Optimizing Chia (*Salvia hispanica* L.) Cultivation in Iran Regions: A Study on Planting Density and Growth Regulator Effects

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Abstract

Chia (*Salvia hispanica* L.) is an annual plant of the Lamiaceae family that has commercial value due to its omega-3 and omega-6 content. Considering the limited cultivation of this plant in Iran, the effect of planting density and growth regulators under Gorgan weather conditions was investigated in this research. This research was conducted as a factorial design based on a randomized complete block design with 3 replications. The treatments included the cultivation distance (50, 60, and 70 cm), growth regulators including ethephon (0, 10, 30, and 60 mg), and daminozide (0, 25, 75, and 150 mg). The measured variables include morphological and yield traits (wet weight and dry weight of leaves, plant height, number of side branches, stem diameter, leaf area, number of inflorescences per plant) and phytochemical traits (total phenol, total flavonoid, mucilage, antioxidant activity by three methods (DPPH, TAOC, and FRAP)). Results indicate significant effects of ethephon and daminozide on plant growth, with differential impacts on morphological traits and reproductive growth. Specific combinations of ethephon and daminozide treatments influenced phytochemical composition, with notable variations in total phenol and flavonoid content. Antioxidant activity varied across treatments and assays, suggesting complex interactions between growth regulators and antioxidant metabolism. Additionally, mucilage production responded to growth regulator applications, with specific combinations yielding the highest mucilage content, notably with 30 ppm ethephon and 25 ppm daminozide at 70 cm row spacing. These findings contribute to optimizing chia cultivation practices and harnessing its potential as a functional food source with diverse health benefits.

Keywords: Ethephon, Daminozide, Phytochemical, Mucilage

Javadifard, F., Kh. Hemmati, N. Hemmati, 2024. Optimizing Chia (*Salvia hispanica* L.) Cultivation in Iran Regions: A Study on Planting Density and Growth Regulator Effects. *Iranian Journal of Plant Physiology,14* (3), 5169- 5179.

Introduction

Chia is an herbaceous and annual plant of the genus Sage and of the Lamiaceae family, which is commercially produced for food and medicinal uses in South American countries, including Argentina and Mexico, as well as Australia and India. This plant is known as chia, Mexican chia,

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Received: June, 2024
Accepted: August, 2024

and black chia (Di Sapio et al., 2012). Its seeds are oval with a diameter of about one millimeter and have brown, gray, white, and black and white colors. Dry chia seeds can be stored for years because they are rich in antioxidants that prevent the degradation of the seed oil (Knez Hrnčič et al., 2019). It performs best in sandy and well-drained soils with low nutrient content, medium salinity, and soil pH of 6 to 8.5 (Zade et al., 2021)and is a short-day plant (Ayerza and Coates, 2005) that grows both domestically and wildly.

The leaves of chia contain an essential oil that repels insects; as a result of this feature, the need for pesticides is reduced. The higher proportion of linoleic acid makes chia an excellent source of omega-3 fats (about 65% of the oil). With chlorogenic acid, caffeic acid, myristin, quercetin, and kaempferol, its seeds are a potential source of antioxidants with cardioprotective, liver, antiaging, and anticancer effects. It has higher beneficial unsaturated fatty acids, gluten-free protein, vitamins, minerals, and phenolic compounds (Sosa et al., 2016). The body requires omega-3 and omega-6 in chia and cannot be synthesized artificially (Ghena and Amany, 2020). Environmental factors such as soil moisture, temperature, and crop density strongly affect seed yield, height, and number of plant branches (Jumrani and Bhatia, 2018). Plant growth regulators (PGRS) are commonly used in cereal management to shorten stems and increase resistance (Hedden and Hoad, 2021). The appropriate dose of growth regulator should be tested for each species to obtain the desired result (Grzeça et al., 2021). Ethephon is a synthetic plant growth regulator with the molecular formula (C2H6CLO3P) used for many groups of plants and reduces stem length growth (Nadeem et al.). Among other plant growth regulators, Daminozide has a systemic action that effectively reduces seedling growth, achieving maximum results in hot periods (Grzeça et al., 2021).

The use of chia seeds as food is increasing worldwide, and research on plant properties is fundamental as the product expands. The cultivation and production of chia seeds currently have not fully met the demand of the world market, and recent studies have focused more on chia seeds and the extraction of their compounds. Also, very few studies have been conducted on its cultivation and response to PGRs. Since this species has a high value and there is little information about the cultivation of this plant in Iran, studies are needed for its commercial use.

Materials and Methods

Plants cultivation

This research was conducted in order to investigate the growth and yield of chia plant in the climatic conditions of Gorgan in the geographical location of E ˝23́ 19 ˚54 and N ˝47́ 49 ˚36 with an altitude of 90 meters above sea level and silt-loam soil (Table 1). The seeds were obtained from the research center of Tehran province, and their germination ability was tested; then, they were planted in the field on May 15, 2021. The statistical design was conducted in a factorial design based on randomized complete blocks in three replications. Four treatments were used, which include row distance (50, 60, and 70 cm), growth regulators such as ethephon (0, 10, 30, and 60 ppm), and daminozide (0, 25, 75, and 150 ppm). The treatments were sprayed on the plant, and after six weeks, sampling was done. Morphological traits were measured in the field, and then the seeds were harvested and transferred to the laboratory to measure phytochemical traits.

