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Research Article

GROWTH, YIELD ATTRIBUTES AND PHYTOCHEMICAL PROPERTIES OF CHIA SEED (*SALVIA HISPANICA*) UNDER VARYING PLANT DENSITIES

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Abstract

Chia (*Salvia hispanica* L.) is a diminutive seed of an annual herbaceous plant. In recent years, the utilization of chia seeds has exhibited a marked increase due to their maximal nutritional and therapeutic values. An experiment was conducted at the Agronomy Research field in the Department of Agronomy and Haor Agriculture, Sylhet Agricultural University (SAU), Sylhet, from 16 November 2022 to 12 April 2023. The objective of this study was to ascertain the growth, yield quality, and phytochemical characteristics of Chia seeds at varying plant densities. The tests were conducted in Randomized Complete Block Design (RCBD) with four replicates. Plant population per unit area regarded as treatments T1 =120 m^{-2} and T2 = 60 m^{-2}. The collected data were evaluated statistically using the R program (version R 4.2.3). The tallest plant height (94.08cm) from T1, maximum count of inflorescence $plant^{-1}$ (42.11) in T2, highest number of leaves $plant^{-1}$ (79.60), highest number of branches $plant^{-1}$ (61.83) was recorded until harvest in T1. The highest seed yield (9.32 Kg/ha), 1000 seed weight (1.38 g) were also recorded in T2 and T1. Additionally other yield attributes such as seeds/floret, floret/inflorescence, number of inflorescence/plants etc. were also found in T1 & T2. Chia seed's overall phenolic content 11.79 mg GAE/g and total flavonoid content 13.37 mg QE/g were both measured after harvest from both treatments. Further study and agricultural advancements will contribute to the cultivation and use of chia seeds in a variety of applications. However, further investigation is necessary to elucidate the bioavailability of chia seeds and the particular extraction procedures related compounds.

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Introduction

Salvia hispanica L., usually called chia, is a yearly herbaceous type plant, originating from Southern Mexico and Northern Guatemala. It belongs to order Lamiales under mint family Labiate, subfamily Nepetoideae, and genus *Salvia*. The appearance of the seed varies from dark, dim, or dark speckled to white (Amato et al., 2017). The seed have between 25% to 40% oil with 60% of it including (omega) ω-3 alpha-linolenic corrosive and 20% of (omega) ω-6 linoleic corrosive. The energy content of chia seeds has been determined to range from 459 to 495 kilocalories per 100 grams. Furthermore, under optimal agronomic conditions, the plant has been observed to yield 500 to 600 kilograms of seeds per acre (Ullah et al., 2016). Various active components like phenolic and essential fatty acids components are present in it. The dynamic components integrate Myricetin, Kaempferol, Quercetin, caffeic acid (Melo et al., 2019). The concentrations of these active chemicals may differ owing to development sites. The environmental fluctuations, climatic changes, supplement availabilities, culture year, or soil characteristics alter the composition of phytochemicals (Melo et al., 2019). In recent years, chia seeds have gained prominence as a globally significant dietary component due to their nutritional value and restorative properties (Coorey et al., 2012). It has been outlined in a few ponders that chia seeds due to the large percentage of fatty acids exhibit can be crucial for health, antioxidant, and antibacterial movement (Caudillo et al., 2008). Chia seeds as a wholesome supplement are gigantic beneficial advantages including aiding the digestive system, boosting good textured skin, bone and muscles develop, lowering the danger of heart illness, diabetes, and so on.

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Today, there are many underused disclosures of nutritious characteristics, phytochemicals, and extraction procedures of chia seeds (Ayerza, 2016). Though chia is a new plant variety in Bangladesh, the financial esteem of chia is higher in both national and international level. So, good production of chia in Bangladesh will boost economic and therapeutic benefits. There is a need to evaluate its cultivation and create adequate management methods of agronomy for optimal growth and output in Bangladesh. Since cultivation is heavily relied on the environment to express its greatest agronomic qualities, studies are vital to evaluate the elements that truly affect the chia output. In this connection, Bangladesh Agricultural Research Institute (BARI) has begun various agronomic management research to estimate the feasibility of chia cultivation in Bangladesh. Chia merits increasing attention because to the wide applicability of its compounds and derivatives. Thus, additional investigations are required to know about chia seed. The aim of this research work is to know the growth characteristics, yield and yield attributes, and phytochemical qualities of chia seed under varied plant densities.

Materials and Methods

Study area and study period

The study was conducted at the Agronomy Research field in the Department of Agronomy and Haor Agriculture, Sylhet Agricultural University (SAU), Sylhet, throughout the period from 16 November 2022 to 12 April 2023.

Land preparation and Treatments

The field was prepared using a - cultivator by ploughing and cross ploughing followed by laddering. Weeds, stubble, and crop residue were removed carefully. The first ploughing was done on 16 November and final land preparation began on 18 November 2022. Treatment was based on plant population per square meter. The treatment strategy was meticulously designed based on the plant population per square meter, with treatments designated T1 where 120 m^{-2} and T2 where 60 m^{-2} were maintained. The unit plot size was 4 m^2 (2 m x 2 m), with line-to-line spacing of 25 cm and a plot-to-plot distance of 50 cm.

