزینی مقرون به صرفه برای بذرهیبرید گران قیمت محسوب می‌شود. این یافته‌ها بر پتانسیل روش پیوند برای بهینه‌سازی تولید خیار، کاهش وابستگی به آفت‌کش‌ها و افزایش سودآوری در کشت‌های تحت پوشش (گلخانه‌ای) تأکید دارند.

Effects of Polyamines and Iron on Iron Absorption, Yield, and Enzymatic Activity in Soybean

Fatemeh Sadat Ghamkhar¹, Seyed Mostafa Hoseini-Mazinani^{1*}, Alireza Pazoki¹, and Mohammad Yousefi²

ABSTRACT

This study evaluated the influence of Polyamines (PAs), applied both individually and in combination with iron, on soybean yield and yield components over the 2021 and 2022 growing seasons. The field experiment was conducted at the research farm of Azad University using a randomized complete block design with three replications. The experimental treatments consisted of foliar applications of 2 mM spermine, spermidine, and putrescine, each with and without 2 mM iron, as well as the combinations of 1 mM of polyamine with 3 mM iron to assess synergistic effects. Additionally, the iron sulfate and a control treatment were included. The results indicated that the treatments significantly affected the growth and yield components (such as plant height, pod number, seed weight, harvest index, seed protein and oil content) and the nutrient contents including Zn, Fe, B, N and Mg. The use of spermidine with iron treatment increased the seed weight by 71% compared to the control. Also, the application of polyamines combined with Fe resulted in a significant increase in the activities of superoxide dismutase (44%), peroxidase (63%), and catalase (43%) enzymes compared to the control. Notably, among the polyamines, spermidine combined with iron had the most significant effects on the studied traits and enzyme activity. These findings suggest that the application of polyamines with iron can significantly improve the soybean yield and yield components.

Keywords: Plant nutrients, Putrescine, Spermidine, Spermine.

INTRODUCTION

Despite an annual production of 271 thousand tons of oilseeds in Iran, the majority of edible oil in the country is imported. Over 90% of domestic oil consumption relies on the foreign sources, highlighting the urgent need for strategic planning to achieve the self-sufficiency in edible oil production. Soybean (*Glycine max* L.) is a critical crop for addressing the human nutritional needs because its seeds contain approximately 40% protein and 20% oil (Sheikhzadeh *et al.*, 2022). Four crops, oil palm, soybean, rapeseed, and sunflower, are collectively responsible for > 91% of all globally traded vegetable oil production,

worth an annual USD 223 billion (Murphy, 2025). Iron is an essential micronutrient for plant growth. Iron deficiency impairs chlorophyll synthesis, leading to chlorosis, which initially appears between the leaf veins and then gradually causes the entire leaf surface to turn yellow, except for the veins. These symptoms typically appear in the young leaves and upper stems, eventually affecting the entire plant (Askary *et al.*, 2017; Briat *et al.*, 2015). Proper management of iron application is crucial, as it significantly influences both plant yield and soil health (Şahin and İşler, 2021). One way to manage the iron in soil and plants is the use of polyamines.

Plant growth regulators, particularly Polyamines (PAs), play a vital role in

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enhancing plant yield and productivity. Polyamines are often regarded as the pseudo-hormonal compounds due to their high concentrations in plant cells compared to traditional plant hormones like abscisic acid, cytokinin, gibberellic acid, and auxin (Alet *et al.*, 2012). Even at milli-molar concentrations, PAs can elicit significant biological responses. They are involved in critical processes such as cell division, gene expression, growth and development, leaf senescence, fruit ripening, DNA and RNA synthesis, protein synthesis, membrane stability, enzymatic regulation, and stress tolerance (Shi and Chen, 2014; Rangan *et al.*, 2014). The primary PAs in plants include the Putrescine (Put), Spermidine (Spd), and Spermine (Spm), which exist in free or bound forms (Pál *et al.*, 2021). The application of PAs during various growth stages, including flowering, root formation, and cell proliferation has demonstrated the beneficial effects on plant metabolism (Rahdari, 2019). However, limited information is available on the role of PAs in mineral nutrient uptake and their impact on the quantitative and qualitative yield of soybeans.

The regulation of plant Iron (Fe) deficiency responses involves multiple signaling molecules, such as Nitric Oxide (NO) (Graziano *et al.*, 2002; Graziano and Lamattina, 2007; Chen *et al.*, 2010), Hydrogen Sulfide (H₂S) (Chen *et al.*, 2015), Carbon Monoxide (CO) (Kong *et al.*, 2010), and Carbon Dioxide (CO₂) (Jin *et al.*, 2009). Among these, NO has been shown to play a critical role in modulating Fe uptake and homeostasis (Graziano *et al.*, 2002; Graziano and Lamattina, 2007; Chen *et al.*, 2010). Notably, exogenous application of polyamines—Putrescine (Put), Spermidine (Spd), and Spermine (Spm)—triggers a rapid and marked increase in NO production in *Arabidopsis* (Zarza *et al.*, 2019), indicating that NO acts as a key intermediary in polyamine-dependent signaling cascades. Further supporting this notion, recent evidence demonstrates that exogenous putrescine enhances NO

accumulation, which in turn activates Fe deficiency responses and facilitates the remobilization of Fe from the cell wall (Zhu *et al.*, 2016). The effects of PAs on plant growth vary depending on the type of PA. For instance, the putrescine promotes the growth through its antioxidant properties and role in cation-anion balance (Tang and Newton, 2005), while the spermidine mitigates the osmotic stress damage in soybeans, improving the growth parameters such as height, fresh weight, and dry weight (Rahdari and Hoseini, 2013).

Given the importance of soybean in agricultural sector of Iran and the challenges associated with iron uptake in high-pH soils of the country, the objective of this research was to investigate the effect of polyamines application on soybean morphological and phytochemical characteristics. Thus, this study explored the role of polyamines in facilitating iron availability and their impact on soybean enzyme activity, yield and nutrient absorption.

MATERIALS AND METHODS

Experimental Site

The study was conducted during 2021 and 2022 crop seasons at the Agricultural and Medicinal Plants Research Center of Imam Khomeini, Azad University. The experimental site was located at an elevation of 825 meters above sea level, with an average annual temperature of 15.3 °C and precipitation of 168 mm. Some weather data are shown in Table 1. Soil samples collected from a depth of 0–30 cm revealed a texture of 38% sand, 34% silt, and 28% clay (Table 1).

Experimental Design and Treatments

The experiment followed a Completely Randomized Block Design (CRBD) with three replications, and included 27 plots. Treatments are shown in Table 2:

Table 1. Characteristics of soil at the experimental farm.^a

EC (dS m ⁻¹)	pH	OC (%)	TNV (%)	Total N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)
3.54	7.21	0.61	16.51	0.056	8.37	217.54	0.69	4.62	0.53	3.23
Year	Min. temperature (°C)		Mean temperature (°C)			Max. temperature (°C)			Rainfall (mm)	
2021	9		14.7			20.3			191	
2022	9.4		15.5			21.5			236	

^a EC: Electrical Conductivity; OC: Organic Carbon; TNV: Total Neutralizing Value; Total N: Total Nitrogen; P: Phosphorus; K: Potassium; Cu: Copper; Mn: Manganese; Zn: Zinc; Fe: Iron.

Table 2. Experimental treatments.

Treatments	Code	Polyamines (mM)	Iron (mM)
Spermine	Spm	2	-
Spermine with iron	Spm+Fe	2	2
Spermine	Spd	2	-
Spermidine with iron	Spd+Fe	2	2
Putrescine	Put	2	-
Putrescine with iron	Put+Fe	2	2
Spermine Spermidine Putrescine with iron	PAs+Fe	1	3
Iron sulfate	Sulfate Fe	-	2
Control	Control	-	-

Crop Management

Tillage and seedbed preparation were conducted before planting. Fertilizers, including 200 kg ha⁻¹ of triple superphosphate and 100 kg ha⁻¹ of potassium sulfate, were applied based on soil analysis. Soybean seeds (Williams cultivar) were sown on May 6, 2021, and May 8, 2022, in plots measuring 8×3 m, with a row spacing of 60 cm and on-row spacing of 5 cm. Foliar applications of PAs were administered at the 8-leaf stage in the morning (before sunrise) using a handheld sprayer. One liter of solution with pH 7 was prepared and used for each plot.

Data Collection and Analysis

Morphological traits, yield components, leaf elemental composition, chlorophyll content, and enzyme activities were measured. To determine the Leaf Area Index (LAI), in each plot, five plants were chosen at random. The Digimizer software was used

to photograph and calculate the area of a single leaf from each selected plant. LAI was then derived by taking the Leaf Area (LA) of an individual plant and dividing it by the Plot Area (P) that the plant occupied. In this study, the planting density was 33 plants per square meter.

$$LAI = \frac{LA}{P} \quad (1)$$

Enzyme Extractions and Assays

Fully expanded young leaves (0.5 g) from soybean plants were sampled and immediately frozen in liquid nitrogen. The frozen samples were ground in 5 mL of Tris buffer solution containing 0.25 M sucrose, 10 mM Tris, and 1 mM EDTA at pH 7.4. The homogenate was then subjected to centrifugation at 4,800 rpm for 15 minutes at 4°C. The resulting supernatant was collected for enzyme assays. The activity of Superoxide Dismutase (SOD) was determined using the SOD Assay Kit-WST (Dojindo Laboratories, Japan). The reaction plate was incubated in a micro-plate



reader at 37°C for 20 minutes, and the absorbance of each reaction mixture was measured at 450 nm. For the Peroxidase Activity (POD) assay, leaf tissues (0.5 g) were ground in liquid nitrogen and homogenized in 5 mL of Tris extraction buffer. The homogenate was then centrifuged at 10,000×g for 10 minutes at 4°C. The POD activity was measured at 290 nm for 15 seconds (A1), followed by incubation of the reaction solution at 37°C and measurement for 135 seconds (A2) using a spectrophotometer. For CAT activity assay, the supernatant was mixed with sodium phosphate buffer (100 mM, pH 7.0) and H₂O₂ (1M), and the CAT activity was measured at 240 nm. One unit of CAT activity was defined as the amount of CAT required to decompose 1 mole of H₂O₂ per minute (Hande Alici and Arabaci, 2016).

After confirming the normality of data (using the Kolmogorov-Smirnov test, Fadlallah *et al.*, 2019) and assessing the residual normality with a Bartlett's test, a combined Analysis Of Variance (ANOVA) was conducted for the data from the two growing seasons. The significance level was set at $P < 0.05$. The analysis was performed by using SAS 9.2 and SPSS software. To determine the significant differences, the means were assessed using Duncan's multiple range test.

RESULTS

The results of the combined ANOVA indicated that the treatment had a significant impact on all studied traits, and the year had a significance effect only on the height and LAI. However, the interaction of year and treatment was not significant.

Morphological Traits

The results of Duncan's multiple range test for the mean comparison showed that the combination of Fe and Polyamines (PAs) treatments resulted in the highest plant height ($P > 0.01$). However, no significant

difference was observed between this treatment and Spermidine with Iron (Spd+Fe). The control treatment showed the lowest plant height. Regarding the shoot fresh weight, the Spd+Fe treatment resulted in the highest mean values ($P > 0.01$). The control treatment, with no iron supplementation, exhibited the lowest shoot fresh weight. Similarly, for shoot dry weight, the Spd+Fe treatment had the highest mean values, significantly outperforming other treatments involving PAs, which showed no significant differences among themselves. Additionally, the treatments containing iron exhibited higher shoot dry weights compared to those without iron (Table 3).

Yield and Yield Components

In terms of mean weight of pods, both spermine with iron (Spm+Fe) and Spd+Fe treatments resulted in the highest mean weight of pods ($P > 0.01$). The control and sulfate Fe treatments had the lowest values. When considering the pod weight with seeds, the Spd+Fe treatment demonstrated the highest values (2161.8 g/plot). The sulfate Fe and control treatments exhibited the lowest mean values (703.5 g and 599 g/plot, respectively). The highest mean number of pods per plot was in the Spd+Fe treatment ($P > 0.01$), significantly outperforming other treatments. The control treatment exhibited the lowest pod number. For mean number of seeds, the Spd+Fe exhibited the highest values ($P > 0.01$). The control, sulfate Fe and Spm treatments, however, showed the lowest mean number of seeds. The mean weight of seeds followed a similar trend, with Spd+Fe showing the highest values (457.9 g/plot), while the control and sulfate Fe again exhibited the lowest values. In terms of 100 seed weight, the treatments of Spd with iron had the highest value ($P > 0.01$) and had no significance difference with Spd and PAs+Fe. However, the Spm treatment had

the lowest value of 100 seeds weight (Table 3).

Physiological Traits

The Spd treatment showed the highest mean leaf area, significantly differing from all other treatments, except Spd+Fe. The control and Put had the lowest mean for leaf area. For leaf area index, the Spd had the highest mean values ($P > 0.01$), which were significantly different from all other treatments. Conversely, the control and Spm treatments exhibited the lowest mean leaf area index. In terms of harvest index, Spd with iron had the highest value ($P > 0.01$), Spm, Spd and Putrescine with Iron (Put+Fe) had no significant difference with Spd+Fe. The control treatment had the lowest value of harvest index (Table 3).

The control treatment exhibited the lowest protein content (25.15%). The Spd+Fe showed the highest protein content (43.92%). Spm+Fe, Spd, Put, Put+Fe and sulfate Fe treatments did not show the significant differences. For seed oil content, the Spd+Fe exhibited the highest mean values (28.29%), while outperforming all other treatments. The control had the lowest oil content (12.59%) (Table 3).

Nutrient Absorption

For Zn content, the Spd+Fe showed the highest mean values. The control treatment exhibited the lowest Zn content. Regarding Fe content, Spd+Fe exhibited the highest mean value (498.4 mg kg⁻¹), the Put and Put+Fe treatments had no significance difference. The control treatment had the lowest Fe content and had no significance difference with Sulfate Fe. In terms of Mg content, Spd+Fe exhibited the highest values, while the control had the lowest Mg content. For nitrogen and boron, the control treatment had the lowest value and had significance difference with all other treatments (Table 3).

Antioxidant Enzymes

A significant ($P < 0.01$) upregulation in the activity of the antioxidant enzymes Superoxide dismutase (SOD), Peroxidase (POD), and Catalase (CAT) was observed in soybean plants treated with Polyamines (PAs) and Iron (Fe) in combination. The Spd+Fe treatment showed the highest SOD and CAT activity, while Put+Fe and Spm+Fe treatments exhibited the highest POD activities (Figure 1).

DISCUSSION

This study revealed that the application of PAs in combination with iron significantly influenced several growth parameters and yield-related traits. The positive effects of PAs, particularly Spd and Put, in promoting growth are consistent with the previous studies that highlighted their role in enhancing plant development through hormonal regulation and stress alleviation. Furthermore, the application of iron was shown to be beneficial in improving pod and seed development, which supports the findings of previous studies emphasizing the importance of Fe in leguminous crops for optimal growth and yield.

Growth Parameters

A study conducted by Amani (2020) investigated the effect of PAs on the plant height and obtained similar observations. The study revealed that foliar application of nutrient solutions containing PAs at different times in soybean plants led to a significant increase in plant height compared to the non-foliar application. This finding highlights the positive effect of nutritional elements in promoting the vegetative growth and enhancing the plant height. PAs, being a nitrogen-containing signaling molecules, play a role in cell division and enlargement,

**Table 3.** Mean comparison of height, shoot fresh weight, shoot dry weight, pod weight and mean number of leaves.^a

Traits		Plant height (cm)	Shoot fresh weight (kg ha ⁻¹)	Shoot dry weight (kg ha ⁻¹)	Mean weight of pods (g m ⁻²)	Mean number of leaves (m ⁻²)
Treatment						
Year	2021	59.9 b	4288.5 a	962.1 a	6.13 a	136.91 a
	2022	67.6 a	4361.1 a	996.9 a	6.44 a	142.59 a
	Significance	**	ns	ns	ns	ns
Polyamines	Spm	51.9 e	3698.7 b	849.0 bc	4.33 e	113.62 de
	Spm+Fe	70.1 bc	4459.5 b	1025.7 bc	9.05 b	169.19 ab
	Spd	63.8 cd	4471.2 b	921.6 bc	7.28 c	132.71 cd
	Spd+Fe	78.4 ab	6627.9 a	1536.3 a	11.01 a	188.06 a
	Put	62.9 cd	3936.3 b	897.9 bc	5.10 e	140.47 c
	Put+Fe	63.0 cd	4367.1 b	1110.6 b	7.40 c	117.22 cde
	PAs+Fe	79.7 a	4470.6 b	809.7 c	6.08 d	162.95 b
	Sulfate Fe	55.5 de	4294.8 b	808.2 c	3.03 f	136.06 cd
	Control	48.4 e	2596.5 c	855.0 bc	3.30 f	97.44 e
	Significance	**	**	**	**	**

Traits		Pod Weight with Seed (g m ⁻²)	Mean number of pods (m ⁻²)	Mean number of seeds (m ⁻²)	Mean weight of seeds (kg ha ⁻¹)	100 Seeds Weight (g)
Treatment						
Year	2021	270.19 a	20.50 a	242.25 a	2637.2 a	124.8 a
	2022	285.56 a	21.03 a	246.69 a	2815.5 a	128.0 a
	Significance	ns	ns	ns	ns	ns
Polyamines	Spm	249.6 e	10.88 f	149.28 e	2374.5 d	98.6 d
	Spm+Fe	458.8 d	25.19 c	265.56 bc	3553.5 b	115.2 c
	Spd	596.3 b	25.08 c	266.40 bc	3184.0 bc	142.8 ab
	Spd+Fe	900.8 a	35.09 a	436.68 a	4487.4 a	148.0 a
	Put	460.0 d	17.79 e	214.92 d	2421.6 d	132.9 b
	Put+Fe	519.2 cd	19.50 d	249.00 c	2518.6 d	134.5 b
	PAs+Fe	581.0 bc	26.36 b	284.55 b	3095.8 c	144.4 ab
	Sulfate Fe	293.1 e	17.03 e	172.89 e	1575.8 e	113.0 c
	Control	24.96 e	9.94 f	158.6 e	1322.0 e	108.0 cd
	Significance	**	**	**	**	**

Traits		Leaf Area (cm ²)	Leaf Area Index	Harvest Index	Protein (%)	Oil (%)
Treatment						
Year	2021	94.74 a	1.476 b	86.9 a	37.12 a	18.18 a
	2022	96.65 a	1.874 a	87.0 a	38.53 a	18.38 a
	Significance	ns	**	ns	ns	ns
Polyamines	Spm	80.24 cd	1.246 d	89.5 ab	37.35 bc	16.4 b
	Spm+Fe	97.42 bcd	1.828 b	88.9 b	40.01 ab	16.29 b
	Spd	152.38 a	2.249 a	89.5 ab	40.72 ab	18.71 b
	Spd+Fe	135.35 ab	1.504 c	90.9 a	43.92 a	28.29 a
	Put	62.48 d	1.836 b	87.4 c	40.47 ab	19.04 b
	Put+Fe	86.65 cd	1.933 b	89.8 ab	39.86 ab	17.42 b
	PAs+Fe	118.62 abc	1.782 b	88.7 bc	34.39 c	17.72 b
	Sulfate Fe	73.90 cd	1.514 c	80.4 d	38.58 abc	18.05 b
	Control	53.72 d	1.186 d	78.2 e	25.12 d	12.59 c
	Significance	**	**	**	**	**

Table 3 continued...

Continued of Table 3. Mean comparison of height, shoot fresh weight, shoot dry weight, pod weight and mean number of leaves.^a

Treatment	Traits	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mg (mg kg ⁻¹)	N (%)	B (mg kg ⁻¹)
Year	2021	46.6 a	319.4 a	43.5 a	1.75 a	109.9 a
	2022	47.9 a	322.0 a	45.1 a	1.75 a	111.2 a
	Significance	ns	ns	ns	ns	ns
Polyamines	Spm	42.3 cd	269.6 cd	45.9 ab	1.97 a	130.4 a
	Spm+Fe	60.8 b	362.6 bc	48.2 ab	1.83 a	109.7 a
	Spd	34.3 d	255.6 cd	42.8 bc	1.84 a	110.4 a
	Spd+Fe	76.0 a	498.4 a	51.0 a	1.87 a	116.3 a
	Put	35.9 d	407.5 ab	43.9 bc	1.84 a	119.1 a
	Put+Fe	55.9 bc	458.1 ab	46.1 ab	1.84 a	133.9 a
	PAs+Fe	42.8 cd	254.4 cd	40.1 cd	1.61 a	97.7 ab
	Sulfate Fe	45.0 cd	217.5 d	43.9 bc	1.91 a	114.6 a
	Control	32.3 d	162.4 d	36.8 d	1.05 b	62.9 b
	Significance	**	**	**	*	*

^a Within columns, means followed by the same letter are not significantly different according to Duncan's multiple range test (0.05). Spd: Spermidine, Spm: Spermine, Put: Putrescine, Fe: Iron, PAs: Polyamines.

**, * and ns: Significance at level 0.01%, 0.05% and not significance, respectively.

thereby stimulating the plant growth (Rawia *et al.*, 2011) and contributing to increase the plant height, leaf number, and fresh and dry leaf weight in plants (Mahqob *et al.*, 2011). These findings are consistent with the results obtained in the current research. Similar outcomes were reported in a study conducted by Mirzashahi *et al.* (2017), where no significant difference in plant height was observed between different treatments of iron application from two sources: iron sulfate and iron folate. Considering the involvement of iron compounds in cell division and growth-related processes, an increase in plant height compared to the control group is not unexpected (Cheng *et al.* 2025). Heidarian *et al.* (2011) reported a 36% increase in soybean seed yield as a result of foliar application of iron. Gülser *et al.* (2019) emphasized that the source and dosage of iron intake, along with their interaction, significantly affected the various traits in soybean plants including plant height, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, and leaf number.

The increase in fresh and dry weight observed in plants treated with Put and Spd

can be attributed to the effects of PAs on improving the growth indices, antioxidant properties, and their protective role in maintaining cell stability (Kausano *et al.*, 2008; Hussein *et al.*, 2006). Similarly, the external application of Spd and Put resulted in increased fresh and dry weight of aerial parts in strawberries (Movahed *et al.*, 2012), which is consistent with the findings of the present research.

Nutrient Uptakes

In this study, the application of PAs was found to increase the nutrient uptake in soybean plants. The increased uptake of elements such as Zn, B, Fe, Mg, and N can have several beneficial effects, including enhancing the pigment content, improving the photosynthetic capacity, and promoting overall plant yield. Previous research has also demonstrated the positive effect of foliar spraying with PA hormones on crop yield. For instance, the foliar spraying of saffron leaves with PA hormones resulted in increased branch yield (Ehsanfar *et al.* 2018). In this study, the increased levels of -

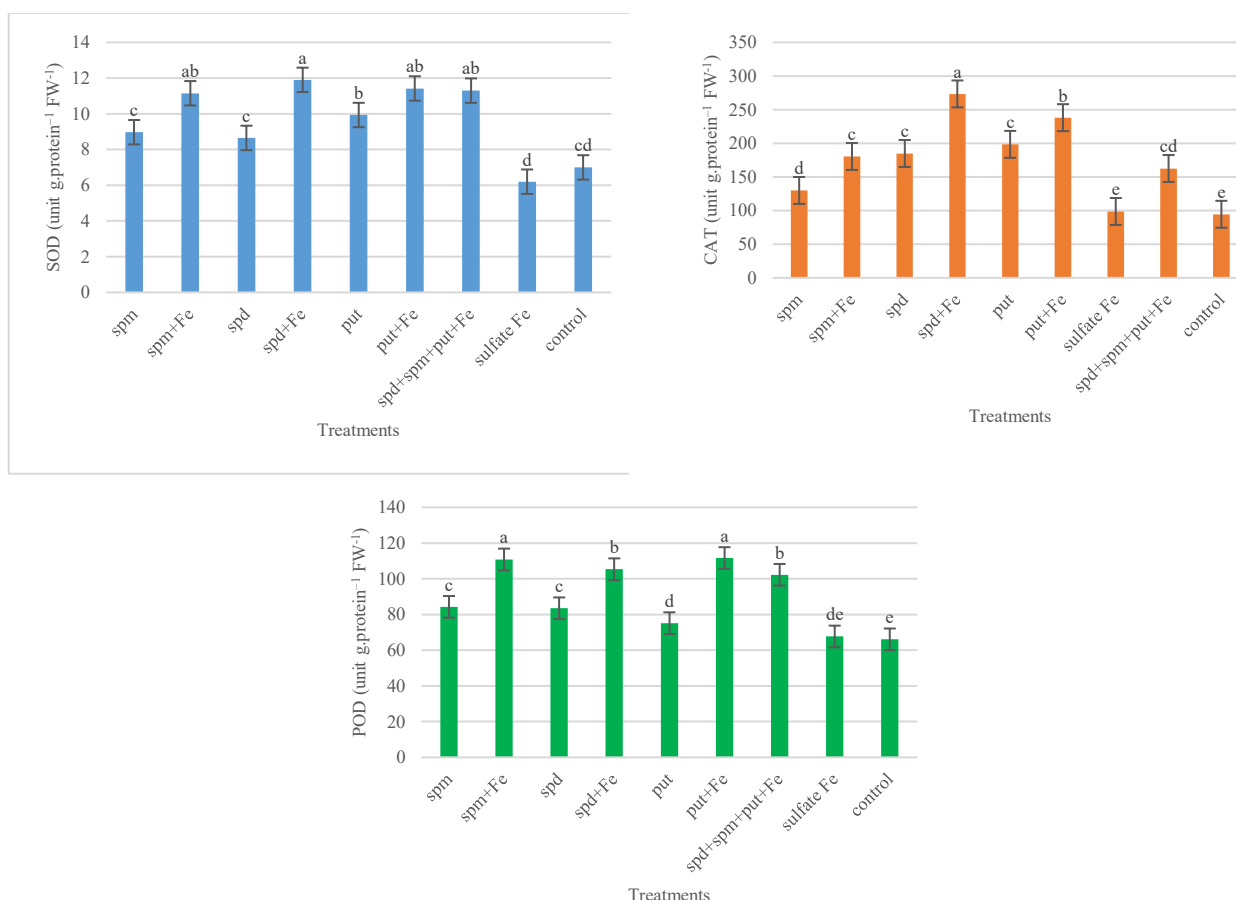


Figure 1. Enzyme activity of soybean under polyamine and iron application.

elements such as K, P, Mg, and Zn observed in plants treated with Spd and foliar sprayed with Put can be another factor contributing to the enhanced growth and seed yield in these treatments, which is consistent with the findings of the present study. Similar outcomes have been reported, where the foliar spraying of Put has led to an increase in specific internal nutrients, such as N, K, P, Fe, and Mg, in marjoram plants (El-Abagy *et al.* 2010).

Seed Quality

In this study, oil and protein contents of soybean seeds are affected by PAs application. Studies on soybean composition have reported variable mean protein and oil contents across different varieties, with

values of 39.8% and 20.5% (Krishna *et al.*, 2003) and 40.2% and 18.3% (Ramteke *et al.*, 2010), respectively. Beyond genetic variation, seed composition is also influenced by positional effects on the plant. Guleria *et al.* (2007) demonstrated that both oil and protein content vary significantly with seed location, finding that seeds at apical positions on the stem axis exhibited higher protein but lower oil content compared to those at basal positions. Consequently, plant architectural traits, such as height, which determines the number and distribution of seed positions, can influence the overall oil and protein content in soybean.

Antioxidant Enzyme Activity

Under stress conditions, plants generate Reactive Oxygen Species (ROS), which can induce oxidative damage. To mitigate this, plants employ a coordinated antioxidant system comprising both non-enzymatic components and enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD) (Abbas *et al.*, 2021; Rashid *et al.*, 2018; Abbas *et al.*, 2022). Evidence indicates that ROS detoxification in response to diverse environmental stresses occurs through a synchronized mechanism (Abbas *et al.*, 2021; Rashid *et al.*, 2018). Within this system, SOD plays a pivotal role by catalyzing the dismutation of Superoxide ($O_2^{\cdot-}$) radicals into Hydrogen Peroxide (H_2O_2) and Oxygen (O_2) (Iftikhar *et al.*, 2022). Nitric Oxide (NO) is a well-established regulator of Fe uptake and homeostasis in plants (Graziano *et al.*, 2002; Graziano and Lamattina, 2007; Chen *et al.*, 2010). Intriguingly, exogenous application of polyamines such as Put, Spd, and Spm rapidly stimulates significant NO production in *Arabidopsis* (Zarza *et al.*, 2019), positioning NO as a central signaling intermediate in polyamine-mediated pathways. Corroborating this, recent findings show that exogenous putrescine induces NO accumulation, which subsequently activates Fe deficiency responses and enhances the remobilization of Fe from the cell wall (Zhu *et al.*, 2016). In the present study, the soybean showed the significant increase in activities of CAT, SOD, and POD that helped the availability of Fe to plant and improved the yield and yield-related traits of soybean.

