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Enhancing Growth, Yield, and Phytochemical Characteristics of *Dracocephalum kotschyi* Boiss through Optimized Planting Density

Sahar Zamani[1], Davood Bakhshi[1]*, Mohammad-Taghi Ebadi[2], Amir Sahraroo[1], and Mehran Fathi [1]

ABSTRACT

Dracocephalum kotschyi Boiss. is a medicinal plant used in various industries due to its high health benefits, antioxidant activity, attractive flavor and aroma. Commercial yield and phytochemicals can be enhanced by cultivation management like suitable plant density. This study investigates the impact of planting density (50×40, 25×40, 12.5×40 cm caused to 5, 10, and 20 plants m^{-2}) on the morpho-physiological traits, yield, trichomes, and phytochemicals profiles of *D. kotschyi*, based on a Randomized Complete Block Design (RCBD) in the Saravarsu region, Guilan province, Iran (2021-2022). The findings reveal a notable fourfold increase in various traits in 20 plants m^{-2} (12.5×40 cm). The highest values for parameters such as plant height (34.66 cm), branch number (14), branch length (9.34 cm), leaf number (138.33), leaf area index (105 mm^{2}), fresh weights of leaves, and stems, as well as total fresh weight (105.1, 98.5, and 203.6 g m^{-2} respectively), dry weights of leaves, stems, total dry yield (26.1, 24.675, and 50.775 g m^{-2} respectively), chlorophyll a, b, total chlorophyll, and carotenoid content (1.038, 0.653, 1.691, and 0.898 mg g^{-1} FW, respectively), secretory trichomes density (14 mm^{-2}), essential oil content and yield (0.633% and 0.322 g m^{-2}, respectively), PAL activity (10.221 nmol g^{-1} FW min^{-1}), antioxidant activity (82.85%), total phenol (1.253 mg g^{-1} DW), and total flavonoid (2.781 mg g^{-1} DW) were observed in 20 plants m^{-2}. In conclusion, a planting density of 20 plants m^{-2} (with spacing 12.5×40 cm) is recommended for achieving optimal commercial yield, and phytochemical production of *D. kotschyi* in the Saravarsu region.

Keywords: Essential oil, Glandular trichomes, Medicinal plants, Cultivation management, Secondary metabolites**.**

INTRODUCTION

Medicinal and aromatic plants are rich Secondary Metabolites (SMs) sources, commonly referred as natural products, renowned for their divers' biological activities, and therapeutic effects in pharmaceutical, food, and cosmetic industries (Zamani *et al*., 2021; Liu *et al*., 2021).

[1] Department of Horticultural Science, Faculty of Agricultural Sciences, University of Guilan, Rasht, Islamic Republic of Iran.
[2] Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
***Corresponding author; Bakhshi-d@guilan.ac.ir**

Dracocephalum kotschyi Boiss is an endangered aromatic and medicinal plant belonging to the Lamiaceae family, is renowned for its abundant essential oils (EOs) and flavonoids, predominantly found in its aerial parts (Fattahi *et al*., 2013). The EO of *D. kotschyi* contains compounds such as limonene, geraniol, citral, and perillaldehyde, while its methanolic extract boasts phenolic compounds like xanthomicrol, luteolin, apigenin, cirsimaritin, and rosmarinic acid (Fattahi *et al*., 2013; Heydari *et al*., 2019). Exhibiting a plethora of medicinal properties including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects, *D. kotschyi* is also utilized for enhancing joint health, immune function, and memory (Zamani et al., 2023), in addition to serving as a flavoring agent in food and tea (Zamani *et al.*, 2023).

Optimizing planting density is vital for maximizing resource utilization and achieving peak yield, a factor influenced by environmental conditions and plant species (Baloch *et al*., 2002). Factors such as temperature, solar radiation, humidity, and soil fertility affect plant growth and nutrient requirements. Interspecies competition intensifies at higher densities, leading to increased humidity, inadequate airflow, and reduced light penetration, thereby increasing the risk of disease and reducing yield. Conversely, lower densities may lead to underutilization of resources and decreased yield. Appropriate density enhances plant establishment, competition against weeds, and light utilization efficiency, and ultimately improves yield and phytochemical production (Copes and Scherm, 2005; Koocheki and Sarmadnia, 2012).

Numerous studies have examined planting density's effects on medicinal plants. Optimal plant height, dry matter, and Essential Oil (EO) yield for *Thymus daenensis* and *T. kotschyanus* were observed at medium to high densities (6 and 8 plants m^{-2}) (Sepahvand *et al*., 2023). *Satureja bachtiarica* Bunge exhibited increased yield but reduced trichome density and EO content with higher planting density (Mirjalili *et al*., 2022). Higher plant density led to increase in fresh and dry biomass as well as EO yield of *Origanum vulgare* (Gerami *et al*., 2018). *Calendula officinalis* at a high density (57 plants m^{-2}) exhibited maximal height, seed weight, chlorophyll, EO content, and yield (Sepehri *et al.*, 2016). *Dracocephalum moldavica* also demonstrated enhanced growth and phytochemical production with increased density (Hashemian Ahmadi and Hadipanah, 2014).

Hence, the current study was undertaken to investigate the influence of planting density on the morphological traits, yield, and phytochemical compounds of the *D. kotschyi*.

MATERIALS AND METHODS

Plant Cultivation

D. kotschyi plants were cultivated in Saravarsu (50° 13' 26" E, 36° 49' 0" N, at 1,430 m) in Rahimabad District, Rudsar County, Guilan Province, Iran. The climatic details of this region are mentioned in Table 1.

Soil samples were obtained from a depth of 0-30 cm; and the physicochemical characteristics are detailed in Table 2. The experiment followed a Randomized Complete Block Design (RCBD) with three densities in three replications. Initially, the designated land was plowed and enriched with 30 ton/ha cow manure. *D. kotschyi* seeds, sourced from Pakan Bazr Company in Isfahan province, were germinated in a greenhouse during late winter. In spring, after levelling the ground and creating ridges and furrows at 40 cm intervals, plots measuring 2×2 meters (4 m^2) were established. Seedlings, at the 6-8 leaf stage were transplanted to the main field. Planting occurred with intervals of 12.5×40, 25×40, and 50×40 cm, equivalent to plant densities of 20, 10, and 5 plants m^{-2}, respectively. Following planting, irrigation intervals were minimized initially to aid plant establishment and prevent soil compaction. Subsequent irrigation schedules were adjusted based on regional conditions and the plant's water requirements. Throughout the growth phase, physical and manual weed removal practices were employed, and no instances of pests or diseases were noted.

