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Pinched sunflowers (*Helianthus annuus* 'Teddy Bear') produce high-quality flowers under high nitrogen fertilizer

Yahya SELAHVARZI [1, 2], Maryam KAMALI [1], Sajede KARIMPOUR [3], Mahdiyeh KHARRAZI [4], Mohammad KARIMI [1]

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Pinched sunflowers (*Helianthus annuus* 'Teddy Bear') produce high-quality flowers under high nitrogen fertilizer

Abstract: This study was investigated the effect of removing the central bud (pinching) and different levels of nitrogen fertilizer urea on some morphological and physiological traits of ornamental sunflower. This study was conducted as a factorial experiment in a randomized complete block design with four replications on ornamental sunflower (*Helianthus annuus* 'Teddy Bear') at Horticulture Farm, Department of Horticulture, Ferdowsi University of Mashhad, Iran, in 2020-2021. The first factor was pinching in two levels (pinching and non-pinching) and the second factor was using urea at four levels (0, 200, 300, and 400 kg ha^{-1}) in the form of water-soluble fertilizer. Results showed that the highest flower dry mass (59.25 g) was observed in pinched plants fertilized by 400 kg ha^{-1} of urea. Besides, the application of a high level of urea fertilizer and pinching treatment increased the amount of total chlorophyll and chlorophyll b. By removing the central bud, the amount of N, P, K, Ca, Zn, and Fe elements in the leaf increased by 1.5, 1.6, 1.3, 1.9, 1.4, and 1.5 times, respectively. Therefore, pinching and the adding of urea fertilizer at 400 kg $ha^{-1}$is recommended for the production of high-quality sunflower plant 'Teddy Bear'.

Key words: flowering period, head diameter, nutrient elements, photosynthesis, plant height

Pincirane sončnice (*Helianthus annuus* 'Teddy Bear') dajejo visoko kakovostna socvetja pri gnojenju z velimi količinami dušikovih gnojil

Izvleček: V raziskavi je bil preučevan učinek odstranjevanja (pinciranja) osrednjega socvetja in različnih odmerkov gnojenja z ureo na nekatere morfološke in fiziološke lastnosti okrasnih sončnic. Raziskava je bila izvedena kot popolni faktorski bločni poskus s štirimi ponovitvami na okrasnih sončnicah (*Helianthus annuus* 'Teddy Bear') na Horticulture Farm, Department of Horticulture, Ferdowsi University of Mashhad, Iran, v rastni sezoni 2020-2021. Prvi dejavnik je obsegal dve ravni pinciranja (pincirano in ne pincirano), drugi dejavnik pa štiri različne odmerke uree (0, 200, 300, and 400 kg ha^{-1}) v obliki vodotopnega gnojila. Rezultati so pokazali, da je bila dosežena največja suha masa socvetij (59,25 g) pri pinciranih rastlinah in uporabi 400 kg ha^{-1} of uree. Večji odmerek uree je pri pinciranih socvetjih povečal vsebnost celokupnega klorofila in klorofila b. Pri odstranitvi osrednega socvetja se je vsebnost N, P, K, Ca, Zn in Fe v listih povečala za 1,5; 1,6; 1,3; 1,9; 1,4 in 1,5 krat. Zaradi naštetega priporočamo pinciranje in gnojenje s 400 kg ha^{-1} uree za vzgojo kakovostnih sončnic 'Teddy Bear'.