Fertilizer application

Urea, TSP, MoP (Muriate of Potash), Gypsum, Sulphur were utilized with 200, 180, 100, 150, 96 (kg/ha), respectively as per Fertilizer Recommendation Guide, BARC (2015), (Sultana et al., 2015). The total amount of TSP (Triple Super Phosphate), MoP, Urea, Gypsum, Sulphur was administered as basal dose during final land preparation. Additionally, 10 tons of cow dung were applied per hectare during the final field preparation. In the final stage of field preparation, half of the urea was applied to the soil, while the remaining fertilizers were side dressed at 24 DAS.

Seed sowing & Thinning

On 20 November 2022 the seeds were sown by continuous sowing method. On 24 November the germination was observed. And 15 days after sowing (DAS), two weeding operations were executed, followed by a thinning procedure that was carried out manually.

Design & statistical analysis

Randomized Complete Block Design (RCBD) was followed with four replications. The data from various parameters obtained, tabulated and statistically analyzed by using R-software (version R 4.2.3).

General observations of experimental field

For the data on yield qualities, 20 plants were randomly picked from each plot before harvesting the. Some images were shot and data were obtained according to the statistical analysis. The growth data including plant height, number of branches/plants, number of leaves/plants, and inflorescence length were taken at defined interval until harvest by destructive sampling approach employing 5 plants for every sampling period from each plot.

Harvesting and processing

At maturity stage, the crop was harvested on 23 February. Prior to harvesting, 20 plants were randomly uprooted for yield parameters viz., number of inflorescence/plants, floret /inflorescence, seeds floret (no.), seed/floret, seeds/inflorescence, weight of 1000-seeds and seed yield was taken from 1m × 1m harvested area from the center of the plots. After harvesting, collected plants were sun dried for 3-4 days to get appropriate seed moisture and optimum seed weight.

Data collection

For yield and yield attributes, before harvesting plot as a whole, 20 sample plants were randomly uprooted from plot to collect data on different yield contributing characters as plant height, Number of leaves, Number of branches, Inflorescence length (cm), Number of inflorescence, Floret/inflorescence, Seed/floret, Number of seeds floret, Plant population, 1000 seeds weight (gm), Seed yield (kg/ha). As a result, total growth and yield contributing characteristics were recorded.

Phytochemical analysis

Determination of Total Phenolic Content

By using Folin Ciocalteau assay, the total content of phenolic was determined by gallic acid standard solution, explained by Singleton *et al.* (1999). The mixture was made with 0.5 ml FCR reagent, 0.5 ml extract or different concentration of standard solution. After 5 min, 5 ml of (7%) Na_2CO_3 solution with 4.5 ml distilled water was added. Hold at room temperature for at least 20 minutes to complete reaction. The absorbance was recorded at 760 nm.

Estimation of Total Flavonoid Content

Total flavonoid evaluated by Aluminum chloride spectrophotometric (Zhishen et al. 1999) with some adjustments. Quercetin solutions were used to produce the standard calibration curve. 1.5 ml of methanol was added to 0.5 ml extract or variable concentration of standard in a test tube. 0.2 ml potassium acetate with 0.2 ml of 10% aluminum chloride solution added with finally 5.6 ml distilled water into test tube and mixed thoroughly. Stabilize by incubated at room temperature to complete response. After 15 min, measure the absorbance at 420 nm.

Statistical analysis of phenolic and flavonoid content

All the studies done in threefold and results recorded as mean ± standard error. The sample concentration was determined using the calibration curve. Calculation of linear correlation coefficient and correlation analysis were done. Using this linear regression equation on a linear line, concentrations of extracts were determined. With the estimated values of concentration of extract, the total phenolics and flavonoids content were computed.

The outcomes of phenolic content were demonstrated as mean value with standard deviation that is in milligram gallic acid equivalents (GAE) per gram of extract. And result of flavonoid content of chia seeds was expressed as mean value with standard deviation that is reported in milligram quercetin equivalents per gram of dry sample.

Results and Discussion

Chia seed growth, yield and phytochemical properties were studied in the experiment, which lasted from November to April. In order to study the characteristics of the chia seed's growth, a lot of data was acquired throughout this experiment. Phytochemical properties of chia seeds, including concentrations of phenolic and flavonoid compounds, as well as attributes associated to yield were also noted. The results of this experiment's description and presentation are given below:

Growth characteristics of Chia seed

Plant height (cm)

The plant height of chia was significantly influenced by two treatments across various growth stages. At 30 DAS, T1 recorded a plant height of 52.33 cm, while T2 measured 51.93 cm, showing no significant difference. However, as the growth progressed, T1 consistently exhibited higher plant height than T2. At 50 DAS, T1 reached 78 cm, whereas T2 was

63 cm. At 70 DAS (harvest), T1 achieved the maximum plant height of 94.08 cm, significantly higher than T2, which measured 78 cm.