CONCLUSIONS

The results of this study demonstrate that the combined application of polyamines (PAs) and iron significantly enhanced morphological traits, yield-related parameters, nutrient uptake, and enzyme

activity. Consequently, the synergistic treatment positively influenced all the measured traits, leading to improved yield and yield components. Notably, the treatments involving Spd+Fe, Spm+Fe, and Put+Fe demonstrated the significant effects on the studied traits. Therefore, the combination of PAs with iron can be utilized as foliar application to enhance the crop morphological traits, yield and yield components, and enzyme activity of soybean.

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تأثیر پلی‌آمین و آهن بر جذب آهن، عملکرد و فعالیت آنزیمی سویا

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چکیده

این مطالعه به بررسی اثر پلی‌آمین‌ها، در ترکیب با آهن و بدون آهن، بر عملکرد و اجزای عملکرد سویا در طول فصول زراعی ۱۴۰۰ و ۱۴۰۱ پرداخت. این آزمایش در مزرعه دانشگاه آزاد اسلامی با استفاده از طرح بلوک‌های کامل تصادفی با سه تکرار انجام شد. تیمارها شامل ۲ میلی‌مولار اسپرمین، اسپرمیدین و پوترسین، با ۲ میلی‌مولار آهن و بدون آهن، و همچنین ترکیبی از ۱ میلی‌مولار پلی‌آمین با ۳ میلی‌مولار آهن بودند. علاوه بر این، ۲ میلی‌مولار سولفات آهن و یک تیمار شاهد نیز در نظر گرفته شد. نتایج نشان داد که تیمارها به طور قابل توجهی بر عملکرد و اجزای عملکرد (مانند ارتفاع گیاه، تعداد غلاف، وزن غلاف (با و بدون دانه)، وزن دانه، شاخص برداشت، محتوای پروتئین و روغن دانه، شاخص سطح برگ) و محتوای مواد مغذی از جمله روی، آهن، بور، نیتروژن و منیزیم تأثیر گذاشتند. استفاده از تیمار اسپرمیدین همراه با آهن، وزن دانه را در مقایسه با شاهد ۷۱ درصد افزایش داد. همچنین، کاربرد پلی‌آمین‌ها همراه با آهن منجر به افزایش قابل توجه فعالیت آنزیم‌های سوپراکسید دیسموتاز (۴۴٪)، پراکسیداز (۶۳٪) و کاتالاز (۴۳٪) در مقایسه با شاهد شد. نکته قابل توجه این است که در بین پلی‌آمین‌ها، اسپرمیدین همراه با آهن بیشترین تأثیر را بر صفات مورد مطالعه و فعالیت آنزیم‌ها داشت. این یافته‌ها نشان می‌دهد که کاربرد پلی‌آمین‌ها همراه با آهن می‌تواند عملکرد و اجزای عملکرد سویا را به طور قابل توجهی بهبود بخشد.

Evaluation of Weed Diversity and Density in White Cabbage Fields in Sakarya Province of Turkey: A New Record for *Orobanche* spp.

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ABSTRACT

In 2022–2023, weed surveys were conducted across 73 randomly selected fields in the districts of Adapazarı, Erenler, Kaynarca, and Pamukova, ensuring coverage of at least 1% of the total cabbage cultivation area. These surveys identified 71 weed species. Among the weeds present, *Stellaria media* (L.) Vill. and *Veronica hederifolia* L. were the most dominant in both survey periods. In the first period, *Capsella bursa-pastoris* (L.) Medik. showed the highest density (5.3907), followed by *Sonchus oleraceus* L., *Stellaria media* (L.) Vill., and *Veronica hederifolia* L., all with 100% frequency of occurrence. In the second period, *Stellaria media* had a density of 39.17, while *Veronica hederifolia* had 8.50. Regarding frequency of occurrence, *Portulaca oleracea* L., *Convolvulus arvensis* L., and *Sonchus oleraceus* L. were the most frequent species, with 83.7, 75.1, and 75.0 %, respectively. The study concluded that *Lamium amplexicaule* L. was the most abundant species (in terms of density) in the first period, while *Veronica hederifolia* L. was the most abundant in the second. However, species dominance varied depending on observation time and whether frequency or density was considered. For the first time in Turkey, *Orobanche* spp. was identified as a parasitic weed on white cabbage during the second survey period.

Keywords: Brassicaceae, *Brassica oleracea* var. *capitata*, *Orobanche* spp., Parasitic weed, Weed survey.

INTRODUCTION

White cabbage (*Brassica oleracea* var. *capitata*), a member of the Brassicaceae family, is among the crops native to Turkey (Al-Shehbaz *et al.*, 2006). It holds a significant place in global agriculture, particularly within the widely cultivated *Brassica* genus. Turkey ranks tenth worldwide in white cabbage production, contributing 860,123 tons (FAO, 2024). Valued for its high nutritional content and low caloric value, white cabbage is a staple in healthy diets.

In Turkey, the Marmara Region, especially Sakarya Province, plays an important role in

cabbage production. Sakarya, contributing 7,857 tons annually, nationally ranks seventh. Its favorable location and proximity to major markets enable efficient distribution (TUIK, 2024).

Global demand for white cabbage is rising due to population growth. In countries with limited arable land, maximizing yield per unit area is essential. However, plant protection challenges, particularly weeds, significantly hinder productivity (Mehdizadeh *et al.*, 2025). Weeds compete with crops for nutrients, water, light, and space, reducing yield and quality (Tepe, 2014; Horwath *et al.*, 2023). In cabbage cultivation, as in other crops, weeds pose a major threat (Akshatha *et*

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al., 2018; Thakur *et al.*, 2023), potentially cutting yields by 50% or more if unmanaged (Al-Khatib and Libbey, 1992). Weeds can also degrade market value due to head deformities or reduced size.

Beyond direct competition, weeds can host pests and pathogens, facilitating their spread (Kızılkaya *et al.*, 2001; Akça and Işık, 2016). Pathogens such as *Alternaria*, *Pythium*, and *Sclerotinia* may cause diseases like damping-off or white mold in adjacent cabbage crops, while pests like aphids and whiteflies often shelter and feed among weedy areas.

Numerous weed species have been identified as particularly problematic in vegetable-growing areas. Key examples include *Amaranthus retroflexus*, *Chenopodium album*, *Convolvulus arvensis*, *Echinochloa crus-galli*, *Orabanche* spp., *Setaria verticillata*, *Sinapis arvensis*, and *Solanum nigrum*, all reported to severely reduce crop yields (Aksoy, 2003; Gürbüz and Uygur, 2007; Aksoy and Uygur, 2008).

This study was conducted in 2022 and 2023 in the white cabbage-growing districts of Adapazarı, Erenler, Kaynarca, and Pamukova in Sakarya Province. Weed surveys were performed in two periods to identify prevalent species, their densities, and frequencies. This research represents the first documented weed survey in white cabbage fields in Turkey, providing essential data for developing effective weed management strategies to sustain yield and crop quality.

MATERIALS AND METHODS

This study was conducted in two periods: October 2022 and January 2023, covering key white cabbage-producing districts in Sakarya: Adapazarı, Erenler, Kaynarca, and Pamukova. Districts were selected based on 2022 cabbage cultivation data from TUIK. Fields were randomly chosen to represent at least 1% of the total cultivated area (Bora and Karaca, 1970), and the number of sampled plots per district is provided in Table 1.

In Pamukova, due to the discontinuation of cabbage farming, only one field was available

for sampling. Environmental variables such as field size, soil type, irrigation, and crop management were recorded to aid interpretation of weed distribution patterns. Meteorological data, including temperature, rainfall, and humidity, were obtained from the Sakarya Provincial Directorate of Meteorology for both seasons.

During the 2022 season, Sakarya experienced moderate temperatures and variable rainfall. From March to October, average temperatures ranged from 8.7 (March) to 23.4°C (July–August), with mean maximum temperatures peaking at 29.5°C in July–August. March recorded the lowest minimum temperature at 4.6°C. Rainfall varied between 49.5 mm in July and 78.3 mm in October, with 5.84 to 10.84 rainy days per month.

The data collected in each visited cabbage field included information on the species of weeds present, their densities, and the frequency of occurrence within each sampled area.

Additionally, the density of each weed species was measured by counting the number of individuals per unit area (0.25 m² quadrates). To eliminate edge effects during the survey studies, ¼ m² quadrates were placed diagonally starting from 10-15 meters inside the field. The number of quadrate placements was determined based on the size of the area, with 12 quadrate placed in areas of 0-0.3 ha, 20 quadrate in areas of 0.3-0.7 ha, and 22 quadrate in areas larger than 0.7 ha (Boz *et al.*, 1993; Boz *et al.*, 2000; Sırma and Kadioğlu, 2010). Weeds within the frames were recorded. Care was taken to ensure that the counting points were not too close to each other. The frequency of occurrence of weeds was calculated using the following formula from Odum (1971). The values obtained from the counts of each weed species in a given area were divided by the total area counted to determine the density of weeds (plants m⁻²) (Güncan, 2019). The frequency of occurrence was calculated with the following formula as the percentage of sampled fields in which each species was found. The heat map representing the abundance of weeds in

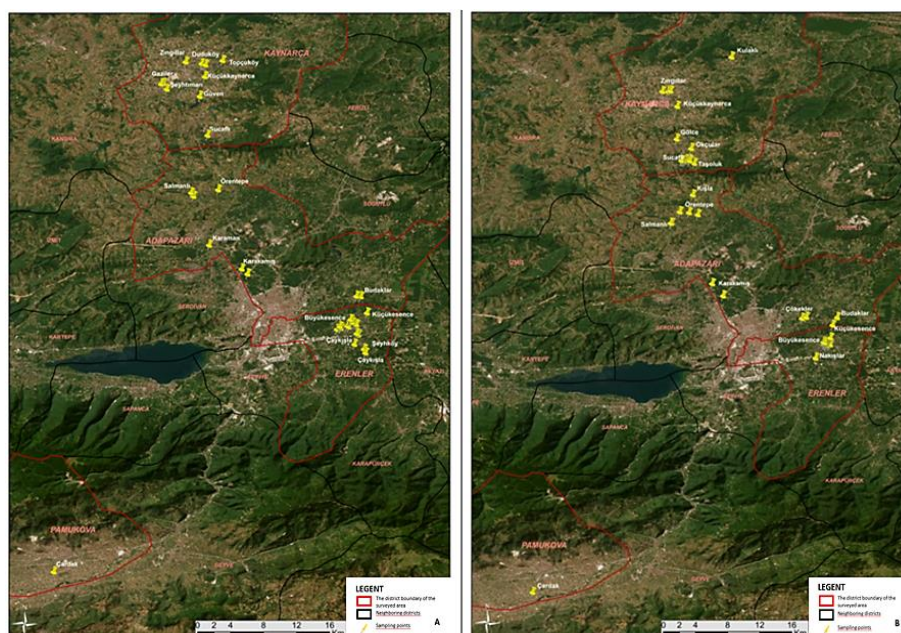


Figure 1. Sampling locations map in white cabbage fields in Sakarya. First period (A) and second period (B).

Table 1. The sampling locations and number of samples collected from white cabbage growing areas in Sakarya Province.

Districts	Number of samples	
	Period 1	Period 2
Adapazarı	8	11
Erenler	15	12
Kaynarca	10	15
Pamukova	1	1
Total	73	

districts were generated with XLSTAT software.

Frequency of Occurance (FO, %) = $n/m \times 100$ (Odum, 1971)

Where, m: Total number of surveyed fields;
n: The number of fields where weed species is detected.

Density = B/n

Where, B: The total number of surveyed fields; n: The number of samples collected.

Most weeds were identified directly in the field at the species level, while others required further identification in the laboratory using herbarium specimens. For this purpose, samples were collected and dried at room temperature, then mounted on sturdy cardboard sheets. Each specimen was labeled with its species name, collection location, date, and relevant data.

Identification was performed using taxonomic keys from Flora of Turkey (Davis, 1965–1989; Hanf, 1983), and Turkish names were based on Uluğ *et al.* (1993).

To characterize weed community diversity in white cabbage fields, three ecological diversity indices were calculated: the Shannon–Wiener Diversity Index (H'), Pielou's Evenness Index (J'), and Margalef's Richness Index (R), following standard ecological methods (Pielou, 1966; Neher and Darby, 2009).

The Shannon–Wiener diversity index was calculated as:

$$H' = -\sum(p_i \times \ln p_i),$$

Where, p_i represents the proportion of individuals belonging to the i th species relative to the total number of individuals recorded in the community.



Pielou's evenness index was calculated as:
 $J' = H'/\ln(S)$,

Where, S represents the Total Number of Species Recorded. This index ranges from 0 to 1, with higher values indicating a more even distribution of individuals among species. Margalef's richness index was calculated as:

$$R = (S - 1)/\ln(N),$$

Where, S represents the Total Number of Species and N represents the Total Number of Individuals Recorded. Higher R values indicate greater species richness relative to the total number of individuals sampled.

RESULTS

As a result of these surveys, a total of 71 weed species belonging to 27 families were identified. Among these, Poaceae ranked first in species diversity with 14 species, followed by Asteraceae with 10 species. Fabaceae, Brassicaceae, and Solanaceae each had five species.

In the first period of the surveys conducted in white cabbage fields across Sakarya Province, a total of 52 weed species were recorded (Table 2). Across Sakarya Province, *Stellaria media* (L.) Vill. showed the highest weed density (35.19 plants m⁻²), followed by *Veronica hederifolia* L. (12.09 plants m⁻²). In terms of Frequency of Occurrence (FO), *V. hederifolia* was recorded in 100% of the surveyed fields, followed by *Lamium amplexicaule* L. with an FO of 93.5%.

At the district level, *Stellaria media* showed the highest density in Adapazarı (25.22 plants m⁻²), while both *V. hederifolia* and *S. media* had a frequency of occurrence (FO) of 100%. In Erenler, *S. media* also had the highest density (32.16 plants m⁻²), whereas *Capsella bursa-pastoris* (L.) Medik. and *V. hederifolia* were recorded in all surveyed fields (FO=100%). In Kaynarca, *V. hederifolia* had the highest density (15.66 plants m⁻²) and was recorded in all surveyed fields (FO = 100%). *Mercurialis annua* L. and *L. amplexicaule* L. also had an FO of 100%. In Pamukova, *S. media* had the highest density (77.66 plants

m⁻²) and occurred in all surveyed fields (FO=100%).

The results of the second-period weed surveys conducted in white cabbage fields across Sakarya province are summarized in Table 3. According to the 2023 growing season data, *S. media* (L.) Vill. had the highest density (39.17 plants m⁻²), followed by *V. hederifolia* L. (8.50 plants m⁻²) Line 141 *Portulaca oleracea* L. (7.55 plants m⁻²). In terms of frequency, *Convolvulus arvensis* L. was the most widespread species with 75.11%, followed by *Sonchus oleraceus* L. (75.07%) and *L. amplexicaule* L. (69.09%).

At the district level, in Adapazarı, *Veronica hederifolia* had the highest density (22.56 plants m⁻²), while *Portulaca oleracea* had 100% frequency. In Erenler, *Stellaria media* dominated in both density (133.63 plants m⁻².) and frequency (100%). In Kaynarca, *Stellaria media* led in density (11.92 plants m⁻²), whereas *Veronica hederifolia* was the most frequent (93.33%). In Pamukova, *C. arvensis* L. was the dominant species with a density of 16.33 plants m⁻² and 100% frequency.

Table 4 categorizes all identified species based on their family, life form, life span, and photosynthetic pathway. According to Table 4, most weeds belonged to Poaceae, Asteraceae, Amaranthaceae, and Brassicaceae. Annual therophytes were dominant, reflecting the disturbed nature of cultivated fields. The majority of species followed the C₃ photosynthetic pathway, while a smaller number (e.g., *Amaranthus retroflexus*, *Portulaca oleracea*, *Setaria* spp.) were C₄ plants, indicating potential competitive advantages under warmer or drier conditions.

In the second survey period conducted in the district of Pamukova, *Orobancha* spp. (broomrape) was detected (Figure 2). This weed was found at a plants m⁻². The incidence in the district is 100%. This is the first time that broomrape has been identified in the cultivation of white cabbage in Turkey.

Table 2. Density and Frequency of Occurrence (FO) of the first season weeds in the districts where white cabbage is intensively cultivated in Sakarya Province.

First period weed species, density and frequency of occurrence in Sakarya Province											
No.	Weed species	Regions								The average density in Sakarya	
		Adapazarı		Erenler		Kaynarca		Pamukova			
		Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO (%)
1.	<i>Abutilon theophrasti</i>	.03	12.5	0	0	0.06	20	0	0	0.0244	8.125
2.	<i>Alopecurus myosuroides</i>	.48	37.5	0.33	20	0.93	70	0	0	0.4399	31.875
3.	<i>Amaranthus retroflexus</i>	.51	12.5	0.10	33.33	0.30	30	6.33	100	1.8141	43.958
4.	<i>Anagallis arvensis</i>		0	0	0	2.42	60	0	0	0.6060	15
5.	<i>Anethum graveolens</i>		0	0.05	13.33	0	0	0	0	0.0127	3.333
6.	<i>Avena fatua</i>	.51	37.5	0.28	20	3.84	80	0	0	1.1637	34.375
7.	<i>Capsella bursa-pastoris</i>	.74	37.5	11.45	100	1.12	20	0	0	5.0798	39.375
8.	<i>Cardamine tenera</i>	.14	12.5	2.05	13.33	0	0	0	0	0.5497	6.458
9.	<i>Chenopodium album</i>	.33	12.5	0.66	53.33	0.48	30	0	0	0.3697	23.958
10.	<i>Cirsium arvense</i>	.59	25	0.66	26.66	0	0	0	0	0.3134	12.916
11.	<i>Cnicus benedictus</i>		0	0.03	13.33	0.18	60	0.66	100	0.2205	43.333
12.	<i>Conium maculatum</i>	.03	12.5	0	0	0.06	20	0	0	0.0244	8.125
13.	<i>Convolvulus arvensis</i>	.11	25	0.50	33.33	0.63	20	0	0	0.3139	19.583
14.	<i>Conyza canadensis</i>	.03	12.5	0	0	0	0	0	0	0.0092	3.125
15.	<i>Crepis sancta</i>	.07	25	0.05	6.66	0.96	40	0	0	0.2736	17.916
16.	<i>Cynodon dactylon</i>	.81	25	0.22	26.66	0.18	10	0	0	0.3042	15.416
17.	<i>Cyperus rotundus</i>		0	0.32	6.66	0	0	0	0	0.0805	1.666
18.	<i>Datura stramonium</i>		0	0	0	0.06	20	0.33	100	0.0984	30
19.	<i>Daucus carota</i>		0	0	0	0.03	10	0	0	0.0075	2.5
20.	<i>Echinochloa crus-galli</i>	.03	12.5	0.01	6.66	0.03	10	0	0	0.0210	7.291
21.	<i>Equisetum arvense</i>		0	0	0	0.15	10	0	0	0.0378	2.5
22.	<i>Euphorbia peplus</i>	.14	25	4.61	80	2.57	90	0	0	1.8335	48.75
23.	<i>Fumaria officinalis</i>	.85	50	0.33	33.33	0.24	30	0	0	0.3583	28.333
24.	<i>Galium aparine</i>	.88	25	0.06	13.33	1.69	40	0	0	0.6634	19.583
25.	<i>Geranium pusillum</i>	.11	25	0.06	20	1.57	70	0	0	0.4386	28.75
26.	<i>Lamium amplexicaule</i>	.40	87.5	2.61	86.66	14.21	100	0.33	100	5.3907	93.541
27.	<i>Lolium temulentum</i>		0	0	0	0.24	20	0	0	0.0606	5
28.	<i>Malva sylvestris</i>	.03	12.5	0	0	0.27	50	0	0	0.0774	15.625
29.	<i>Matricaria chamomilla</i>	.03	12.5	0.01	6.66	0	0	0	0	0.0134	4.791
30.	<i>Medicago sativa</i>		0	0	0	0.42	10	0	0	0.1060	2.5
31.	<i>Mercurialis annua</i>	.51	50	1.06	73.33	4.15	100	0	0	1.4344	55.833
32.	<i>Papaver rhoeas</i>	.88	12.5	0.20	13.33	0.69	40	0	0	0.4473	16.458
33.	<i>Physalis angulata</i>		0	0.05	13.33	0.03	10	0	0	0.0202	5.833
34.	<i>Poa annua</i>	.62	50	0.37	20	1.87	20	0	0	1.4703	22.5
35.	<i>Polygonum aviculare</i>	.40	12.5	5.96	46.66	0	0	0	0	1.5933	14.791
36.	<i>Ranunculus repens</i>	.81	12.5	0.03	13.33	0.21	20	0	0	0.2652	11.458
37.	<i>Raphanus raphanistrum</i>	.11	25	0.18	33.33	0.27	40	0	0	0.1425	24.583
38.	<i>Rumex obtusifolius</i>	.22	25	0.54	60	0.39	50	0	0	0.2896	33.75
39.	<i>Sambucus ebulus</i>		0	0	0	0.0018	10	0	0	0.00045	2.5
40.	<i>Senecio vulgaris</i>	.34	100	0.13	100	0.03	100	0	0	0.125	75
41.	<i>Setaria verticillata</i>		0	0.001	6.666	0	0	0	0	0.00025	1.6665
42.	<i>Setaria viridis</i>	.013	75	0.0074	46.66	0.0018	10	0	0	0.0055	32.915
43.	<i>Solanum nigrum</i>	.0046	25	0	0	0.0075	40	0	0	0.0030	16.25
44.	<i>Sonchus oleraceus</i>	.069	100	0.12	100	0.077	100	0.0018	10	0.066	100
45.	<i>Sorghum halepense</i>		0	0.0021	13.33	0.0037	20	0	0	0.00145	8.33
46.	<i>Stellaria media</i>	.57	100	2.01	100	0.35	100	0.44	100	1.13	100
47.	<i>Taraxacum officinale</i>		0	0.0074	46.66	0.0056	30	0	0	0.0032	19.165
48.	<i>Trifolium</i> spp.		0	0.0042	26.66	0.018	100	0	0	0.0055	31.66
49.	<i>Urtica urens</i>		0	0.0010	6.66	0	0	0	0	0.00025	1.665
50.	<i>Veronica hederifolia</i>	.004	100	0.705	100	0.979	100	0.030	100	0.67	100
51.	<i>Vicia sativa</i>		0	0	0	0.011	60	0	0	0.0027	15
52.	<i>Xanthium strumarium</i>		0	0	0	0.0018	10	0	0	0.00045	2.5

**Table 3.** Density and Frequency of Occurrence (FO) of the second season weeds in the districts where white cabbage was intensively cultivated in Sakarya Province.

Second period weed species, density and frequency of occurrence in Sakarya Province											
No.	Weed species	Regions								Average density in	
		Adapazarı		Erenler		Kaynarca		Pamukova		Sakarya	
		Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO (%)
1.	<i>Abutilon theophrasti</i>	0.40	36.36	0.13	33.33	0.10	13.33	0	0	0.1623	20.7575
2.	<i>Agropyronrepens</i>	1.18	54.54	0	0	0	0	0	0	0.2972	13.6363
3.	<i>Amaranthus retroflexus</i>	0.75	63.63	0.38	58.33	0.07	6.66	1	100	0.5539	57.1590
4.	<i>Anagallis arvensis</i>	0.05	9.09	0.13	25	0.03	13.33	0	0	0.0570	11.8560
5.	<i>Anethum graveolens</i>	0.02	9.09	0	0	0	0	0	0	0.0067	2.2727
6.	<i>Avena fatua</i>	0.27	9.09	0.02	8.33	0.92	66.66	0	0	0.3069	21.0227
7.	<i>Beta vulgaris</i> var. <i>altissima</i>	0.27	27.27	0	0	0	0	0	0	0.0675	6.8181
8.	<i>Capsella bursa-pastoris</i>	2.83	45.45	3	16.66	0.24	20	0	0	1.5208	20.5303
9.	<i>Chenopodium album</i>	0.51	45.45	0.41	50	1.59	46.66	3	100	1.3816	60.5303
10.	<i>Cirsium arvense</i>	0	0	6.11	50	0	0	0.33	100	1.6111	37.5
11.	<i>Cnicus benedictus</i>	0.02	9.09	0	0	0.14	13.33	0.33	100	0.1251	30.6060
12.	<i>Convolvulus arvensis</i>	2.83	45.45	2.91	75	5.98	80	16.33	100	7.0175	75.1136
13.	<i>Crepis sancta</i>	0.10	36.36	0	0	0.15	26.66	0	0	0.0665	15.7575
14.	<i>Cynodon dactylon</i>	0	0	0.16	33.33	0.17	26.66	0.66	100	0.2521	40
15.	<i>Cyperus rotundus</i> L.	0.35	9.09	0	0	0.05	6.66	14.33	100	3.6843	28.9393
16.	<i>Datura stramonium</i>	0.32	27.27	0.36	41.66	0.01	6.66	0	0	0.1757	18.9015
17.	<i>Daucus carota</i>	0	0	0	0	0.26	33.33	0	0	0.0657	8.3333
18.	<i>Digitaria sanguinalis</i>	0.10	27.27	0	0	0.03	6.66	0	0	0.0357	8.4848
19.	<i>Echinochloa crus-galli</i>	1.45	54.54	0.13	25	0.01	6.66	0	0	0.4039	21.5530
20.	<i>Euphorbia peplus</i>	0.13	36.36	0.97	16.66	0.47	40	0	0	0.3952	23.2575
21.	<i>Fumaria officinalis</i>	0.27	27.27	0	0	0.82	33.33	0	0	0.2737	15.1515
22.	<i>Geranium pusillum</i>	0.43	36.36	0.16	8.33	1.73	33.33	0.66	100	0.7506	44.5075
23.	<i>Heliotropium dolosum</i>	0.37	18.18	0.02	8.33	0.01	6.66	1	100	0.3559	33.2954
24.	<i>Hibiscus sabdariffa</i>	0	0	1.36	33.33	0.05	13.33	0.33	100	0.4367	36.6666
25.	<i>Hordeum vulgare</i>	0.51	9.09	0	0	2.43	26.66	0	0	0.7380	8.9393
26.	<i>Lamium amplexicaule</i>	1.02	36.36	4.44	66.66	5.36	73.33	2	100	3.2099	69.0909
27.	<i>Lolium temulentum</i>	0	0	0	0	2.68	6.66	0	0	0.6710	1.6666
28.	<i>Malva sylvestris</i>	0.08	27.27	0	0	0.28	46.66	0	0	0.0904	18.4848
29.	<i>Mentha pulegium</i>	0	0	0	0	0.21	13.33	0	0	0.0526	3.3333
30.	<i>Mercurialis annua</i>	0.40	36.36	2.61	75	5.38	73.33	0	0	2.1006	46.1742
31.	<i>Orobancha</i> spp.	0	0	0	0	0	0	7.66	100	1.9166	25
32.	<i>Papaver rhoeas</i>	0	0	0.02	8.33	0	0	0	0	0.0069	2.0833
33.	<i>Phaseolus vulgaris</i>	0.10	18.18	0	0	0	0	0	0	0.0270	4.5454
34.	<i>Physalis angulata</i>	0.10	9.09	0	0	0.82	26.66	0	0	0.2331	8.9393
35.	<i>Plantago major</i>	0.13	9.09	0	0	0	0	0	0	0.0337	2.2727
36.	<i>Poa annua</i>	0.62	27.27	0.11	25	1.96	66.66	0	0	0.6744	29.7348
37.	<i>Polygonum aviculare</i>	0.05	18.18	0.02	8.33	0.03	6.66	0	0	0.0292	8.2954
38.	<i>Portulaca oleracea</i>	4.62	100	7.02	75	2.57	60	16	100	7.5570	83.75
39.	<i>Raphanus raphanistrum</i>	0.10	18.18	0.02	8.33	0.19	26.66	1	100	0.3322	38.2954
40.	<i>Rumex obtusifolius</i>	0.21	27.27	0.77	41.66	0.15	20	0	0	0.2879	22.2348
41.	<i>Scrophularia nodosa</i>	0.02	9.09	0	0	0	0	0	0	0.0067	2.2727
42.	<i>Senecio vulgaris</i>	0.51	27.27	1.08	41.66	0.01	6.66	0	0	0.4035	18.9015
43.	<i>Setaria verticillata</i>	0	0	0	0	0	0	0.66	100	0.1666	25
44.	<i>Setaria viridis</i>	0.18	18.18	0	0	0	0	0	0	0.0472	4.5454
45.	<i>Sinapis arvensis</i>	0	0	0.13	25	0.61	20	0	0	0.1882	11.25
46.	<i>Solanum nigrum</i>	0.94	72.72	1.08	41.66	0.36	20	0	0	0.5994	33.5984
47.	<i>Solanum tuberosum</i>	0.81	27.27	0	0	0	0	0	0	0.2027	6.8181
48.	<i>Sonchus asper</i>	0.13	27.27	0	0	0.03	13.33	0	0	0.0425	10.1515

Table 3 continued...