Ključne besede: cvetenje, premer koška, hranila, fotosinteza, višina rastlin

1 Department of Horticultural Science and Landscape Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2 Corresponding author, e-mail: selahvarzi@um.ac.ir
3 Department of Horticultural Science and Landscape Engineering, Shirvan Faculty of Agriculture, University of Bojnord, Bojnord, Iran
4 Ornamental Plants Biotechnology Research Department, Research Institute for Industrial Biotechnology, Iranian Academic Centre for Education, Culture and Research (ACECR), Mashhad, Iran

1 INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an annual plant belonging to the Asteraceae family. This plant is native to North America and has medicinal, nutritional, and ornamental uses (Sehrawat et al., 2003) beside of its usage as a biodegradable source in biodiesel fuels (Saba et al., 2016). According to the specialized institute of cut flowers, in some sunflower cultivars, such as 'Pro-Cut Gold' and 'Sunrich Lemon', the stems are so long. In contrast, other sunflower cultivars produce lateral shoots and have short stems and uniform flowers (Dole, 2002). Removing the central bud (pinching) is considered one way to stimulate the plant to produce lateral branches and increase the number of stems per plant (Wien, 2015; Cheema, 2018). Depending on the stage of plant growth, pinching can be beneficial or harmful for plants (Smakel, 2006), as pinching of the different sunflower cultivars at the right time enhanced flower production three to four times (Wien, 2012a). However, pinching delays flowering and reduces flower size (Cheema, 2018) and the formation of flowers for 7-10 days (Wajid et al., 2007). Wien (2016b) reported that pinching the 'Sunrich Orange' cultivar, led to the production of smaller flowers but appropriate stem length. The smaller size of the flower, but with the marketable stem length, allows the florists to use them in arranging the flower bouquets properly. The study results of Badge and Panchbhai (2018) revealed that pinching the African marigold (*Tagetes erecta* L.) plants (15 days after transplanting) lead to the production of maximum flower yield in comparison to other treatments. The maximum nitrogen, phosphorus, and potassium content and uptake, as well as yield parameters, were obtained by pinching the plants 15 days after transplanting and foliar application of gibberellic acid at 300 mg l^{-1} (Badge et al., 2015). Prakash et al. (2016) reported that pinching the African marigold (*Tagetes erecta*) affects the plant height, number of lateral branches, number of flowers, and number of days to 50 % of flowering.

Adequate nutrition with essential elements, especially with nitrogen, is very important for the successful development of plants. Nitrogen is an essential nutrient that plays a role in the structure of various proteins, enzymes, coenzymes, nucleic acids, and cytochromes (Hassegawa et al., 2008), as well as, involving in the cell division and expansion, thereby increasing leaf length and width (Kumari, 2011, Lehri et al., 2011). Besides, this element plays a crucial role in the formation of chlorophyll and has a vital function in supplying carbohydrates and photosynthesis (Wajid et al., 2007). The effect of nitrogen on plant growth and development has often been linked to increased photosynthesis because the appropriate amount of nitrogen determines plant yield (Mekonnen et al., 2002). Studies indicated that the increase in growth and yield of the sunflower plant is dependent upon the adequate supply of nitrogen (Ali et al., 2004; Ali, 2015). The results of Oad et al. (2018) study indicated that sunflower plants treated with foliar application of urea (1 %) after 35 days of sowing in addition to recommended soil applied urea (130 kg ha^{-1}) led to the highest plant height, head diameter, grains per head, seed index, and grain yield. Ali et al. (2014) reported that the application of 80 kg ha^{-1} nitrogen fertilizer resulted in an increased plant height and head diameter of the sunflower plants. In another study, a significant increment in crop growth, biomass, dry matter production, and biological yield resulted in 100 kg ha^{-1} of N rate application (Saifullah, 1996), but Handayati and Sihombing (2019) recommended the application of 150 kg ha^{-1} nitrogen for the cultivation of this plant.

Considering the effect of pinching and nitrogen on the reproductive and vegetative traits of the sunflower plant, the present experiment was aimed to investigate the effect of removing the central bud (pinching) and different levels of nitrogen fertilizer (urea) on flowering, flower size, plant height, and other morphological and physiological traits of ornamental sunflower (*Helianthus annuus* 'Teddy Bear').

2 MATERIALS AND METHODS

The field experiment was conducted at Research Farm, Department of Horticulture, Ferdowsi University of Mashhad, Iran, in 2020-2021. Before planting, chemical analysis of the soil was done at an upper 0-30 cm zone, the results of which are shown in Table 1.