Morphological Traits Measurement

The aerial parts of *D. kotschyi* plants were randomly harvested from each plot at the full flowering stage (maximum phytochemicals accumulation). Plant height and branch length were measured using a ruler (cm). The branch and leaf number were quantified numerically. Leaf area index was calculated using grid paper. Yield indices, such as fresh and dry weights of leaves, stems, and total weight, were measured using a digital balance (g m^{-2}).

Chlorophylls and Carotenoids Content

To measure chlorophyll and carotenoid content, 0.1 g of the sample was finely ground with 1 ml of 80% acetone, and the resulting mixture was refrigerated at 4°C in darkness for 24 hours. Subsequently the absorbance was recorded at wavelengths 470, 645, and 663 nm. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated using the formulas and reported as mg g^{-1} of fresh weight (Lichtenthaler and Buschmann, 2001):

$$Chl\ a\ (\text{mg g}^{-1}\ \text{FW}) = (12.25\ A_{663} - 2.79\ A_{645})\ (V/W\ 1000) \quad (1)$$

Chl b (mg g^{-1} FW)= (21.5 A_{645}−5.10 A_{663}) (V/W 1000) (2)

Total Chl (mg g^{-1} FW) = Chl a+ Chl b (3)

Carotenoids (mg g^{-1} FW)= [(1000 A_{470}−1.82 $Chla$−85.02 $Chlb$)/198] (V/W 1000) (4)

Which, V= Acetone Volume (ml) and W= Sample Weight (g).

Glandular Trichrome Density Measurement

Leaf samples measuring 2-3 mm^2 were cleansed with ethanol and distilled water. Subsequently, the dried samples were fixed on metal bases using conductive glue and coated with a thin layer of gold using a Coater (SBC121, KYKY Company, China). Finally, the samples were examined using a scanning electron microscope (SEM XL 30, Philips, Netherlands). Trichome density was determined by counting the number of trichomes within a 1 mm^2 area.

EO Content and Yield (EOC and EOY)

For EO extraction, 20 g of dried and powdered plant material was hydro-distilled using a Clevenger apparatus for three hours. The resulted EO samples were stored at 4°C until analysis (Zamani *et al*., 2023). EO content and yield were determined using Equations 5 and 6, respectively:

EO content (%)= (EO weight/Dry matter weight)×100 (5)

EO yield (g m^{-2}) = EO content×Total dry weight/100 (6)

Phenylalanine Ammonia Lyase (PAL) Enzyme Activity

For enzyme extraction, 0.1 g of fresh plant material was homogenized in 1 ml phosphate buffer (pH= 7). Subsequently, 250 µl of the enzyme extract was combined with 250 µl of 10 mM sodium borate buffer (pH= 8.8), 250 µl distilled water, and 250 µl phenylalanine (50 mmol, 250 µl) and read at 290 nm using a UV/VIS spectrophotometer (PG Instrument Ltd, Leicester, UK). PAL activity was determined by the Beer-Lambert law with an extinction coefficient of 9630 µ^{-1}cm^{-1} and expressed as nmol g^{-1} FW min^{-1} (Saunders and McClure’s, 1974).

Total Phenol Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AOA)

TPC (mg Gallic acid g^{-1} DW) was quantified using methanolic extract (1:10) by the Folin-Ciocalteu method at 760 nm (Singleton *et al*., 1999). TFC (mg Quercetin g^{-1} DM) was determined using the aluminium chloride method at 506 nm (Du *et al*., 2009). The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method at 517 nm and determined as the percentage of DPPH inhibition using Equation (7) (Chiou *et al*., 2007; Zamani *et al.*, 2023):

$$\text{Free radical scavenging percentage} = [(A_{control} - A_{sample})/A_{control}]\times 100 \qquad (7)$$

$A_{control}$: Absorbance of DPPH, A_{sample}: Absorbance of plant extract+DPPH.

Statistical analysis

The experiment followed a Randomized Complete Block Design (RCBD) with three density treatments replicated three times. Mean data were compared using the LSD test at $P< 0.01$ that analyzed with SAS statistical software (Ver. 9). Correlation and regression analysis were performed using SPSS Ver. 26.

RESULTS AND DISCUSSION

Effect of Plant Density on the Morphological Characteristics of *D. kotschyi*

The plant density significantly influenced the plant morpho-physiological traits ($P< 0.01$) (Table 3). Increasing the density from 5 to 20 plants m^{-2} resulted in a 2-3fold enhancement in the morpho-physiological characteristics of *D. kotschyi*. Specifically, the highest values for plant height (34.66 cm), branch number (14), branch length (9.34 cm), leaf number (138.33), and leaf area index (105 mm^2) were observed in plants cultivated at a density of 20 plants m^{-2}. Conversely, the lowest density (5 plants m^{-2}) exhibited the lowest values for the investigated indices (Figure 1).

Increased plant height due to higher plant density is linked to competition for solar radiation. Dense plants populations reduce light penetration, triggering intensified competition for sunlight. Additionally, under shaded conditions with limited light availability, the production of growth hormones, such as auxin increases, resulting in heightened plant height (Arvin and Firouzeh, 2018). Similar to our findings, the positive effects of increasing the cultivation density on the plant height have been reported in ginseng (Liu et al., 2021), stevia (Btru *et al*., 2017), and peppermint (Mansoori, 2014). In higher planting density, the number of plants per unit area increases, resulting in a greater leaf count per unit area. In our study, an augmentation in plant density resulted in an increased Leaf Area Index (LAI) in *D. kotschyi* plants. Optimal density levels caused faster leaf growth and led to an augmented leaf area index. This increase in leaf area positively impacts the photosynthesis rate, influencing various growth parameters, including yield (Li *et al*., 2019). These findings align with research conducted on sunflowers (Li *et al*., 2019) and marigolds (Sepehri *et al*., 2016). While greater spaces between plants (low densities) lead to an average increase in individual development, but accompany with a decrease in the total potential yield (Moldovan *et al*., 2022).