Morphological traits

This study investigated some morphological traits such as seed weight, stem diameter, lateral branch number, leaf area, number of flowers, and chlorophyll (Table 2).

Preparation of extract

To prepare the methanolic extract of seeds, 10 ml of 80% methanol was added to one gram of the dried seeds powder, placed on a shaker for 24 hours, and then filtered.

Total phenol determination

To measure total phenol, 20 µl of the prepared extract was mixed with 1.16 ml of distilled water

Table 1
Soil characteristics of the tested field

Soil type	Silt %	Clay %	Sand %	EC $dS.m^{-1}$	pH
Silt-loam	59.5	26	14.5	1538	5.42

Table 2
Morphological traits in chia plant

Measured parameters	Measurement method	Unit
Seed weight	Digital balance accuracy of 0.1 g (WT2003G)	gram
Stem diameter	Digital calliper (model)	millimeter
Height	Meter	centimeter
Lateral branches	Numeration	number
Leaf area	Leaf Area Index Meter (DELTA-T)	square centimetre
Chlorophyll	Chlorophyll meter (SPAD-502)	-

and 100 µl of Folin–Ciocalteau. After 5 minutes, 300 µl of 1 M sodium carbonate was added to the mixture. The final mixture was kept in a water bath at 40°C for 30 minutes. The absorbance of the samples was read at a wavelength of 760 nm by a spectrophotometer (WPA-S800). The results were represented in mg of gallic acid equivalents per g (Vl, 1999)

Total flavonoids determination

The aluminum chloride colorimetric method was used to calculate the total flavonoid (Chang et al., 2002). First, 500 µl of the methanolic extract was mixed with 100 µl of 10% aluminum chloride. Then 100 µl of 1 M potassium acetate, 1.5 ml of methanol, and 2.8 ml of distilled water were added, respectively. The mixture was placed in a dark place for 30 minutes and finally read at a wavelength of 415 nm. Results were represented in mg of rutin equivalents per g.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

1 ml of methanolic extract was mixed with 1 ml of DPPH reagent with a concentration of 0.1 mM. The final mixture was kept in the dark for 30 minutes, and its absorbance was read at 517 nm wavelength (Lee et al., 2003). The free radicals scavenging was calculated according to the following equation (Lee et al., 2003). In this equation, AC is DPPH radical absorption, and AS is DPPH absorption of the sample.

Formula 1: (DPPH= 100 (1-As/Ac))

TAOC (Total Antioxidant Capacity) assay

To measure the antioxidant activity using the TAOC method, it is necessary to prepare a stock solution. 0.38 grams of sodium phosphate, 0.078 grams of ammonium molybdate, and 3.36 ml of sulfuric acid were mixed and brought to a volume of 100 ml with distilled water, then one milliliter of the stock prepared with 100 µL of the extract was combined and placed in a water bath at 95 °C for 90 minutes. After cooling, the sample was read at a wavelength of 695 nm (Sun et al., 2011).

FRAP (Ferric Reducing Antioxidant Power Assay)
To prepare the FRAP reagent, the following three solutions were first prepared:
- 0.31 g of sodium acetate was mixed with 160 µL of acetic acid and made up to 100 mL with distilled water.
- 16 mg of TPTZ was mixed with 17 µL of 37% hydrochloric acid and made up to 5 mL with distilled water.
- 16 mg of iron chloride was dissolved in 50 mL of distilled water.

Solutions 1, 2, and 3 were mixed with a ratio of 10:1:1 and 1.5 mL of this solution was combined

Table 3
Mean comparison of morphological traits of chia medicinal plant (*Salvia hispanica* L.)