The statistical analysis indicated a significant difference in plant height between treatments at later growth stages. The trend suggests that T1 had a more favorable impact on chia growth compared to T2. The differences observed could be attributed to variations in treatment conditions, possibly due to the influence of phytochemicals or other growth-promoting factors.

The results indicate that plant height in chia was significantly influenced by the applied treatments, with T1 consistently showing superior growth compared to T2. The significant differences observed at later growth stages suggest that T1 provided more favorable conditions for plant development. This could be attributed to better nutrient uptake, enhanced metabolic activity, or the presence of growth-promoting compounds in the treatment. Result specified that relatively the growth rate of plant height from T2 (60 m^{-2}) was lower than T1 (120 m^{-2}) due to higher plant densities. As competition increases in plants, it results in decreases. Similar conclusions were also found in (Masudul et al., 2016), who conducted a study on effect of planting time and yield of chia.

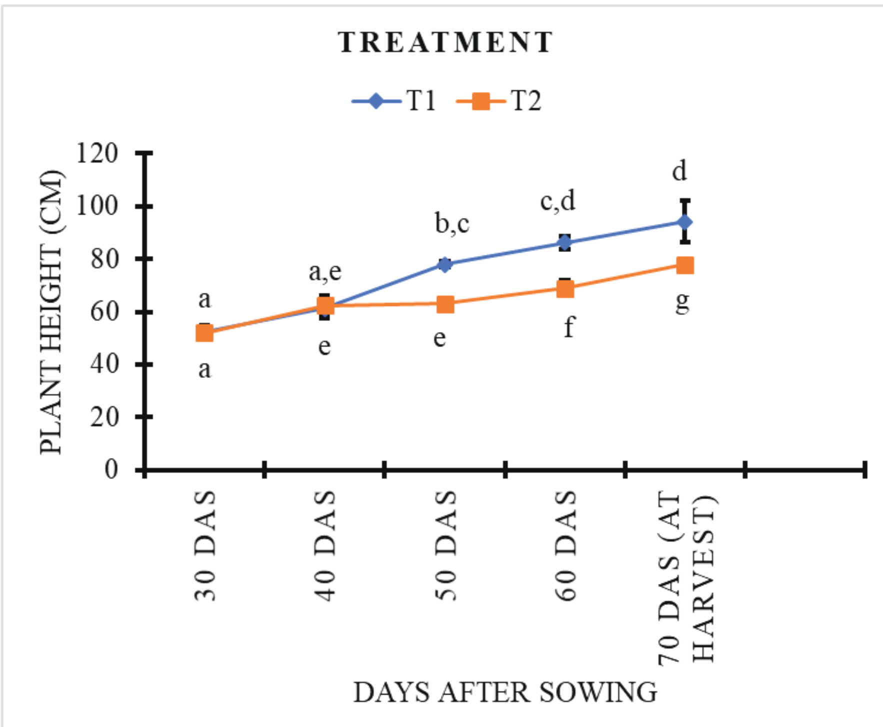

Figure 1. Plant height of chia under two treatments across growth stages. Values are in mean ±SE. Here, N=40, where each treatment comprises of four replications and each replication consists of 10 plants. Small letters above the line represent multiple comparisons within the T_1 (Days after sowing) and small letters below the line represents multiple comparisons within the T_2 (Days after sowing). Different letters in each values indicate P<0.05 either within the treatment or between the treatments (T_1 and T_2).

Quantity of leaves plant^{-1}

Significant effects of treatments on the number of leaves per plant were observed at different growth stages. At 30 DAS, T1 recorded an average of 25.83 leaves, while T2 had a slightly higher count (28.35 leaves). A significant increase was observed at 40 DAS, with T1 reaching 60.08 leaves, which was higher than T2 (45.83 leaves). At 50 DAS, T2 exhibited the highest leaf count (74.85 leaves), surpassing T1 (69.98 leaves). However, at 60 DAS, T1 recorded the peak number of leaves (79.6 leaves), while T2 declined to 64.5 leaves. By the time of harvest (70 DAS), both treatments exhibited a reduction, with T1and T2 recording 52.3 and 52.48 leaves, respectively.

The statistical analysis indicated significant differences among the treatments across different growth stages, as represented by different superscripts in the graphical representation figure 2. Treatments followed distinct growth patterns, with T1 maintaining a more consistent increase in leaf production, peaking at 60 DAS, while T2 exhibited fluctuations with an early peak at 50 DAS.

The results suggest that T1 promotes a more stable leaf development pattern, while T2 shows an early peak followed by a decline. These differences may be attributed to the specific effects of the treatments on plant growth dynamics. The differences of results might happen due to the development rate of the plant & by the environmental situation. Similar findings also available from (Alamgir et al., 2015), in their study they also found the same thing that number of leaves declines at harvest. Moosavi et al. (2012) also reported that delay in sowing decreased substantially the leaf number, plant height and yield.