Effect of Plant Density on the Fresh and Dry Yield of the *D. kotschyi*

As indicated in Table 3, the plant density significantly affected the fresh and dry yield of the *D. kotschyi* ($P< 0.01$). Reduced planting intervals led to an augmentation in the fresh and dry matter of the *D. kotschyi* plant. The highest fresh weights of leaves, stems, and the total (105.1, 98.5, and 203.6 g m^{-2}, respectively) and dry weights of leaves, stems, and total biomass (26.1, 24.675, and 50.775 g m^{-2}, respectively) were observed at a density of 20 plants m^{-2}, which were 3-4 times higher than plants cultivated at a density of 5 plants m^{-2} (Figure 2).

The correlation between higher plant density and increased biomass production, with peak yield achieved at the highest density (20 plants m^{-2}). Alteration in density affects various factors, and in a density with minimized competition between the plants to receive moisture, light and nutrients, maximum photosynthesis, photosynthetic pigments and growth is achieved. This is the optimal density which leads to maximum yield and depends on the plant species and environmental conditions. The increased density more than the optimum usually decreases most growth parameters and photosynthesis (Dai *et al*., 2022; Sepehri *et al*., 2016). In *Satureja bachtiarica* Bunge, the maximum plant yield was achieved at the highest plant density (80,000 plants/ha) (Mirjalili *et al*., 2022). Similarly, in *Dracocephalum moldavica*, an increase in the plant density enhanced the weight with the highest fresh (8,260.2 kg) and dry yield (2,787.5 kg ha^{-1}) were observed at a cultivation distance of 10 cm (Hashemian Ahmadi and Hadipanah, 2014). It was consistent with study on peppermint, where fresh and dry biomass yield and leaves significantly increased with increasing plant density from 8 to 20 plants m^{-2} (Mansoori, 2014). Optimum plant density provides favorable condition, leading to increased dry matter accumulation. This phenomenon is observed in medicinal plants such as *Thymus daenensis* (Jasemi *et al*., 2019) and *Calendula officinalis* (Sepehri *et al*., 2016).

Effect of Plant Density on Photosynthetic Pigments of the *D. kotschyi*

According to Table 4, the plant density treatment significantly influenced chlorophyll b, total chlorophyll, and carotenoid levels at a 1% significance level, however had no impact on chlorophyll a. The maximum chlorophyll a, b, total chlorophyll, and carotenoid content (1.038, 0.653, 1.691, and 0.898 mg g^{-1} FW, respectively) were observed in the 20 plants m^{-2} treatment, representing an increase of up to 3-fold compared to the lowest density. Conversely, the lowest pigment content was noted at 5 plants m^{-2} (Figure 3).

Photosynthetic pigments, including chlorophyll a, b, and carotenoids, play direct and indirect roles in photosynthesis, crucial for light absorption and energy regeneration (Tohidi *et al*., 2020). Consistent with our findings, planting density impacted photosynthetic pigments in *Perilla frutescens*. Increased plant density leads to higher content of photosynthetic pigments due to increased leaf number and area (as the source of photosynthetic pigments) (Wu *et al*., 2020). *Thymbra spicata* and *Calendula officinalis* showed higher chlorophyll a, b, and carotenoid with increased row distance (Malekmaleki *et al*., 2022; Sepehri *et al*., 2016). On the other hand, increased planting density (especially more than optimum density) reduced plant photosynthetic capacity [net Photosynthetic rate (Pn)], stomatal conductance (Gc), and leaf chlorophyll content (Dai *et al*., 2022). Additionally, *Levisticum officinale* and *Coriandrum sativum* exhibited decreased chlorophyll with increased density (Atteya *et al*., 2021; Arvin and Firouzeh, 2018). Generally, leaf pigment levels are influenced by factors like density, nutrition, species type, environment conditions, and diseases, impacting photosynthesis and yield (Tohidi *et al*., 2020; Cui *et al*., 2018).

Effect of Plant Density on Glandular Trichomes of the *D. kotschyi*

Scanning electron microscope images revealed various types of trichomes in the *D. kotschyi* leaves including non-glandular (protective) and glandular (secretory) trichomes. Non-glandular trichomes were unicellular or multicellular, non-branched, sharp-pointed, bent, or erect types. Secretory trichomes were observed in two forms: peltate and capitate. In this experiment, non-glandular and glandular trichomes were observed in all three densities, but with an increase in cultivation density, the number of secretory trichomes increased from 10 numbers mm^{-2} in the lowest treatment to 14 mm^{-2} in the highest planting density (Figure 4).

Glandular trichomes, essential secretory structures in mint plants, are abundant in aerial organs and play a crucial role in producing therapeutic compounds like terpenoids (EOs) and phenolic compounds (Feng et al., 2021; Tozin et al., 2015). Understanding their morphology and response to factors like cultivation density is essential for medicinal plants, as changes in trichome structures and EO accumulation can occur due to environmental adaptations (Tuttolomondao et al., 2016). In our study, an increase in plant density led to a higher number of secretory trichomes on *D. kotschyi* leaves, likely due to the increased leaf number (providing more space for trichome accumulation). Similar findings were observed in *Artemisia cina,* where glandular trichome number correlated with leaf number (Herawati *et al*., 2020). In Oregano, trichome density increased at the middle density (150×50 cm) but no significant difference was noted across density levels (Tuttolomondao *et al*., 2016). Reports suggest that high-density conditions, coupled with resource limitations like water, may induce slight stress, affecting trichome numbers. Glandular trichomes density of *Artemisia annua* increased with slight water stress (50% field capacity), while it decreased by 25% of the field capacity (Shi *et al*., 2018). Optimal growth traits and secretory structure development were observed at a density of 20 plants m^{-2}. Overall, secretory trichome numbers are influenced by various factors, including growth conditions and plant species (Tozin *et al*., 2015).