Ethephon (ppm)	Daminozide (ppm)	row spacing (cm)	height (cm)	stem diameter (mm)	leaf surface	Number of inflorescences	seed weight (g)	Leaf dry weight (g)
0	0	50	223^{d-f}	25.85^{a-h}	189.72^{a}	80^{xy}	7.28^{q}	57^{a}
0	0	60	241^{a}	28.32^{a-d}	166.50^{cd}	85^{xy}	8.80^{o}	40.33^{i-l}
0	0	70	230.33^{b}	25.60^{a-f}	184.37^{a}	91^{y}	4.33^{v}	42.66^{f-j}
0	25	50	216^{h-j}	25.26^{b-h}	160.94^{d-j}	97^{wx}	4.38^{v}	52^{b}
0	25	60	181.33^{v-x}	16.63^{l}	167.02^{cd}	84^{xy}	3.73^{x}	38.33^{j-n}
0	25	70	189^{q-s}	26.98^{a-h}	173.76^{b}	97.66^{wx}	4.35^{v}	43^{f-i}
0	75	50	216^{h-j}	28.71^{a-c}	152.75^{h-j}	151.33^{l}	9.41^{m}	50.66^{bc}
0	75	60	210.33^{l-m}	17.70^{k-l}	167.13^{cd}	141^{n}	3.09^{y}	39.66^{i-m}
0	75	70	213.33^{j-l}	29.07^{ab}	142.34^{m-p}	313^{b}	17.33^{b}	38^{k-n}
0	150	50	210^{l-m}	24.44^{b-i}	142^{m-p}	120^{rs}	12.37^{e}	49.66^{b-d}
0	150	60	217.66^{g-i}	24.36^{b-i}	155.22^{g-j}	140^{n}	8.37^{o}	42.66^{f-j}
0	150	70	187.66^{r-t}	21.87^{h-k}	171.77^{bc}	235^{e}	10.80^{j}	32^{p}
10	0	50	213^{j-l}	21.87^{h-k}	164.16^{d-f}	119^{s}	7.33^{q}	50^{b-d}
10	0	60	204.33^{n}	23.43^{d-j}	155.85^{g-j}	95^{x}	5.10^{u}	33^{o-p}
10	0	70	224^{c-e}	21.22^{g-k}	165.56^{cd}	200^{i}	11.99^{f}	42^{g-k}
10	25	50	200.33^{o}	22.40^{f-k}	142.39^{m-p}	125^{q}	11.66^{g}	49.66^{b-d}
10	25	60	184.33^{t-v}	17.43^{k-l}	163.55^{d-f}	104^{v}	9.38^{n}	36.66^{l-o}
10	25	70	187^{s-u}	19.48^{i-l}	131.43^{st}	192.66^{j}	9^{p}	42^{g-k}
10	75	50	200.33^{e-g}	25.95^{a-h}	165.67^{cd}	89^{xy}	3.28^{xy}	50^{b-d}
10	75	60	213^{j-l}	27.59^{a-f}	158.88^{e-h}	116^{t}	8.80^{o}	35.66^{m-p}
10	75	70	200.33^{e-g}	23.42^{d-j}	165.34^{c-e}	212.66^{h}	10.85^{i}	46^{d-g}
10	150	50	180^{wx}	26.04^{a-h}	152.67^{h-j}	99^{w}	4.13^{v-w}	47^{c-f}
10	150	60	211^{k-m}	27.44^{a-f}	137.03^{o-s}	133^{p}	7.01^{r}	38^{k-n}
10	150	70	193^{p}	23.21^{d-j}	133.04^{r-t}	136^{o}	7^{r}	48^{b-e}
30	0	50	200^{o}	24.56^{b-i}	149.72^{j-l}	122.66^{qr}	6.33^{s}	48^{b-e}
30	0	60	212.66^{j-l}	30.82^{a}	142.77^{m-o}	82^{A}	3.73^{x}	41.66^{g-k}
30	0	70	180.66^{v-x}	28.09^{a-e}	113.45^{wx}	256^{d}	10.85^{i}	42^{g-k}
30	25	50	180^{wx}	25.53^{b-h}	134.82^{q-t}	135^{o-p}	6.29^{s}	33.33^{o-p}
30	25	60	225^{cd}	22.62^{f-k}	151.49^{j-l}	83^{xy}	3.33^{xy}	40^{i-m}
30	25	70	219.66^{f-h}	23.52^{c-j}	140.25^{n-q}	53^{z}	2.19^{z}	41^{h-l}
30	75	50	216.33^{h-j}	16.60^{l}	147.96^{k-m}	133^{p}	6^{st}	41.66^{g-k}
30	75	60	212^{k-l}	17.47^{k-l}	158.16^{f-i}	104^{v}	7.33^{q}	40.33^{i-l}
30	75	70	227^{bc}	25.08^{b-h}	151.57^{j-k}	99^{w}	4.41^{v}	49^{b-e}
30	150	50	183.33^{u-w}	23.23^{d-j}	123.95^{uv}	147^{m}	9.74^{k}	32^{p}
30	150	60	214.33^{i-k}	22.51^{f-k}	133.78^{q-t}	102^{b}	7.23^{q}	33^{o-p}
30	150	70	225.33^{cd}	24.73^{b-i}	108.91^{x}	381^{a}	25.90^{a}	51^{bc}
60	0	50	165^{y}	23.90^{b-i}	145.04^{l-n}	185^{k}	4.45^{v}	52^{b}
60	0	60	191^{p-r}	28.98^{ab}	134.52^{q-t}	227^{f}	11^{h}	35^{n-p}
60	0	70	192^{q-p}	24.26^{b-i}	113.73^{wx}	286^{c}	15.66^{c}	48^{b-e}
60	25	50	177.66^{x}	27.27^{a-g}	123.25^{u-v}	139.33^{n}	9.62^{l}	49.66^{b-d}
60	25	60	187^{r-t}	22.94^{d-j}	120.44^{v}	133.66^{o-p}	8^{p}	34^{n-p}
60	25	70	180^{wx}	27.48^{a-f}	117.50^{v-w}	88^{y}	3.20^{xy}	49^{b-e}
60	75	50	186.66^{s-u}	26.30^{a-h}	155.77^{g-j}	136^{o}	9.33^{n}	49^{b-e}
60	75	60	190.33^{p-s}	24.40^{b-i}	135.96^{p-t}	90^{y}	5.80^{tu}	38^{k-n}
60	75	70	191^{p-r}	18.47^{j-l}	154.21^{h-j}	110^{u}	8.80^{o}	49^{b-e}
60	150	50	181.66^{v-w}	28.16^{a-d}	138.97^{n-r}	200^{i}	11^{c}	52^{b}
60	150	60	187.33^{r-t}	24.26^{b-i}	129.48^{t-u}	104^{r}	5.12^{u}	32.66^{o-p}
60	150	70	189^{qs}	24.44^{b-i}	151.91^{i-k}	221^{g}	14.12^{d}	45^{e-g}

with 50 µL of extract. After vortexing, it was incubated at 40°C in a water bath for four minutes. After cooling, the absorption number of the samples compared to the control was read at a wavelength of 593 nm (Biskup et al., 2013).