This experiment was conducted as a factorial experiment in a randomized complete block design with four replications on ornamental sunflower (*Helianthus annuus* 'Teddy Bear'). The first factor was removing or not removing the central bud (pinching); and the second factor was applied in four levels of adding urea fertilizer ($CO(NH_2)_2$): 0, 200, 300, and 400 kg ha^{-1} in the form of water-soluble fertilizer. The sunflower seeds were purchased from the Dutch Hemogenetic Company and sown in April 2020. Four weeks later, the seedlings with four true leaves were planted at spacing 50 × 20 cm. Ten days after transplanting, urea fertilizer was applied three times (weekly) with irrigation water according to the mentioned levels. Then, one month after transplanting, the pinching treatment was applied.

During the experiment, the number of days to flowering (vegetative period), and the duration of the flowering period (flowering period) were recorded. The number of flowers per plant was counted, and the head

Table 1: The physical and chemical properties of the soil

Depth	Soil Texture	Sand	Clay	Loam	pH	EC	N	P	K	Fe	Zn	Ca
(cm)			(%)			(dS m^{-1})			(mg kg^{-1})			
0-30	Sandy loam	40	33	27	7.5	1.3	610	606	6251	24716	52	29371

diameter and the stem diameter of each treatment were measured with a digital caliper. In 50 % of the flowering stage, the number of leaves per plant and the plant height were calculated. At this stage, the rates of photosynthesis and transpiration were also measured using a portable photosynthesis system (Li-6400) from 9:00 to 11:00 AM under natural conditions.

Fresh leaf tissue was used for the measurement of chlorophyll contents. 0.2 g fresh leaf was crushed in 10 ml of methanol 96 %. The resulted solution was filtered through Whatman filter paper and then centrifuged at 2500 rpm for 10 minutes. The supernatant optical absorption was then read at 653, and 666 nm using a spectrophotometer (model CE2502, BioQuest, UK) method (Sukran et al., 1998). Finally, the chlorophyll pigments were obtained using the following equations:

$$Chl_a\ (\mu g.ml^{-1}) = 15.65\ A_{666} - 7.340\ A_{653}$$
$$Chl_b\ (\mu g.ml^{-1}) = 27.05\ A_{653} - 11.21\ A_{666}$$
$$Chl_{Total} = Chl_a + Chl_b$$

After applying the treatments, at the beginning of the reproductive phase, N, P, K, Zn, Fe, and Ca elements in the sunflower leaf were measured. The amount of nitrogen in the plant was measured using the Kjeldahl method (Bremmer and Mulvaney, 1982). Concentrations of P, K, Ca, Zn, and Fe were analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin-Elmer Optima 5300 DV) in plant samples (Van de Wiel, 2003).

The flowers were collected and dried during the flowering period to record the flower dry mass. After flowering, the leaf area was measured using the leaf area meter (Model Li-Cor-1300, USA). The specific leaf area (SLA = leaf area leaf dry $mass^{-1}$) and the leaf area ratio (LAR = leaf area total dry $mass^{-1}$) were also calculated. To measure the dry mass of plant components (stems, roots, leaves, and flowers) and the total dry mass, the plant samples were dried at 70 °C until the sample mass was held constant. Then the dry mass of different plant parts was recorded.

2.1 DATA ANALYSIS

Data were analyzed with One Way ANOVA using JMP® (v.8) software (SAS institute, 1989-2021), and means were compared based on the LSD test at the 5 % of probability level.

3 RESULTS

The results of ANOVA revealed that urea application and pinching have significant effects on different traits of the sunflower plant including mineral uptake, vegetative and generative traits, photosynthesis and transpiration rate, chlorophyll contents, and dry mass of different parts of sunflower (data not shown).