Effect of Plant Density on EO Content (EOC) and Yield (EOY) of the *D. kotschyi*

As indicated in Table 4, plant density significantly influenced both the EOC and EOY ($P< 0.01$). With an increase in plant density, both the plant EOC and EOY showed an upward trend. The maximum EOC and EOY (0.633% and 0.322 g m^{-2}, respectively) were recorded in the 20 plants m^{-2} treatment (Figure 5).

EOs valued for their diverse medicinal properties, have extensive applications across the pharmaceutical, food, and cosmetic industries (Masyita *et al*., 2022). Increasing cultivation density in *Levisticum officinale* led to higher EO content, with optimal yield obtained at a distance of 15 cm (Atteya *et al*., 2021). *Satureja bachtiarica* showed increased EO yield at higher plant densities (80,000 plants ha^{-1}) (Mirjalili *et al*., 2022). *Dracocephalum moldavica* achieved the highest EO yield at a cultivation distance of 10 cm (Hashemian Ahmadi and Hadipanah, 2014). EO content in *Thymus daenensis* and *Calendula officinalis* increased with cultivation density (Jasemi *et al*., 2019; Sepehri *et al*., 2016). Higher planting density boosts Essential Oil (EO) yield in savory plants due to elevated biomass production in dense conditions. Since EO is mainly stored in leaves and flowering branches, plants cultivated at high density, yielding more leaves and flowering branches, also exhibit higher EO yield (Abbaszadeh *et al*., 2014). These findings are consistent with our observations. In *Thymbra spicata*, a row distance of 20 cm resulted in the highest EO content and yield (Malekmaleki *et al*., 2022). Coriander plants exhibited increased EO content and yield with higher density (Arvin and Firouzeh, 2018). Various physiological and environmental factors influence EO biosynthesis and accumulation, including geographical location, agricultural techniques, plant type, and harvest time (Tuttolomondao *et al*., 2016). Plant density plays a crucial role in maximizing crop quality, biomass, and EO yield, as observed in our study and others.

Effect of Plant Density on PAL Enzyme Activity and Antioxidant Compounds of the *D. kotschyi*

The plant density exerted a significant impact on antioxidant activity, total phenol, and flavonoid content ($P < 0.05$), but did not influence PAL enzyme activity (Table 4). As illustrated in Figure 5, the highest PAL activity (10.221 nmol g^{-1} FW min^{-1}), antioxidant activity (82.85%), total phenol (1.253 mg g^{-1} DW), and total flavonoid (2.781 mg g^{-1} DW) were observed in the 20 plants m^{-2} treatment. Conversely, the lowest the investigated traits were noted at a density of 5 plants m^{-2}.

Phenylalanine Ammonia-Lyase (PAL), a crucial enzyme, contributes to the formation of antioxidant phenylpropanoid compounds like phenolic and flavonoid compounds which aid plant defense mechanisms (Zamani *et al*., 2021). These compounds have diverse biological activities including antioxidant, antimicrobial, anticancer, and anti-inflammatory properties (Maina *et al*., 2021). PAL activity is influenced by various factors including nutrition, light, environmental changes, density, stresses, etc. which can enhance gene expression, enzyme production and phenolic productions (Zamani *et al*., 2021; Medda *et al*., 2020). Studies on *Linum usitatissimum* L., have shown the increasing plant density enhances PAL enzyme activity in specific cultivars (Gao *et al.*, 2018). Alterations in planting density impact the availability of growth factors, influencing plant competition for optimal growth and subsequently phytochemicals such as phenolic compounds and antioxidants (Tazeh *et al*., 2016). Consistent with these findings, studies indicated a positive correlation between higher plant density and elevated phenol and flavonoid levels, as well as increased antioxidant activity. For instance, in ginseng, higher and medium planting densities were associated with the highest ginsinoside production (Liu *et al*., 2021). Increasing planting density also led to an increase in rosmarinic acid content in *Perilla* increases (Wu *et al*., 2020). In *Levisticum officinale*, total phenol and antioxidant activities increased with higher density (Atteya *et al*., 2021). High planting density in some studies limits several factors like light, water, and nutrients, and leads to plant competition, and induced stress can stimulate PAL enzyme activity and alter phytochemicals (Maina et al., 2021). However, in our study, it seems that the high density of plants provided the maximum availability of nutrients and the growth requirements of the plant, thus the plant has optimal growth, and it produces high phytochemical compounds.

Correlation and Regression Analysis of Measured Traits

Positive and significant correlations were observed among growth traits, yield, photosynthetic pigments, glandular trichomes and phytochemical traits in *D. kotschyi* (Table 5), which are crucial for plant production and breeding programs. Elevated plant density led to increased growth traits, positively correlated with yield, indicating the contribution of vegetative organs to overall yield enhancement. Morpho-physiological factors showed a positive correlation with photosynthetic pigments, influenced by increased leaf number and area (as the source of photosynthetic pigments) due to density increment, leading to higher fresh and dry matter production. A study on *Levisticum officinale* found a significant positive correlation between growth characteristics, fresh and dry weight, and chlorophyll (Atteya *et al*., 2021). Additionally, positive correlations were noted between fresh weight, dry matter weight, photosynthetic pigment, and active substances (Table 5), suggesting enhanced pigment levels contribute to increased biomass, enzyme activity, and phytochemical production. Similar findings were observed in studies on *Mentha piperita* and *Satureja sahendica* Bornm, emphasizing the importance of growth traits in Essential Oil (EO) production (Ostadi *et al*., 2023; Abbaszadeh *et al*., 2014). Positive correlations between EO yield and overall fresh and dry yield were reported in thyme (Sepahvand *et al*., 2016). Similar positive correlations between growth traits and plant yield were also observed in *Stevia Rebaudiana* (Btru *et al*., 2017). Since secretory trichomes are biosynthesis and storage places of essential oil, their density is positively correlated with essential oil and is affected by several factors including planting density (Esmaeili *et al*., 2019). In *Origanum majorana*, more and larger secretory trichomes with higher EO content were observed in high light intensity (Shafiee-Hajiabad *et al*., 2015). Table 5 reveals a positive correlation between the PAL enzyme and antioxidant compounds, emphasizing its role in phenylpropanoid and antioxidant synthesis. Similar findings were reported by Zamani *et al*. (2021) for *Cynara scolymus*, highlighting PAL's influence on phenylpropanoid compounds. Atteya *et al*. (2021) found a strong positive correlation between phenol and antioxidant activity in *Levisticum officinale* across various cultivation densities. Increased density likely enhances antioxidant activity through elevated phenolic compounds, which inhibit free radicals directly and enhance hydrogen donation potential (Ghimire *et al*., 2021).