Mucilage determination

One gram of the seed powder was mixed with 20 mL of distilled water, and after 24 hours of rest in the refrigerator, it was filtered. Then, 10 mL of the filtered solution was added to 20 mL of 95% alcohol. The mixture was placed in the refrigerator for 24 hours, and finally, it was filtered, and the sediments were weighed (Vl, 1999). Next, the obtained mucilage was dried in an oven at a temperature of 50 °C. The measurement of extraction efficiency was calculated based on the initial weight of the seed

ANOVA analysis

The variance analysis was done using the SAS software (version 9.1), and the means were measured using the LSD test. Graphs were drawn using Excel software.

Results

Chia growth

According to this research, the application of ethephon and daminozide has led to a decrease in the height of the chia plant. As indicated in Table 3, the lowest height was related to the 60 ppm of ethephon in the 50 cm row spacing (165 cm) and the highest height was related to the control (223 cm). Applying the interaction effects of 30 ppm of ethephon and 150 ppm of daminozide treatment at 70 cm row spacing reduced the leaf area (108.91). On the other hand, the number of inflorescences of the chia plant was increased in this treatment (381), which shows that by reducing the vegetative effect of the plant by the applied treatments, its reproductive growth has increased. Using PGRs not only has a negative effect on the number of inflorescences of the plant but also increases it. The highest leaf area, leaf dry weight, and the lowest number of inflorescences were observed in the control. The highest stem diameter (30.82 cm) was observed at 30 ppm of ethephon with 60 cm of row spacing. The lowest (16.60 cm) was seen in the 30 ppm of ethephon and 25 ppm of daminozide at 50 cm of row distance, which was not significantly different from 25 ppm of daminozide treatment with 60 cm of row spacing (16.63 cm). Also, the highest weight of 1,000 seeds (25.90 g) was recorded in 30 ppm of ethephon and 150 ppm of daminozide at 70 cm of row spacing**,** and the lowest weight of 1,000 seeds (3.09 g) was recorded in 75 ppm of daminozide with 60 cm of row spacing (Table 3).

Total phenol

The means comparison of seed total phenol showed that the highest amount of total phenol was related to the interaction effects of 60 ppm of ethephon and 25 ppm of daminozide with 70 cm of row spacing (17.02 mg. g^{-1} DW), which was not significantly different from 75 ppm of daminozide at 70 cm of row spacing (14.38 mg. g^{-1} DW), as well as the interaction effects of 30 ppm of ethephon at 50 cm of row spacing and two concentrations of daminozide (75 and 150 ppm). Also, the lowest amount of total phenol related to 10 ppm of ethephon with 70 cm of row spacing (5.26 mg. g^{-1} DW) (Fig. I).

Total flavonoids

In this study, the maximum amount of total flavonoids was recorded in the interaction treatment of 10 ppm of ethephon with 50 cm of row spacing (1.09 mg. g^{-1} DW), which was not significantly different from the interaction treatments of 30 ppm of ethephon with 60 cm of row spacing and 10 ppm of daminozide at 50 cm of row spacing. Also, with the increase of ethephon concentration, the flavonoid compounds decreased. The lowest total flavonoid content was observed in the 60 ppm of ethephon treatment with 70 cm of row spacing (0.39 mg. g^{-1} DW). Different concentrations of daminozide and its interactions with other treatments did not cause significant differences in the total flavonoid content of seeds (Fig. II).

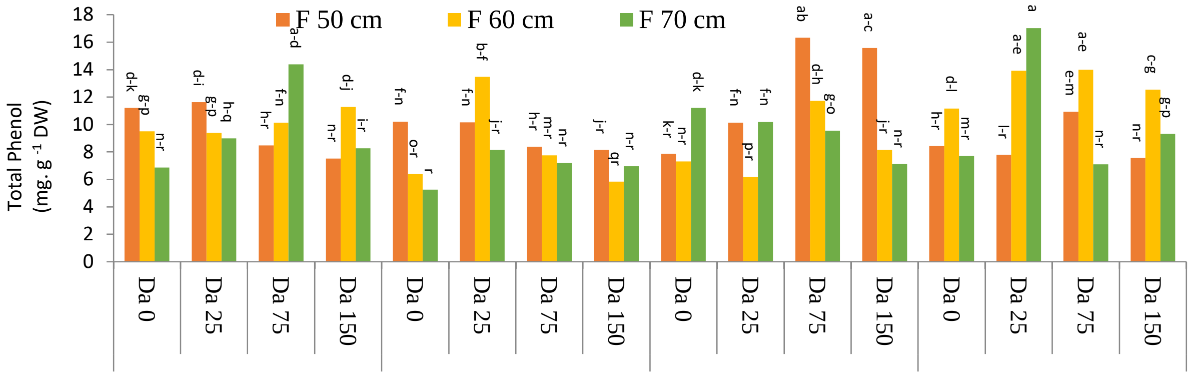

Fig. I. Effect of daminozide, ethephon, and row spacing on total phenol of chia seed.