3.1 ELEMENT UPTAKE

The element content of sunflower shoots was affected by urea application and pinching, and not by their interaction. The use of urea led to an increase in N content in shoots as well as P, K, Ca, Zn, and Fe contents. As the amount of urea fertilizer increased, the accumulation of these elements in the shoots also increased. Using urea fertilizer at 400 kg ha^{-1} induced mineral accumulation 2.4, 2.6, 1.7, 2.3, 4.1, and 4.2 times more than the control for N, P, K, Ca, Zn, and Fe, respectively (Table 2). Contrariwise, these element contents decreased when pinching was applied. Pinched plants had 1.5, 1.6, 1.3, 1.9, 1.4, and 1.5 times less amount of N, P, K, Ca, Zn, and Fe than non-pinched sunflower plants, respectively (Table 2).

3.2 VEGETATIVE TRAITS

The interaction of pinching × urea fertilizer had a significant effect on vegetative traits including plant height, stem diameter, leaf number, leaf area, SLA, and LAR. Sunflower plants had the biggest height (153 cm) when grown using 400 kg ha^{-1} of urea fertilizer and not pinched, while pinched plants without urea fertilizing showed the lowest height (129 cm). The same results were obtained for stem diameter growth with 28.98 and 20.90 mm, respectively. The leaf number increased by urea application and pinching (27.5-30.0), whereas, the lowest number of leaf production (13.0) was recorded in non-pinched plants without urea. The biggest leaf area (16130.25 cm^2) showed in 400 kg ha^{-1} of urea applica-

Table 2: The simple effect of pinching and urea fertilizer treatments on element content in sunflower shoots

Factors	Treatments	N	P	K	Ca	Zn	Fe
				(mg kg^{-1})			
Urea fertilizer (kg ha^{-1})	0	940.00d*	820.00d	12346.0d	9167.0d	8.0000d	93.000d
	100	1088.00c	968.00c	16934.0c	11939.3c	11.3750c	108.500c
	200	1212.50b	1094.00b	19746.0b	20584.0b	17.8750b	333.875b
	400	2292.00a	2172.00a	20847.8a	20798.0a	32.8750a	393.125a
Pinching	-	1659.50a	1539.50a	19499.4a	20623.1a	20.3750a	277.000a
	+	1106.75b	987.50b	15437.4b	10621.1b	14.6875b	187.250b

*Means followed by similar letters in each trait and for each factor didn't have any significant difference based on LSD test ($p \leq 0.01$)

tion and pinching treatment, while non-pinched plants grown without urea fertilizer expanded their leaf to the minimum amount (8953.65 cm^2). SLA was the highest when 400 kg ha^{-1} of urea fertilizer with pinching (302.46 cm^2 g^{-1}) and 300 kg ha^{-1} of urea fertilizer without pinching (301.37 cm^2 g^{-1}) was applied and the lowest amount of SLA was shown in the 300 kg ha^{-1} of urea application with pinching (266.60 cm^2 g^{-1}) treatment (Table 3). The highest amount of the LAR was obtained in two treatments include 400 and 300 kg.ha^{-1} of urea fertilizer + pinching (73.64 and 72.97 cm^2.g^{-1}, respectively), and the lowest amount was recorded for plants with no urea fertilizing with (62.11 cm^2.g^{-1}) or without (62.36 cm^2 g^{-1}) pinching (Table 3).

3.3 GENERATIVE TRAITS

We obtained the highest number of flowers (77.75) in pinched plants fertilized by 400 kg ha^{-1} of urea fertilizer, and non-pinched plants produced the less flower number (21.25-26.00) in all levels of urea fertilizer (Table 4, A). The head diameter had the highest amount (146.68-15.1.59 mm) when pinching was not applied in plants of urea fertilizer in 0, 200, and 300 kg ha^{-1}, and the lowest amount (97.42 mm) was recorded in the pinched plants with no urea using. The number of days to first flower appearance and the duration of the flowering stage were affected by urea fertilizer, that is, the increase in urea levels led to prolongation of the vegetative and generative period and low levels of urea stimulate the entering to and shortening of the generative stage. Duration of the flowering stage also was increased by pinching up to 6 days compared to the non-pinched plants (Table 4, B).