Continue of Table 3. Density and Frequency of Occurrence (FO) of the second season weeds in the districts where white cabbage was intensively cultivated in Sakarya Province.

Second period weed species, density and frequency of occurrence in Sakarya Province											
No.	Weed species	Regions								Average density in	
		Adapazari		Erenler		Kaynarca		Pamukova		Sakarya	
		Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO	Plant m ⁻²	FO (%)
49.	<i>Sonchus oleraceus</i>	1.70	63.63	1.41	83.33	1.01	53.33	5	100	2.2842	75.07575
50.	<i>Sorghum halepense</i>	1.27	27.27	1.58	25	0	0	0.66	100	0.8800	38.0681
51.	<i>Spinacia oleracea</i>	1.43	9.09	0	0	0.01	6.66	0	0	0.3624	3.93939
52.	<i>Stellaria media</i>	11.13	90.90	133.63	100	11.92	66.66	0	0	39.1759	64.3939
53.	<i>Taraxacum officinale</i>	0.02	9.09	0	0	0.03	6.66	0	0	0.0155	3.9393
54.	<i>Trifolium</i> spp.	0	0	0	0	0.01	6.66	0	0	0.0043	1.6666
55.	<i>Triticum</i> spp.	0.02	9.09	0	0	1.10	20	0	0	0.2830	7.2727
56.	<i>Urtica urens</i>	0	0	0	0	0.21	6.66	0	0	0.0526	1.6666
57.	<i>Veronica hederifolia</i>	22.56	72.72	2.30	66.66	9.14	93.33	0	0	8.5033	58.1818
58.	<i>Vicia sativa</i>	0	0	0	0	0.03	13.33	0	0	0.0087	3.3333
59.	<i>Vicia faba</i>	0	0	0	0	0.01	6.66	0	0	0.0043	1.6666
60.	<i>Xanthium strumarium</i>	0.40	9.09	0.02	8.33	0.49	13.33	0	0	0.2311	7.6893
61.	<i>Zea mays</i>	0.02	9.09	0	0	0	0	0	0	0.0067	2.2727
49.	<i>Sonchus oleraceus</i>	1.70	63.63	1.41	83.33	1.01	53.33	5	100	2.2842	75.07575
50.	<i>Sorghum halepense</i>	1.27	27.27	1.58	25	0	0	0.66	100	0.8800	38.0681
51.	<i>Spinacia oleracea</i>	1.43	9.09	0	0	0.01	6.66	0	0	0.3624	3.93939
52.	<i>Stellaria media</i>	11.13	90.90	133.63	100	11.92	66.66	0	0	39.1759	64.3939
53.	<i>Taraxacum officinale</i>	0.02	9.09	0	0	0.03	6.66	0	0	0.0155	3.9393
54.	<i>Trifolium</i> spp.	0	0	0	0	0.01	6.66	0	0	0.0043	1.6666
55.	<i>Triticum</i> spp.	0.02	9.09	0	0	1.10	20	0	0	0.2830	7.2727
56.	<i>Urtica urens</i>	0	0	0	0	0.21	6.66	0	0	0.0526	1.6666
57.	<i>Veronica hederifolia</i>	22.56	72.72	2.30	66.66	9.14	93.33	0	0	8.5033	58.1818
58.	<i>Vicia sativa</i>	0	0	0	0	0.03	13.33	0	0	0.0087	3.3333
59.	<i>Vicia faba</i>	0	0	0	0	0.01	6.66	0	0	0.0043	1.6666
60.	<i>Xanthium strumarium</i>	0.40	9.09	0.02	8.33	0.49	13.33	0	0	0.2311	7.6893
61.	<i>Zea mays</i>	0.02	9.09	0	0	0	0	0	0	0.0067	2.2727



Figure 2. Orobanche on non-harvested (A) and harvested (B) cabbage plants.

**Table 4.** The family, life form, life span, and photosynthetic pathway of weeds identified in cabbage fields.

Plant Name	Familya	Life form ^a	Life span ^b	C3/C4
<i>Abutilon theophrasti</i>	Malvaceae	Th	A	C4
<i>Agropyron repens</i>	Poaceae	G	P	C3
<i>Alopecurus myosuroides</i>	Poaceae	Th	A	C3
<i>Amaranthus retroflexus</i>	Amaranthaceae	Th	A	C4
<i>Anagallis arvensis</i>	Primulaceae	Th	A	C3
<i>Anethum graveolens</i>	Apiaceae	Th	A	C3
<i>Avena fatua</i>	Poaceae	Th	A	C3
<i>Beta vulgaris</i> var. <i>altissima</i>	Amaranthaceae	H	Bi	C3
<i>Capsella bursa-pastoris</i>	Brassicaceae	Th	A	C3
<i>Cardamine tenera</i>	Brassicaceae	Th	A	C3
<i>Chenopodium album</i>	Amaranthaceae	Th	A	C3
<i>Cirsium arvense</i>	Asteraceae	H	P	C3
<i>Cnicus benedictus</i>	Asteraceae	Th	A	C3
<i>Conium maculatum</i>	Apiaceae	Th	A/Bi	C3
<i>Convolvulus arvensis</i>	Convolvulaceae	H	P	C3
<i>Conyza canadensis</i>	Asteraceae	Th	A	C3
<i>Crepis sancta</i>	Asteraceae	Th	A	C3
<i>Cynodon dactylon</i>	Poaceae	G	P	C4
<i>Cyperus rotundus</i>	Cyperaceae	G	P	C4
<i>Datura stramonium</i>	Solanaceae	Th	A	C3
<i>Daucus carota</i>	Apiaceae	H	Bi	C3
<i>Digitaria sanguinalis</i>	Poaceae	Th	A	C4
<i>Echinochloa crus-galli</i>	Poaceae	Th	A	C4
<i>Equisetum arvense</i>	Equisetaceae	G	P	C3
<i>Euphorbia peplus</i>	Euphorbiaceae	Th	A	C3
<i>Fumaria officinalis</i>	Papaveraceae	Th	A	C3
<i>Galium aparine</i>	Rubiaceae	Th	A	C3
<i>Geranium pusillum</i>	Geraniaceae	Th	A	C3
<i>Heliotropium dosolum</i>	Boraginaceae	Th	A	C3
<i>Hibiscus sabdariffa</i>	Malvaceae	Th	A	C3
<i>Hordeum vulgare</i>	Poaceae	Th	A	C3
<i>Lamium amplexicaule</i>	Lamiaceae	Th	A	C3
<i>Lolium temulentum</i>	Poaceae	Th	A	C3
<i>Malva sylvestris</i>	Malvaceae	H	Bi	C3
<i>Matricaria chamomilla</i>	Asteraceae	Th	A	C3
<i>Medicago sativa</i>	Fabaceae	H	P	C3
<i>Mentha pulegium</i>	Lamiaceae	H	P	C3
<i>Mercurialis annua</i>	Euphorbiaceae	Th	A	C3
<i>Orobancha</i> spp.	Orobanchaceae	Holoparasite	A	C3
<i>Papaver rhoeas</i>	Papaveraceae	Th	A	C3
<i>Phaseolus vulgaris</i>	Fabaceae	Th	A	C3
<i>Physalis angulata</i>	Solanaceae	Th	A	C3
<i>Plantago major</i>	Plantaginaceae	H	P	C3
<i>Poa annua</i>	Poaceae	Th	A	C3
<i>Polygonum aviculare</i>	Polygonaceae	Th	A	C3
<i>Portulaca oleracea</i>	Portulacaceae	Th	A	C4
<i>Ranunculus repens</i>	Ranunculaceae	H	P	C3
<i>Raphanus raphanistrum</i>	Brassicaceae	Th	A	C3
<i>Rumex obtusifolius</i>	Polygonaceae	H	P	C3
<i>Sambucus ebulus</i>	Adoxaceae	G	P	C3
<i>Scrophularia nodosa</i>	Scrophulariaceae	G	P	C3

^aTh: Therophyte, G: Geophyte, H: Halophyte. ^b A: Annual, B: Biannual, P: Perannual.

Table 4 continued...

Continued of Table 4. The family, life form, life span, and photosynthetic pathway of weeds identified in cabbage fields.

Plant Name	Family ^a	Life form ^a	Life span ^b	C3/C4
<i>Senecio vulgaris</i>	Asteraceae	Th	A	C3
<i>Setaria verticillata</i>	Poaceae	Th	A	C4
<i>Setaria viridis</i>	Poaceae	Th	A	C4
<i>Sinapis arvensis</i>	Brassicaceae	Th	A	C3
<i>Solanum nigrum</i>	Solanaceae	Th	A	C3
<i>Solanum tuberosum</i>	Solanaceae	G	P	C3
<i>Sonchus asper</i>	Asteraceae	Th	A	C3
<i>Sonchus oleraceus</i>	Asteraceae	Th	A	C3
<i>Sorghum halepense</i>	Poaceae	G	P	C4
<i>Spinacia oleracea</i>	Amaranthaceae	Th	A	C3
<i>Stellaria media</i>	Caryophyllaceae	Th	A	C3
<i>Taraxacum officinale</i>	Asteraceae	H	P	C3
<i>Trifolium</i> spp.	Fabaceae	H	A/P	C3
<i>Triticum</i> spp.	Poaceae	Th	A	C3
<i>Urtica urens</i>	Urticaceae	Th	A	C3
<i>Veronica hederifolia</i>	Plantaginaceae	Th	A	C3
<i>Vicia faba</i>	Fabaceae	Th	A	C3
<i>Vicia sativa</i>	Fabaceae	Th	A	C3
<i>Xanthium strumarium</i>	Asteraceae	Th	A	C3
<i>Zea mays</i>	Poaceae	Th	A	C4

^aTh: Therophyte, G: Geophyte, H: Halophyte. ^b A: Annual, B: Biannual, P: Perannual.

The heat map generated with XLSTAT represents weed abundance in surveyed fields in Sakarya. The colors black, red, and green indicate different population densities. In the map red tones represent low density (Figure 4).

The bar charts in Figure 4 illustrate the biodiversity indices in four districts. Shannon Index (H') values for biodiversity in four districts: Adapazarı, Erenler, Kaynarca and Pamukova. The Shannon Index measures species diversity, with higher values indicating greater biodiversity. Pamukova has the highest Shannon Index (2.08), represented by the letter indicating that its biodiversity is significantly higher compared to the other districts. Both Adapazarı (1.757) and Kaynarca (1.717) have similar Shannon Index values, indicating no significant difference in their biodiversity levels. However, these values are lower than those of Pamukova. Erenler has the lowest Shannon Index (1.036), indicating significantly lower biodiversity compared to the other districts. Overall, the graph emphasizes that Pamukova has the highest biodiversity while Erenler has

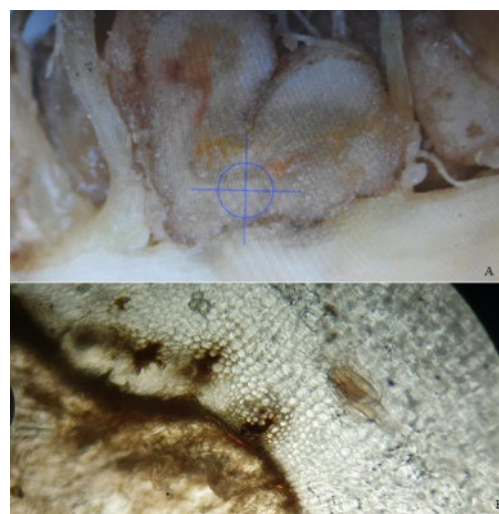


Figure 3. Stereomicroscopic and microscopic evidence of *Orobanche* sp. attachment to the root of white cabbage (*Brassica oleracea* var. *capitata*). (A) Stereomicroscopic view showing the attachment site of the parasite on the host root and the haustorial connection region. (B) Microscopic view of the host-parasite interface, showing close tissue association at the attachment site.

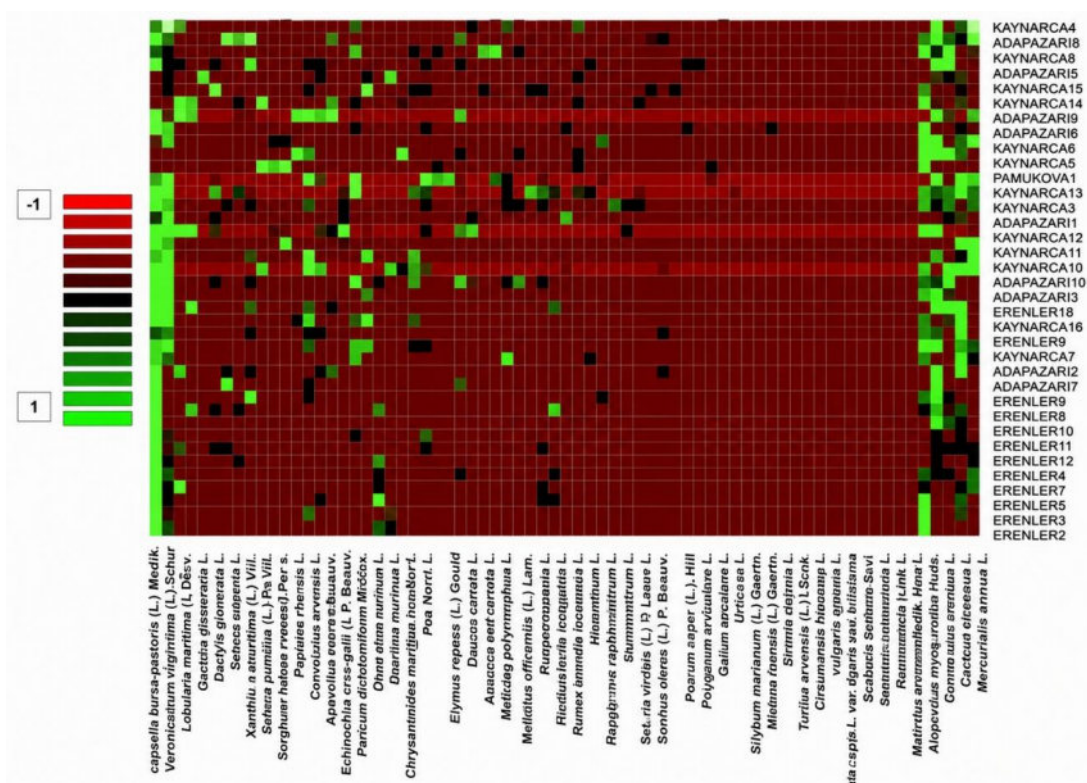


Figure 4. Heat map showing weed abundance in survey areas.

the lowest, with Adapazarı and Kaynarca in between (Figure 5).

DISCUSSION

Sustainable weed management is becoming increasingly important, as weeds cause significant yield and quality losses across all agricultural sectors. Effective control depends on understanding weed biology, distribution, host relationships, and competitiveness. Although numerous studies focus on major crops, research on weeds in white cabbage fields is limited in Turkey, despite its role in crop rotation and allelopathic potential. In this pioneering study, 73 fields were surveyed over two growing seasons (2022–2023), marking the first weed survey in Turkey's white cabbage production areas.

During the surveys in Sakarya, 71 weed species belonging to 27 families were identified, reflecting a diverse and complex

weed community that may affect crop performance. These findings are consistent with those of Hwang *et al.* (2014), who recorded 71 weed species belonging to 25 families in China, comprising 39 annual, 16 biennial, and 16 perennial species. In their study, the most species-rich families were Compositae (20 species), Poaceae (8), Cruciferae (6), and Polygonaceae (5). Furthermore, ten dominant weed species belonging to six families accounted for 50% of the total, with *Portulaca oleracea* (8.07%), *Digitaria ciliaris* (7.54%), and *Chenopodium album* (5.73%) among the most prevalent species.

In Sakarya, *S. media* (39.17%) and *V. hederifolia* (100% frequency) were notably competitive. *S. media* is known for competing strongly with plant, reducing crop yield and quality (Kumar *et al.*, 2021), while *V. hederifolia*'s adaptability makes it a persistent problem (Bourgeois *et al.*, 2019). Their dominance, especially in the first

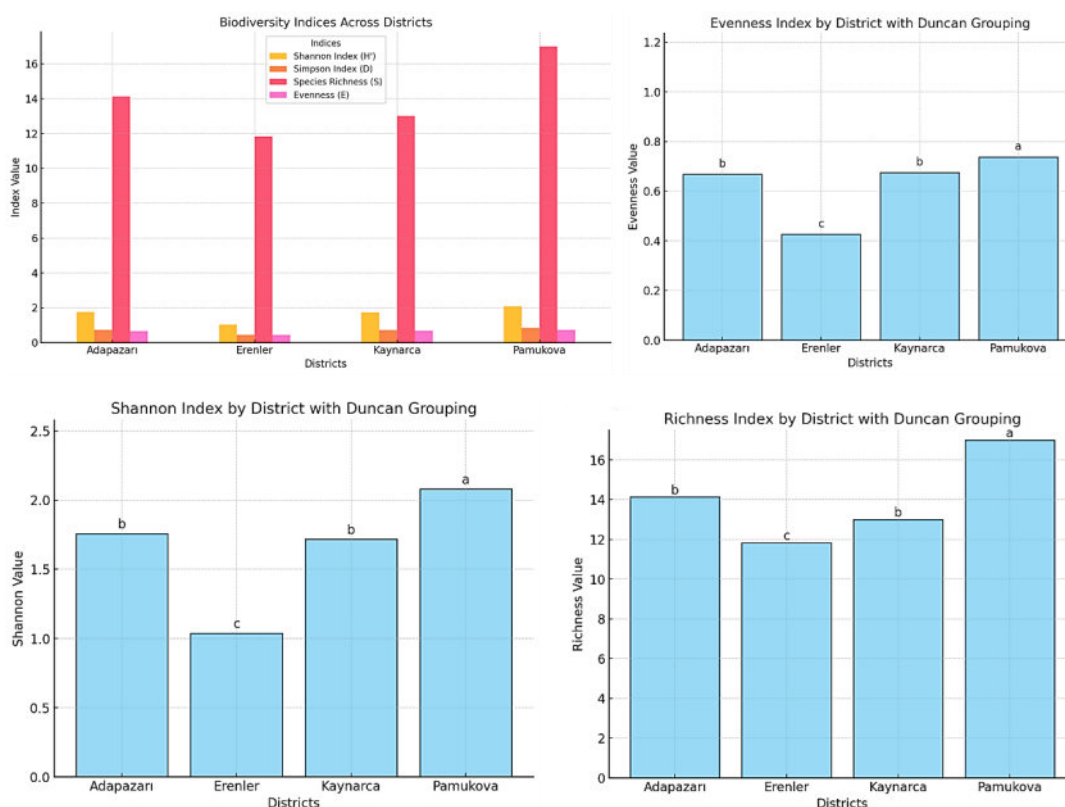


Figure 5. Biodiversity indice values comparisons and Duncan results. Bars followed by different lowercase letters (a–c) are significantly different according to Duncan's multiple range test at $P < 0.05$, whereas bars sharing the same letter are not significantly different.

period, where both appeared in all plots, underscores the importance of Integrated Weed Management (IWM), which combines mechanical, cultural, and chemical methods (Buhler, 2016).

In the second survey, *V. hederifolia* had the highest density in Adapazari (22.56 plants m^{-2}), and *Portulaca oleracea* was present in 100% of plots. This aligns with Çal Büyükdemir and Kara (2020), who reported its prevalence in sugar beet due to drought tolerance and high seed production. In Erenler, *S. media* showed the highest density (133.63 plants m^{-2}), and frequency (100%), consistent with its resilience across various soils. Holm *et al.* (1997) also reported its dominance in cooler climates, as seen in Kaynarca. In Pamukova, *Convolvulus arvensis* was both dense (16.33) and widespread (100%), confirming its

persistence as an invasive perennial with a deep root system (Heap, 2023).

District-level differences were evident, such as the dominance of *V. hederifolia* in Kaynarca and the exceptional density of *S. media* in Pamukova. This spatial variability highlights the need for localized management strategies suited to specific ecological conditions (Liebman and Davis, 2000). In Erenler, *C. bursa-pastoris* also reached 100% frequency and is known to host pests that affect cabbage health and yield (Storkey *et al.*, 2012).

Overall, *S. media* and *V. hederifolia* were the most prevalent weeds in white cabbage fields. Similar observations were made by Oerke (2006) in Germany, where *C. album* and *Sinapis arvensis* were dominant. This suggests regional weed dominance varies based on environmental and agricultural practices. Likewise, Veisi *et al.* (2020) in Iran



identified *V. hederifolia* as a major weed in vegetable crops, confirming its adaptability across different cropping systems.

Another significant outcome of the study was the first confirmed detection of *Orobanch* spp. parasitizing white cabbage in Turkey, marking a novel host-parasite relationship. Laboratory analysis verified its root attachment, suggesting risks of nutrient depletion and yield loss. Similar associations have been reported in other *Brassica* crops globally (Qasem and Foy, 2007; Piowarczyk, 2012); and recent studies note the expanding host range of *Orobanch* spp. (Kaçan, 2019; Chedadi *et al.*, 2021; Güzel and Doğan, 2022).

CONCLUSIONS

This study presents the first weed survey in white cabbage fields in Turkey, identifying 71 species from 27 families across Sakarya Province. *S. media* and *V. hederifolia* were the most dominant weeds, with significant densities and frequencies across districts. The results emphasize the need for localized integrated weed management strategies.

A major finding was the first detection of *Orobanch* spp. parasitizing white cabbage in Turkey, indicating a novel host-parasite relationship. Given its potential to cause significant yield losses, this finding underscores the importance of regular monitoring and effective control measures. Overall, the study contributes valuable baseline data for sustainable weed management in white cabbage production systems.

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ارزیابی تنوع و تراکم علف‌های هرز در مزارع کلم سفید در استان ساکاریای ترکیه: گزارشی
جدید از گونه‌های ***Orobanché***

بهادیر شین، و او مر اومیت اوکچو

چکیده

در سال‌های ۲۰۲۲-۲۰۲۳، به‌منظور اطمینان از پوشش حداقل ۱ درصد از کل سطح زیر کشت کلم، بررسی‌هایی در خصوص علف‌های هرز در ۷۳ مزرعه که به‌صورت تصادفی در مناطق آداپازاری (Adapazarı)، ارنلر (Erenler)، کاینارجا (Kaynarca) و پاموکوا (Pamukova) انتخاب شده بودند، انجام شد. در این بررسی‌ها، ۷۱ گونه علف هرز شناسایی شد. در میان علف‌های هرز موجود، گونه‌های *Veronica* و *Stellaria media* (L.) Vill. *hederifolia* L. در هر دو دوره بررسی، غالب‌ترین گونه‌ها بودند. در دوره نخست، گونه *Capsella bursa-pastoris* (L.) Medik بیشترین تراکم (۵/۳۹۰۷) را نشان داد و پس از آن گونه‌های *Sonchus oleraceus* L.، *Stellaria media* (L.) Vill. و *Veronica hederifolia* L. قرار گرفتند که همگی دارای فراوانی وقوع ۱۰۰ درصد بودند. در دوره دوم، تراکم گونه *Stellaria media* برابر با ۳۹/۱۷ و تراکم گونه *Veronica hederifolia* برابر با ۸/۵۰ بود. از نظر فراوانی وقوع، گونه‌های *Portulaca oleracea* L.، *Convolvulus arvensis* L. و *Sonchus oleraceus* L. با مقادیر به‌ترتیب ۸۳/۷، ۷۵/۱ و ۷۵/۰ درصد، بیشترین فراوانی را داشتند. این مطالعه به این نتیجه رسید که گونه *Lamium amplexicaule* L. در دوره اول (از نظر تراکم) فراوان‌ترین گونه بوده است، در حالی که گونه **Veronica hederifolia* L. در دوره دوم بیشترین فراوانی را داشته است. با این حال، گونه غالب بسته به زمان مشاهده و اینکه فراوانی یا تراکم مد نظر قرار گرفته باشد، متفاوت بود. برای نخستین بار در ترکیه، گونه‌های ***Orobanché*** در دومین دوره پایش، به عنوان علف هرز انگل روی کلم سفید شناسایی شدند.

Polymorphisms of the Self-Incompatibility Locus of Sweet Cherry (*Prunus avium* L.) Species from the VNIISPK Bio-Resource Collection

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ABSTRACT

Self-incompatibility is a common evolutionary mechanism of flowering plants. Sweet cherry (*Prunus avium* L.) has a gametophytic type of self-incompatibility. Most of sweet cherry cultivars are self-incompatible, although self-compatible genotypes also occur. This study presents data of S genotype investigation of 39 sweet cherry cultivars from VNIISPK bio-resource collection. To identify S genotypes, PCR with consensus (PaConsI and PaConsII) and allele-specific (S₁, S₂, S₃, S₄, S₅, S₆, S₇, S₈, S₉, S₁₀, S₁₁, S₁₂, S₁₃, S₁₄ and S₁₆) primers was done. The S genotype was fully identified for 33 cultivars. Then, 7 S alleles (S₁, S₃, S₄, S₅, S₆, S₉, and S₁₃) were detected in the genomes of the investigated sweet cherries. According to the results of allele-specific amplification, 'Venera' (S₁/S₄), 'Zolotuhinskaya' (S₄/S₆) and 'Viskochka' (S₃/S₄) were suspected to have unique or mutant alleles of the gene. The cultivars with fully determined S-genotypes were distributed into nine incompatibility groups (III, VI, VII, IX, XV, XVII, XIX, XXI, and XLV).

Keywords: Allele-specific primers, Consensus primers, Allele-specific primers.

INTRODUCTION

Self-incompatibility is one of the most important and widespread mechanisms used by flowering plants to prevent self-fertilization and, consequently, to provide the genetic diversity of populations. Also, sweet cherry, like other fruit crops, has too high commercial value and getting high yields is the most important task.

Sweet cherry, as the other Rosacea, has a gametophytic type of self-incompatibility and the success of its pollination is dependent on the interaction of two genes, S-Allele Specific Ribo-nuclease (S-RNase) and S Haplotype-Specific F-Box Protein (SFB) (Yamane *et al.*, 2003). S-RNase gene presented with numerous alleles, expresses into stylar tissue and non-selectively takes up into the cytoplasm of the pollen tube.

Fertilization is possible only when S-RNases of pistil and pollen are different. In opposite way, SFB triggers off cytotoxicity of self S-RNase and the pollen tube growth is arrested. To explain specific inhibition of the growth of self-pollen tubes, two models of genes interaction, an inhibitor model and a receptor model, have been proposed. Presently, the inhibitor model is the most favoured. According to this model, all S-RNases enter pollen tubes, but inhibitors in the pollen tube cytoplasm inhibit the activity of non-self S-RNases, as opposed to the self S-RNase. So, S locus F-box like protein and S haplo-type-specific F-box like protein are considered as good candidates for the role of some general inhibitor molecule to take place in detoxification of non-self S-RNase (Wang *et al.*, 2003; Matsumoto *et al.*, 2019).

To determine the S-RNase gene

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polymorphism, a set of primers for amplification of both variable and high conserved fragments were designed that made possible the PCR-based identification of *S* alleles (Tao *et al.*, 1999; Wiersma *et al.*, 2001; Sonneveld *et al.*, 2001; Sonneveld *et al.*, 2003). The development of PCR-based methods stimulated the identification of numerous *S* alleles and determination of the *S*-genotype of a wide number of sweet cherry cultivars. As a result, alleles *S*₁ to *S*₁₆ (Wiersma *et al.*, 2001; Sonneveld *et al.*, 2001; Sonneveld *et al.*, 2003), *S*₁₇ to *S*₂₂ (De Cuyper *et al.*, 2005; Schuster *et al.*, 2007; Gisbert *et al.*, 2008), *S*₂₄ (Wunsch *et al.*, 2004), *S*₂₇, *S*₃₀ (Vaughan *et al.*, 2008) and *S*₃₇, *S*₃₈ (Szikriszt *et al.*, 2013) were described.