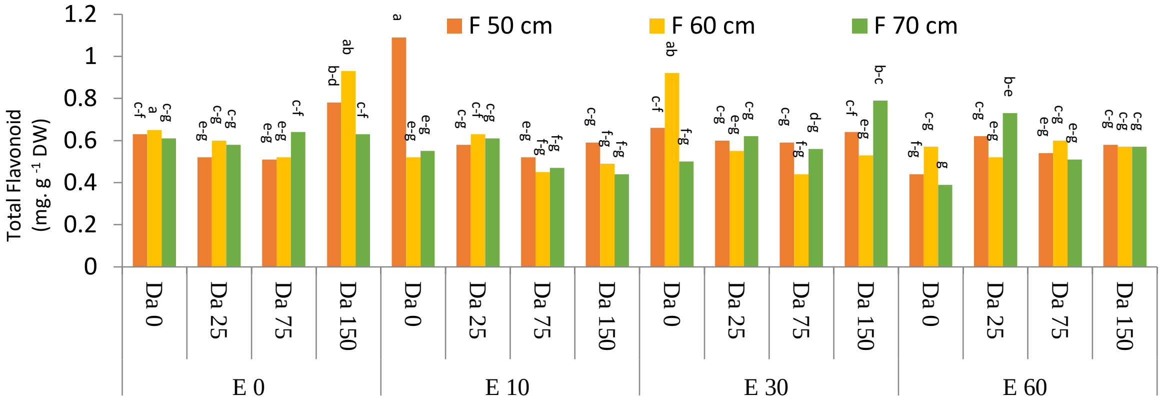

Fig. II. Effect of daminozide, ethephon, and row spacing on total flavonoid of chia seed.

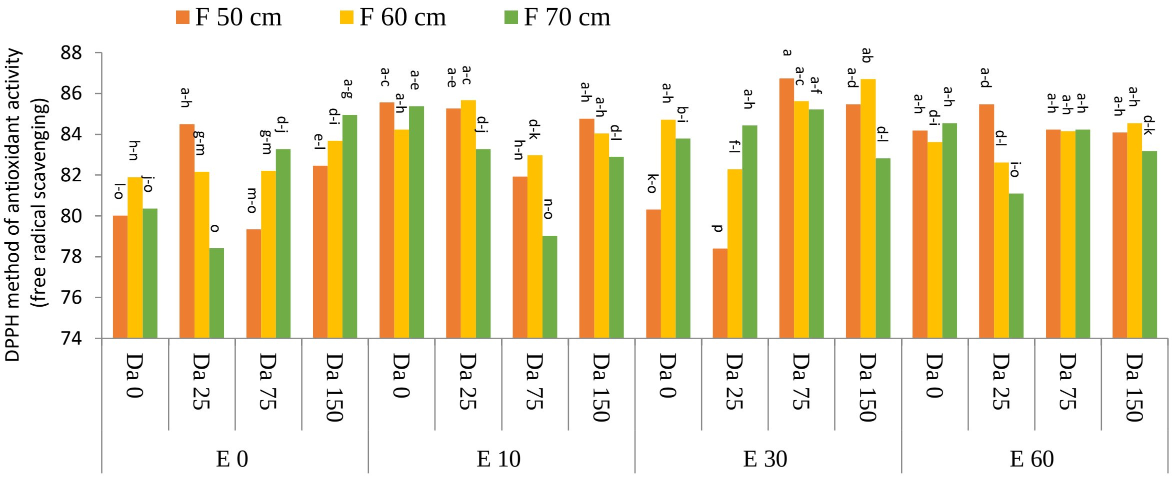

Fig. III. Effect of daminozide, ethephon and row spacing on antioxidant activity using DPPH method of chia seed.

DPPH assay

According to the means comparison results, the highest percentage of seed antioxidant activity was measured in the treatment of the combination effects of 30 ppm of ethephon, 75 ppm of daminozide with 50 cm of row spacing (86.74%), and the lowest was related to the interaction effects of 30 ppm of ethephon, 25 ppm of daminozide at 50 cm of row spacing (78.4%), which was not significantly different from 25 ppm of daminozide treatment with 70 cm of row spacing (78.42%). Based on the results, by increasing the amount of daminozide, antioxidant