The multivariate regression analysis was employed to explore the relationship between EO Yield (EOY), as a dependent variable (Y), and its affecting traits, treated as independent variables (X). The regression findings indicated that the Total Dry Weight (TDW) of the entire plant, with an explanation coefficient of 98%, and the EO Content (EOC), with an explanation coefficient of 94.8%, emerged as the most influential determinants of EOY (Equation 8):

$$Y= -0.232+0.005X_1+0.468X_2 \quad (8)$$

Where, Y= EO yield (g m^{-2}), X_1= Total dry yield of the whole plant (g m^{-2}), X_2= EO content (%).

Given that EO yield is affected by both plant dry weight and EO content, augmenting dry weight and enhancing the EO content due to plant density has consistently resulted in heightened EO yield, as shown in various studies (Ostadi *et al*., 2023; Sepahvand *et al*., 2016; Abbaszadeh *et al*., 2014).

CONCLUSIONS

Based on the results, plant density significantly affected the most measured traits and the highest plant height, branch number, branch length, leaf area index, fresh and dry yield, photosynthetic pigments, secretory trichomes density, EO content and yield, PAL activity, antioxidant activity, total phenol, and total flavonoid were observed in high density (20 plants m^{-2}). Generally, *D. kotschyi* plant cultivation with distances of 12.5×40 cm (a density of 20 plants m^{-2}) due to the optimal growth conditions (nutrients, water, light, etc.), causes maximum growth and phytochemicals production. Therefore, this cultivation interval is recommendable for the economic production of the *D. kotschyi* plant in similar ecological regions.

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Table 1. Climatic characteristics of Saravarsu Region during growth season in 2021-2022.

Months	Minimum temperature (°C)	Mean temperature (°C)	Maximum temperature (°C)	Average relative humidity (%)	Total rainfall (mm)
April	6.09	12.92	19.75	71	37.7
May	9.14	15.02	20.91	77.5	46.9
June	12.96	18.92	24.88	78	28.1
July	16.08	21.37	26.67	80	57.4
August	16.04	20.89	25.74	79	45.4
September	14.7	19.79	24.88	77.5	53.3
Annual mean	7.68	13.32	18.96	72	680.5

Table 2. Soil physicochemical characteristics of experimental field.

OM (%)	pH	EC (ds/m)	Clay (%)	Silt (%)	Sand (%)	Texture	N (%)	P (ppm)	K (ppm)
1.91	7	0.36	22.8	21.2	56	Sandy clay loam	0.2	255	6.4

Table 3. ANOVA of plant density effect on morpho-physiological and yield of *D. kotschyi*.

		Mean Square										
SOV	df	PLH	BN	BL	LN	LAI	LFW	SFW	TFW	LDW	SDW	TDW
Block	2	0.44 $^{n.s}$	3.11 $^{n.s}$	1.3 $^{n.s}$	108.33 $^{n.s}$	47.44 $^{n.s}$	124.3 $^{n.s}$	104.64 $^{n.s}$	428.24 $^{n.s}$	11.29 $^{n.s}$	9.15 $^{n.s}$	25.18 $^{n.s}$
Density	2	308.77**	45.44**	23.7**	7033.33**	1596.77**	3970.5**	4136.82**	16155.9**	289.99**	318.62**	1215.89**
Error	4	7.27	0.61	0.60	66.66	23.44	191.7	78.29	452.91	12.9	5.643	30.91
CV	-	11.61	7.99	12.65	9.24	6.08	21.46	15.79	17.65	20.12	18.49	20.04

*, **: Significant differences at 5 and 1% ; $^{n.s}$: no significant difference

SOV: Source Of Variances; df: Degree of freedom; CV: Coefficient of Variation; PLH: Plant Height; BN: Branch Number; BL: Branch Length; LN: Leaf Number; LAI: Leaf Area Index; LFW: Leaf Fresh Weight; SFW: Stem Fresh Weight; TFW: Total Fresh Weight; LDW: Leaf Dry Weight; SDW: Stem Dry Weight; TDW: Total Dry Weight.

Table 4. ANOVA of plant density effect on biochemical and phytochemical traits of *D. kotschyi*.

		Mean Square									
SOV	df	Chl a	Chl b	Chl t	Car	EOC	EOY	PAL	TPC	TFC	AOA
Block	2	0.01$^{n.s}$	0.001 $^{n.s}$	0.017 $^{n.s}$	0.001 $^{n.s}$	0.003 $^{n.s}$	0.001 $^{n.s}$	0.017 $^{n.s}$	0.013 $^{n.s}$	0.005 $^{n.s}$	0.046 $^{n.s}$
Density	2	0.109 $^{n.s}$	0.151**	0.518**	0.225**	0.016**	0.057**	0.246 $^{n.s}$	0.213*	1.186*	8.208*
Error	4	0.024	0.001	0.024	0.003	0.0002	0.001	0.038	0.022	0.077	0.585
CV	-	19.087	10.464	12.893	9.299	3	21.86	1.983	14.81	12.8	0.948

*, **: Significant differences at 5 and 1% ; $^{n.s}$: no significant difference.

SOV: Source of Variances; df: Degree of freedom; CV: Coefficient of Variation; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl t: Chlorophyll total; Car: Carotenoid; EOC: EO Content; EOY: EO Yield; PAL: Phenylalanine Ammonia Lyase enzyme; TPC: Total Phenol Content; TFC: Total Flavonoid Content; AOA; Antioxidant Activity.

Table 5. Correlation between morpho-physiological and phytochemical traits of *D. kotschyi*.