The reported results of the *S*-alleles identifications were summarized and analyzed, and sweet cherry genotypes were grouped on the basis of the received PCR data (Tobutt *et al.*, 2001; Tobutt *et al.*, 2004; Schuster, 2012, 2017, 2020; 2024).

The last overview of self-incompatibility genotypes of cultivated sweet cherries was published by Schuster in 2024, containing information on 1,700 genotypes and 26 different alleles (*S*₁, *S*₂, *S*₃, *S*₄, *S*₅, *S*₆, *S*₇, *S*₉, *S*₁₀, *S*₁₂, *S*₁₃, *S*₁₄, *S*₁₆, *S*₁₇, *S*₁₈, *S*₁₉, *S*₂₁, *S*₂₂, *S*₂₄, *S*₂₇, *S*₃₀, *S*₃₇, *S*₃₈ and three mutated alleles *S*_{3'}, *S*_{4'}, *S*_{5'}). Some 1583 genotypes were divided into 72 self-incompatibility groups. Twenty-six cultivars having a unique *S* allele combination were included in group 0 (universal donors). Ninety-one self-compatible genotypes were included in group SC (Self-Compatible) (Schuster *et al.*, 2024).

In this study, to estimate the *S*-*RNases* gene polymorphisms of cultivars from the VNIISPK sweet cherry collection, PCR was performed with consensus (PaConsI and PaConsII) and allele-specific (*S*₁, *S*₂, *S*₃, *S*₄, *S*₅, *S*₆, *S*₇, *S*₉, *S*₁₀, *S*₁₂, *S*₁₃, *S*₁₄, and *S*₁₆) primers. A total of 39 sweet cherry genotypes were investigated. For 33 of them, *S*-genotype was determined completely, and partially for 4 cultivars. In the case of two cultivars, 'Bahor' and

'Naslazhdenie', PCR with allele-specific primers was fruitless. So, we could conclude that none of the tested *S* alleles was present in their genomes. Also, surprisingly, in the result of amplification with primers specific to *S*₁ and *S*₄ alleles, the size of the received PCR products was different from what was expected for the three cultivars. There are, probably, two not yet described alleles present in these genomes. In the genomes of the investigated cultivars, the presence of 7 *S* alleles (*S*₁, *S*₃, *S*₄, *S*₅, *S*₆, *S*₉, and *S*₁₃) was estimated. Cultivars with fully determined *S*-genotypes belong to nine incompatibility groups (III, VI, VII, IX, XV, XVII, XIX, XXI, and XLV).

MATERIALS AND METHODS

Thirty-nine genotypes, cultivars and selections, from VNIISPK sweet cherry collection were studied in this work (Table 1). The majority of cultivars originate from Russia, but some of them are from Ukraine or Belarus.

Genomic DNA was extracted from young leaves by CTAB method, with modifications for tissue containing high polysaccharide and polyphenol components (Porebski *et al.*, 1997).

To investigate polymorphisms of the *S*-*RNases* gene of chosen sweet cherry genotypes, PCR with consensus (PaConsI and PaConsII) (Sonneveld *et al.*, 2003) and allele-specific (*S*₁, *S*₂, *S*₃, *S*₄, *S*₅, *S*₆, *S*₇, *S*₉, *S*₁₀, *S*₁₂, *S*₁₃, *S*₁₄ and *S*₁₆) (Sonneveld *et al.*, 2001; Sonneveld *et al.*, 2003) primers were performed. For amplification, set of PCR reagents with Hot Start Taq polymerase (Dia-m, Kat. KH017-002-21, Russia) was used. PCR was carried out in 25 µL total reaction mix containing 50 ng of genomic DNA, PCR reaction buffer supplemented with 2 mM MgCl₂, 1U of Taq polymerase, 0.5 mM dNTP and 0.5 µM of each primer. PCR conditions were: Initial denaturation at 95°C for 5 minutes, 35 cycles at 95°C for 30 seconds, primer annealing temperature 55-58°C for 30 seconds, elongation at 72°C for

1 minute, followed by a final elongation at 72 °C for 5 min.

Amplification products were analysed by 1.4 % (w/v) agarose gel and electrophoresis was stained with ethidium bromide and observed under ultraviolet light. Size of amplification products were estimated visually by comparison with markers of molecular weight Step50 plus (Biolabmix, Russia) and Step100 Long (Biolabmix, Russia).

RESULTS

This study presents the results of investigation of the *S*-genotype of 39 cultivars from sweet cherry VNIISPK collection. VNIISPK (Russian Research Institute of Fruit Crop Breeding) is one of the oldest horticultural institutions in Russia, founded in 1845 (<https://vniispk.ru>). The bio-resource collection of the institute includes fruit, berry, and ornamental crops. At present, the bio-resource collection of the VNIISPK includes more than 1800 varieties, more than 2500 elite and selected forms, and hybrid fund is about 25 thousand genotypes (<https://vniispk.ru/pages/unu>).

Most of the tested sweet cherry cultivars originate from Russia. Four cultivars, namely, ‘Gostinets’, ‘Sopernitsa’, ‘Medunitsa’, and ‘Minchanka’, originate from Belarus. Another 4 cultivars, i.e. ‘DonetskiiVelikan’, ‘Vasilisa’, ‘Alenushka’, and ‘Naslazhdenie’, originate from Ukraine. Also, one cultivar from a private collection from Ukraine (‘Sweet Cherry from Donetsk’) was tested.

At the first step, PCR was performed with consensus primers PaConsI and PaConsII created Sonneveld (Sonneveld *et al.*, 2003). These two pairs of primers were designed to amplify regions of the two introns of the *S-RNase* gene. In the result of amplification with consensus primers, a set of PCR products ranged from 303 to 523 bp for PaConsI primers, and from 577 to 2,383 bp for PaConsII primers (Sonneveld *et al.*, 2003). Application of consensus primers

allows identifying some alleles in genomes, including *S*₃ and *S*₆ alleles, and predicting other alleles. Exploitation of the consensus primers gives an opportunity to reduce the number of checked alleles. But, allele-specific amplification is necessary to avoid mistakes.

In this study, the *S*₃ allele was detected using PaConsI primers in the genomes of 21 tested sweet cherries. The *S*₆ allele was identified using PaConsII primers in the genomes of 19 sweet cherries (Table 1). Received data were confirmed by *S*₃ and *S*₆ allele-specific amplification.

Based on data of PCR with consensus primers, amplification with *S*₁, *S*₂, *S*₄, *S*₅, *S*₇, *S*₉, *S*₁₀, *S*₁₂, *S*₁₄ and *S*₁₆ allele specific primers was performed.

The alleles *S*₁, *S*₂, *S*₅, *S*₉, and *S*₁₀ more frequently occur in genomes of sweet cherries and that is why they were tested at first. As a result of the analysis, only alleles *S*₁, *S*₄, *S*₅ and *S*₉ were detected in the genomes of the investigated cultivars. The *S*₄ allele was detected in the genomes of 16 genotypes. The *S*₁, *S*₅, and *S*₉ alleles were present in the genomes of few sweet cherry cultivars (*S*₁ allele in 2 cultivars, *S*₅ allele in 5 cultivars, and the *S*₉ allele was detected in 1 cultivar) (Table 1). Surprisingly, in the result of amplification with primers specific to *S*₁ and *S*₄ alleles, the size of the three PCR products, i.e. one in amplification with *S*₁ primers and two with *S*₄ primers, was different from the expected one (Figure 1). The expected size of PCR products after allele-specific amplification with *S*₁ and *S*₄ primers, was 820 b.p. for both primer pairs. The amplification with *S*₁ primers with the DNA of ‘Venera’ cultivar results in the PCR product size approximately 100 bp less than the predicted one. Also, the amplification with *S*₄ allele specific primers with DNA of ‘Zolotuhinskaya’ and ‘Viskochka’ results in synthesis of PCR product size approximately 200 bp less than what was expected. In these two cases, specific amplification took place. To avoid mistakes, PCRs were repeated. In the case of *S*₄ allele specific PCR, the size of amplified products with DNA

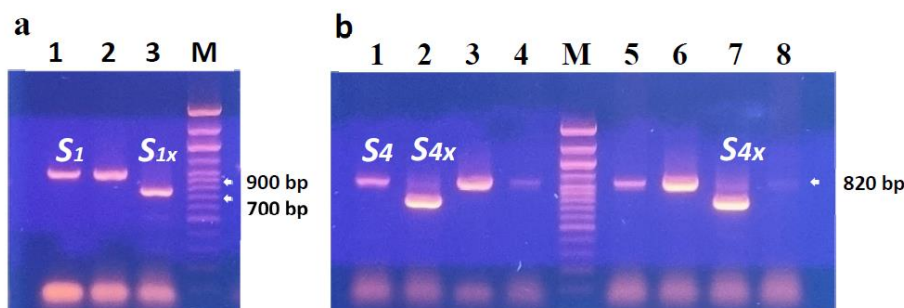


Figure 1. PCR amplification of cultivars used S_1 and S_4 allele specific primers: (a) PCR with S_1 allele specific primers. 1–‘BrianskayaRosovoya’, 2–‘Gostinits’, 3– ‘Venera’, M- markers of molecular weight Step100 Long (Biolabmix, Russia); (b) PCR with S_4 allele specific primers. 1–‘Irinka’, 2– ‘Zolotuhinskaya’, 3–‘Medunitsa’, 4–‘PamiatiZhukova’, 5– ‘Lubimitsa Astahova’, 6– ‘Alenushka’, 7– ‘Viskochka’, 8– ‘PamiatiChernishevskogo’, M- Markers of molecular weight Step100 Long (Biolabmix, Russia).

‘Zolotuhinskaya’ and ‘Viskochka’ was identical, which also excludes mistakes of amplification.

The results of the amplification using consensus primers PaConsI and PaConsII of these genotypes were also not entirely typical. No PCR product was detected after amplification with PaConsI primers using DNA of ‘Zolotuhinskaya’ cultivar (Figure 2). The size of the PCR product after amplification with Venera DNA was around 500 bp, which could suggest the presence of multiple alleles, including S_1 , S_4 and S_6 . The amplification with DNA from ‘Viskochka’ resulted in two PCR products, one approximately 300 bp and the other around 500 bp (Figure 2). The detection of a 300 b.p. PCR product indicates the presence of the S_3 allele in the genome. This was also

confirmed by allele-specific amplification. Thus, the sizes of the PCR products amplified using PaConsI primers corresponded to alleles S_1 , S_3 , S_4 and S_6 . The only unexpected result was the absence of PCR products when ‘Zolotuhinskaya’ DNA was used as the template.

As expected, the sizes of the PCR products obtained using PaConsII primers corresponded to alleles S_1 , S_3 , S_4 and S_6 (Figure 3). The detection of a 570 bp product indicated the presence of allele S_6 in the ‘Zolotuhinskaya’ genome. Products with sizes of 800–900 bp indicated the presence of alleles S_1 and S_3 , and products with sizes of 900–1,000 bp indicated the presence of alleles S_3 and S_4 (Sonneveld *et al.*, 2003). All results were rechecked and refined using allele-specific primers.

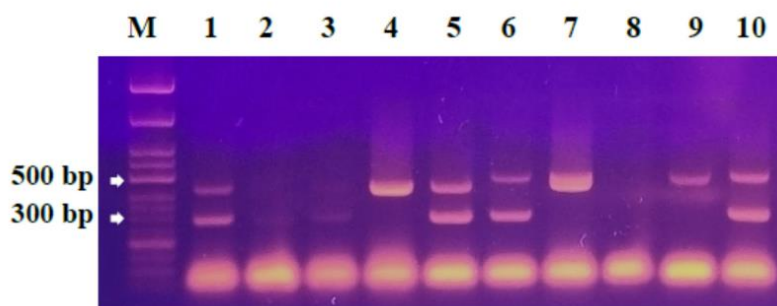


Figure 2. PCR amplification of cultivars used consensus PaConsI primers. 1– ‘Cheremashnaya’, 2– ‘Raditsa’, 3– ‘Sopernitsa’, 4– ‘Venera’, 5– ‘Viskochka’, 6– ‘Kompaktnaya’, 7– ‘Irinka’, 8– ‘Zolotuhinskaya’, 9– ‘Medunitsa’, 10– ‘Lena’, M- Markers of molecular weight Step50 Plus (Biolabmix, Russia).

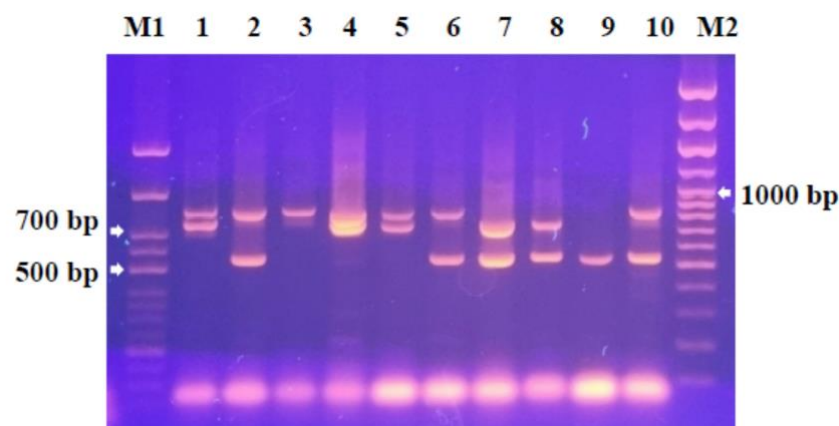


Figure 3. PCR amplification of cultivars used consensus PaConsII primers. 1– ‘Cheremashnaya’, 2– ‘Raditsa’, 3– ‘Sopernitsa’, 4– ‘Venera’, 5– ‘Viskochka’, 6– ‘Kompaktnaya’, 7– ‘Irinka’, 8– ‘Zolotuhinskaya’, 9– ‘Medunitsa’, 10– ‘Lena’, M1– Markers of molecular weight Step50Plus (Biolabmix, Russia); M2– Markers of molecular weight Step100 Long (Biolabmix, Russia).

Alleles S_7 , S_{12} , S_{13} , S_{14} , and S_{16} are rarely detected in sweet cherry genomes. Cultivars with not yet established S genotype were tested by PCR with these allele specific primers last. S_{13} allele was detected in the genomes of three cultivars: ‘Kormilitsa’, ‘Iput’ and ‘Mak’. Alleles S_7 , S_{12} , S_{14} , and S_{16} were not identified in the tested genomes. The S genotype of the two cultivars ‘Bahor’ and ‘Naslazhdenie’ was unclear because no one of the tested S alleles was detected in their genomes. The S genotype of the four cultivars ‘Odrinka’, ‘Preludia’, ‘Minchanka’, and ‘Valentina’ was partially identified. Further investigation of their S allele polymorphisms is necessary.

As a result of the performed analysis, seven S alleles (S_1 , S_3 , S_4 , S_5 , S_6 , S_9 , and S_{13}) were detected in the genomes of the investigated sweet cherry cultivars. Alleles S_2 , S_7 , S_{10} , S_{12} , S_{14} , and S_{16} were not present in any tested genotypes. For two cultivars ‘Bahor’ and ‘Naslazhdenie’, PCR with allele-specific primers did not produce any results, although PCR with consensus primers was successful. For four cultivars, only one S allele was identified. The S-genotype of the three genotypes needs further study due to atypical amplification with S_1 and S_4 allele specific primers. The S-genotype of the ‘Venera’ cultivar is shown in Table 1 as S_{1x}/S_4 . S-genotypes of cultivars

‘Zolotuhinskaya’ and ‘Viskochka’ are shown as S_{4x}/S_6 and S_3/S_{4x} , respectively. For 30 sweet cherry cultivars, self-incompatible groups were determined. Nine incompatibility groups (III, VI, VII, IX, XV, XVII, XIX, XXI, and XLV) were detected. The data is summarized in Table 1.

DISCUSSION

Sweet cherry (*Prunus avium* L.) is an important fruit crop. Most sweet cherries are self-incompatible. Sweet cherry has a gametophytic type of self-incompatibility controlled by S-Allele Specific Ribonuclease (S-RNase) and S Haplotype-Specific F-Box Protein (SFB). As we wrote above, self-incompatibility is a very significant mechanism in the evolution of flowering plants. Self-incompatibility matters in the prevention or reduction of self-fertilization. Knowledge of the S-genotype of cultivars gives us the opportunity to predict the effectiveness of fertilization. At the same time, the S genotype of sweet cherry cultivars is almost never taken into account in the breeding process. Therefore, some rare S alleles can be lost. Maintenance of sweet cherry cultivars containing rare and unique S alleles in their genomes is important.

**Table 1.** Results of S-genotyping of sweet cherry cultivars.

Cultivar	S allele														S-genotype	Incomp. group
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₉	S ₁₀	S ₁₂	S ₁₃	S ₁₄	S ₁₆			
Odrinka			+												S ₃	-
Kormilitsa				+							+				S ₄ /S ₁₃	XLV
PamiatyAstahova			+			+									S ₃ /S ₆	VI
Fatezh			+			+									S ₃ /S ₆	VI
Mak			+								+				S ₃ /S ₁₃	XIX
Brianskaya Rosovaya	+			+											S ₁ /S ₄	IX
Donetskii Velikan				+				+							S ₄ /S ₉	XXI
Gostinets	+			+											S ₁ /S ₄	IX
Zaria Vostoka			+			+									S ₃ /S ₆	VI
Iput			+								+				S ₃ /S ₁₃	XIX
Pamiaty Nikitina				+		+									S ₄ /S ₆	XVII
Geroinya			+		+										S ₃ /S ₅	VII
Krasnaya Gorka				+		+									S ₄ /S ₆	XVII
Tutchevka				+		+									S ₄ /S ₆	XVII
SeianetsChernishevskogo					+	+									S ₅ /S ₆	XV
Sweet cherry from Donetsk					+	+									S ₅ /S ₆	XV
Cheremashnaya			+	+											S ₃ /S ₄	III
Raditsa			+			+									S ₃ /S ₆	VI
Sopernitsa			+		+										S ₃ /S ₅	VII
Venera	x			+											S _{1x} /S ₄	-
Dar Cheliabinsku			+	+											S ₃ /S ₄	III
Kompactnaya			+			+									S ₃ /S ₆	VI
Irinka				+		+									S ₄ /S ₆	XVII
Zolotuhinskaya				x		+									S _{4x} /S ₆	-
Medunitsa				+		+									S ₄ /S ₆	XVII
Lena			+			+									S ₃ /S ₆	VI
Aelita					+	+									S ₅ /S ₆	XV
Preludia			+												S ₃	-
Pamiati Zhukova				+		+									S ₄ /S ₆	XVII
Lubimitsa Astahova			+	+											S ₃ /S ₄	III
Alenushka			+	+											S ₃ /S ₄	III
Viskochka			+	x											S ₃ /S _{4x}	-
Bahor															-	-
Pamiati Chernishevskogo			+	+											S ₃ /S ₄	III
Naslazhdenie															-	-
Brianochka			+			+									S ₃ /S ₆	VI
Minchanka						+									S ₆	-
Valentina			+												S ₃	-
Vasilisa			+			+									S ₃ /S ₆	VI

x: The amplification with allele specific primers was successful, but the size of the PCR product was different from expected.

The main aim of this study was to investigate S-genotype polymorphisms of sweet cherry cultivars and selections from the VNIISPK fruit crop bio resource collection. The sweet cherry collection includes not only cultivars that originate from Russia, but also those from Belarus and Ukraine. Previously, the analysis of *S-RNase* gene polymorphisms of cultivars of

VNIISPK breeding was performed (Bezlepkina *et al.*, 2020), so these genotypes were not included in the investigation. However, this data should be considered when analysing the VNIISPK collection of sweet cherry genotypes generally.

This paper represents data of S genotype analysis of 39 sweet cherry cultivars. The presence of thirteen S alleles in genotypes

was checked. Seven of them (S_1 , S_3 , S_4 , S_5 , S_6 , S_9 , and S_{13}) were detected. Alleles S_3 , S_4 , and S_6 were the most common. The S_3 , S_4 , and S_6 alleles were detected in the genomes of 21, 16, and 19 cultivars, respectively. The S_1 , S_5 , S_9 , and S_{13} alleles were identified in the genomes of sweet cherry collection much less frequently. They were detected in 2, 5, 1, and 3 genotypes, respectively.

In a previous study (Bezlepina *et al.*, 2020), we tested nine sweet cherry cultivars of VNIISPK breeding. They were ‘Adelina’ (S_3/S_5), ‘Poezia’ (S_3/S_5), ‘Malish’ (S_6), ‘PodarokOrlu’ (S_9), ‘OrlovskayaRozovaya’ (S_6/S_x), ‘OrlovskayaYantarnaya’ (S_6), ‘OrlovskayaFeia’ (S_3/S_5), ‘Trosnianskaya’ (S_5/S_6), and ‘Siana’ (S_3/S_6).

Considering the data of the previous S genotype investigation, allele S_3 is present in 25 out of 48 tested genotypes of the VNIISPK sweet cherry collection. This is 52% of the investigated genomes. The allele S_4 was detected in 16 out of 48 genomes. This is 33% of the tested genotypes. In 50% of the investigated sweet cherry genomes, the allele S_6 was detected. The S_5 allele is the next one by the presence in the genomes of sweet cherry cultivars from the VNIISPK collection. The S_5 allele was detected in 19% of the genotypes. The S_3 , S_4 , S_5 and S_6 alleles were more prevalent in the genomes of sweet cherry cultivars. Each of the S_1 , S_9 and S_{13} alleles were detected in 4% of the investigated genomes (Figure 4).

S_1 , S_3 , S_4 , S_5 , S_6 , and S_9 alleles are the most common in sweet cherry genotypes worldwide (Schuster *et al.*, 2024). In this regard, the presence of S_3 , S_4 , S_5 , and S_6 alleles in a large number of sweet cherry genotypes from the VNIISPK bio-resource collection was expected. The S_1 , S_9 , and S_{13} alleles are relatively poorly represented in the genomes of sweet cherry cultivars from the VNIISPK bio-resource collection. However, the most interesting cultivars are sweet cherries, in the genome of which unique alleles of the *S-RNase* gene have been identified. These are ‘Venera’ (S_{1x}/S_4), ‘Zolotuhinskaya’ (S_{4x}/S_6), ‘Viskochka’ (S_3/S_{4x}) and ‘Orlovskaya Rozovaya’ (S_6/S_x). As well as cultivars in which allelic set of the *S-RNase* gene has not been fully determined that may indicate the presence of rarer alleles of the gene in the genome. In the process of selective breeding for a complex of economically valuable traits, the allelic polymorphism of the *S-RNase* gene is most likely to decrease. In this regard, the preservation of sweet cherry genotypes with rare and unique *S* alleles is of great importance.

Also, in the result of S genotype analysis presented in this article, incompatibility groups were established for 30 sweet cherry cultivars. A total of nine incompatibility groups were identified. There are III (S_3/S_4), VI (S_3/S_6), VII (S_3/S_5), IX (S_1/S_4), XV (S_5/S_6), XVII (S_4/S_6), XIX (S_3/S_{13}), XXI (S_4/S_9), and

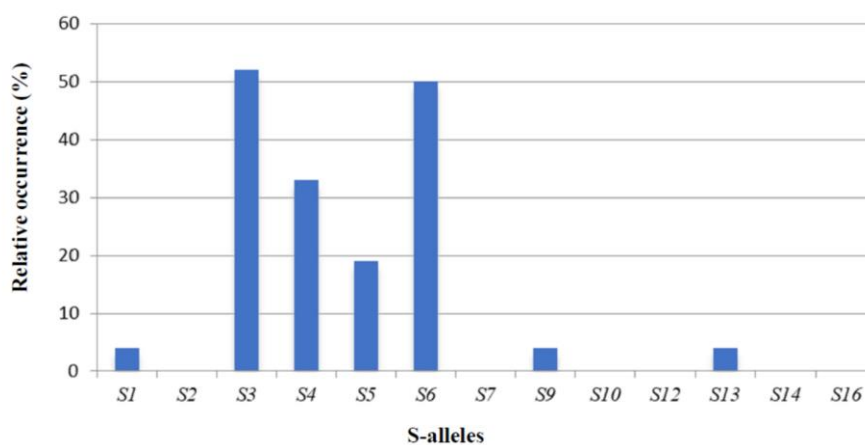


Figure 4. The frequency of S-alleles in the VNIISPK sweet cherry collection.



XLV (S_4/S_{13}) incompatibility groups. The sweet cherry cultivars of VNIISPK breeding tested previously belong to the VI, VII, and XV in-compatibility groups (Bezlepkina *et al.*, 2020). The incompatibility groups III (S_3/S_4), VI (S_3/S_6), and XVII (S_4/S_6) are the most numerous. This was expected due to the frequent occurrence of the S_3 , S_4 , and S_6 alleles in the genomes of the investigated sweet cherry cultivars.

CONCLUSIONS

To summarize, a total of 39 cultivars were tested. The presence of thirteen S alleles was checked, and seven of them (S_1 , S_3 , S_4 , S_5 , S_6 , S_9 , and S_{13}) were detected in the sweet cherry's genomes. The S_3 , S_4 , S_5 , and S_6 alleles were present in 52%, 33%, 19%, and 50% of the investigated genomes, respectively. The S_1 , S_9 , and S_{13} alleles were identified in the genomes of sweet cherry collection much less frequently. 'Venera' (S_{1x}/S_4), 'Zolotukhinskaya' (S_{4x}/S_6) and 'Viskochka' (S_3/S_{4x}) are suspected to have unique or mutant alleles of the gene. In 6 genotypes, the allele set of the gene was not fully determined, that may indirectly indicate the presence of more rarely-occurring alleles of the gene in the genome. Cultivars with fully established S-genotypes belong to nine incompatibility groups (III, VI, VII, IX, XV, XVII, XIX, XXI, and XLV). The most numerous incompatibility groups are III (S_3/S_4), VI (S_3/S_6), and XVII (S_4/S_6).

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پلی مورفیسم های جایگاه خودناسازگاری در گونه گیلاس شیرین (*Prunus avium* L.) از
مجموعه منابع زیستی VNIISPK

النا بزلپکینا، و الکساندرا گولیا اوا

چکیده

خودناسازگاری یک سازوکار تکاملی رایج در گیاهان گل دار است. گیلاس شیرین (*Prunus avium* L.) دارای نوع گامتوفیتی خودناسازگاری است. بیشتر ارقام گیلاس شیرین خودناسازگار هستند، هرچند ژنوتیپ های خودسازگار نیز وجود دارند. این مطالعه داده های مربوط به بررسی ژنوتیپ S در ۳۹ رقم گیلاس شیرین از مجموعه منابع زیستی VNIISPK



را ارائه می‌دهد. برای شناسایی ژنوتیپ‌های S، واکنش زنجیره‌ای پلیمراز (PCR) با استفاده از پرایمرهای عمومی (PaConsl و PaConslI) و پرایمرهای اختصاصی آلل (S1 تا S14 و S16) انجام شد. ژنوتایپ S برای ۳۳ رقم به‌طور کامل شناسایی شد. سپس، ۷ آلل S (شامل S1، S3، S4، S5، S6، S9 و S13) در ژنوم گیلاس‌های شیرین مورد مطالعه شناسایی گردید. بر اساس نتایج تکثیر اختصاصی آلل (allele-specific amplification)، احتمال وجود آلل‌های منحصربه‌فرد یا جهش‌یافته‌ی این ژن در ارقام «S1x/S4» (Venera)، «S4x/S6» (Zolotuhinskaya) و «S3/S4x» (Viskochka) مطرح شد. ارقام با ژنوتیپ‌های S کاملاً تعیین‌شده، در نه گروه ناسازگاری (III، VI، VII، IX، XV، XVII، XIX، XXI و XLV) طبقه‌بندی شدند.