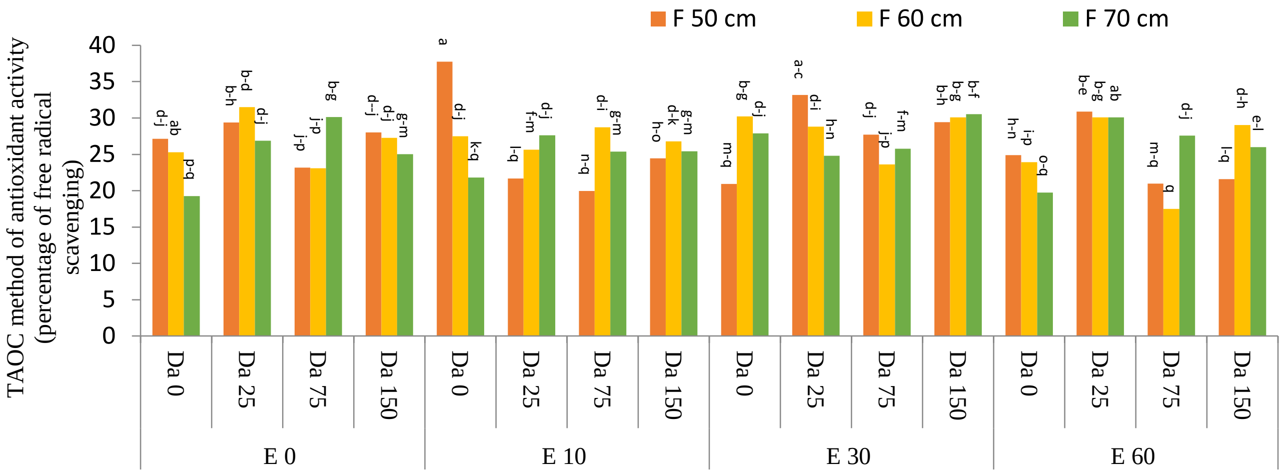

Fig. IV. Effect of daminozide, ethephon and row spacing on antioxidant activity using TAOC method of chia seed.

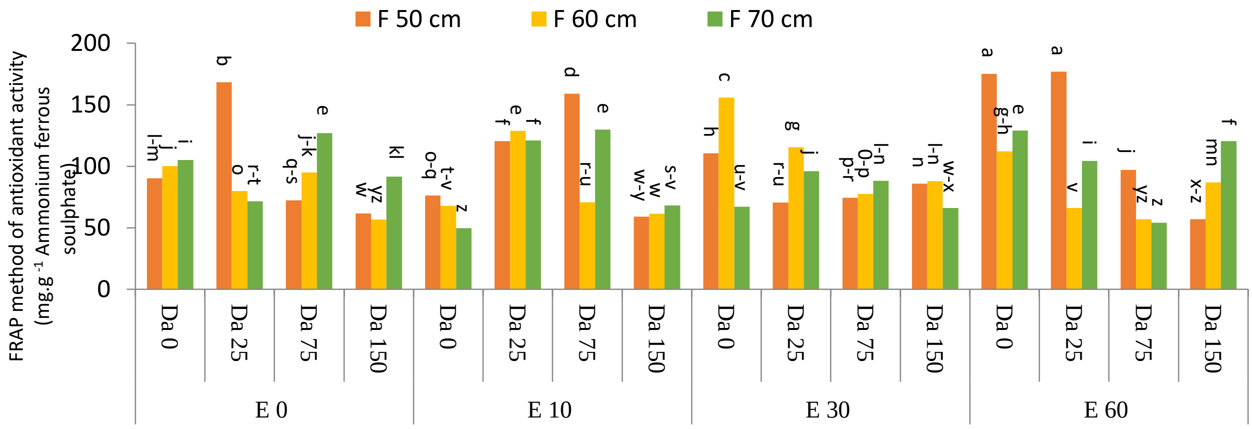

Fig. V. Effect of daminozide, ethephon, and row spacing on antioxidant activity using FRAP method of chia seed.

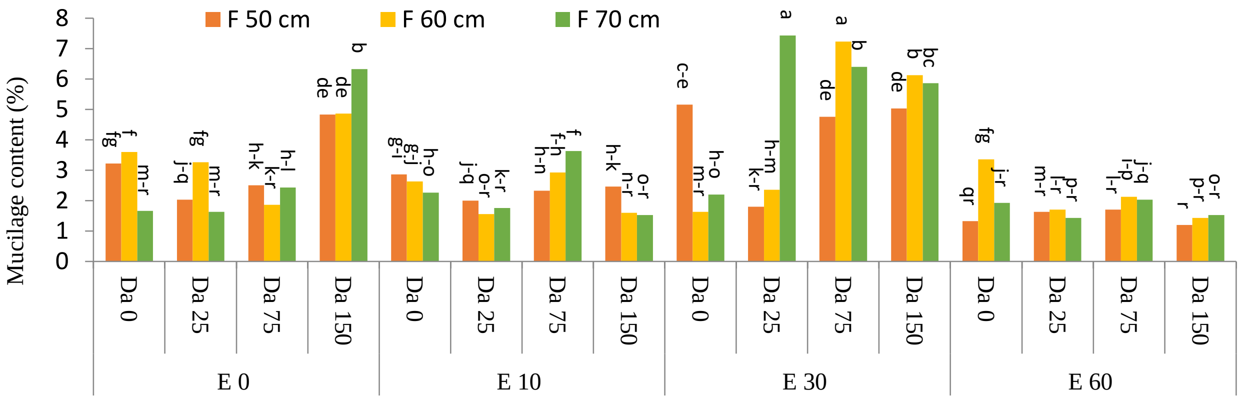

Fig. VI. Effect of daminozide, ethephon, and row spacing on mucilage content of chia seed.

activity increases, but its interaction with ethephon can change its performance (Fig. III).