	PLH	BN	BL	LN	LAI	LFW	SFW	TFW	LDW	SDW	TDW	Chl a	Chl b	Chl t	Car	GTs	EOC	EOY	PAL	TPC	TFC	AOA
PLH	1																					
BN	0.935**	1																				
BL	0.866**	0.905**	1																			
LN	0.907**	0.961**	0.902**	1																		
LAI	0.944**	0.955**	0.954**	0.954**	1																	
LFW	0.901**	0.832**	0.862**	0.911**	0.92**	1																
SFW	0.93**	0.915**	0.782*	0.944**	0.88**	0.887**	1															
TFW	0.909**	0.904**	0.8**	0.948**	0.88**	0.892**	0.988**	1														
LDW	0.912**	0.843**	0.842**	0.889**	0.93**	0.984**	0.86**	0.848**	1													
SDW	0.819**	0.818**	0.723*	0.867**	0.82**	0.844**	0.878**	0.928**	0.806**	1												
TDW	0.904**	0.937**	0.856**	0.962**	0.941**	0.898**	0.951**	0.963**	0.88**	0.903**	1											
Chl a	0.855**	0.825**	0.742*	0.704*	0.816*	0.641	0.749*	0.721*	0.682*	0.632	0.763*	1										
Chl b	0.951**	0.928**	0.887**	0.939**	0.938**	0.907**	0.926**	0.948**	0.884**	0.935**	0.931**	0.794*	1									
Chl t	0.969**	0.924**	0.858**	0.865**	0.924**	0.815**	0.883**	0.879**	0.824**	0.824**	0.893**	0.949**	0.945**	1								
Car	0.906**	0.924**	0.862**	0.942**	0.921**	0.844**	0.946**	0.953**	0.814**	0.862**	0.968**	0.823**	0.925**	0.922**	1							
GTs	0.93**	0.941**	0.898**	0.982**	0.948**	0.909**	0.963**	0.963**	0.88**	0.864**	0.963**	0.768*	0.946**	0.904**	0.979**	1						
EOC	0.839*	0.761*	0.78*	0.83*	0.793*	0.813**	0.855**	0.881**	0.748*	0.854**	0.801**	0.687*	0.908**	0.841**	0.875**	0.894**	1					
EOY	0.887**	0.909**	0.828**	0.957**	0.914**	0.893**	0.946**	0.969**	0.862**	0.953**	0.973**	0.736*	0.952**	0.881**	0.963**	0.967**	0.887**	1				
PAL	0.894**	0.909**	0.942**	0.929**	0.96**	0.866**	0.722*	0.824**	0.869**	0.731*	0.872**	0.562	0.88**	0.872**	0.905**	0.943**	0.826**	0.876**	1			
TPC	0.803*	0.809**	0.707*	0.833*	0.735*	0.681*	0.726*	0.88**	0.619	0.792*	0.784*	0.69*	0.856**	0.816**	0.878**	0.883**	0.93**	0.856**	0.783*	1		
TFC	0.804*	0.832**	0.724*	0.9**	0.834**	0.834**	0.819**	0.94**	0.798**	0.938**	0.943**	0.648	0.882**	0.806**	0.935**	0.920**	0.85**	0.981**	0.803**	0.823**	1	
AOA	0.914**	0.9**	0.797*	0.9**	0.927**	0.873**	0.894**	0.902**	0.891**	0.859**	0.955**	0.833**	0.894**	0.911**	0.943**	0.928**	0.79*	0.944**	0.882**	0.744*	0.927**	1

*, **: Significant differences at 5 and 1%.

PLH: Plant Height; BN: Branch Number; BL: Branch Length; LN: Leaf Number; LAI: Leaf Area Index; LFW: Leaf Fresh Weight; SFW: Stem Fresh Weight; TFW: Total Fresh Weight; LDW: Leaf Dry Weight; SDW: Stem Dry Weight; TDW: Total Dry Weight; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl t: Chlorophyll total; Car: Carotenoid; GTs: Glandular Trichomes; EOC: EO Content; EOY: EO Yield; PAL: Phenylalanine Ammonia Lyase enzyme; TPC: Total Phenol Content; TFC: Total Flavonoid Content; AOA: Antioxidant Activity.

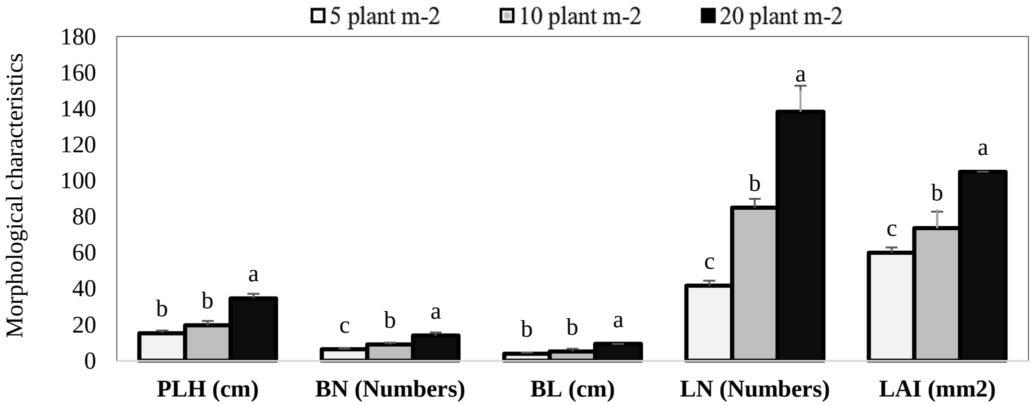

Figure 1. Morpho-physiological characteristics of *D. kotschyi* under different plant densities. PLH: Plant Height; BN: Branch Number; BL: Branch Length; LN: Leaf Number; LAI: Leaf Area Index. Means with same letters had no significant different at P< 0.01.