Study of the Morphological Fruit Quality, Total Polyphenol Content, and Antioxidant Activity in Four Mulberry Species (*Morus* spp.) in Malatya-Turkey

Muharrem Ergun^{1*}, Harun Yoncaci¹, and Zahide Süslüoğlu¹

ABSTRACT

The present study evaluated the morphological and biochemical characteristics of fruits from four widely distributed mulberry species, i.e. *M. alba*, *M. nigra*, *M. rubra*, and *M. laevigata*, in Turkey, in order to inform species selection and promote the utilization of underexploited genotypes for food and nutraceutical applications. The fruits and juice were analyzed for size, weight, juice yield, Total Soluble Solids (TSS), Titratable Acidity (TA), pH, colour (CIELAB coordinates), Total Polyphenol Content (TPC), and antioxidant activity (DPPH assay). Significant interspecific variation was observed for most traits. *M. rubra* and *M. laevigata* produced the largest and heaviest fruits, with *M. rubra* also presenting the highest juice yield (57.15%). Colour analysis separated pale-coloured *M. alba* from the anthocyanin-rich, dark-pigmented *M. nigra* and *M. rubra*. *M. alba* and *M. nigra* presented the highest levels of TSS at 19.93% and 19.64%, respectively. However, *M. alba* had the lowest levels of TA and TPC. In contrast, *M. nigra* had the highest TPC (1608.40 mg GAE 100 g⁻¹ fw). The antioxidant activity (DPPH) of the three dark-pigmented mulberries (*M. nigra*, *M. rubra*, and *M. laevigata*) was found to be similar to each other but higher than that of the pale-coloured *M. alba*. These findings highlight the potential of the dark-pigmented mulberry species as valuable sources of bioactive compounds for the development of functional foods.

Keywords: Juice yield, *Morus alba*, *Morus laevigata*, *Morus nigra*, *Morus rubra*, Titratable acidity, Total soluble solids.

INTRODUCTION

Mulberry (*Morus* spp.), a member of the Moraceae family, is a perennial deciduous tree cultivated across tropical, subtropical, and temperate regions (Sánchez-Salcedo *et al.*, 2015; Chauhan *et al.*, 2018). Nineteen species are currently recognized worldwide (Butt *et al.*, 2008; Ercisli and Orhan, 2008). It is hypothesised that mulberries first originated in the Himalayan foothills, from which they have adapted to a wide range of ecological zones, from sea level to 4,000 m (Pel *et al.*, 2017; Yuan *et al.*, 2017). Within the geographical limits of Turkey, *M. alba* is predominant, accounting for approximately 95% of the population; in contrast, *M. rubra* (3%) and *M. nigra* (2%) are observed to

occur with comparatively lower frequency (Ercisli, 2004). *M. laevigata*, distinguished by its substantial red-black fruits, has recently attracted interest for cultivation in select regions.

Mulberries represent a multipurpose crop of nutritional, medicinal, and economic importance. Beyond their role in sericulture and animal husbandry, the fruits are consumed fresh or processed into jams, juices, and desserts (Salih *et al.*, 2022). Their popularity can be attributed to their nutritional and functional properties. Mulberries provide carbohydrates, dietary fiber, minerals, and vitamins, while being relatively low in calories (Jan *et al.*, 2021). They are also rich in polyphenols, particularly anthocyanins, flavonols, and

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flavanols, which contribute to strong antioxidant capacity (Hassimotto *et al.*, 2008; Natić *et al.*, 2015). Dark-pigmented species such as *M. nigra* and *M. rubra* typically accumulate more anthocyanins and phenolic acids, whereas pale-coloured *M. alba* is characterised by glycosylated flavonoids (Chan *et al.*, 2020). These compounds are associated with protection against oxidative stress, metabolic disorders, and cardiovascular risks (Ahmad *et al.*, 2022; Batiha *et al.*, 2023).

Mulberries also hold significant cultural and medicinal value. Traditional uses of fruits and extracts include treatment of oral wounds, anaemia, and respiratory conditions, while mulberry leaves are employed against diabetes, hypertension, and wounds (Abbasi *et al.*, 2014; Younus *et al.*, 2016; Thaipitakwong *et al.*, 2018). Such practices reinforce their dual role as food and medicine across Asia and the Mediterranean.

Fruit quality traits, however, are influenced not only by genetics but also by environment. Studies worldwide show that warm, humid climates enhance sugar accumulation but reduce acidity, whereas cooler or drier conditions promote phenolic and anthocyanin synthesis as well as smaller fruit size (Aljane *et al.*, 2016; Lou *et al.*, 2024; Rong *et al.*, 2024). These regional patterns underline the need to evaluate mulberries under defined climatic conditions. The continental climate of Malatya, Turkey, thus provides a valuable setting for assessing interspecific differences while enabling comparisons with studies from other regions.

Despite increasing interest in mulberries, most prior studies have either focused on a single species or compared accessions grown under variable environments, making it difficult to separate genetic from environmental influences. The present study is novel in simultaneously evaluating four widely distributed *Morus* species (*M. alba*, *M. nigra*, *M. rubra*, and *M. laevigata*) cultivated under uniform environmental conditions in Turkey. This design minimises

climatic and soil-related confounding factors, allowing robust identification of species-specific differences. In addition, the use of multivariate analyses provides new insights into relationships among morphological, physicochemical, and antioxidant traits.

The objectives of this study were threefold: firstly, to characterise interspecific variation in fruit quality traits; secondly, to examine relationships among morphological, biochemical, and antioxidant properties; and thirdly, to provide guidance for the utilisation of mulberry fruits in food and nutraceutical applications.

MATERIALS AND METHODS

Climatic Conditions of the Study Area

The study area has a continental climate characterised by hot, dry summers and cold, snowy winters. The mean annual temperature is 13.6°C, while maximum temperatures reach 27 to 30°C in June and July. Annual precipitation averages 400–500 mm (Malatya İl Kültür ve Turizm Müdürlüğü, 2025).

Plant Material and Sampling

The study was conducted in the Battalgazi District of Malatya, Turkey (38–39° N, 38–39° E; 900 m asl). Four mulberry species (*M. alba*, *M. nigra*, *M. rubra*, and *M. laevigata*) were evaluated. For each species, ten healthy and productive trees were selected. Approximately 1.5 kg of fully ripe fruits were harvested per tree, randomly sampled from all parts of the canopy. Samples were transported under refrigerated conditions (5°C) to the laboratory for analysis. All measurements were performed on 10 replicates per species, with each replicate consisting of individual fruits (for weight and size parameters) or pooled 50-g fruit samples (for juice, biochemical, and colour analyses).

Morphological Mulberry Fruit Characteristics

Fruit length and width were measured with a digital calliper, and fresh weight was recorded with a precision electronic balance. Dry weight was determined by drying the sample at 65 °C (Cemeroğlu, 1992). Fruit skin colour was assessed using a portable colorimeter (Linshang Technology, model LS172, China), with CIELAB colour coordinates L^* , a^* , and b^* recorded.

Chemical Mulberry Fruit Juice Characters

Juice was extracted and filtered through cheesecloth. Juice yield was calculated as the ratio of the juice weight to the fresh fruit weight. Total Soluble Solids (TSS) content was measured using a digital refractometer (A. Krüss Optronic GmbH, model DR6100-T, Germany), while Titratable Acidity (TA) was determined by titrating with 0.1N NaOH to pH 8.2 and expressed as malic acid equivalent. The pH and colour of the juice were measured using a digital pH meter (Kyoto Electronics Manufacturing Co., Ltd., model AT-510, Japan) and the CIELAB scale (L^* , a^* , b^*), respectively.

Mulberry Fruit Total Polyphenol Composition

The quantification of Total Polyphenol Content (TPC) was performed employing Folin-Ciocalteu's reagent, as described by Slinkard and Singleton (1977), with a minor modification. The reaction mixture contained 0.02 mL of extract, 3.9 mL of distilled water, 0.25 mL of reagent, and 0.75 mL of Na_2CO_3 . After a 2-h dark incubation, the absorbance was measured at 745 nm using a UV-vis spectrophotometer (Analytik Jena GmbH, SPECORD 200 PLUS, Germany). Results were expressed as mg Gallic Acid Equivalent (GAE) per 100 g fresh weight (fw).

Mulberry DPPH Activity

Antioxidant activity was determined using the DPPH radical-scavenging assay, as described by Blois (1958). An aliquot of 0.2

mL of extract was mixed with 2 mL of 0.1 mM DPPH solution and incubated at room temperature for 30 min in the dark. Absorbance was recorded at 517 nm. The inhibition (%) of DPPH radicals was calculated as:

$$\text{Inhibition percentage (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 and A_1 represent the absorbance of the control and sample, respectively. Results were expressed in %.

Statistical Analysis

Data analysis was performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis Of Variance (ANOVA) and Duncan's multiple range test were applied to compare mean values across species at a significance level of $P \leq 0.05$. Pearson correlation coefficients were calculated to evaluate relationships among variables. Principal Component Analysis (PCA) and hierarchical cluster analysis were conducted based on Euclidean distances to explore multivariate patterns among species.

RESULTS

Fruit Morphological Characteristics

Morphological traits such as fruit size, weight, and juice yield are key factors influencing consumer preference, marketability, and yield potential. Among the four *Morus* species evaluated, *M. rubra* exhibited the greatest fruit width (19.29 mm), while *M. laevigata* produced the longest fruits (37.47 mm) (Figure 1). In contrast, *M. alba* and *M. nigra* yielded more compact fruits with moderate widths and shorter lengths (15.09 and 24.83 mm in *M. alba*, and 16.45 and 23.49 mm in *M. nigra*), consistent with prior reports of size variability across *Morus* species (Ercisli, 2004; Orhan *et al.*, 2020; Gnanesh *et al.*, 2023).

Similar trends were observed for fruit weight. *M. laevigata* and *M. rubra* exhibited the highest fresh weights (6.19 g and 6.14 g, respectively), while *M. alba* produced the

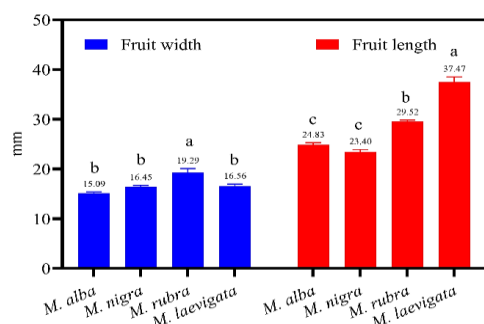


Figure 1. Fruit width and length of the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

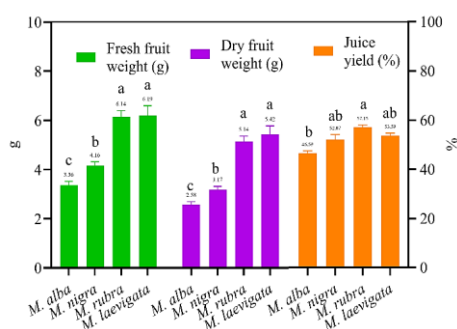


Figure 2. Fresh, dry weight, and juice yield of the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

lightest fruits (3.36 g) (Figure 2). Dry weight followed comparable patterns. *M. rubra* exhibited the highest juice yield (57.15%), which was significantly higher than that of *M. alba* but statistically similar to those of *M. nigra* and *M. laevigata*. These results suggest that the three dark-pigmented *Morus* species were particularly suitable for fresh consumption and juice production.

Fruit and Juice Colorimetric Attributes

Fruit and juice colour parameters (L^* , a^* , b^*) differed significantly among species (Figures 3 and 4). *M. alba* presented the

highest fruit L^* value (66.47), indicative of its light skin. In contrast, the remaining samples demonstrated significantly lower L^* values, measuring less than 20, suggesting that the fruit skins were of a darker hue. Negative a^* values for *M. alba* (-3.00) reflected its greenish hue, whereas positive a^* values in other species indicated red pigmentation linked to anthocyanins. Lower b^* values (0.375–0.94) in *M. rubra*, *M. nigra*, and *M. laevigata* also indicated darker, anthocyanin-rich pigmentation, in line with earlier findings (Özgen et al., 2009; Ercisli and Orhan, 2007, 2008).

Juice colour followed a similar pattern. *M. alba* exhibited the highest juice L^* value (39.12), a negative a^* value (-2.39), and a higher b^* value (5.76), resulting in a light, greenish-yellow colour. In contrast, juices from *M. rubra*, *M. nigra*, and *M. laevigata* were darker and reddish, reflecting their higher anthocyanin content. These colour differences further support the classification of *Morus* fruits based on pigment profiles and their potential functional food value.

Fruit Chemical Properties

Total Soluble Solids (TSS), Titratable Acidity (TA), and pH are important determinants of flavour and processing suitability. As shown in Figure 5, both *M. alba* and *M. nigra* presented the highest TSS values (19.93% and 19.64%, respectively), indicating higher sugar content, which is desirable for fresh consumption, drying and sweet products. In contrast, *M. rubra* (11.48%) and *M. laevigata* (6.54%) exhibited significantly lower TSS values, making them more suitable for processing.

Acidity profiles also varied markedly (Figure 5). *M. nigra* exhibited the highest TA (11.57%) and lowest pH (3.85), contributing to its pronounced tartness. Conversely, *M. alba* had the lowest TA (1.52%) and highest pH (6.10), consistent with its mild flavour. *M. rubra* and *M. laevigata* displayed intermediate acidity values. These species-specific acidity

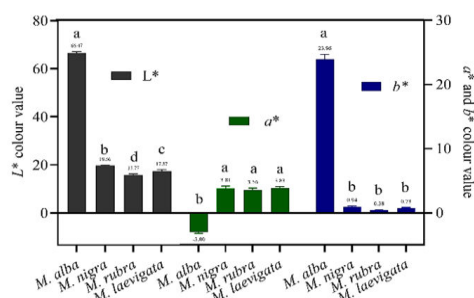


Figure 3. CIE L^* a^* b^* fruit colour space values for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

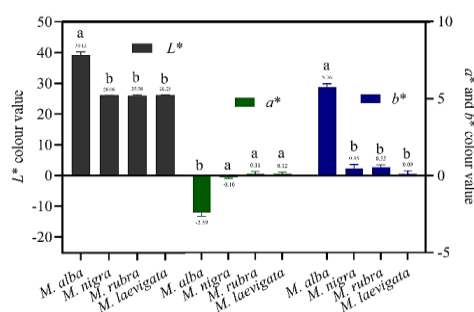


Figure 4. CIE L^* a^* b^* juice colour space values for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

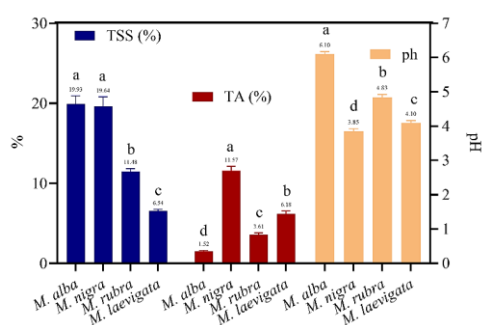


Figure 5. Total Soluble Solids (TSS), Titratable Acidity (TA), and pH values of for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

et al., 2009; Gundogdu *et al.*, 2011) and provide guidance for cultivar selection based on target flavour attributes.

The ecology of mulberry plants has been demonstrated to exert a significant impact on the TSS and acidity present in the fruit. Mulberries from tropical regions of China, where warmer climates favoured higher sugar accumulation but lower acidity compared to cooler regions (Lou

et al., 2024). In contrast, mulberries grown off-season, corresponding to shorter and cooler summers, typically present lower sugar content but especially higher acidity (Liu *et al.*, 2022), aligning more closely with our observations in *M. nigra*.

Total Polyphenol Content and DPPH Antioxidant Activity

Total Polyphenol Content (TPC) and antioxidant activity (DPPH assay) exhibited significant interspecific differences (Figure 6). *M. nigra* had the highest TPC (1608.40 mg GAE 100 g⁻¹ fw), followed by *M. rubra*, *M. laevigata*, and *M. alba*. Correspondingly, dark-pigmented mulberries (*M. nigra*, *M. rubra*, and *M. laevigata*) presented higher DPPH radical-scavenging activity compared to the pale-coloured mulberry (*M. alba*).

These results corroborate the strong association between dark pigmentation, polyphenol richness, and antioxidant capacity, as reported in previous studies (Imran *et al.*, 2010; Ercisli *et al.*, 2010). The findings highlight especially *M. nigra* and *M. rubra* as promising candidates for functional food and nutraceutical applications.

The high phenolic and antioxidant levels observed in *M. nigra* and *M. rubra* under the Malatya climate may result from protective mechanisms in the plants, which enhance anthocyanin production to serve as antioxidants and UV protectants (Li and Ahammed, 2023). Conversely, mulberries cultivated in tropical Asian regions often display higher sugar levels but comparatively lower phenolic concentrations (Lou *et al.*, 2024), illustrating the strong role of climatic adaptation in shaping biochemical traits.

profiles align with previous studies (Özgen



Correlation Parameters and Multivariate Analysis

"Significant morphological, colorimetric, and biochemical relationships among the species were evident from the correlation matrix (Figure 7). Dry and fresh fruit weight exhibited strong positive correlations ($r=0.99$), consistent with the findings of Ercisli and Orhan (2007), who reported significant correlations between water content and biomass accumulation in fleshy fruits. Notably, the fruit colour parameters (L^* , a^* , b^*) are almost perfectly correlated ($r>0.95$), which reflects the coordinated regulation of the anthocyanin biosynthesis pathways established by Gundogdu *et al.*, (2011). The correlation between TA and pH ($r=-0.85$) was consistent with previous studies on organic acid metabolism, which associate lower pH values with higher levels of malic and citric acid (Meng *et al.*, 2024). Contrary to expectations, the TSS exhibited a negligible correlation with TPC ($r=0.06$). This phenomenon is likely attributable to interspecific contrasts: *M. alba* exhibits a combination of high TSS and low TPC, while dark-fruited species demonstrate the opposite, with higher TPC but lower TSS. Such cross-species patterns reduce the overall correlation.

Clustering and PCA Analysis

The hierarchical clustering heat-map analysis revealed distinct phenotypic and biochemical differences among the four mulberry species (Figure 8). The heat-map clearly delineated the species into two primary clusters. The first cluster consisted solely of *M. alba*, which was distinguished by its significantly higher fruit lightness (L^*) and lower a^*/b^* colour values, indicating a lighter fruit colour compared to the other species. Additionally, *M. alba* exhibited lower TPC and DPPH antioxidant activity, suggesting reduced biochemical

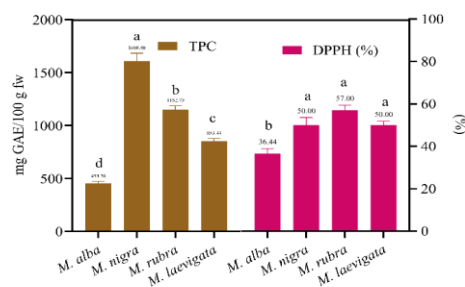


Figure 6. Total Polyphenol Content (TPC) and DPPH radical-scavenging activity for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

potency relative to the other species. The second cluster was characterised by the aggregation of *M. nigra*, *M. rubra*, and *M. laevigata*, primarily due to the presence of shared characteristics, including higher TPC, greater fruit dimensions, and stronger antioxidant properties. Within this cluster, *M. nigra* and *M. rubra* were more closely related, likely due to their exceptionally high TPC levels and notable DPPH activity. *M. laevigata*, while still within this group, displayed intermediate traits, including moderate fruit size and slightly lower acidity (TA), which may explain its slight divergence from the other two species.

The 16 traits were grouped into three broad categories by the x-axis dendrogram, indicating common physiological or biochemical activities (Figure 8). Cluster I included juice colour L^* , a^* , b^* , fruit colour a^* , pH, and TSS, primarily associated with sensory quality, ripeness, and sugar metabolism. These traits were tightly clustered due to their co-regulation during fruit ripening and pigmentation processes, particularly in the biosynthesis of anthocyanins and flavonoids (Giovanelli and Buratti, 2009). Cluster II consisted of DPPH activity, juice yield, and fruit width, which indicate antioxidant capacity (DPPH) and processing characteristics. This cluster -

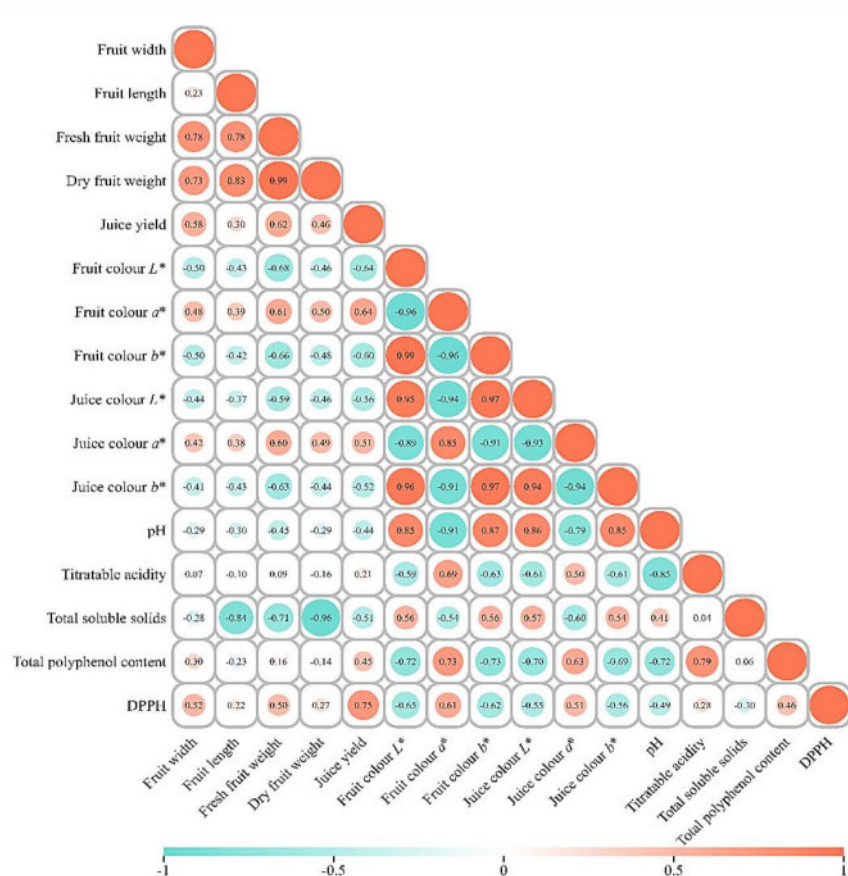


Figure 7. Heat-map of Pearson correlation coefficients among morphological and biochemical traits of the four mulberry species. Colour scale indicates Pearson correlation coefficient (r): Red= Positive, Blue= Negative, White= No correlation.

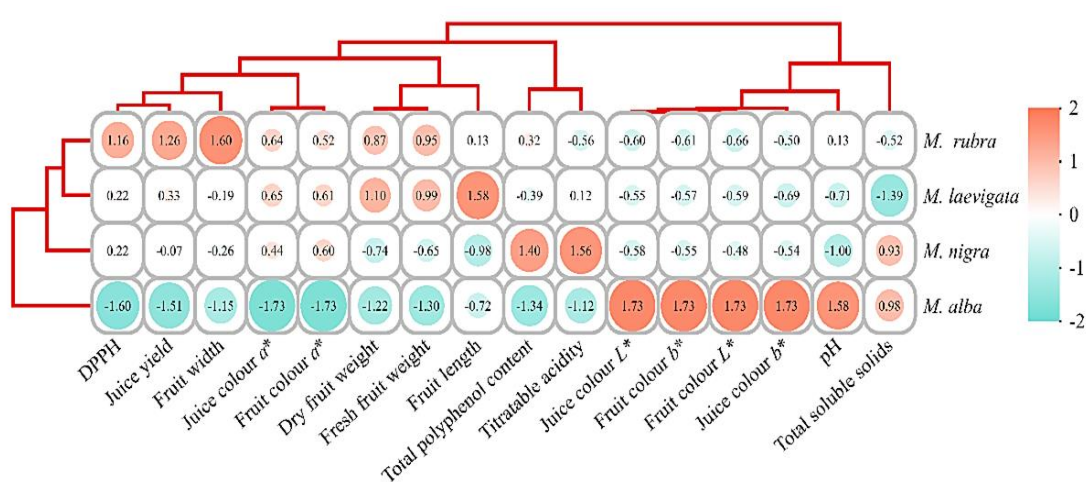


Figure 8. Hierarchical clustering heat-map of the four mulberry species based on morphological and biochemical traits. Colour scale represents normalized trait values (Z-score), from low (blue) to high (red). "

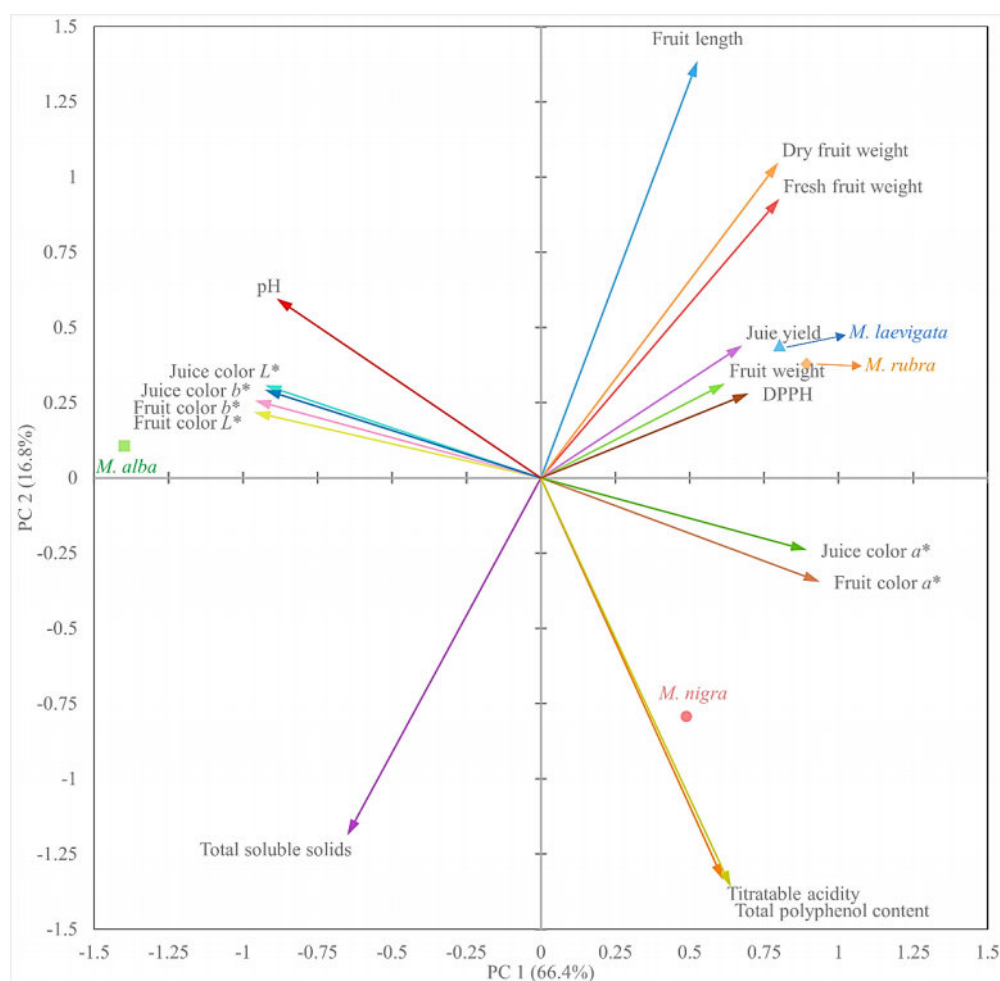


Figure 9. Principal Component Analysis (PCA) biplot of the four mulberry species based on morphological and biochemical traits.

lends further support to the prevailing hypothesis that increased fruit size and juice content are concomitant with elevated levels of bioactive compounds (Özgen *et al.*, 2009). Cluster III included fruit length, fresh and dry fruit weight, and TPC, which are traits associated with biomass and nutritional value. These are the most significant traits for breeding programs aimed at improving yield and promote health (Mohammadi and Prasanna, 2003).

The first and two Principal Components (PC1 and PC2) accounted for 83.2% of the total variance, with PC1 contributing 66.4% and PC2 contributing 16.8%. PC1 was positively correlated with TA, TPC, juice colour a^* , and fruit colour a^* , suggesting that this component reflects fruit acidity and

antioxidant potential. Conversely, PC1 was negatively correlated with TSS, fruit colour L^* , and juice colour L^* , which are indicative of fruit sweetness and brightness. The strong association of *M. nigra* with TA and TPC suggests that this species has a superior antioxidant profile. This finding is consistent with previous reports emphasizing the higher phenolic and anthocyanin content in *M. nigra* fruits (Ercisli and Orhan, 2007; Özgen *et al.*, 2009).