3.4 CHLOROPHYLL CONTENTS, PHOTOSYNTHESIS, AND TRANSPIRATION RATE

The content of chlorophyll$_b$ and total chlorophyll was affected by the interaction of urea fertilizer × pinching, while the chlorophyll content was not influenced by interaction but was affected by simple effect of them. The plants which were grown under 400 kg ha^{-1} of urea fertilizer with (0.28 µg g^{-1} FM) or without pinching (0.24 µg

Table 3: The interaction effect of pinching × urea fertilizer on vegetative traits of the sunflower plant

Pinching	Urea fertilizer (kg ha^{-1})	Plant height (cm)	Stem diameter (mm)	Leaf number	Leaf area (cm^2)	SLA** (cm^2 g^{-1})	LAR** (cm^2 g^{-1})
-	0	139.33abc*	25.49abcd	13.00b	8953.65d	294.81bc	62.36c
-	200	143.66abc	26.25abc	22.00ab	9976.30cd	298.51ab	69.07ab
-	300	147.00ab	26.37abc	21.00ab	12407.76b	301.37a	72.97a
-	400	153.00a	28.98a	30.33a	9856.37cd	273.78cd	64.12bc
+	0	129.00c	20.90d	22.33ab	11416.25bc	276.35c	62.11c
+	200	133.00bc	21.45cd	27.50a	12969.25b	296.16ab	69.29ab
+	300	138.00abc	23.02bcd	26.66a	12813.25b	266.60e	65.21b
+	400	147.33ab	28.01ab	30.00a	16130.25a	302.46a	73.64a

*Means followed by similar letters in each trait do not have any significant difference based on the LSD test ($p \leq 0.01$)
**SLA: The specific leaf area, LAR: The leaf area ratio

Table 4: The interaction effect of pinching × urea fertilizer (A) and simple effect of them (B) on generative traits of the sunflower plant

(A)				(B)			
Pinching	Urea fertilizer (kg ha^{-1})	Flower number	Head diameter (mm)			Day to1st flowering (day)	Duration of flowering (day)
-	0	23.50d*	146.68a	Urea fertilizer (kg ha^{-1})	0	44.0000D	32.3750D
-	200	21.25d	151.59a		200	46.1250C	34.1250C
-	300	26.00d	148.68a		300	48.7500B	36.7500B
-	400	25.50d	119.27b		400	52.7500A	40.7500A
+	0	45.50c	97.42c	Pinching			
+	200	46.75c	106.84bc		-	44.9375A	32.9375B
+	300	63.50b	111.24bc		+	50.8750A	39.0625A
+	400	77.75a	110.04bc				

*Means followed by small (interaction effect) and capital (simple effect) letters in each trait does not have a significant difference based on the LSD test ($p \leq 0.01$)

g^{-1} FM) had the highest amount of chlorophyll b and the lowest was related to not using urea fertilizer for pinched and non-pinched plants (0.11-0.13 µg g^{-1} FM). In the same manner, total chlorophyll content was the highest in non-pinched plants treated by 400 kg ha^{-1} of urea fertilizer (0.45 µg g^{-1} FM), and the lowest amount was recorded in the pinched and non-pinched plants without urea fertilizing (0.25-0.26 µg g^{-1} FM)(Table 5, A). Unlike the chlorophyll$_b$ and total chlorophyll, the amount of chlorophyll only was affected by urea fertilizer and pinching. Urea fertilizer at 300 kg ha^{-1} (0.19 µg g^{-1} FM) and pinching (0.17 µg g^{-1} FM) provoked chlorophyll$_a$ accumulation. There was a trend for photosynthesis and transpiration rate, increasing urea levels from zero to 400 kg ha^{-1} enhanced the amounts of photosynthesis from 6.19 to 11.39 µmol mol^{-1} CO_2 and transpiration rate from 1.44 to 2.65 mmol.mol^{-1} H_2O, respectively. Pinching significantly led to a decrease in photosynthesis and transpiration rate (Table 5, B).