TAOC assay

The highest antioxidant activity of seeds by the TAOC method was seen in the treatment of 10 ppm of ethephon with 50 cm of row spacing

(37.74%), which statistically has no significant difference with the treatment of 30 ppm of ethephon and 25 ppm of daminozide at 50 cm of row spacing interaction effects (33.17%). Also, the lowest amount was recorded in control at 70 cm row spacing (19.26%) (Fig. IV).

FRAP assay

The maximum percentage of seed antioxidant activity by FRAP method was observed in the treatment of the interaction effects of 60 ppm of ethephon and 25 ppm of daminozide with 50 cm of row spacing (176.83 mg.g-1 ammonium ferrous sulphate), which was not significantly different from the effects of 60 ppm of ethephon at 50 cm of row spacing (175.24 mg.g-1 ammonium ferrous sulphate) and the lowest amount was recorded in 10 ppm of ethephon treatment with 70 cm of row spacing (49.75 mg. g^{-1} ammonium ferrous sulphate). Also, based on the results, the high concentration of daminozide decreased the antioxidant activity However, as the amount of ethephon increased, the antioxidant activity also increased. The results were influenced by daminozide and ethephone (Fig. V).

Mucilage determination

According to the mean comparison of chia seed mucilage, the interaction effect of 30 ppm of ethephon treatment with 25 ppm of daminozide at 70 cm of row spacing had the highest percentage of mucilage (7.43%), which is statistically was not significantly different from the interaction effect of 30 ppm of ethephon and 75 ppm of daminozide treatment at 60 cm of row spacing (7.23%). The application of ethephon treatment in low (10 ppm) and high (60 ppm) concentrations and its interaction with daminozide and row spacing had a negative effect on the amount of mucilage. However, the application of daminozide increased the amount of mucilage compared to the control in different row spacing. Also, its interaction with 30 ppm of ethephon showed an increasing trend in mucilage (Fig. VI).

Correlation

Based on the correlation results, a positive and significant correlation was observed between total flavonoid trait and antioxidant activity by the TAOC method. Scientific Fact: Flavonoids are well-known secondary metabolites in plants and are recognized for their antioxidant properties. They can scavenge free radicals, which are reactive molecules that can cause oxidative stress in cells. Numerous studies have demonstrated the positive correlation between flavonoid content and antioxidant activity in various plant species.

The antioxidant activity by the TAOC method negatively correlated with the amount of chlorophyll. While chlorophyll is essential for photosynthesis and the production of energy in plants, an excess of it can lead to the generation of reactive oxygen species (ROS). Antioxidants play a crucial role in neutralizing ROS. Therefore, a negative correlation between antioxidant activity and chlorophyll content aligns with the concept that higher antioxidant activity may be needed to counteract potential oxidative stress caused by elevated chlorophyll levels.

Also, the correlation between plant height and mucilage was positive. On the other hand, the antioxidant activity of the FRAP method was negatively correlated with the amount of mucilage and plant height. The negative correlations observed between antioxidant activity (FRAP method) and mucilage/plant height might be attributed to resource allocation within the plant. Plants often allocate resources (such as energy and nutrients) based on their growth strategies. Understanding the specific biochemical pathways involved in antioxidant production and the synthesis of mucilage can provide insights into these trade-offs (Fig. VII).

Discussion

Chia is an essential crop due to its rich secondary metabolite content, including omega-3 fatty acids, antioxidants, and phytochemicals. The modulation of secondary metabolites is of great interest to researchers and farmers aiming to enhance chia's nutritional and pharmaceutical potential. Daminozide and ethephon are two commonly used chemicals in agriculture, known