PC2 was positively associated with fruit length, dry and fresh fruit weight, juice yield, and pH. These traits are primarily linked to fruit size and productivity. The vectors for *M. laevigata* and *M. rubra* were located in the positive quadrant of PC2, indicating their association with larger fruits

and higher juice yield. This supports earlier findings that *M. laevigata* typically produces larger and juicier fruits compared to other *Morus* species (Gozlekci *et al.*, 2015). On the other hand, *M. alba* was separated negatively along PC1 and PC2, indicating lower TPC, acidity, and fruit size. The fruit and juice colour (L^* , b^*) were also closely associated with *M. alba*, supporting the characterization of its fruits as lighter-coloured and less acidic (Redha *et al.*, 2023).

The biplot showed a clear separation among the four *Morus* species based on the PCA axes: *M. nigra* was strongly associated with TA, TPC, and darker colour attributes (juice/fruit colour a^*), highlighting its potential for functional food and nutraceutical applications. *M. rubra* and *M. laevigata* were positioned closely along PC1 and PC2, showing high juice yield, pH, and fruit dimensions, making them suitable for fresh consumption and juice production. *M. alba* was negatively correlated with most traits linked to fruit quality and bioactivity. It may be better suited for dried fruit or culinary use where lower acidity and mild flavour are desired.

DISCUSSION

This study demonstrated significant interspecific variation in fruit quality traits among the four mulberry species grown in Malatya, Turkey. The results show that *M. rubra* and *M. nigra* had the highest TPC and antioxidant activity, supporting their use in functional food and nutraceutical applications. *M. alba*, with its high sugar content and mild acidity, was better suited for fresh consumption, drying or culinary use, while *M. laevigata* offered potential for fresh consumption and juice production due to its large fruit size.

The ecology of mulberry plants has been demonstrated to exert a significant impact on the TSS and acidity present in the fruit. Mulberries from tropical regions of China, where warmer climates favoured higher

sugar accumulation but lower acidity compared to cooler regions (Lou *et al.*, 2024). In contrast, mulberries grown off-season, corresponding to shorter and cooler summers, typically present lower sugar content but especially higher acidity (Liu *et al.*, 2022), aligning more closely with our observations in *M. nigra*.

The high phenolic and antioxidant levels observed in *M. nigra* and *M. rubra* under the Malatya climate may result from protective mechanisms in the plants, which enhance anthocyanin production to serve as antioxidants and UV protectants (Li and Ahammed, 2023). Conversely, mulberries cultivated in tropical Asian regions often display higher sugar levels but comparatively lower phenolic concentrations (Lou *et al.*, 2024), illustrating the strong role of climatic adaptation in shaping biochemical traits.

These findings corroborate the strong association between dark pigmentation, polyphenol richness, and antioxidant capacity, as reported in previous studies (Imran *et al.*, 2010; Ercisli *et al.*, 2010). The findings highlight especially *M. nigra* and *M. rubra* as promising candidates for functional food and nutraceutical applications.

Our results provide practical guidance for cultivar selection and highlight the combined influence of genetics and regional climate on mulberry fruit quality. Further studies should explore the specific anthocyanin profiles and their bioavailability, as well as the effects of different processing methods on the retention of bioactive compounds.

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مطالعه کیفیت مورفولوژیک میوه، محتوای کل پلی فنول و فعالیت آنتی اکسیدانی در چهار گونه توت (*Morus* spp*) در مالاتیای ترکیه

محرم ارگون، هارون یونجاجی، و زاهده سوسلو اوغلو

چکیده

پژوهش حاضر به ارزیابی ویژگی‌های ریخت‌شناختی و بیوشیمیایی میوه‌های چهار گونه توت با پراکنش وسیع در ترکیه — شامل *M. alba**، *M. nigra**، *M. rubra** و *M. laevis** — پرداخت؛ هدف از این پژوهش، فراهم‌سازی اطلاعات لازم برای انتخاب گونه‌ها و ترویج بهره‌برداری از ژنوتیپ‌هایی است که تاکنون کمتر مورد استفاده قرار گرفته‌اند و پتانسیل کاربرد در صنایع غذایی و محصولات فراسودمند (نوتراسوتیکال (nutraceutical) را دارند. میوه‌ها و آبمیوه از نظر اندازه، وزن، بازده آبمیوه، کل مواد جامد محلول (TSS)، اسیدیته قابل‌تیتراسیون (TA)، pH، رنگ (مختصات CIELAB)، محتوای کل پلی‌فنول (TPC) و فعالیت آنتی‌اکسیدانی (آزمون DPPH) مورد تجزیه و تحلیل قرار گرفتند. تنوع قابل‌توجهی میان‌گونه‌ای در بیشتر صفات مشاهده شد. گونه‌های *M. rubra** و *M. laevis** بزرگ‌ترین و سنگین‌ترین میوه‌ها را تولید کردند و *M. rubra** همچنین بیشترین بازده آبمیوه (۱۵/۵۷ درصد) را به خود اختصاص داد. تحلیل رنگ، گونه *M. alba** با رنگ روشن را از گونه‌های *M. nigra** و *M. rubra** که دارای رنگدانه تیره و سرشار از آنتوسیانین هستند، متمایز کرد. گونه‌های *M. alba** و *M. nigra** به ترتیب با مقادیر ۱۹/۹۳٪ و ۱۹/۶۴٪، بالاترین میزان TSS را نشان دادند؛ با این حال، گونه *M. alba** کمترین میزان TA و TPC را داشت. در مقابل، گونه *M. nigra** بیشترین میزان TPC ۴۰/۱۶۰۸ میلی‌گرم GAE در هر ۱۰۰ گرم وزن تازه را داشت. مشخص شد که فعالیت آنتی‌اکسیدانی (DPPH) سه گونه توت با رنگدانه تیره (*M. rubra**، *M. nigra** و *M. laevis**) مشابه یکدیگر، اما بیشتر از گونه *M. alba** با رنگ روشن (Pale-coloured) بوده است. این یافته‌ها بر پتانسیل گونه‌های توت با رنگدانه تیره به عنوان منابع ارزشمند ترکیبات زیست‌فعال برای تولید غذاهای فراسودمند تأکید می‌کنند.

Temperature-Dependent Development of *Exorista larvarum* (Diptera: Tachinidae): An Efficient Candidate for Biological Pest Control

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ABSTRACT

To optimize the mass rearing of *E. larvarum* and improve its application in IPM programs, the developmental times of this parasitoid were investigated under seven constant temperatures (15-35°C) in laboratory conditions. We applied two linear and 26 nonlinear models to describe the temperature-dependent development rate of *E. larvarum*. The Lower Temperature Threshold (T_0) and Thermal Constant (K) were estimated to be 5.09°C and 389.41 Degree-Days (DD) using the ordinary linear model and 9.73°C and 292.74 DD using the Ikemoto model, respectively. Among the nonlinear models, Performance-2, Beta, Janisch/Kontodimas, Analytis-1, and Analytis-3/Kontodimas were the best models to describe the temperature-dependent development rate of the parasitoid. The estimated Fastest Developmental Temperature (T_{fast}) values obtained from the Performance-2 and Janisch/Kontodimas models were 33.2 and 31.4°C, respectively, which were close to the observed T_{fast} (32.5°C). Our findings can help to gain new and valuable insights into the biology of *E. larvarum* and provide essential information that can be incorporated into forecasting models of this parasitoid.

Keywords: Bio-control, Degree day, Developmental rate, Temperature thresholds, Tachinid fly.

INTRODUCTION

Understanding insect adaptation to climatic conditions is pivotal for effective pest management (Bale *et al.*, 2002; Kang *et al.*, 2009). Temperature is a critical climatic factor with profound effects on the development of pests and their natural enemies. Consequently, calculating temperature-dependent development and thermal requirements utilizing thermal models is important for creating forecast models. This practice not only facilitates the comprehension of thermal adaptations among natural enemies but also their synchrony with hosts. Thermal requirements, often utilized as a predictive basis, become integral components in the strategic implementation of Integrated Pest Management (IPM) systems (Haghani *et al.*,

2007a, b). Moreover, thermal requirements provide some essential information on the biology of natural enemies, enhancing their effectiveness as biological control agents (Haghani *et al.*, 2009; Iranipour *et al.*, 2010; Pakyari *et al.*, 2011; Walker, 2011; Baek *et al.*, 2014; Park *et al.*, 2016; Mirhosseini *et al.*, 2018; Farazmand *et al.*, 2020; Yazdanpanah *et al.*, 2022).

While factors such as nutrition, humidity, temperature and photoperiod significantly influence development rate and should be considered when applying predictive models (Gilbert and Raworth, 1996), thermal traits may vary across species, populations, and developmental stages (Honek, 1999). Nevertheless, laboratory-derived estimations of thermal requirements remain valuable for identifying temperature ranges conducive to stable population growth (Pilkington and

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Hoddle, 2006). This information is particularly useful for identifying geographical areas where undesirable temperature circumstances may impede the establishment of permanent populations of biological control agents (Hoelmer and Kirk, 2005).

Exorista larvarum (Linnaeus, 1758) (Diptera: Tachinidae), a polyphagous gregarious larval endo-parasitoid of Lepidoptera, is well known as an effective candidate for biological control of forest and agricultural lepidopterous defoliators (Grenier, 1988; Cerretti and Tschorsnig, 2010; Benelli et al., 2017; Dindo and Nakamura, 2018). This parasitoid can be mass-produced, and the availability of various mass-rearing techniques for this tachinid makes it a suitable candidate for industrial-scale production and release against its host pests (Benelli et al., 2018).

An important step in the mass production of parasitoids under laboratory conditions and their inundative release in the field, as well as a fundamental requirement for any biological pest control program, involves evaluating the optimum temperature for the development of parasitoids (Meirelles et al., 2015). Temperature, as one of the most important environmental factors (Taylor, 1981; Gilbert and Ragworth, 1996; Gorji et al., 2008; Johnson et al., 2016; Amjad Bashir et al., 2022), significantly affects the abundance, distribution, immature development, adult emergence, fecundity, longevity, and parasitism capability of parasitoids (Liu et al., 2012). Also, temperature can be utilized in specifying optimal circumstances for parasitoids, timing of their release, forecasting the incidence of their host pests, knowledge of temperature effects on pests or parasitoids, and their adjustment to climatic circumstances plays a pivotal role in the success or failure of a bio-control program (Agbodzavu et al., 2020; Moradi et al., 2023). Because of its importance relative to other environmental factors, temperature is frequently used as input in mathematical models that are vigorous tools for describing

the effect of temperature on insect development and predicting their population growth potential (Mirhosseini et al., 2017). The application of thermal models promises for monitoring natural enemies and is invaluable in the implementation of pest management programs (Paes et al., 2018; Sampaio et al., 2021; Malekera et al., 2022).

Currently, various linear and nonlinear models are usually utilized to calculate the development of insect pests and natural enemies (Ranjbar Aghdam et al., 2009; Mirhosseini et al., 2018) and determine the relationship between temperature and their development rate (Worner, 2008). The Thermal Constant (K), expressed in degree-days above the developmental zero, represents the amount of physiological time required for an insect to complete its development (Campbell et al., 1974). This parameter can be estimated accurately using linear models, which assume a constant rate of development within a specific temperature range. However, because insect development deviates from linearity at temperature extremes—both high and low—nonlinear models are essential for accurately determining the optimum temperature for development and the upper thermal threshold. Thermal thresholds and optimum temperature significantly impact the entire main life processes of poikilothermic organisms (ectothermic organisms) due to the limitations imposed by temperature on their biological performance (Roy et al., 2002).

Given the critical influence of temperature on the development and efficiency of natural enemies, the present research aimed to assess the thermal requirements and developmental responses of *E. larvarum* under constant temperatures. This exploration serves as a prerequisite for the mass rearing and effective deployment of *E. larvarum* in biological control programs. Although the only study that has previously examined the effect of temperature on the biological parameters of *E. larvarum* is that by Simoes (2004), our research offers novel insights by employing two linear models and

several nonlinear models to evaluate the influence of temperature on the development of this promising parasitoid. The results of this study could be beneficial for designing an exhaustive program to manage different lepidopterous defoliators in forest and agricultural systems, thereby enhancing the application of this parasitoid in future IPM programs.

MATERIALS AND METHODS

Insect Cultures

In 2019, a laboratory stock colony of *E. larvarum* was initially established using adults reared from larvae and pupae of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). The host material collected from forested areas in northern Iran, Guilan Province, Rezvanshahr, Paresar, Sandian (37° 34' 16.94" N, 49° 7' 27.30" E, 5 m asl). The emerged parasitoids were identified using the key of Tschorsnig and Herting (1994), which is widely recognized for its reliability in tachinid identification (Karami *et al.*, 2023). To reduce inbreeding risk, we periodically introduced field-collected individuals from wild populations into the colony during 2021. The colony was maintained in the Entomology Laboratory of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran, using the last-instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), as a factitious host for continuous rearing. This developmental stage was chosen as the most suitable one for parasitism by *E. larvarum* according to Mellini *et al.* (1993), and Dindo *et al.* (2003). The greater wax moth larvae were reared on an artificial diet (Campadelli, 1987) at 30±1°C, 65±5% RH and in total darkness. This host is widely used in parasitoid rearing due to its proven suitability for development, compatibility with low-moisture artificial diets that reduce contamination risk, and greater convenience and safety compared to natural hosts

(Campadelli, 1988; Mellini and Coulibaly, 1991; Delobel and Laviolette, 1969). The adults of parasitoid were maintained in clear and cubic Plexiglas cages (30×30×30 cm, with 80–100 adults per cage) in a growth chamber at 26±1°C, 65±5% RH, and a photoperiod of 16: 8 (L:D) hour. The adult tachinids were fed on sugar cubes, cotton balls soaked in a honey and water solution (20% honey) and distilled water in drinking cups with soaked cotton (Dindo *et al.*, 1999; Dindo *et al.*, 2007; Depalo *et al.*, 2010; Benelli *et al.*, 2018; Dindo *et al.*, 2019; Martini *et al.*, 2019; Dindo *et al.*, 2021). The sugar cubes and drinking cups were changed weekly, but soaked cotton balls were renewed 4–5 times per week.

Experimental Setup

To start the experiment, last-instar larvae of *G. mellonella* were exposed in a cage to 5–7-day-old mated females of *E. larvarum*, at a ratio of 2–3 larvae per female. Females of this age were selected because they exhibit higher oviposition rates (Dindo *et al.*, 1999). The parasitized larvae were removed from the cage approximately one hour after exposure, after 4–5 eggs had been laid on their body surface. These larvae were then transferred individually into ventilated 0.2 mL micro tubes (this maintenance procedure minimizes dropping out eggs from the body surface of the larvae) and placed at seven constant temperatures of 15, 20, 26, 30, 32.5, 34 and 35±1°C, relative humidity of 65±5% and a photoperiod of 16: 8 (L:D) hour. in growth chambers. After 24 hours, the surplus eggs were removed, and only one egg was kept on the surface body of each larva, and a total of 100 parasitized larvae were randomly selected as a cohort for each temperature. Each parasitized larva was considered a replicate. The development of the remaining eggs was checked daily until hatching. Following egg hatching and subsequent penetration of the parasitoid larvae into the host larvae, each parasitized larva was transferred individually into a



transparent cylindrical plastic container (10 cm in diameter×8 cm in height). The containers were fitted with lids containing numerous small round holes (1 mm in diameter) for ventilation. These larvae were not provided with any food throughout the experimental period. The subsequent stages were spent in these plastic containers in the same conditions as above. To confirm the successful penetration of the newly hatched parasitoid larvae and determine the duration and mortality of the immature stages of *E. larvarum*, monitoring was carried out daily and the wax moth larvae were examined under a stereomicroscope until the parasitoids reached adulthood.

Data Analysis and Thermal Modeling

Prior to analysis, the data were tested for normality and found to meet the assumptions of parametric analysis. A one-way ANOVA was conducted to evaluate the effect of temperature on the total developmental time of *E. larvarum*, using SPSS software (version 27.0). Mean comparisons were performed using Tukey's HSD test at a significance level of $P < 0.05$. To describe the development rate of *E. larvarum*, we assessed the efficiency of two linear and 26 nonlinear models, utilizing ArthroThermoModel (ATM) software (Table 1) (Mirhosseini et al., 2017).

Excluding temperature 34°C from data analysis due to being beyond the linear portion of developmental rate and its elimination is essential for the accurate assessment of T_0 (Ikemoto and Takai, 2000). The linear models were used to estimate the Lower Temperature Threshold (T_0) and Thermal Constant (K) of *E. larvarum*. Three criteria including Sum of Squared Error (SSE), adjusted Coefficient of Determination (R^2_{adj}), and Akaike Information Criterion (AIC) (Table 1) were used to assess the nonlinear models. All fitted nonlinear models were ranked using AIC, as the best statistical criterion (Akaike, 1974), and the model(s) with the smallest

value of this parameter was considered the best model for describing the temperature-dependent development of parasitoid.

RESULTS

The parasitoid successfully completed its development at all tested temperatures except 35°C. At 35°C, none of the eggs hatched; therefore, no developmental data obtained at this temperature. Temperature had a significant effect on total developmental time of *E. larvarum* ($F = 4410.778$; $df = 5, 313$; $P < 0.001$). The mean total developmental time decreased until reaching 32.5 °C, after which it increased at 34°C (Table 2). Individuals reared at 15°C had the longest total developmental time. In contrast, those reared at the temperature range of 20–34°C showed a significantly shorter total developmental time, ranging from 23.04 to 15.33 days, respectively (Table 2).

The relationship between temperature and the parasitoid developmental rate was described using various linear and nonlinear thermal models. Table 3 shows the estimated Thermal Constant (K) and Lower Temperature Threshold (T_0) values for the immature stages of *E. larvarum* utilizing two linear models. The ordinary model produced a higher thermal constant estimate and a lower T_0 estimate compared to the Ikemoto model. Additionally, the Ikemoto model exhibited a higher adjusted coefficient of determination (R^2_{adj}) than the other linear model, indicating a slightly greater confidence in the parameter estimates provided by this model.

The nonlinear models for the total developmental time of *E. larvarum* were assessed and ranked by AIC criterion (Table 4). According to AIC rankings (Akaike, 1974), the Performance-2, Beta, Janisch/Kontodimas, Analytis-1 and Analytis 3/Kontodimas models provided more accurate descriptions of the temperature-dependent developmental rates

Table 1. Linear and nonlinear models for fitting to the development rate of *Exorista larvarum* as a function of temperature.

Model	Equation ^a	Reference
Ordinary linear model	$R(T) = a + bT$	(Campbell <i>et al.</i> 1974)
Ikemoto linear model	$DT = K + T_0 D$	(Ikemoto and Takai 2000)
Pradhan-Taylor	$R(T) = R_m \times \exp\left[\frac{-1}{2} \left(\frac{T - T_m}{T_\sigma}\right)^2\right]$	(Pradhan 1945, Taylor 1981)
Davidsons logistic	$R(T) = \frac{K}{1 + e^{(a-bT)}}$	(Davidson 1942, 1944)
Logan-6	$R(T) = \psi [e^{\rho T} - e^{(\rho T_U - \tau)}], \quad \tau = \frac{T_U - T}{\Delta T}$	(Logan <i>et al.</i> 1976)
Hilbert and Logan	$R(T) = \psi \left[\frac{(T - T_0)^2}{((T - T_0) + D)^2} \right] - e^{-\left(\frac{T_U - (T - T_0)}{\Delta T}\right)}$	(Hilbert and Logan 1983)
Lactin-1	$R(T) = e^{\rho T} - e^{(\rho T_U - \frac{T_U - T}{\Delta})}$	(Lactin <i>et al.</i> 1995)
Lactin-2	$R(T) = e^{\rho T} - e^{(\rho T_U - \frac{T_U - T}{\Delta})} + \lambda$	(Lactin <i>et al.</i> 1995)
Logan-10	$R(T) = a \left[\frac{1}{1 + K e^{-\rho T}} - e^{-\tau} \right], \quad \tau = \frac{T_U - T}{\Delta T}$	(Logan <i>et al.</i> 1976)
Analytis-1	$R(T) = P \delta^n (1 - \delta)^m, \quad \delta = \frac{T - T_0}{T_U - T_0}$	(Analytis 1977, 1980)
Analytis-2	$R(T) = [P \delta^n (1 - \delta)]^m, \quad \delta = \frac{T - T_0}{T_U - T_0}$	(Analytis 1977, 1980)
Analytis-1/Allahyari	$R(T) = P \delta^n (1 - \delta^m), \quad \delta = \frac{T - T_0}{T_U - T_0}$	(Allahyari 2005, Zahiri <i>et al.</i> 2010)
Analytis-3	$R(T) = a(T - T_0)^n (T_U - T)^m$	(Analytis 1977, 1980)
Briere-1	$R(T) = aT(T - T_0)(T_U - T)^{\frac{1}{2}}$	(Briere <i>et al.</i> 1999)
Briere-2	$R(T) = aT(T - T_0)(T_U - T)^{\frac{1}{n}}$	(Briere <i>et al.</i> 1999)
Analytis-3/Kontodimas	$R(T) = a(T - T_0)^2 (T_U - T)$	(Kontodimas <i>et al.</i> 2004)

^a T = Temperature, R = Development Rate, D = Development Time, T_0 = Lower Temperature Threshold, T_U = Upper Temperature Threshold, T_{opt} = Optimum Temperature (Equals T_{fast} in the text). Other notations are model constants. For more details on the concepts of the parameters see Mirhosseini *et al.* (2017).

Table 1 Continued...

Continued of Table 1. Linear and nonlinear models for fitting to the development rate of *Exorista larvarum* as a function of temperature.

Model	Equation ^a	Reference
Janisch/Rochat	$R(T) = \frac{2c}{a(T-T_0) + b(T_U-T_0)}$	(Rochat and Gutierrez, 2001)
Sharpe and DeMichele	$R(T) = \frac{T e^{(\phi - \Delta H_A^*/T)/R}}{1 + e^{(\Delta S_L - \Delta H_L/T)/R} + e^{(\Delta S_H - \Delta H_H/T)/R}}$	(Sharpe and DeMichele, 1977)
Sh and DeMichele/Schoolfield	$R(T) = \frac{\rho_{(25^\circ C)} \frac{T}{298} \exp[\frac{\Delta H_A^*}{R} (\frac{1}{298} - \frac{1}{T})]}{1 + \exp[\frac{\Delta H_L}{R} (\frac{1}{T_{1/2L}} - \frac{1}{T})] + \exp[\frac{\Delta H_H}{R} (\frac{1}{T_{1/2H}} - \frac{1}{T})]}$	(Schoolfield et al., 1981)
Sh and DeMichele/Kontodimas	$R(T) = \frac{T \exp(a - b/T)}{1 + \exp(c - d/T) + \exp(f - g/T)}$	(Kontodimas et al., 2004)
Polynomial (cubic)	$R(T) = a_0 T^3 + a_1 T^2 + a_2 T + a_3$	(Harcourt and Yee, 1982)
SSI model	$R(T) = \frac{\rho_\phi \frac{T}{T_\phi} \exp[\frac{\Delta H_A}{R} (\frac{1}{T_\phi} - \frac{1}{T})]}{1 + \exp[\frac{\Delta H_L}{R} (\frac{1}{T_L} - \frac{1}{T})] + \exp[\frac{\Delta H_H}{R} (\frac{1}{T_H} - \frac{1}{T})]}$	(Ikemoto, 2005; 2008)
Performance-1	$R(T) = c(1 - e^{-K_1(T-T_0)})(1 - e^{K_2(T-T_U)})$	(Shi et al., 2011)
Performance-2	$R(T) = m(T - T_0)(1 - e^{K_2(T-T_U)})$	(Shi et al., 2011)
Wang	$R(T) = \frac{m[1 - \exp(K_1(T - T_0))][1 - \exp(K_2(T - T_U))]}{1 + \exp(-c(T - T_0))}$	(Wang et al., 1982)
Ratkowsky	$\sqrt{R(T)} = c(T - T_0)(1 - e^{K(T-T_U)})$	(Ratkowsky et al., 1983)
Beta	$R(T) = r_m \left(\frac{T_U - T}{T_U - T_{opt}} \right) \left(\frac{T - T_0}{T_{opt} - T_0} \right)^{\frac{T_{opt} - T_0}{T_U - T_{opt}}}$	(Yin et al., 1995)

^a T = Temperature, R = Development Rate, D = Development Time, T_0 = Lower Temperature Threshold, T_U = Upper Temperature Threshold, T_{opt} = Optimum Temperature (Equals T_{fast} in the text). Other notations are model constants. For more details on the concepts of the parameters see Mirhoseini et al. (2017).

Table 2. Developmental time in days (mean±SE) of *Exorista larvarum* reared on *Galleria mellonella* at seven constant temperatures.^a

Temperature (°C)	Total developmental time (Day)	N
15	55.06±0.37 ^a	52
20	23.04±0.24 ^b	76
26	17.43±0.15 ^c	61
30	15.92±0.11 ^{cd}	75
32.5	15.13±0.13 ^d	52
34	15.33±0.33 ^d	3
35	No eggs hatched	ND

^a Means followed by different letters in the column of total developmental time are significantly different (Tukey's test; $P < 0.05$). N: sample size.

Table 3. Parameters of two linear regression models and r^2 values for temperature-dependent developmental rates *Exorista larvarum* reared on *Galleria mellonella*.^a

Method	Equation	r^2_{adj}	P	T_0 (°C)	K (DD)
Ordinary	$R = -0.013075 + 0.002568T$	0.88	< 0.01	5.09	389.41
Ikemoto and Takai	$DT = 292.744 + 9.7275D$	0.90	< 0.01	9.73	292.74

^a T_0 : Lower Temperature Threshold (No measurable development is detected); K: Thermal Constant (Total effective temperature).

Table 4. All fitted nonlinear models for temperature-dependent total developmental time of *Exorista larvarum* reared on *Galleria mellonella*.

Model	SSE	R^2_{adj}	AIC	Rank
Pradhan-Taylor	0.007	0.92	-3416.36	6
Davidsons logistic	—	—	—	—
Logan-6	—	—	—	—
Hilbert and Logan	—	—	—	—
Lactin-1	0.011	0.88	-3273.94	10
Lactin-2	0.008	0.91	-3382.98	8
Logan-10	—	—	—	—
Analytis-1	0.006	0.93	-3452.49	4
Analytis-2	—	—	—	—
Analytis-1/Allahyari	—	—	—	—
Analytis-3	—	—	—	—
Briere-1	0.007	0.92	-3409.34	7
Briere-2	—	—	—	—
Analytis-3/Kontodimas	0.006	0.93	-3450.59	5
Janisch/Kontodimas	0.006	0.94	-3476.04	3
Janisch/Rochat	0.008	0.90	-3350.90	9
Sharpe and DeMichele	—	—	—	—
Sh and DeMichele/Schoolfield	—	—	—	—
Sh and DeMichele/Kontodimas	—	—	—	—
Polynomial (cubic)	—	—	—	—
SSI model	—	—	—	—
Performance-1	—	—	—	—
Performance-2	0.005	0.94	-3505.10	1
Wang	—	—	—	—
Ratkowsky model	—	—	—	—
Beta model	0.006	0.94	-3481.40	2

**Table 5.** Parameters of the five best nonlinear models for temperature-dependent developmental rates of *Exorista larvarum* reared on *Galleria mellonella*.^a

Rank	Model	Parameter	Values	Temperature	Observed development time	Estimated development time
1	Performance-2	K_2	0.0444 (0.001582, 0.08722)	15	55.06	51.31
		T_0 (°C)	11.26 (10.65, 11.86)	20	23.04	24.10
		T_U (°C)	45 (40.86, 49.14)	26	17.43	16.82
		m	0.00708 (0.003448, 0.01071)	30	15.92	15.50
				32.5	15.13	15.61
				34	15.33	16.07
2	Beta	T_0 (°C)	9.662 (8.661, 10.66)	15	55.06	51.18
		T_U (°C)	45	20	23.04	24.15
		T_{opt} (°C)	30.35 (30.03, 30.67)	26	17.43	16.65
		r_m	0.06463 (0.06398, 0.06529)	30	15.92	15.48
				32.5	15.13	15.77
				34	15.33	16.38
3	Janisch/Kontodimas	D_{min}	24.52 (24.08, 24.95)	15	55.06	48.41
		K	0.01503 (0.0127, 0.01736)	20	23.04	24.52
		λ	0.2211 (0.2089, 0.2333)	26	17.43	16.67
		T_{opt} (°C)	20 (fixed at bound)	30	15.92	15.59
				32.5	15.13	15.57
				34	15.33	15.69
4	Analytis-1	P	0.5086 (0.1284, 0.8888)	15	55.06	47.29
		T_0 (°C)	5.626 (4.04, 7.212)	20	23.04	24.76
		T_U (°C)	45 (35.87, 54.13)	26	17.43	16.85
		m	1.14 (0.3958, 1.883)	30	15.92	15.42
		n	2 (fixed at bound)	32.5	15.13	15.61
				34	15.33	16.20
5	Analytis-3/Kontodimas	T_0 (°C)	5.013 (4.335, 5.69)	15	55.06	46.40
		T_U (°C)	43.92 (42.83, 45.01)	20	23.04	24.91
		a	7.472e-06 (6.492e-06, 8.451e-06)	26	17.43	16.96
				30	15.92	15.40
				32.5	15.13	15.51
				34	15.33	16.06

^a The values in the parentheses represent 95% confidence intervals. For more details on concepts of the parameters see Mirhosseini et al. (2017).

of *E. larvarum* total immature stages compared to others (Table 5).