3.5 DRY MASS OF PLANT ORGANS

Leaf, head, root, and total dry mass of sunflower was affected by the interaction of urea fertilizer × pinching, as the highest amount of them was recorded on pinched plants were fertilized by 400 kg ha^{-1} of urea fertilizer, 53.33, 59.25, 39.99, and 219.04 g, respectively. Non-

Table 5: The interaction effect of pinching × urea fertilizer on chlorophyll b and total chlorophyll content (A) and simple effect of them on photosynthesis, transpiration rate and Chlorophyll a content (B) in sunflower plant

(A)				(B)				
Pinching	Urea fertilizer (kg ha^{-1})	Chlorophyll$_b$ content (µg g^{-1} FM)	Total chlorophyll content (µg g^{-1} FM)			Chlorophyll$_a$ content (µg g^{-1} FM)	Photosynthesis (µmol.mol^{-1} CO_2)	Transpiration rate (mmol mol^{-1} H_2O)
-	0	0.11c*	0.25d	Urea fertilizer (kg ha^{-1})	0	0.13C	6.19D	1.44D
-	200	0.15bc	0.30cd		200	0.15BC	7.67C	1.78C
-	300	0.11c	0.37b		300	0.19A	8.96B	2.08B
-	400	0.24a	0.45a		400	0.17AB	11.39A	2.65A
+	0	0.13c	0.26d	Pinching				
+	200	0.16bc	0.31c		-	0.15B	9.07A	2.09A
+	300	0.18b	0.31c		+	0.17A	8.09B	1.88B
+	400	0.28a	0.42ab					

*Means followed by small (interaction effect) and capital (simple effect) letters in each trait does not have a significant difference based on the LSD test ($p \leq 0.01$)

pinched plants had the lowest amount of leaf (30.37 g), head (33.75 g), root (22.78 g), and total (143.57-153.70 g) dry matter. Indeed, the total dry matter was not much significantly affected by urea fertilizer levels (Table 6, A).

Urea fertilizer and pinching had a significant effect on stem dry mass as a simple effect. The application of urea at 300 kg ha^{-1} induced the highest dry matter in the stem, while the lowest amount was detected when no urea fertilizer was used. Pinching increased stem dry mass to 1.2 times (62.03 g) in comparison with non-pinching (Table 6, B).

The percentage of dry matter allocation in different parts of the plant showed no distinctive difference between treatments. On average, the highest to the lowest percentage of dry matter allocation were for stem (32.7 %), head (26.0 %), leaf (23.4 %), and root (17.9 %), respectively (Table 7).

Table 7: The percentage of dry matter allocation in different parts of the sunflower plant under different levels of urea fertilizer and pinching

Pinching	Urea fertilizer (kg ha^{-1})	Dry mass (%)			
		Leaf	Head	Stem	Root
-	0	20.5	27.0	33.2	19.2
-	200	20.6	28.2	32.2	19.0
-	300	25.6	23.1	35.7	15.6
-	400	25.6	24.0	34.1	16.2
+	0	22.8	26.8	32.3	18.1
+	200	22.8	27.8	30.7	18.8
+	300	22.7	28.0	30.4	18.9
+	400	26.5	22.8	33.0	17.7
Mean		23.4	26.0	32.7	17.9