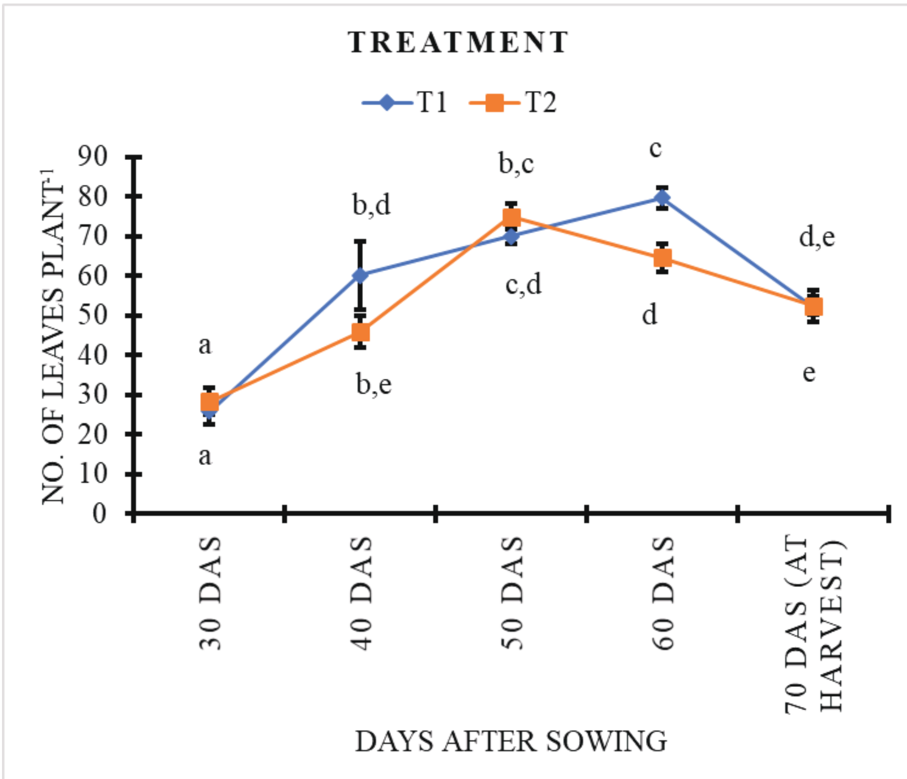

Figure 2. Effect of treatments on leaf number in chia plants. Values are in mean ±SE. Here, N=40, where each treatment comprises of four replications and each replication consists of 10 plants. Small letters above the line represent multiple comparisons within the T_1 (Days after sowing) and small letters below the line represents multiple comparisons within the T_2 (Days after sowing). Different letters in each values indicate $P<0.05$ either within the treatment or between the treatments (T_1 and T_2).

Number of branches plant-1

The results revealed that both treatments (T1 and T2) exhibited an increasing trend in the number of branches up to 60 DAS, followed by a decline at harvest (70 DAS). At 30 DAS, T1 had 14.28 branches, while T2 recorded a slightly higher value of 15.43 branches. A significant increase was observed at 40 DAS, with T1 reaching 33.05 branches and T2 at 25.4 branches. The trend continued at 50 DAS, where T1 (56.13) had more branches than T2 (45.08). The highest number of branches was recorded at 60 DAS, with T1 reaching 61.83 and T2 at 54.98. However, at 70 DAS (harvest stage), a decline was observed, with T1 decreasing to 27.55 and T2 to 31.25 branches. The statistical analysis (as indicated by the different letter groupings in the figure 3) shows significant differences among the treatments at various growth stages. T1 generally exhibited a higher number of branches compared to T2, particularly at later growth stages (50 and 60 DAS). However, the differences became less pronounced at harvest. The decline in the number of branches at 70 DAS may be attributed to plant senescence and resource allocation toward seed development.

These findings suggest that treatment T1 was more effective in promoting branch formation than T2, particularly during the mid-growth stages (50-60 DAS). The differences between treatments could be due to variations in nutrient uptake, environmental factors, or treatment-specific effects on plant growth dynamics.

At 70 DAS the number of branches declines might be due to the nonexistence of leaves. As varying plant densities were present between treatments that slightly affects the branch number. In addition, number of branches plant^{-1} is the result of genetic frame of the crop and environmental condition that plays a remarkable role towards the final seed yield of the crop (Masudul et al., 2016). The same results were also found by (Alamgir et al., 2019), reported that varies in genetic makeup and planting time might be affected the branching stage.