The Lower Temperature Threshold (T_0) for the total immature stages of the parasitoid was estimated using the Beta nonlinear model, yielding a result consistent

with that obtained from the Ikemoto linear model. On the other hand, the low temperature thresholds estimated by Analytis-1 and Analytis-3/Kontodimas were approximately equivalent to the estimate provided by the ordinary linear model. The

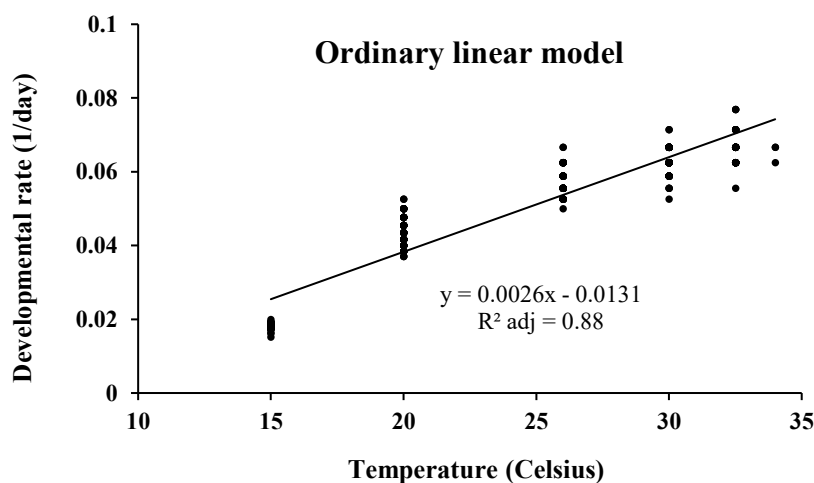


Figure 1. Observed total immature stages development rate of *Exorista larvarum* reared on *Galleria mellonella* (dots) and the ordinary linear model (line).

maximum value of this parameter was estimated by the Performance-2 nonlinear model.

The Upper Temperature Threshold (T_U) values were overestimated, and none of the above-mentioned nonlinear models offered a realistic and precise estimate of this crucial temperature. The T_U values estimated by these models for total immature stages were between 43 and 45°C, which are higher than the observed data, because eggs were not able to hatch at 35°C.

In Table 5, the observed development time was compared to the development time estimated by the five best nonlinear models at six constant temperatures. Notable point in this comparison is the clear proximity between the estimated and observed development time values. Figures 1 and 3 illustrate the relationship between temperature and the developmental rate of total immature stages of *E. larvarum*, as modeled by the ordinary linear model and the five best nonlinear models, respectively. Additionally, Figure 2 presents the effect of

temperature on the developmental time of total immature stages of *E. larvarum* based on the Ikemoto linear model.

Fastest Developmental Temperature (T_{fast}) values calculated by the fitted nonlinear models for *E. larvarum* are displayed in Table 6. The estimated T_{fast} values derived from the Performance-2 and Janisch/Kontodimas models were in close accordance with the observed T_{fast} .

DISCUSSION

To the best of our knowledge, no previous studies have estimated the thermal requirements for the immature *E. larvarum* development. Therefore, our findings provide fundamental information on the biology of this parasitoid, which will result in its more successful application as an efficient biocontrol agent against different lepidopterous defoliators in both forest and agricultural ecosystems.

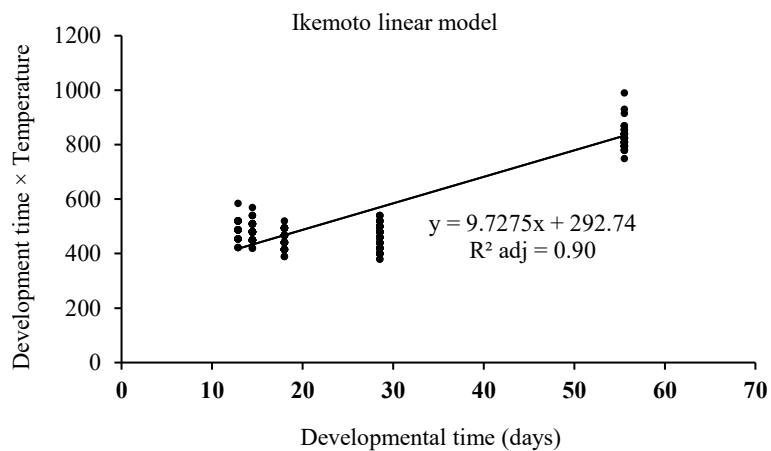


Figure 2. Observed total immature stages development time of *Exorista larvarum* reared on *Galleria mellonella* (dots) and the Ikemoto linear model (line).

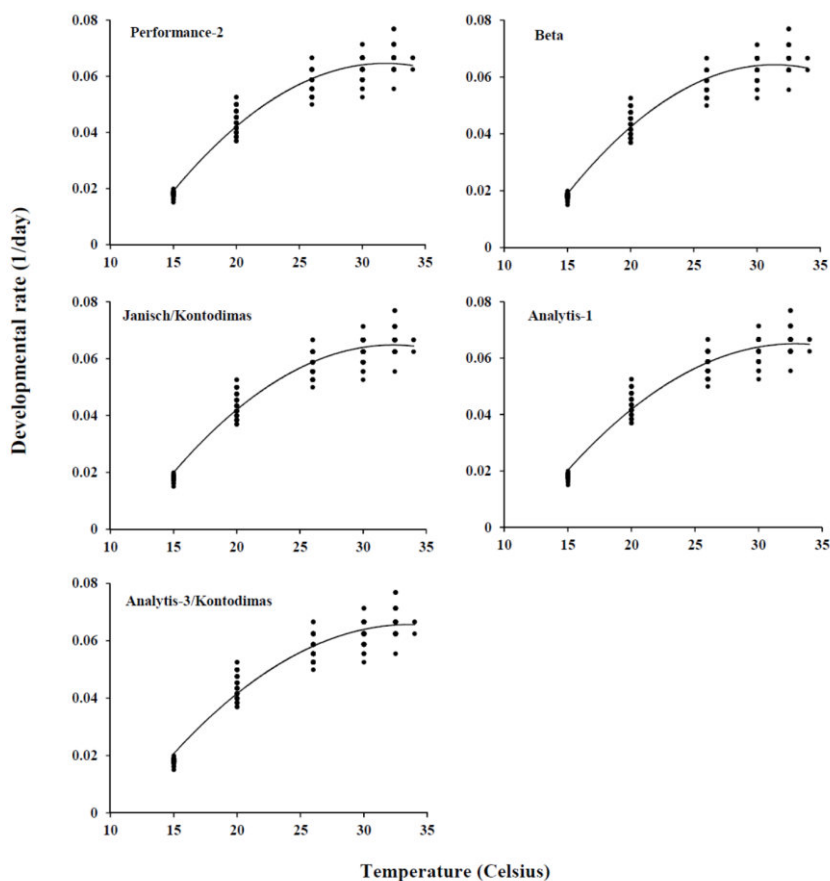


Figure 3. Observed total immature stages development rate of *Exorista larvarum* reared on *Galleria mellonella* (dots) and the five best nonlinear models (lines).

In this study, development was observed across all temperatures, except at 35°C,

showing that this temperature lies beyond the conducive range for the development of

Table 6. T_{fast} estimated by fitted nonlinear models for *Exorista larvarum* reared on *Galleria mellonella*.

Model	T_{fast}	Developmental time (Day)
Pradhan-Taylor	30.7	15.37
Lactin-1	30.4	21.56
Lactin-2	31.1	14.96
Analytis-1	30.7	15.38
Briere-1	30.9	15.19
Analytis-3/Kontodimas	31	15.34
Janisch/Kontodimas	31.4	15.54
Janisch/Rochat	31	15.28
Performance-2	33.2	15.79
Beta	30.3	15.47

E. larvarum. Combining calculates from both linear and nonlinear models offered an accurate prediction of the first appearance of *E. larvarum* adults in forest and agricultural ecosystems. This analysis revealed that this parasitoid could develop within a temperature range of 15–34°C, a favorable feature for its potential role as a biological control agent in management programs. The extensive geographic distribution of *E. larvarum* across various provinces of Iran, each with diverse climatic conditions, is a clear witness of this claim (Modarres Awal, 1994; Karimpour *et al.*, 2005; Saeidi, 2011; Ghahari, 2017; Karami *et al.*, 2023).

The results obtained demonstrated a significant impact of temperature on the developmental time of this parasitoid. The duration of total immature stages exhibited a negative correlation with temperature up to 32.5°C, and then increased. Conversely, the development rate increases as the temperature rises, reaching its peak and, subsequently, declining to zero at the Upper Temperature Threshold (T_U) (Mirhosseini *et al.*, 2017).

The assessment of temperature thresholds and thermal constants for the development of natural enemies can considerably contribute to the choice of the most appropriate natural enemy to be utilized in various environmental circumstances (Perdikis and Lykouressis, 2002).

The observed linear relationship between temperature and the development rate of *E. larvarum* across the 15–32.5°C range indicates strong thermal dependence in the biology of this parasitoid. Estimation of the

Lower Temperature Threshold (T_0) and the Thermal Constant (K) using both the ordinary and Ikemoto linear models confirmed this trend. However, the Ikemoto model demonstrated greater accuracy, as evidenced by a higher R^2_{adj} coefficient, for modeling temperature-dependent development in this species. These findings align with previous studies on other natural enemies, where the Ikemoto model often provides a better predictive performance (Aghdam *et al.*, 2009; Jafari *et al.*, 2022). The reliable estimation of T_0 and K are essential for predicting field development rates and optimizing mass-rearing or biological control strategies under varying thermal conditions.

The value of the Lower Temperature Threshold (T_0) for the *E. larvarum* immature stages was found to be 9.7°C using the Ikemoto model, which was in agreement with Foerster and Doetzer (2002) who reported 9.3°C as a lower threshold temperature from eggs to adulthood for another tachinid, *Peleteria robusta* (Wiedman). Lower values for the low temperature threshold were estimated by Walker (2011) and Park *et al.* (2016) for *Chaetophthalmus dorsalis* (Malloch) (7.3 and 7.4°C for females and males, respectively) and *Exorista japonica* (Townsend, 1909) (7.8°C), respectively. The thermal constant calculated with the Ikemoto linear model for total development of *E. larvarum* [292.7 Degree-Days (DD)] was lower than previously reported values for other tachinid flies: *E. japonica* (370.4 DD) (Park *et al.*, 2016), *P. robusta* (457.5 DD)



(Foerster and Doetzer, 2002), and *C. dorsalis* (366.7 and 333.3 DD for females and males, respectively) (Walker, 2011). The differences among their results and our reports may be due to the difference in the host and parasitoid species, the difference in rearing techniques and experimental conditions, along with differences in data analysis procedure.

The temperature maximizing developmental rate, commonly termed the Fastest Developmental Temperature (T_{fast}), may not necessarily align with the temperature that maximizes overall population fitness, as the benefits of accelerated development may be counterbalanced by increased mortality rates. In the present study, the maximum developmental rate was recorded at 32.5°C among all the examined temperatures, and can be intended as the observed fastest developmental temperature. Some of the nonlinear models can be applied directly to calculate T_{fast} (Kontodimas et al., 2004; Mojib-Haghighadam et al., 2019). The T_{fast} values calculated by Performance-2 and Janisch/Kontodimas for the parasitoid immature stages were 33.2 and 31.4°C, respectively. These two models appear to offer realistic values of T_{fast} compared with other models. In other words, T_{fast} denotes the temperature at which the developmental rate reaches its maximum and the developmental time is minimized (Moallem et al., 2017; Shamakhi et al., 2018). From a biological control perspective, a shorter pre-adult period is considered advantageous for agents such as parasitoids, as it reduces generation time and can enhance population growth potential. This trait is particularly valuable for improving the efficiency and rapid establishment of parasitoid populations in the field (Milenovic et al., 2023; Wyckhuys et al., 2024).

To choose the best nonlinear model(s) for describing temperature-dependent developmental rates of *E. larvarum* total immature stages, the adjusted R^2 (R^2_{adj}), Akaike Information Criterion (AIC), and estimated temperature-related biological

parameters (Akaike, 1974; Kvalseth, 1985; Kontodimas et al., 2004) were used. As the best statistical criterion to validate models, the lowest value of AIC was used to rank the fitted nonlinear models (Akaike, 1974; Larranaga and Bielza, 2014). Moreover, the nonlinear models are frequently verified by observed developmental data and biological criteria (Zahiri et al., 2010; Soltani Orang et al., 2014; Aghdam and Nemati, 2020; Yazdanpanah et al., 2022). In the present research, the observed development time was compared to the estimated development time because a usual manner for evaluating the precision of the calculated crucial temperatures is according to their comparison with experimental data (Kontodimas et al., 2004). Notable point in this comparison is a close agreement was observed between the estimated and observed developmental times, indicating that the models provided relatively accurate and reliable estimates. According to our findings, the nonlinear Performance-2, Beta, Janisch/Kontodimas, Analytis-1, and Analytis 3/Kontodimas models best described the developmental rate of *E. larvarum*. The calculated T_{fast} by Performance-2 model was similar to the observed T_{fast} (32.5°C).

In conclusion, the results demonstrated that *E. larvarum* could be an effective biocontrol agent in forest and agricultural ecosystems over a vast range of temperatures. Evidently, parameters calculated in the present research were derived in the laboratory under totally determined climatic circumstances, despite the reality that parasitoids are subjected to more complicated and oscillating circumstances in their natural environment. However, the results of this study can provide a starting point for developing a model applicable to the Iranian population of *E. larvarum*. When combined with other ecological data, such a model could be valuable for the development and implementation of pest management programs.

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رشد وابسته به دمای *Exorista larvarum* (Diptera: Tachinidae)، گزینه‌ای توانمند برای کنترل بیولوژیک آفات

آمنه کرمی، علی اصغر طالبی، یعقوب فتحی پور، و محمد مهرآبادی

چکیده

برای بهینه‌سازی پرورش انبوه (Diptera: Tachinidae) *Exorista larvarum* (Linnaeus, 1758) و توسعه کاربرد آن در برنامه‌های مدیریت تلفیقی، طول مرحله‌ی رشد پیش از بلوغ این پارازیتوئید در شرایط آزمایشگاهی در هفت دمای ثابت ($15-35^{\circ}\text{C}$) مورد بررسی قرار گرفت. برای توصیف نرخ رشد وابسته به دمای *E. larvarum*، از دو مدل خطی و ۲۶ مدل غیرخطی استفاده شد. آستانه پایین دمایی (T_0) و ثابت گرمایی (K) با استفاده از مدل خطی ordinary به ترتیب $5/09^{\circ}\text{C}$ و $389/41$ روز-درجه (DD) و با استفاده از مدل خطی Ikemoto and Takai به ترتیب $9/73^{\circ}\text{C}$ و $292/74$ DD برآورد شد. مدل‌های غیرخطی Analytis-3/Kontodimas و Analytis-1، Janisch/Kontodimas، Beta، Performance-2 به ترتیب بهترین مدل‌ها برای توصیف نرخ رشد وابسته به دمای این پارازیتوئید تعیین شدند. دمای برآورد شده بیشینه رشد (T_{fast}) توسط مدل‌های Performance-2 و Janisch/Kontodimas به ترتیب $33/2$ و $31/4^{\circ}\text{C}$ محاسبه شد که نزدیک‌ترین مقادیر را به T_{fast} مشاهده شده ($32/5^{\circ}\text{C}$) داشتند. یافته‌های این پژوهش می‌تواند بینش جدید و ارزشمندی درباره زیست‌شناسی *E. larvarum* ارائه دهد و اطلاعات اساسی فراهم کند که می‌تواند در مدل‌های پیش‌آگاهی این پارازیتوئید گنجانده شود.

Urea-Formaldehyde Nano-encapsulation Enhances *Bunium persicum* Essential Oil's Toxicity against *Brevicoryne brassicae*

Masoud Heidary¹, Shahriar Jafari¹, Javad Karimzadeh², Maryam Negahban³, Jahanshir Shakarami¹, and Mehran Rezaei^{4*}

ABSTRACT

This study investigated the aphicidal activity of both pure and nanoencapsulated *Bunium persicum* (Boiss.) Fedtsch Essential Oils (EO) against *Brevicoryne brassicae* L.. The EO obtained through hydrodistillation and analyzed by Gas Chromatography and Mass Spectrophotometry (GC-MS), contained γ -terpinen (36.62%), p -cymene (18.41%), carvacrol (13.60%), and cuminaldehyde (13.50%) as its major components. Nanocapsules were synthesized via in situ polymerization of an oil-in-water emulsion and characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM), revealing spherical particles with a median diameter of 10.88 nm and a low Polydispersity Index (PDI) of 0.057. Bioassays demonstrated that the nanoencapsulated formulation exhibited significantly higher toxicity than the pure EO, with lower contact LC_{50} and LC_{90} values of 365.43 and 1908.46 $\mu\text{L L}^{-1}$ water, respectively, compared to the pure EO (1030.40 and 3977.08 $\mu\text{L L}^{-1}$ water). Similarly, the fumigant LC_{50} and LC_{90} values for the nanocapsules (23.15 and 59.49 $\mu\text{L L}^{-1}$ air) were significantly lower than those of the pure EO (35.07 and 79.59 $\mu\text{L L}^{-1}$ air). The findings suggest the potential of nanoencapsulated *B. persicum* EO in integrated pest management for controlling cabbage aphid.

Keywords: Aphid, Biocompatible insecticide, Efficacy, Encapsulated, Nanoparticle.

INTRODUCTION

The Brassicaceae (or Cruciferae) family, the largest within the order Brassicales, comprises 12–15 tribes, 338–360 genera, and approximately 3,709 species distributed globally (excluding Antarctica). This family includes economically significant species cultivated for food, fodder, medicine, oil production, and ornamental purposes (Raza *et al.*, 2020). However, brassica crops face substantial yield losses due to insect pests, particularly sap-sucking insects. The

cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), is one of the most serious insect pests of cruciferous crops throughout the world. This insect pest species inflicts direct damage by feeding on plant sap and indirectly by transmitting viral pathogens, posing a significant threat to cruciferous crops (Canassa *et al.*, 2020; Patel *et al.*, 2024).

Conventional management of *B. brassicae* relies heavily on synthetic insecticides, which, despite their efficacy in boosting crop yields, have led to the development of widespread resistance in aphid populations

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and significant environmental contamination (Ahmad and Akhtar, 2013; Nematollahi et al., 2014). Documented resistance includes key insecticide groups such as neonicotinoids (e.g., imidacloprid, thiamethoxam), pyrethroids (e.g., cypermethrin, deltamethrin, bifenthrin), and organophosphates (e.g., profenofos, chlorpyrifos) (Ahmad and Aslam, 2005; Ahmad and Akhtar, 2013). Given these challenges, there is an urgent need for sustainable solutions, such as biopesticides, to address the environmental and resistance concerns linked to synthetic chemicals (Moharramipour and Negahban, 2014; da Silva et al., 2023).

Plant-derived Essential Oils (EOs) have emerged as promising alternatives to synthetic insecticides for pest management due to their broad-spectrum bioactivity and eco-friendly properties (Abdelgaleil et al., 2009; Campolo et al., 2018; Rezaei et al., 2019). These complex volatile mixtures, typically obtained through hydrodistillation or steam extraction of aromatic plants, contain diverse bioactive constituents including monoterpenes, sesquiterpenes, and oxygenated derivatives such as alcohols, aldehydes, ketones, esters, phenols, and lactones (Abbassy et al., 2009; Abdelgaleil et al., 2009; Lopez and Pascual-Villalobos, 2010; Campolo et al., 2018). EOs exhibit multiple modes of action against insect pests, demonstrating contact toxicity, fumigant activity, repellency, and antifeedant effects when applied through conventional application methods (Ahmadi et al., 2018; Rezaei et al., 2019). Their biological activity stems from both vapor-phase toxicity and cuticular penetration upon topical application (Abbassy et al., 2009; Khanavi et al., 2017; Campolo et al., 2018). Particularly against *B. brassicae*, numerous studies have documented the efficacy of various EOs and their constituent compounds through these mechanisms (Jahan et al., 2016; Heidary et al., 2022; da Silva et al., 2023). For instance, EO from *Schinus terebinthifolius* (Raddi) (Brazilian pepper tree) demonstrated superior

insecticidal and repellent activity against *B. brassicae* compared to *Eucalyptus citriodora* (Hook) and *Cymbopogon winterianus* (Jowitt). Notably, while effectively controlling the aphid pest, the *S. terebinthifolius* EO simultaneously attracted *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), a key parasitoid of *B. brassicae* (da Silva et al., 2023). However, it is reported that EO of *Elettaria cardamomum* (L.) Maton is an appropriate compound against *B. brassicae* than other tested EOs, including *Cinnamomum zeylanicum* Blume, *Citrus sinensis* (L.) Osbeck, *Foeniculum vulgare* Mill., and *Thymus carmanicus* Jalas (Jahan et al., 2016).

Iran, with its rich biodiversity of over 8,200 vascular plant species, approximately 2300 of which are medicinal or aromatic, provides a valuable resource for such bioactive compounds (Mozaffarian, 2012). The Apiaceae family is particularly noteworthy for producing secondary metabolites with demonstrated insecticidal properties (Thiviya et al., 2022). Among these, *Bunium persicum* (Boiss.) Fedtsch. (Persian cumin), a perennial aromatic herb indigenous to the Irano-Turanian region, produces schizocarpic fruits containing potent EOs (Nickavar et al., 2014). These characteristically aromatic brown seeds serve dual roles as both a traditional culinary spice and a source of bioactive compounds, with their EO profile exhibiting notable insecticidal activity against various agricultural and medicinal pests, including *Sitotroga cerealella* Olivier (Nouri Ganbalani et al., 2021), *Tribolium castaneum* (Herbst) (Moravvej et al., 2011), *Trichoplusia ni* (Hübner) (Khanavi et al., 2017), *Anopheles stephensi* Liston (Sanei-Dehkordi et al., 2016; Vatandoost et al., 2018), *Culex quinquefasciatus* Say (Perinelli et al., 2022), and *Culex pipiens* L. (Sanei-Dehkordi et al., 2016).

Despite their pesticidal potential, EOs face practical limitations including high volatility, poor water solubility, and rapid environmental degradation (Abdelgaleil et

al., 2009; Heidary *et al.*, 2022). Nanoencapsulation technology addresses these challenges by enhancing compound stability, improving bioavailability, and enabling the controlled release of active ingredients (Nuruzzaman *et al.*, 2016; Granata *et al.*, 2018; Kumar *et al.*, 2019). Although several studies have investigated the efficacy of pure EOs from various plant species against *B. brassicae* (e.g., Jahan *et al.*, 2016; da Silva *et al.*, 2023), research on nanoformulated EOs for aphid control remains limited. To bridge this knowledge gap, the present study aimed to: (1) Characterize the chemical composition of *B. persicum* EO, (2) Develop and optimize a nanoencapsulated formulation, (3) Analyze nanocapsule morphology and particle size distribution, and (4) Conduct a comparative evaluation of the aphicidal efficacy between pure and nanoencapsulated EOs against *B. brassicae*.

MATERIALS AND METHODS

Plant and Insect Rearing

Flowering kale, *Brassica oleracea* var. *acephala*, cv. Pigeon F₁ Red FHB 511 (Takii Europe B.V., De Kwakel, Netherlands) was cultivated in clay pots (12 cm diameter×12 cm height) filled with a sterilized growth medium consisting of soil, leaf mold, manure, peat moss, and humic acid (30:10:10:3:1, v/v). Plants were maintained in a greenhouse at 25±5°C, 60±5% Relative Humidity (RH), and a 16:8-hour Light:Dark (L:D) photoperiod, with no pesticide application. The original population of *B. brassicae* was collected from canola fields in PirBakran region (32° 28' N, 51° 33' E; 1610 m altitude), Isfahan Province, central Iran. Culture of apterae *B. brassicae* was maintained on 5-week-old *B. oleracea* in ventilated cages (40×40×40 cm) under constant environmental conditions (25±1°C, 65±5% RH, and 16:8 hours L:D photoperiod). The plants were watered regularly and replaced with new ones as

needed. The aphids were subsequently reared for more than five generations on *B. oleracea* before treatment.

Bunium Persicum Essential Oil

The fruits of *B. persicum* were collected in May 2018 from cultivated fields in Khur and Biabanak County (33° 46'N, 55° 05'E, Isfahan Province, Iran). A voucher specimen (#16059) was identified and deposited in the Herbarium of Isfahan Research and Education Center for Agriculture and Natural Resources (Isfahan, Iran). The plant material was air-dried at room temperature (23-25°C) for five days and stored in darkness until distillation. The EO was extracted from dried plant samples (50 g each) via hydrodistillation (material-to-water ratio of 1:10 w/v; 4 hours distillation) using a modified Clevenger-Type apparatus. Anhydrous sodium sulfate was used to remove water after extraction. The EO yield was 4% (w/w) on a dry weight basis. The resulting EO was transferred into sealed glass vials and stored at 4°C in a refrigerator until bioassay analysis (Rezaei *et al.*, 2019). For experimental use, the pure EO was diluted in distilled water with Tween 80 (0.5% v/v) as an emulsifier.

Chemical Analysis of Essential Oil

The chemical composition of *B. persicum* EO was analyzed using Gas Chromatography-Mass Spectrometry (GC/MS) (Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass selective detector; Agilent Technologies, Palo Alto, CA, USA). Separation was achieved using an HP-5MS capillary column (30 m×0.25 mm, 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 0.8 mL min⁻¹. The oven temperature was initially held at 60°C for 2 minutes, then increased to 280 °C at a rate of 4°C min⁻¹. A split injection mode (split ratio 1:50) was used with a manual



injection volume of 0.1 μL . Individual compounds were identified by comparing their Retention Indices (RI) and mass spectra with those reported in the literature (Adams, 2007).

Nanocapsule Preparation of Essential Oil

Nanocapsules were prepared via in-situ polymerization using an Oil-in-Water (O/W) emulsion method (Rochmadi *et al.*, 2010). The core material consisted of *B. persicum* EO, while the nanocapsule shell was formed from urea and formaldehyde (37%). Tween 80 (2%) served as the emulsifier. Also, castor oil (10%) and potassium silicate were used as synergists. Initially, a Urea-Formaldehyde (U-F) pre-polymer solution was prepared, homogenized, and then mixed with Tween 80 and the EO. To facilitate the encapsulation of EO droplets within the U-F shell, the solution pH was adjusted to 3.0. Stable nanocapsules formed after 4 h of reaction. The nanocapsule formulation containing *B. persicum* EO was prepared with the active ingredient at an 8% concentration. The resulting suspension was cooled to room temperature, filtered, and finally dehydrated by freeze-drying (Ting *et al.*, 2010; Zhang *et al.*, 2016). The yield and efficiency of nanoencapsulation and oil loading content were calculated according to the following equations (Khoee and Yaghoobian, 2009).

$$\text{Nanoencapsulation yield \%} = \left(\frac{N}{C}\right) \times 100$$

Where, C is the weight of the produced nanocapsules (g) and N is the weight of the initial materials (g).

$$\text{Nanoencapsulation efficiency \%} = \left(\frac{A}{B}\right) \times 100$$

Where, A is the weight of the loaded EO (g) within the nanocapsules and B is the weight of the EO used in the experiment (g).

$$\text{Oil loading content \%} = \left(\frac{A}{D}\right) \times 100$$

Where, A is the weight of the loaded EO (g) within the nanocapsules and B is the

weight of the nanocapsules in the experiment (g).