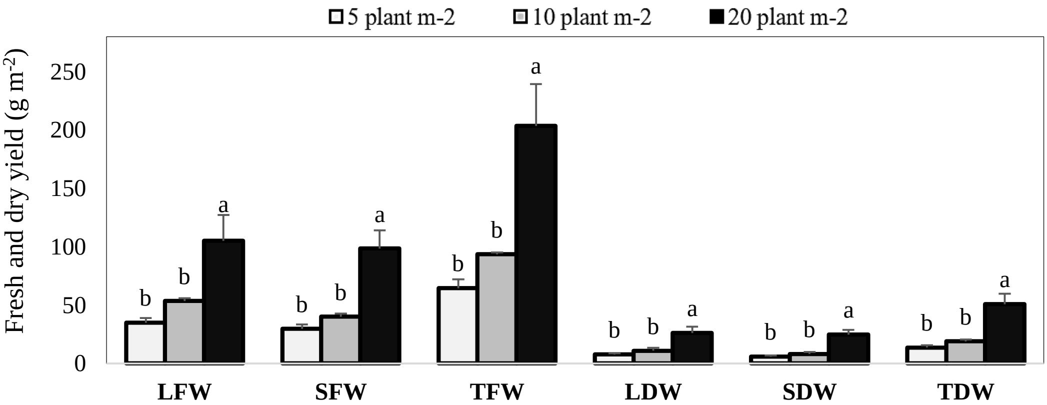

Figure 2. Fresh and dry yield of *D. kotschyi* under different plant densities. LFW: Leaf Fresh Weight; SFW: Stem Fresh Weight; TFW: Total Fresh Weight; LDW: Leaf Dry Weight; SDW: Stem Dry Weight; TDW: Total Dry Weight. Means with same letters had no significant different at P< 0.01.

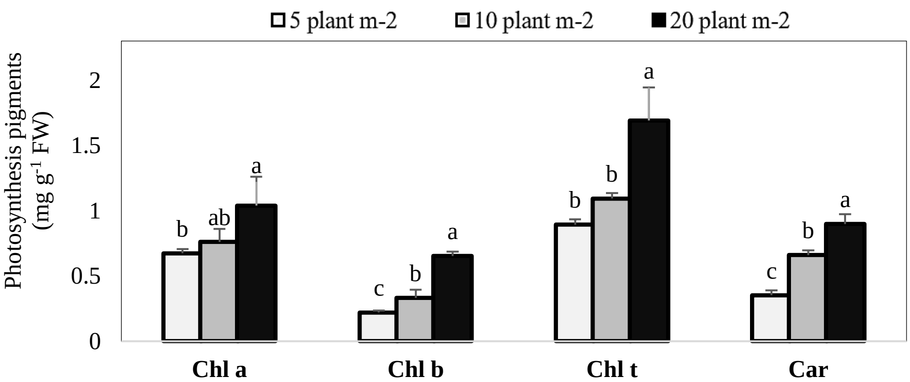

Figure 3. Chlorophyll and Carotenoid pigments of *D. kotschyi* under different plant densities. Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl t: Chlorophyll total; Car: Carotenoid. Means with same letters had no significant different at P< 0.01.