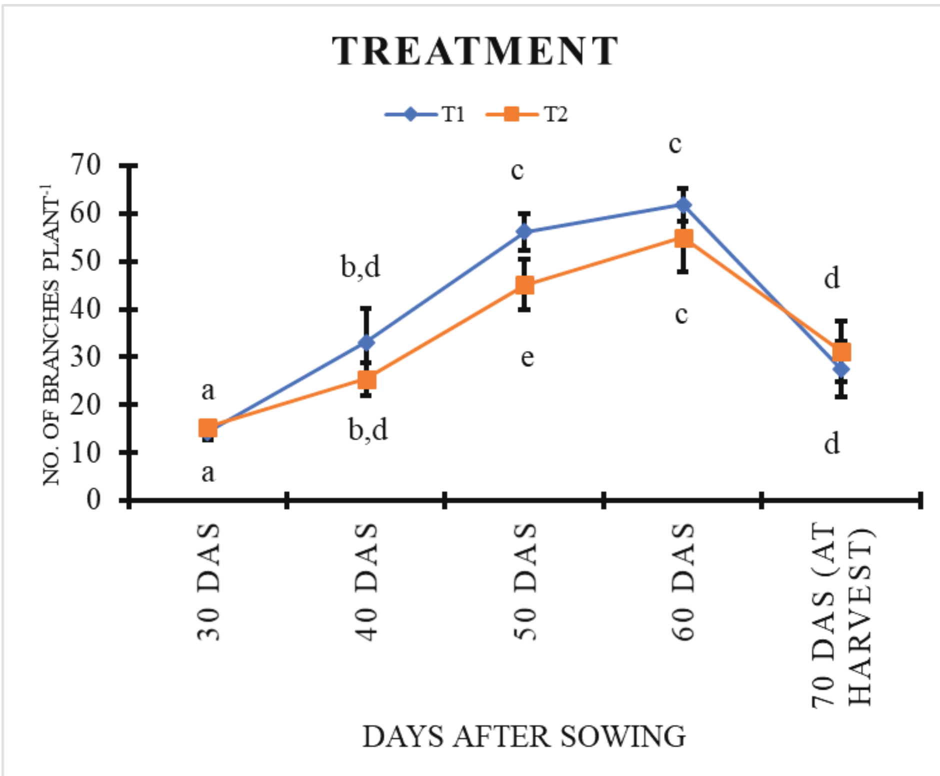

Figure 3. Effect of treatments on number of branches per chia plant. Values are in mean ±SE. Here, N=40, where each treatment comprises of four replications and each replication consists of 10 plants. Small letters above the line represent multiple comparisons within the T_1 (Days after sowing) and small letters below the line represents multiple comparisons within the T_2 (Days after sowing). Different letters in each values indicate P<0.05 either within the treatment or between the treatments (T_1 and T_2).

Inflorescence length (cm)

At 30 and 40 DAS, no inflorescence was observed in either treatment. However, by 50 DAS, inflorescence started developing, with T1 showing an average length of 40.99 cm and T2 slightly higher at 42.11 cm. The trend continued at 60 DAS, where T1 recorded 39.27 cm and T2 recorded 40.77 cm. At harvest (70 DAS), the inflorescence length slightly decreased, with T1 measuring 38.02 cm and T2 at 39.75 cm. Statistical analysis indicated that there was no significant difference between T1 and T2 at any stage of growth, as shown by the same letter groupings in figure 4. The highest inflorescence length was observed at 50 DAS, after which it showed a slight decline toward harvest. These findings suggest that both treatments resulted in similar inflorescence development patterns, with no substantial advantage of one treatment over the other in terms of final inflorescence length. Result indicated that T2 shows higher performance on inflorescence length than T1. This may be due to higher branching rates and the plant population as well.

This findings aligns with Rahman et al. (2023), suggest that closer spacing and higher NPK levels may contribute to increased inflorescence length, possibly due to enhanced nutrient availability and reduced competition for resources. Whereas, Shorna et al. (2024) found that significant variations in the main inflorescence length among different nitrogen fertilizer applications. Plants in the control group exhibited relatively shorter main inflorescence. They also found that the moderate nitrogen fertilizer application can influence inflorescence elongation. Other factors, such as genetic potential, environmental conditions, or plant compensatory mechanisms, may have played a role in determining inflorescence length.