Essential oil Nanocapsule Size and Morphology

The morphology and surface characteristics of the nanocapsules were analyzed using Transmission Electron Microscopy (TEM) (Carl Zeiss-EM10C, 100 kV, Carl Zeiss AG, Oberkochen, Germany). The mean diameter (z-average) and Poly-Dispersity Index (PDI) were determined by Dynamic Light Scattering (DLS) using a Nanophox 90-246 V nanoparticle size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany), which employs Photon Cross-Correlation Spectroscopy (PCCS) (Moghimi *et al.*, 2018).

BIOASSAY

Contact Toxicity

The contact toxicity of pure and nanocapsulated formulations of *B. persicum* EO against *B. brassicae* adults was evaluated using the leaf-dipping method (Rezaei and Moharramipour, 2019). To obtain synchronized aphids for the experiments, apterous females were individually placed on flowering kale leaf discs (5 cm diameter) in ventilated Petri dishes (9 cm diameter) containing a 5 mL layer of 2% agar solution to produce offspring. After 24 hours, the adults were removed, and the nymphs were allowed to develop into adults. The presence of exuviae was used to determine when molting happened.

Preliminary tests were conducted to determine appropriate concentrations (inducing 10–90% mortality at logarithmic intervals) of pure and nanocapsulated *B. persicum* EOs using 2-day-old *B. brassicae* adults. Based on these tests, the selected concentrations for the main experiment were 103, 272, 717, 1886, and 4964 ppm for the pure EO and 33, 104, 326, 1026, and 3226 ppm for the nanocapsulated EO. The different solutions of both raw and

nanocapsulated *B. persicum* EO were prepared by dispersing a known quantity of each in a volume of distilled water.

For bioassays, twenty 2-day-old *B. brassicae* adults were placed on a flowering kale leaf disc (5 cm diameter). After 15–30 minutes (allowing aphids to settle), the leaf discs were dipped into the respective EO concentrations for 5 seconds. The treated leaf discs were then placed in ventilated Petri dishes (9 cm diameter) with a 5 mL layer of 2% agar solution. Parafilm was used to seal the dishes and prevent aphid escape. Controls consisted of distilled water (with 0.5% Tween 80 for pure EO and without Tween 80 for nanocapsulated EO).

Mortality was assessed after 72 hours (Heidary *et al.*, 2022), with aphids considered dead if they showed no movement after gentle prodding with a fine brush (Khoobdel *et al.*, 2017; Rezaei and Moharramipour, 2019). Each concentration was replicated five times, and all bioassays were conducted under controlled conditions (25±1°C, 65±5% RH, and a 16:8 hour L:D photoperiod).

Fumigant Toxicity

The fumigant toxicity of pure and nanocapsulated *B. persicum* EO against apterous *B. brassicae* females was evaluated using sealed 400 mL glass vials. Similar to the contact toxicity bioassays, preliminary tests were conducted with 2-day-old adults to determine effective concentrations.

For our experiments, flowering kale leaf discs (5 cm diameter) were placed on a 7.5 mL layer of 2% agar solution. Synchronized *B. brassicae* females were carefully transferred onto the leaf discs using a soft paintbrush. Filter paper strips (Whatman no. 1, 5×2 cm) were treated with the pure or nanocapsulated EOs at designated concentrations and attached to the inner vial walls to prevent direct insect-pesticide contact.

In the main bioassay, the tested concentrations were 12.47, 20.80, 34.65,

57.72, and 96.17 µL L⁻¹ air for the pure EO and 7.65, 13.32, 23.22, 40.45, and 70.50 µL L⁻¹ air for the nanocapsulated EO. Untreated filter papers served as controls (Ahmadi *et al.*, 2018). The vials were sealed with parafilm, and each treatment was replicated five times. The mortality and bioassay conditions were the same as those used for the contact toxicity.

Statistical Analysis

The Dynamic Light Scattering (DLS) data were analyzed using the Cumulants analysis (mono-modal and Gaussian distribution). The distribution of particles was calculated by Polydispersity Index (PDI) as follows:

$$PDI = \left(\frac{\sigma}{d}\right)^2$$

Where, δ and d denote the standard deviation and mean diameter of particles, respectively (Fang *et al.*, 2004). The mortality was evaluated using the Abbott correction formula for the natural mortality in untreated controls (Abbott, 1925). Generalized Linear Models (GLMs) with the binomial family were applied to the bioassay data. In particular, the dose-response data were analyzed using logistic regression (binomial errors), where regression lines were fitted to dose-mortality data on a log (of concentration)-logit (of mortality) scale, and the estimated Lethal Concentrations (LC) and associated 95% Confidence Intervals (CI) were then calculated from the estimated linear regression parameters. Because of non-parallel regression lines, the toxicity ratio test (lethal dose ratios) was used to compare LCs, instead of overlapping CIs, based on the statistical power of the ratio test and its better type I error rates (Wheeler *et al.*, 2006; Crawley, 2012). All statistical analyses were performed in R 3.6.1 (R Development Core Team).

**Table 1.** Chemical composition of *Bunium persicum* essential oil.^a

Compounds	RT (Min)	Value (%)	RI	KI
α -Thujene	5.38	0.49	923	924
α -Pinene	5.55	1.57	930	932
β -Pinene	6.47	3.11	969	974
α -Terpinen	7.58	0.26	1014	1017
ρ -Cymene	7.80	18.41	1022	1024
Limonene	7.91	7.12	1027	1029
γ -Terpinen	8.79	36.62	1060	1059
Trans-2-Caren-4-ol	10.43	0.55	1119	-
Borneol	12.19	0.52	1175	1169
γ -Terpineol	13.05	0.41	1202	1199
Thymol methyl ether	14.10	0.16	1227	1235
Cuminaldehyde	14.56	13.50	1239	1239
2-Caren-10-al	16.00	1.96	1274	-
Acetic acid, chloro-, n-decyl ester	16.24	0.12	1280	-
Carvacrol	16.62	13.60	1289	1298
β -Caryophyllene	20.30	0.53	1416	1419
Ledene	22.98	0.12	1499	1490
γ -Bisabolene	23.98	0.24	1538	1533

^a RT: Denotes the Retention Time for each compound; RI: Denotes the Retention Index for each compound, KI: Kovats Retention Index. The Retention Index (RI) refers to the Kovats Retention Index (KI), which is a standardized, dimensionless parameter used in Gas Chromatography (GC) to identify volatile compounds. It is calculated based on the retention times of a series of n-alkanes (straight-chain hydrocarbons) under the same chromatographic conditions (Vernin *et al.*, 1992; Marques *et al.*, 2000).

RESULTS

Chemical Analysis of Essential Oil

The GC-MS analysis identified 28 constituents in *B. persicum* EO, with 18 compounds present at concentrations > 0.1% (Table 1). The oil was dominated by terpenes and benzenes, with γ -terpinene (36.62%) being the most abundant constituent, followed by ρ -cymene (18.41%), carvacrol (13.60%), cuminaldehyde (13.50%), limonene (7.12%), β -pinene (3.11%), 2-caren-10-al (1.96%), and α -pinene (1.57%).

Nanocapsule Size, Morphology, and Formulation Characteristics

Transmission Electron Microscopy (TEM) analysis (16700 $\times$ magnification) revealed that nanocapsulated *B. persicum* EO formed distinct core-shell structures with near-spherical morphology (Figure 1), confirming

successful encapsulation. The nanocapsules exhibited slightly irregular surface topography. Size distribution analysis showed nanoparticle diameters ranging from 8.11 nm (10th percentile) to 14.62 nm (90th percentile) (Figure 2), with median diameter (x_{50}) of 10.88 nm, Volume Mean Diameter (VMD) of 11.17 nm, and Sauter Mean Diameter (SMD) of 10.60 nm. The corresponding polydispersity indices were 0.057 (x_{50}), 0.054 (VMD), and 0.060 (SMD), indicating a narrow size distribution.

In the nanocapsule polymerization process, the yield and efficiency of nanoencapsulation and oil loading content were 91, 92, and 88.22%, respectively.

Contact and Fumigant Toxicity

Logistic regression revealed significant dose-response relationships for both pure and nanocapsulated EO formulations in both contact and fumigant toxicity tests ($P < 0.001$ for all comparisons). For contact toxicity, the models showed strong fits (McFadden's

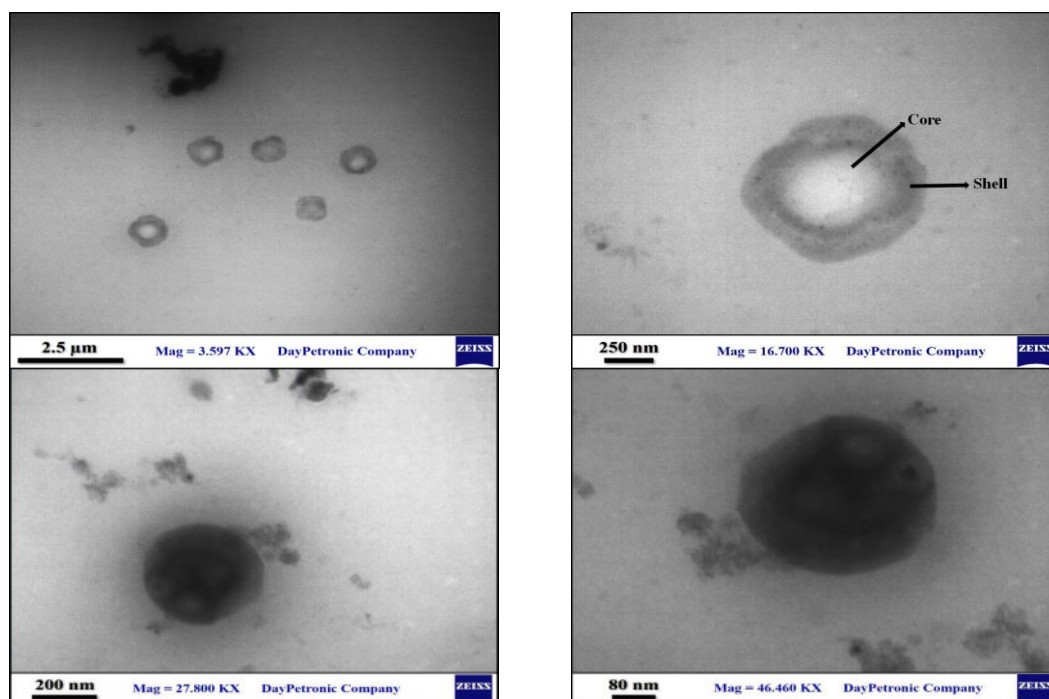


Figure 1. Transmission electron microscopy images of nanocapsulated essential oil of *Bunium persicum* with different magnifications between 3.597×10^3 and 4.646×10^4 .

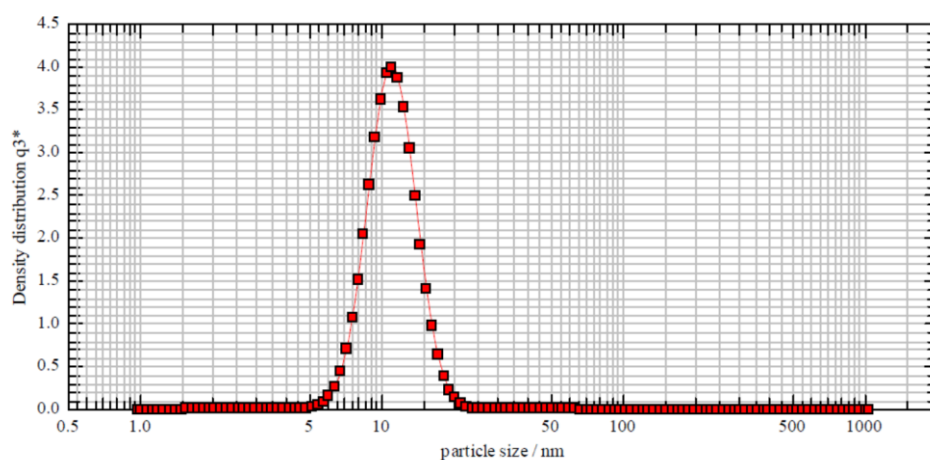


Figure 2. Size distribution of nanocapsule particles of *Bunium persicum* essential oil.

$R^2 = 0.833$ and 0.879 for pure and nanocapsulated EO, respectively) with significant Z-values (12.06 and 12.28). Similarly, fumigant toxicity models demonstrated excellent fits ($R^2 = 0.924$ and 0.921) with significant z-values (12.20 and 12.29) (Table 2).

Toxicity ratio tests indicated significantly greater potency of nanocapsulated EO across

all lethal concentrations (LC_{10} - LC_{90}) for both test methods (Table 4). For contact toxicity, nanocapsulated EO required substantially lower concentrations (69.97-1908.46 ppm) compared to pure EO (266.96-3977.08 ppm) to achieve equivalent mortality levels (Table 3). This enhanced efficacy was similarly evident in fumigant tests, where nanocapsulated EO showed



lower required concentrations (9.01-59.49 $\mu\text{L L}^{-1}$ air) versus pure EO (15.45-79.59 $\mu\text{L L}^{-1}$ air) (Table 3).

Dose-response curves (Figures 3 and 4) consistently demonstrated superior toxicity of nanocapsulated EO across the entire tested range (0.01-99.99% mortality), confirming the enhanced insecticidal efficacy of the encapsulated formulation through both exposure routes.

DISCUSSION

This study demonstrated the insecticidal efficacy of both pure and nanoencapsulated formulations of *B. persicum* EO against *B. brassicae* using standardized contact and fumigant bioassay methods. Our findings align with previous reports of this EO's toxicity against other insect pests (Moravvej et al., 2011; Khanavi et al., 2017; Perinelli et al., 2022). To our knowledge, this work

provides the first evidence of *B. persicum* EO's activity against *B. brassicae* and represents the first evaluation of its nanoencapsulated formulation for this pest. The rationale for selecting *B. persicum* EO was based on: (1) Documented insecticidal properties against various pests (Sanei-Dehkordi et al. 2016; Nouri Ganbalani et al. 2021); (2) Potential for enhanced efficacy through nanoformulation (Kumar et al., 2019; Heidary et al., 2022); (3) Scalability of production; (4) Widespread distribution and cultivation feasibility in Iran (Thiviya et al., 2022); and (5) Its favorable environmental and human safety profile (Moharramipour and Negahban, 2014; Campolo et al., 2018). However, the high volatility of the EO limits their practical application in pest management programs, necessitating the development of stabilization strategies.

Table 2. Logistic regression of dose-response data of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults.

Bioassay procedure	Formulation	Fitted model ^a				
		a (SE)	b (SE)	Z-value ^b	P value <	R ² _{McF} ^c
Contact	Pure EO	-11.29 (0.94)	1.63 (0.13)	12.06	0.001	0.833
	Nanocapsulated EO	-7.84 (0.65)	1.33 (0.11)	12.28	0.001	0.879
Fumigation	Pure EO	-7.08 (0.59)	2.68 (0.22)	12.20	0.001	0.924
	Nanocapsulated EO	-5.03 (0.42)	2.25 (0.18)	12.29	0.001	0.921

^a $= \frac{e^{a+bx}}{1+e^{a+bx}}$, where x and y represent natural logarithm of concentration (ppm) and the proportion of dead adult, respectively. ^b The standard normal deviate. ^c McFadden's R-squared.

Table 3. Contact and fumigant toxicity of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults.

Bioassay procedure	Formulation	Lethal concentration (95% CI) in ppm or $\mu\text{L L}^{-1}$ air ^a (n= 5)			
		LC ₁₀	LC ₂₅	LC ₅₀	LC ₉₀
Contact	Pure EO	266.96 a (205.65 - 346.55)	524.48 a (436.23 - 630.58)	1030.40 a (882.86 - 1202.59)	3977.08 a (3018.89 - 5239.39)
	Nanocapsulated EO	69.97 b (50.86 - 96.27)	159.91 b (127.68 - 200.28)	365.43 b (303.62 - 439.83)	1908.46 b (1378.10 - 2642.93)
Fumigant	Pure EO	15.45 A (13.19 - 18.09)	23.27 A (20.87 - 25.95)	35.07 A (32.18 - 38.12)	79.59 A (68.02 - 93.06)
	Nanocapsulated EO	9.01 B (7.55 - 10.76)	14.45 B (12.80 - 16.30)	23.15 B (21.04 - 25.47)	59.49 B (49.74 - 71.08)

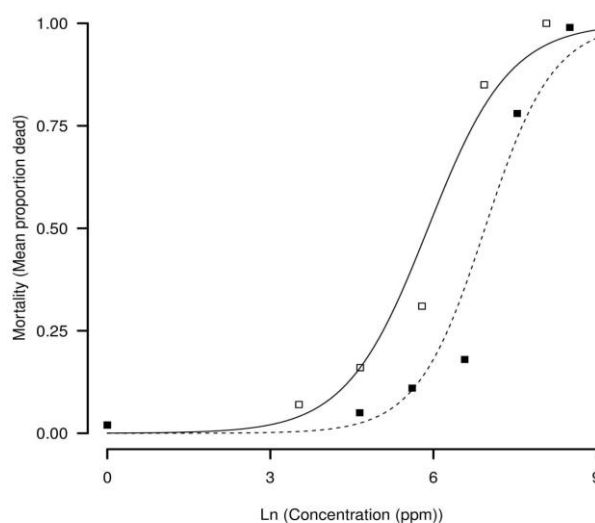
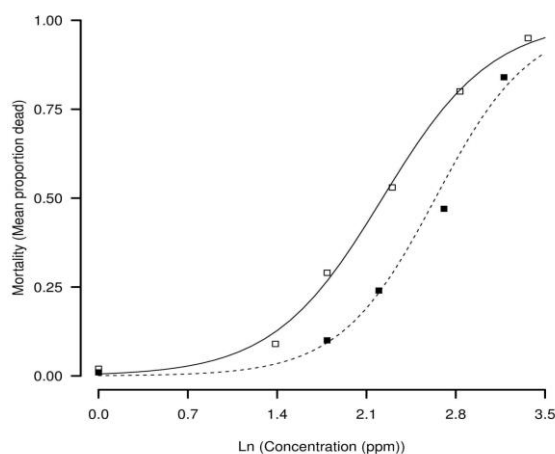
^a Units for contact and fumigant lethal concentrations are ppm and $\mu\text{L L}^{-1}$ air, respectively.

^{A-B} and ^{a-b}: Values marked with different small or capital letters within columns are significantly (P< 0.05) different (based on ratio test; see Table 4).

Table 4. Relative toxicity ratios of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults.

Bioassay procedure	Lethal dose ratio (95% CI) of Nanocapsulated EO:Pure EO ^a			
	LC ₁₀	LC ₂₅	LC ₅₀	LC ₉₀
Contact	0.266 (0.176 - 0.402)	0.305 (0.228 - 0.409)	0.351 (0.275 - 0.447)	0.463 (0.301 - 0.712)
Fumigant	0.584 (0.460 - 0.740)	0.621 (0.527 - 0.730)	0.660 (0.581 - 0.751)	0.747 (0.589 - 0.947)

^a If the 95% CI (Confidence Interval) of the ratio includes 1, then the lethal doses are not significantly different.

**Figure 3.** Dose-response curves of contact toxicity of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults. Dashed (filled-square) and solid (empty-square) lines (points) represent pure and nanocapsulated essential oil of *B. persicum*, respectively.**Figure 4.** Dose-response curves of fumigant toxicity of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults. Dashed (filled-square) and solid (empty-square) lines (points) represent pure and nanocapsulated essential oil of *B. persicum*, respectively.



Our study successfully developed stable nanocapsules containing *B. persicum* EO with favorable physicochemical properties. The Polydispersity Index (PDI) values for the nanocapsulated *B. persicum* EO (0.057, 0.054, and 0.060) fell within the mid-range (0.05–0.7), indicating a moderately uniform particle size distribution. PDI values below 0.05 indicate a highly monodisperse system, while those exceeding 0.7 reflect a very broad size distribution, which is unsuitable for Dynamic Light Scattering (DLS) analysis. Mid-range PDI values are compatible with various size distribution algorithms, ensuring reliable characterization (Danaei et al., 2018).

Nanoencapsulation enhances pesticide efficacy by improving the availability of active ingredients, through slow release and increased cuticular absorption, to target arthropod pests (Nuruzzaman et al., 2016; Kumar et al., 2019). The controlled release of pesticide actives depends on the physicochemical properties of the encapsulation material, including its composition, nanoparticle morphology (shape and size), polymorphism, shell thickness, porosity, encapsulation efficiency, and payload capacity (Kah et al. 2013). Notably, nano-pesticides exhibit greater efficiency due to their high surface area-to-volume ratio, which reduces the required dosage (Nuruzzaman et al. 2016). Furthermore, nanoencapsulation not only protects the active ingredient but also facilitates its sustained release (Kah et al. 2013; Kah and Hofmann, 2014). In this study, nanocapsulated *B. persicum* EO exhibited well-defined core-shell structures with near-spherical morphology and irregular external surfaces. Spherical nanoparticles are optimal for controlled-release mechanisms, as they promote uniform active ingredient diffusion (Perlatti et al. 2013).

Our results demonstrated significantly greater insecticidal efficacy of nanocapsulated *B. persicum* EO compared to its pure form against *B. brassicae* in both contact and fumigant toxicity bioassays.

This observed enhancement aligns with previous reports of superior pesticidal activity in nanoencapsulated EOs from *Allium sativum* L., *Rosmarinus officinalis* L., *Achillea millefolium* L., and *Cuminum cyminum* L. (Yang et al. 2009; Ziaee et al. 2014; Khoobdel et al. 2017; Ahmadi et al. 2018), likely attributable to improved nanoparticle mobility facilitating deeper tissue penetration. Nanoformulations address key limitations of pure EOs by enabling controlled release of active compounds (enhancing bioavailability) while reducing non-target toxicity (Khoobdel et al. 2017; Granata et al. 2018). The urea-formaldehyde polymer matrix used in our nanocapsules offers additional environmental benefits through microbial degradation into plant-available nitrogen (Hayatsu, 2014; Gonzalez-Hurtado et al., 2017). However, further validation under different conditions is needed to assess environmental impacts on nanocapsule performance, along with evaluation against other pest species to determine broad-spectrum applicability. Future studies should also investigate Lethal Time (LT) to assess the long-term stability and efficacy of the nanocapsulated *B. persicum* EO.

The present study identified γ -terpinene as the dominant compound in *B. persicum* EO, followed by *p*-cymene, carvacrol, and cuminaldehyde, collectively representing 82.13% of the total phytochemical profile. These findings align with previous reports by Sanei-Dehkordi et al. (2016) and Nouri Ganbalani et al. (2021), though with notable compositional variations. Khanavi et al. (2017) reported similar major constituents (γ -terpinene: 45%; *p*-cymene: 15%; cuminaldehyde: 18%) along with significant limonene content (11%), while Nickavar et al., (2014) identified cuminaldehyde, *p*-cymene, and γ -terpinene as primary components without detecting limonene as a major constituent. Such variability in EO composition likely stems from differences in plant provenance, growing conditions (climate and season), and extraction

methodologies (Ahmadi *et al.*, 2018; Campolo *et al.*, 2018; Rezaei *et al.*, 2019).

γ -Terpinene (4-methyl-1-(1-methylethyl)-1,4-cyclohexadiene), a monoterpene isomer that differs from α - and β -terpinene by its distinctive double bond configuration, is a well-characterized acetylcholinesterase inhibitor that contributes to the insecticidal activity of EOs (Lopez and Pascual-Villalobos 2010). Its bioactivity has been demonstrated across multiple insect species, including larvicidal effects against *Anopheles anthropophagus* Xu & Feng (Diptera: Culicidae) (LC_{50} = 29.21 ppm; Zhu and Tian 2011) and both *Aedes aegypti* (L.) and *A. albopictus* (Skuse) (Diptera: Culicidae) (LC_{50} = 30.7 and 29.8 ppm, respectively; Cheng *et al.* 2009). The compound also shows significant toxicity against lepidopteran (*Spodoptera littoralis* (Boisd.) fourth instar larvae) and hemipteran (*Aphis fabae* Scopoli adults) insect pests (Abbassy *et al.*, 2009). Given this broad-spectrum activity, the γ -terpinene-rich EO of *B. persicum* represents a promising botanical insecticide candidate for *B. brassicae* control. Further research under semi-field conditions is warranted to evaluate its practical efficacy and potential integration into Integrated Pest Management (IPM) programs.

In both pure and nanocapsulated *B. persicum* EO bioassays, the fumigant method exhibited significantly higher toxicity than the contact method. Several studies have reported the fumigant activity of *B. persicum* EO against various insect pests (Sanei-Dehkordi *et al.*, 2016; Nouri Ganbalani *et al.*, 2021), while others have documented both fumigant and contact toxicity (Moravvej *et al.*, 2011; Khanavi *et al.*, 2017; da Silva *et al.*, 2023). Consistent with our findings, Khanavi *et al.* (2017) observed greater fumigation toxicity of *B. persicum* EO compared to contact toxicity in *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). The authors suggested that the contact toxicity of the EO may primarily arise from cuminaldehyde, which showed the weakest activity in fumigation assays.

Notably, Abdelgaleil *et al.* (2009) demonstrated the contact activity of cuminaldehyde against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), linking its efficacy to a significant inhibitory effect on Acetylcholinesterase (AChE) activity.

CONCLUSIONS

Screening for insecticidal effects of EOs offers an opportunity to develop novel pesticides based on their chemical properties and toxicity, while minimizing the adverse environmental impacts and harm to non-target organisms associated with synthetic insecticides (Abbassy *et al.*, 2009; Ahmadi *et al.*, 2018; Campolo *et al.*, 2018). Our findings suggest that nanocapsulated *B. persicum* EO has significant potential as a botanical insecticide and could play a valuable role in IPM programs targeting *B. brassicae*, owing to its dual fumigant and contact activity. Additionally, since *B. persicum* has a long history of use in herbal medicine, it likely poses fewer risks to human health compared to conventional chemical insecticides. Nevertheless, further field and semi-field studies are recommended to evaluate the compound's efficacy under real-world conditions. Evaluating the cost-effectiveness of nanocapsulated *B. persicum* EO compared to conventional methods is also recommended for assessing its practical potential.

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نانوکپسول اوره-فرمالدهید سمیت اسانس *Bunium persicum* را در برابر *Brevicoryne brassicae* افزایش می دهد

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مهران رضایی

چکیده

این مطالعه، فعالیت حشره کشی اسانس های خالص و فرمولاسیون نانوکپسول زیره سیاه، *Bunium persicum* (Boiss.) Fedtsch را روی شته مومی کلم بررسی نمود. اسانس گیاهی از طریق تقطیر با آب جداسازی شد و توسط کروماتوگرافی گازی و طیف سنجی جرمی (GC-MS) تجزیه و تحلیل شد. ترکیبات اصلی شامل گاما-تریپنین (۳۶/۶۲٪)، پارا-سایمن (۱۸/۴۱٪)، کارواکرول (۱۳/۶٪) و کومین آلدئید (۱۳/۵٪) بود. نانوکپسول ها از طریق پلیمریزاسیون درجا به روش امولسیون روغن در آب سنتز شدند و با پراکندگی نور دینامیکی (DLS) و میکروسکوپ الکترونی عبوری (TEM) ویژگی های آن ها شناسایی شد که شکل ذرات کروی با قطر متوسط ۱۰/۸۸ نانومتر و شاخص پراکندگی ذرات (PDI) برای میانه برابر با ۰/۰۵۷ بود. نتایج زیست سنجی نشان داد که فرمولاسیون نانوکپسول سمیت به مراتب بالاتری نسبت به اسانس خالص نشان می دهد. مقادیر LC₅₀ و LC₉₀ سمیت تماسی فرمولاسیون نانوکپسول (به ترتیب ۳۶۵/۴۳ و ۱۹۰۸/۴۶ میکرولیتر بر لیتر آب) در مقایسه با اسانس خالص (۱۰۳۰/۴۰ و ۳۹۷۷/۰۸ میکرولیتر بر لیتر آب) پایین تر بود. به صورت مشابه، مقادیر LC₅₀ و LC₉₀ سمیت تدخینی برای نانوکپسول (۲۳/۱۵ و ۵۹/۴۹ میکرولیتر بر لیتر هوا) به صورت معنی داری پایین تر از اسانس خالص (۳۵/۰۷ و ۷۹/۵۹ میکرولیتر بر لیتر هوا) بود. نتایج نشان دهنده پتانسیل اسانس نانوکپسول *B. persicum* برای بکارگیری در برنامه های مدیریت تلفیقی آفات شته مومی کلم می باشد.

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