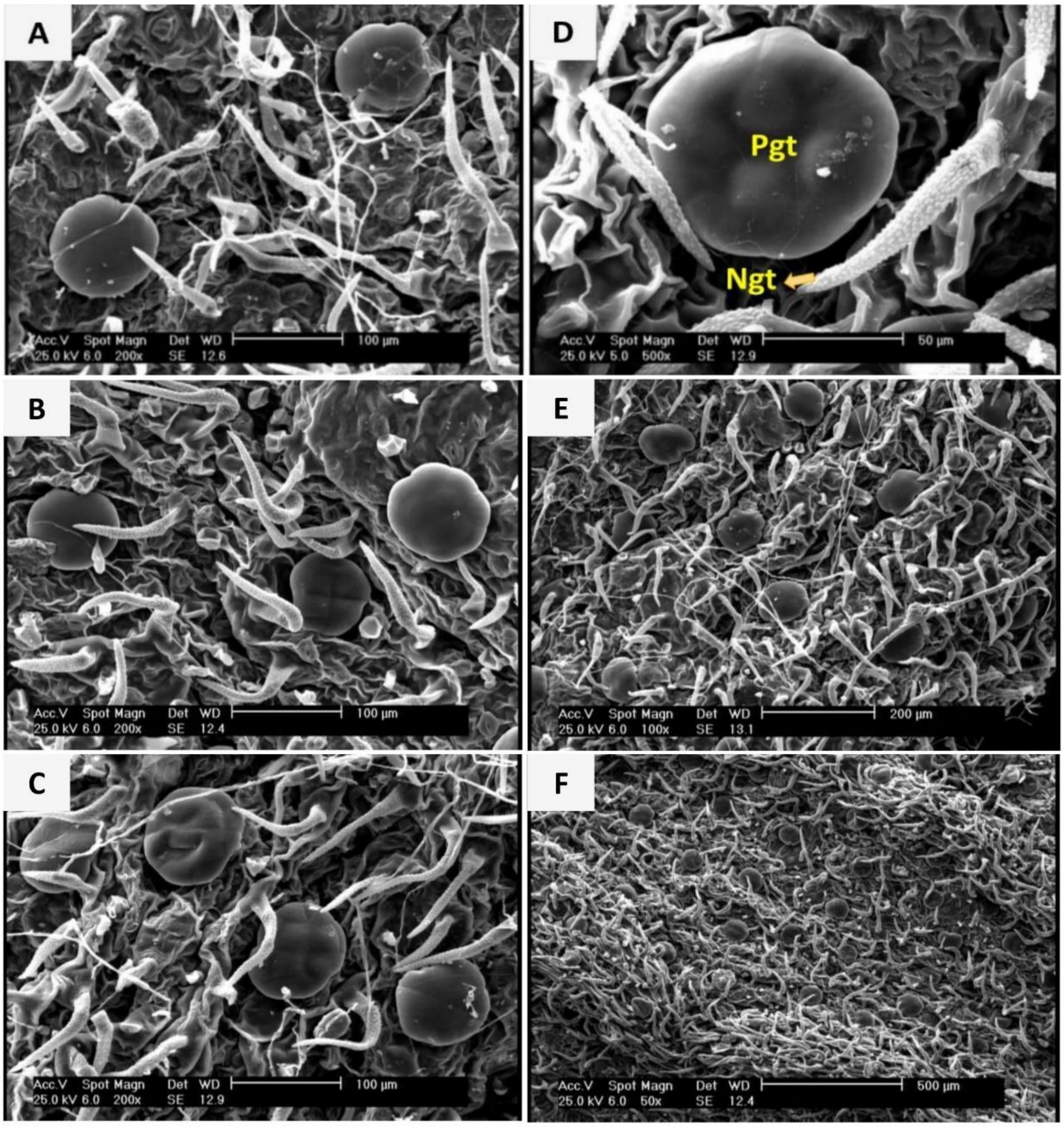

Figure 4. SEM images of *D. kotschyi* leaf under different plant densities. Distribution of Peltate glandular trichomes (Pgt), and Non-glandular trichomes (Ngt) of plants cultivated with densities at 5 (A, D), 10 (B, E), and 20 plant m^{-2} (C, F); Scale: A, B, C: 100 µm; D: 50 µm; E: 200 µm; F: 500 µm.

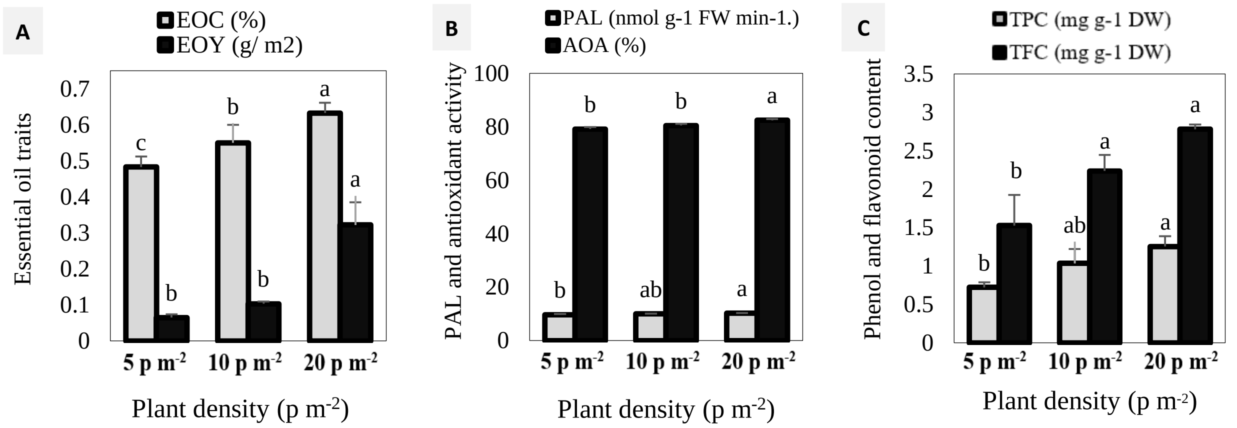

Figure 5. EOC (EO Content); EOY: EO yield (A); PAL: Phenylalanine ammonia lyase enzyme; AOA; Antioxidant activity (B); and TPC: Total Phenol Content; TFC: Total flavonoid content (C) of *D. kotschyi* under different plant densities. p m^{-2}: Plant per m^2. Means with same letters had no significant different at P< 0.05.

افزایش رشد، عملکرد و ویژگی‌های فیتوشیمیایی زرین گیاه (*Dracocephalum kotschyi* Boiss) حاصل از تراکم کشت بهینه

سحر زمانی، داوود بخشی، محمدتقی عبادی، امیر صحرارو، و مهران فتحی

چکیده

زرین گیاه (*Dracocephalum kotschyi* Boiss.) گیاه دارویی است که به دلیل داشتن خواص درمانی، فعالیت آنتی اکسیدانی، طعم و عطر جذاب در صنایع مختلف مورد استفاده قرار می‌گیرد. عملکرد تجاری و تولید ترکیبات فیتوشیمیایی را می‌توان با مدیریت کشت مانند انتخاب تراکم کشت مناسب افزایش داد. این مطالعه، با هدف بررسی تراکم بوته (فواصل 40×50، 40×25 و 40×12/5 سانتی‌متر به ترتیب با تراکم 5، 10 و 20 بوته در متر مربع) بر صفات مورفولوژیکی، عملکرد، کرک‌های ترشحی و ترکیبات فیتوشیمیایی زرین گیاه، بر اساس طرح بلوک‌های کامل تصادفی (RCBD) در منطقه سراورسو استان گیلان در سال‌های 01–1400 انجام شد. نتایج نشان داد که تراکم 20 بوته در مترمربع (40×12/5 سانتی‌متر) سبب افزایش 4 برابری صفات مورد بررسی شد و بالاترین مقادیر ارتفاع بوته (34/66 سانتی‌متر)، تعداد شاخه (14)، طول شاخه (9/34 سانتی‌متر)، تعداد برگ (138/33)، شاخص سطح برگ (105 میلی‌مترمربع)، وزن تر برگ‌ها و ساقه‌ها و کل (105/1، 98/5 و 203/6 گرم بر متر مربع)، وزن خشک برگ‌ها و ساقه‌ها و کل (26/1، 24/675، و 50/775 گرم در متر مربع) کلروفیل a ، b، کلروفیل کل و محتوای کاروتنوئید (به ترتیب 1/038، 0/653، 1/691 و 0/898 میلی‌گرم در گرم وزن تر)، تراکم کرک‌های ترشحی (14 عدد در میلی‌مترمربع)، محتوای اسانس و عملکرد اسانس (به ترتیب 0/633٪ و 0/322 گرم بر مترمربع)، فعالیت آنزیم PAL(10/221 نانومول بر گرم وزن خشک در دقیقه)، فعالیت آنتی اکسیدانی (82/85 درصد)، فنل کل (1/253 میلی‌گرم بر گرم وزن خشک) و فلاونوئید کل (2/781 میلی‌گرم بر گرم وزن خشک) در 20 بوته در مترمربع مشاهده شد. در نتیجه، تراکم 20 بوته در متر مربع (40×12/5 سانتی‌متر) به منظور افزایش عملکرد و تولید تجاری ترکیبات فیتوشیمیایی زرین گیاه در منطقه سراورسو توصیه می‌شود.