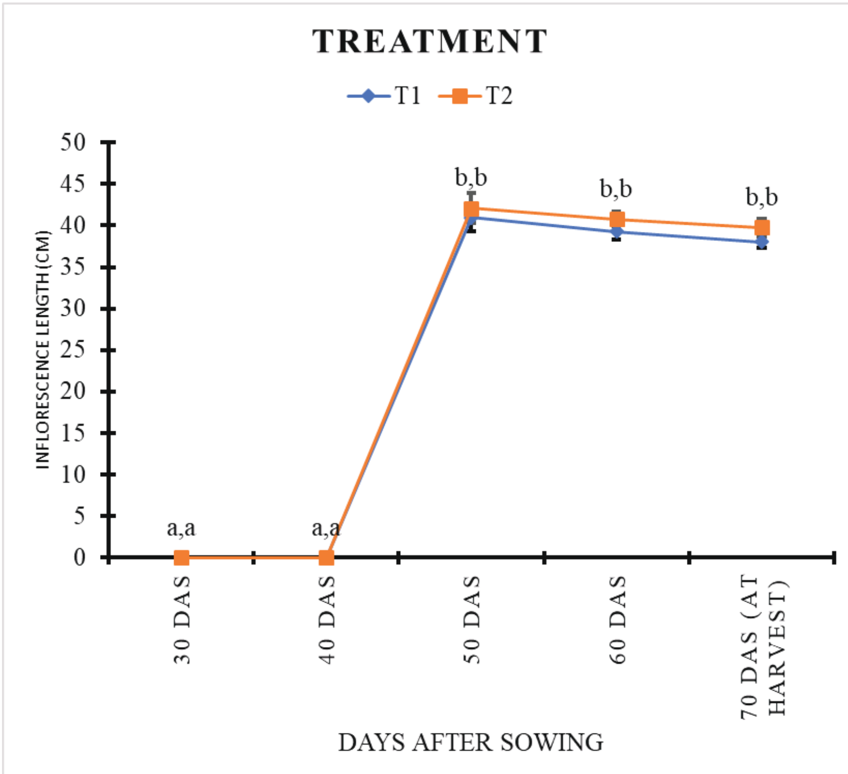

Figure 4. Effect of treatments on inflorescence length (cm) of chia at different growth stages. Values are in mean ±SE. Here, N=40, where each treatment comprises of four replications and each replication consists of 10 plants. Different letters in each values indicate $P<0.05$ either within the treatment or between the treatments (T_1 and T_2).

Yield and yield contributing features of Chia seed

Number of inflorescence plant^{-1}

Significant differences found in the number of inflorescence plant^{-1} (Table 1). The uttermost number of inflorescence plant^{-1} counted (19.61) when plant population was 60 m^{-2} in (T2). When the plant population in (T1) was 120 m^{-2}, the smallest number of inflorescence/plant (14.08) was perceived which was not statistically comparable to T2. The number of inflorescences per plant is a crucial determinant of the final yield, which is significantly influenced by the initial yield. Due to floret maturation and the possibility of inflorescence to be broken at that time, there may be less inflorescence than usual. Masudul et al. (2016) published similar findings, demonstrating that planting timing had a substantial impact on the quantity of inflorescences.

Figure 5. Inflorescence of the Chia plant.

Florets/inflorescence

Significant variation was not observed in florets/inflorescence (Table 1). Maximum floret/inflorescence was recorded 16.93 from T2 (60 m^{-2}). And the minimum floret/inflorescence was 16.25 from T1 (120 m^{-2}) that was statistically identical with T2. Result implied that floret/inflorescence was higher due to the existence of maximum plant population 120 m^{-2} in (T1). Comparable findings also obtained by Masudul et al. (2016), reported that maximum plant population ensuing in good number of floret/inflorescences.

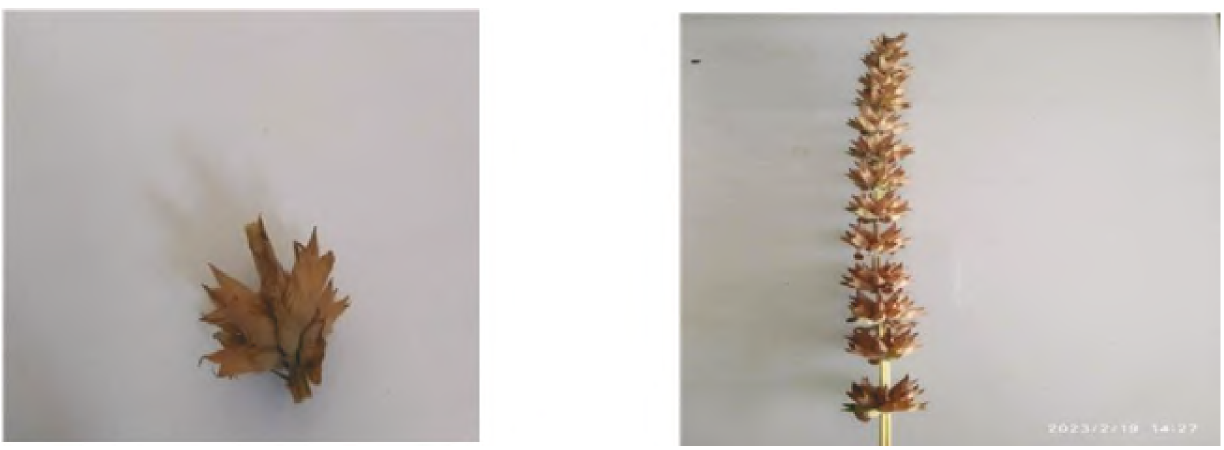

Figure 6. Floret/inflorescence of the Chia plant.

Seed floret (no.)

No significant difference was seen between treatments in terms of the no of seeds florets (Table 1). The maximum number of seed floret (18.88) was recorded when the plant population was 60 m^{-2} in (T2). Additionally, the smallest number of seeds florets (16.44) was found when the plant population in (T1) was 120 m^{-2}, which was statistically identical as (T2). The results uncovered that maximum seed floret (no.) was noted from T2 (60 m^{-2}) due to the highest branch number and number of inflorescence $plant^{-1}$. Masudul et al., (2016) reported that lower number of seeds floret was found in case of January planting may be due to a smaller number of branches, soil requirements. In the case of February planting, plants fail to generate any ripen inflorescence. Thus produce less seed/floret.

Figure 7. Seed floret (no.) in the Chia plant

Seed/inflorescence

Significant differences were not observed in case of seed/inflorescence between treatments (Table 1). Maximum seed/inflorescence was obtained (33.06) from T2 when plant population was 60 m^{-2}. Minimum seed/inflorescence was obtained (30.44) from T1 when plant population was 120 m^{-2}. As well as both treatments were statistically similar as well. This may occur due to the availability of nutrients. Similar findings were acquired by Masudul et al., (2016), who investigated that planting time also affects seed/ inflorescence number.