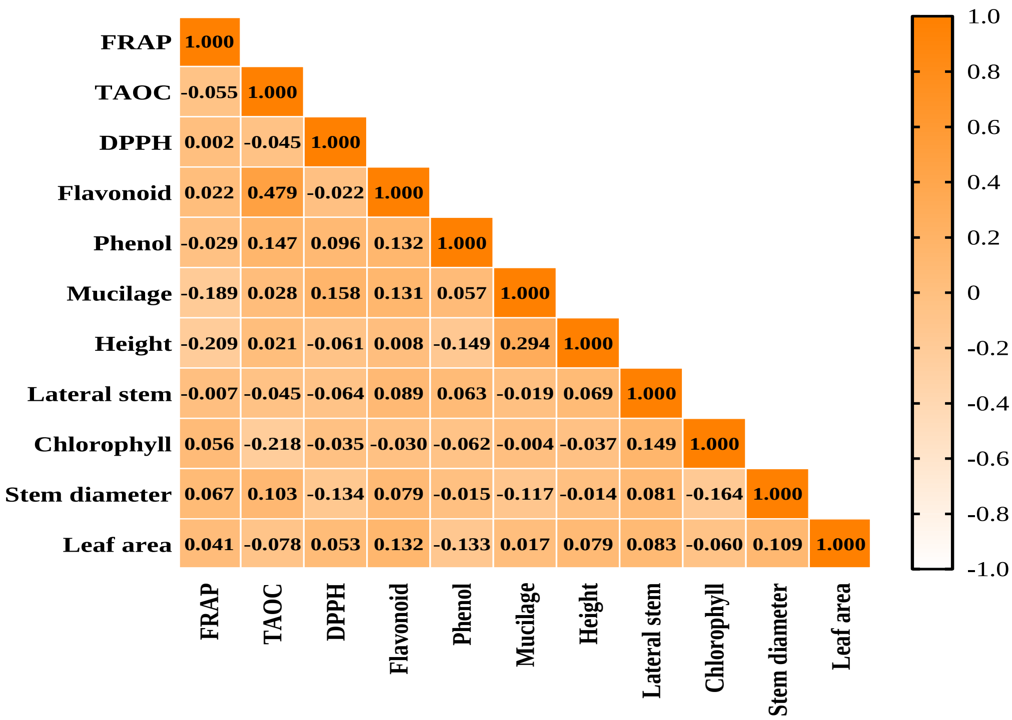

Fig. VII. Correlation table of morphological and biochemical traits of *Salvia hispanica* L.

for their ability to influence plant growth and development. Understanding their impact on secondary metabolites in chia plants is essential for optimizing chia cultivation and product quality. Based on the results of this research, the application of ethephon and daminozide has led to a decrease in the height of the chia plant. Daminozide primarily affects the gibberellin biosynthesis in plants, which results in reduced internode elongation and increased lateral branching. This altered growth pattern may influence the allocation of resources within the plant and potentially affect secondary metabolite production. The research found that stevia seedlings treated with daminozide had shorter internodes than the control. Daminozide 20 ppm showed the most substantial effect (55.5%) in shortening the seedling stem, so the seedlings in this treatment have fewer nodes (1.1±6.5) compared to other treatments (Saptari et al., 2022). Applying the interaction effects of 30 ppm ethephon and 150 ppm daminozide treatment at 70 cm row spacing reduced the leaf area (108/291 cm).

In contrast, the number of inflorescences (381) and seeds (25.90) had the highest number in this treatment. The leaves that expand after applying growth retarders are often smaller but thicker than the previous leaves. The use of growth retarders prevents the negative effects of stress by reducing the water consumption of the whole plant. This process is done by creating smaller leaves, less leaf surface, and a small number of pores or smaller leaf pores (Ullah et al., 2018).

Modulating plant growth by daminozide may influence the production of flavonoids and antioxidants. The mechanisms underlying daminozide and ethephon's effects on the biosynthesis of secondary metabolites need to be well documented. The present study investigated these PGRs on total phenol, flavonoid, and antioxidant activity. According to our results, the interaction of daminozide and ethephon could increase the amount of total phenol. While increasing total flavonoid, ethephon played a more critical role. Yamazaki et al. (Yamazaki et al., 2021)indicated that using ethephon could enhance flavonoids of *Vitis vinifera* in cell culture. Other researchers indicated that ethephon activated most of the phenylpropanoid pathway genes and markedly increased the accumulation of several phenylpropanoid compounds, including flavonoids and anthocyanins in Tartary buckwheat hairy root(Song et al., 2024). Also, the expressions of most genes in the flavonoid biosynthetic pathway were significantly induced by ethephon treatment (Li et al., 2017). Ethephon's ability to release ethylene can increase chia plants' flavonoid and phenol content(Yan et al., 2023). Ethylene is a known regulator of flavonoid biosynthesis. Therefore, ethephon application can be used strategically to boost flavonoid content in chia(Yildirim et al., 2024). Ethylene exposure can induce stress in plants, increasing the production of secondary metabolites like phenolic compounds, including total phenols and flavonoids. The interaction between daminozide and ethephon can result in synergistic or antagonistic effects on secondary metabolite production(Ritonga et al., 2023). This depends on the specific secondary metabolite and the timing and dosage of the PGR application.

According to the results, the row spacing of 60 cm generally performs better in chia plants. In the proper planting distance, the maximum yield will be obtained by increasing the plant's benefit from environmental factors; using the proper planting distance can obtain the desired yield (Gardner et al., 2017). By regulating the plant density per unit area and planting arrangement, the effect of inter-species and intra-species competition is minimized, the use of growth factors is maximized, and the maximum seed yield is achieved. Sedghi et al. (Sedghi et al., 2008)reported that a density less than optimal in sunflowers causes a decrease in yield and an increase in weeds, as well as a density higher than the optimal level reduces the yield of seeds and seed oil. Shading the lower leaves causes rot and susceptibility to pests and diseases. In another study, plant density and planting date have been reported to be significant in basil seed yield (Sadeghi et al., 2009).

Conclusion

Daminozide and ethephon's effects on chia plants' phytochemical content are multifaceted and often indirect. The interplay between growth regulation and secondary metabolite production is complex, making it essential to determine optimal dosages, timings, and other cultivation practices for achieving the desired outcomes. Further research and experimentation are needed to gain a more precise understanding of how these plant growth regulators can be harnessed to enhance chia plants' nutritional and pharmaceutical potential.

Funding

This research was funded by the Gorgan Agricultural Science and Natural Resources.

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