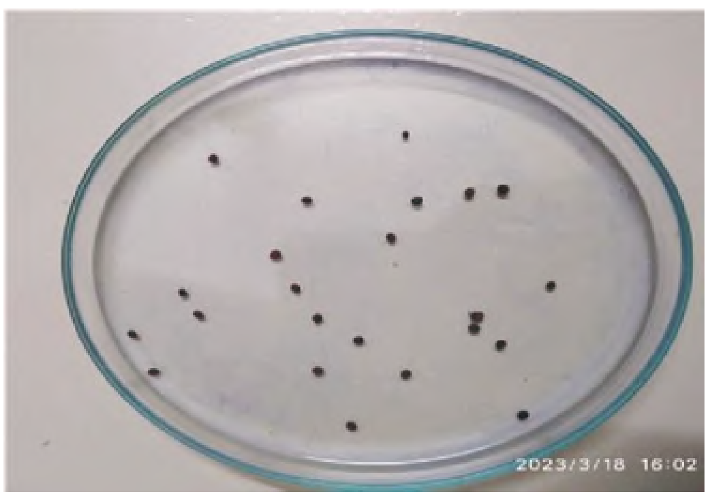

Figure 8. Seed/inflorescence in the Chia plant

1000 seed weight (g)

Significant differences were found in terms of 1000 seed weight (g) between treatments (Table 1). The uttermost 1000 seed weight was obtained (1.38g) when plant population was 120 m^{-2} in (T1). And the lowest 1000 seed weight was reported (1.27g) when plant population was 60 m^{-2} in (T2) that do not differ statistically with (T1). This could differ depending on the size of the seeds. According to Ayerza and Coates (2005), a crop's yield possibility is best demonstrated by 1000 seed weight, which conveys the extent of seed development, an important factor in determining output.

Seed yield (kg ha^{-1})

Seed yield is the factor that determines final yield. Notable difference was observed regarding yield of seed between treatments (Table 1). The maximum yield of seed was found 9.32 kg ha^{-1} when plant population was 60 m^{-2} in (T2). Meanwhile, lowest yield of seed was 7.31 kg ha^{-1} when plant population was 120 m^{-2} in (T1), which was statistically dissimilar with T2. Although the plant population of T1 was higher (120 m^{-2}) but T2 (60 m^{-2}) had the maximum seed output. This may have appeared because of certain crop morphological environments. Such elements as planting season, seeding method, and environmental variable quantity may be liable. In addition, the highest branch count, maximum inflorescence count, number of seeds per floret, and florets per inflorescence were also noted in T2. As a result, T2 produced the highest seed productivity.

Comparable findings were also obtained by (Ayerza and Coates 2005) who reported that the seed yield was significantly differed by growth characteristics as well as based on the planting time. Higher seed yield (103 kg/ha) was found from November planting (Masudul et al., 2016). They found that the alteration on seed yield may be due to planting program, soil & environmental conditions etc.

Table 1. Yield and yield contributing attributes of chia seed

Treatment	Inflorescence plant^{-1}	Florets/ Inflorescence	Seeds floret (No.)	Seeds/ floret	Seeds/ inflorescence	1000 seed wt. (g)	Seed yield (kg/ha)
T1 (120 m^{-2})	14.08b	16.25a	16.44a	3.31a	30.44a	1.38a	7.31b
T2 (60 m^{-2})	19.61a	16.93a	18.88a	2.87a	33.06a	1.27b	9.32a
CV (%)	5.89	5.92	12.07	9.75	11.73	2.62	12.24
LSD $_{(0.05)}$	2.23	3.53	3.61	0.67	8.38	0.07	1.78

Results of total phenolic content

The outcomes of phenolic content were written as mean value and standard deviation that is reported in gallic acid equivalents (GAE) using unit's mg/g of extract in (Table 2). In the current study, the phenolic content was found 11.79 mg GAE/g. Caudillo et al. (2008) discovered that the phenolic content ranged from 0.66 to 1.63 mg GAE/g which is not equivalent with this finding. This could be due to the experimental procedure or could be different concentration and absorbance that were utilized. Caudillo et al. (2008) analyzed the phenolic compounds on chia seed extracts obtained by stirring with ethanol at room temperature for 24 hours. They discovered values of 8.8 g GAE/kg of dry sample, indicating that phenolic compounds present in chia seed would have a lower polarity and thus be more easily extracted in less polar solvents.

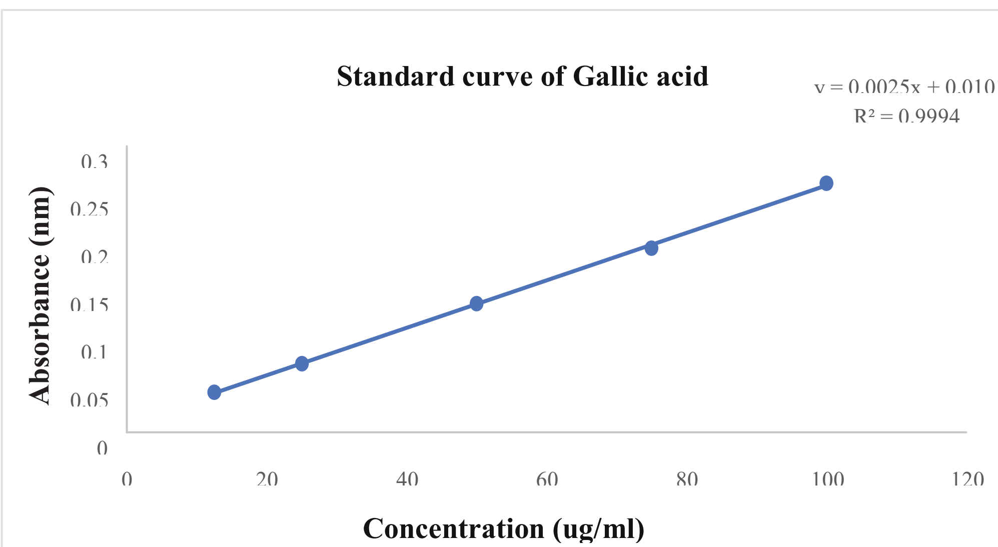

Figure 9. Straight curve of Gallic acid solution

Table 2. Total phenolic content of extracts

Sample Absorbance	Calculated Conc. (ug/ml)	Mean ± SD
0.041	12.47	
0.039	11.66	11.79 ± 0.61
0.037	11.26	

Results of total flavonoid content

The flavonoid content recorded 13.37 mg QE/g from (Table 3). (Shahidi and Naczk 1990) who reported that in their research the flavonoid content was 1.17 mg QE/g. Whereas the outcome is not aligned with current research. Lin and Tang (2007) reported that they found total flavonoid content of chia seed extracts by different ethanol concentrations were 0.007 and 0.018 g/kg QE. These differences may be due to the extraction method or to the genetic and agronomic conditions (Chandrasekara and Shahidi 2010).

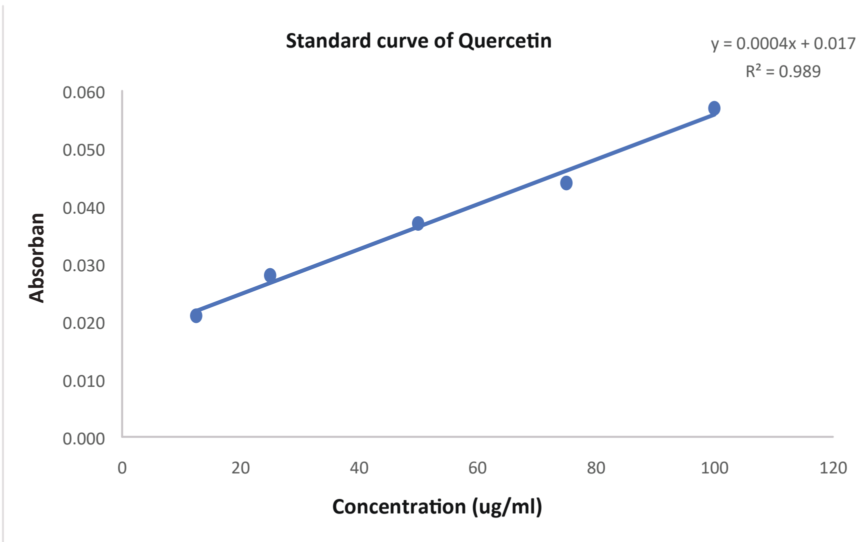

Figure 10. Linearity curve of quercetin solution.

Table 3. Total flavonoid content of extracts

Sample absorbance	Calculated Conc. (ug/ml)	Mean ± SD
0.023	15.91	
0.022	13.37	13.37 ± 2.54
0.021	10.83	

The differences in total phenolic and flavonoid content between this study and the others could be due to two reasons: (a) The samples used in this study and in the others are harvested from diverse places, and it has been proven that growing locations significantly influence the configuration of chia seeds (Ayerza, 2013). (b) The approaches used for the removal of phenolic and flavonoid complexes in different researches vary as well as different extraction procedures have been shown to considerably influence the phytochemical properties of chia seed (Scapin et al., 2016).

Conclusion

The current study demonstrates the growth, yield characteristics and phytochemical components of chia seeds to observe their interactions. Growth parameters such as plant height, leaf number, branch number, and inflorescence length were observed to evaluate yield performance. Furthermore, the phytochemical analysis displays variations between phenolic and flavonoid content. The finding suggests that a correlation between growth features may lead to the accumulation of bioactive compounds. Thereby, chia seed provides high flavonoid content than the phenolic content in this study which can influence soil microbial communities by promoting beneficial microbes and suppressing harmful ones, thus enhancing plant health and productivity. In that way, these phytochemical components contribute to the growth and yield characteristics. In development conditions, higher yield performance was examined, as well as these conditions also increased phytochemical content, enhancing the nutritional and functional potential of chia seeds.

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