4 DISCUSSION

Besides the high cost of chemical fertilizer, the environmental impacts of their application are the important reason for the need to determine the exact amount of fertilizers. In our experiment, the interaction of different amounts of urea fertilizer (CO $(NH_2)_2$) and the removal of apical bud (pinching) had distinctive results on sunflower 'Teddy Bear' growth and development. The nitrogen fertilizer that used in this experiment is quite soluble and converts to ammonia in several days. So, as expected, a rise in the urea levels caused an increase in the nitrogen uptake. Similarly, the uptake of nitrogen is enhanced in broccoli plants when nitrogen fertilizer amounts increase (Vagen, 2003). In addition, the amount of P, K, Zn, Fe, and Ca was enhanced in the sunflower shoots by increasing the urea levels (Table 2). Confirmed results were reported by Karitonas (2003) and Yildirim et al. (2007) on broccoli plant, that an increase in the uptake of P, K, Fe, and Ca were shown by adding nitrogen fertilizer. Similarly, lettuce and tomato plants which were foliar sprayed by urea had higher amounts of N and K (Padem and Alan, 1995), and N, K, and Fe (Alan and Padem, 1994), respectively. All studied nutrient elements (i.e., N, P, K, Ca, Zn, and Fe) play several important functions and critical roles within plants; metabolism, and catabolism processes, so, increasing in their uptake by plants can explain the significant differences in the studied traits in this experiment. The availability of nitrogen in the soil increase

Table 6: The interaction effect of pinching × urea fertilizer (A) and simple effect of them (B) on the dry mass of different parts of the sunflower plant

(A)

Pinching	Urea fertilizer (kg ha^{-1})	Leaf dry mass (g)	Head dry mass (g)	Root dry mass (g)	Total dry masst (g)
-	0	30.37e*	33.75e	22.78e	143.57c
-	200	33.42de	37.14de	28.50cde	144.42c
-	300	41.17ab	45.75bcd	30.88bcd	170.03bc
-	400	36.00cde	40.00cde	25.07de	153.70c
+	0	41.31bcd	45.90bcd	32.84bc	183.78b
+	200	43.79bc	48.66bc	36.04ab	187.16b
+	300	48.06ab	53.40ab	35.70ab	196.48ab
+	400	53.33a	59.25a	39.99a	219.04a

(B)

		Stem dry mass (g)
Urea fertilizer (kg ha^{-1})	0	53.83B
	200	55.60AB
	300	60.87A
	400	57.19AB
Pinching	-	51.71B
	+	62.03A

*Means followed by small (interaction effect) and capital (simple effect) letters in each trait does not have any significant difference based on the LSD test ($p \leq 0.01$)

RuBisCO contents in leaves, even though some climate and soil factors including light, air humidity, and soil pH showed considerable influences on the fraction of nitrogen allocated to RuBisCO regionally (Luo et al., 2021). Many scientists believe that the higher uptake of essential nutrients by plants as a result of the urea application is related to the positive influence of nitrogen on the chemical properties of the soil (Malhi et al., 2006; Haydon et al., 2007; Choudhury et al., 2011; Ai et al., 2017; Adekiya et al.,2018; Pasley et al., 2019). Ewulo et al. (2009) stated the possible reason for this is related to more microbial soil activity induced by urea application that causes more production and mineralization of organic matter in the soil. The reduction in the soil pH is another probable reason for higher element uptake by urea application that is shown in the Adekiya et al. (2018) report. As the sunflower plants like the slightly acidic soils, this reduction in pH can improve elements uptake as the soil pH of the experiment site was close to neutral, 7.5 (Table 1).

'Teddy Bear' cultivar of sunflower is a dwarf cultivar and mature plants grow up maximum 140 cm. Urea application up to 400 kg ha^{-1} had a positive influence on plant height, and pinching reduced its effect. The suppressive effect of pinching on the plant height has been previously reported for different cultivars of sunflowers (Wien, 2016b; Cheema, 2018). Increasing the plant height and the stem diameter by using urea fertilizer is related to more leaf area production (Milford et al. 2000), while increasing the amount of chlorophyll in the sunflower leaves, followed by increasing photosynthesis and dry matter production, is closely related to higher uptake of various elements, including iron and zinc. It has been reported that iron is involved in the structure of chlorophylls, cytochrome, and nitrogenase enzymes, and zinc is involved in the activity of enzymes associated with chlorophyll formation and consequently increase photosynthesis, accelerating the formation of growth compositions such as tryptophan as the raw material of auxins (Haydon et al., 2007). Enhanced dry matter production in non-pinched plants under more urea fertilizer, is probably due to increased water and mineral absorption by extended roots and rapid growth (Solangi et al., 2015). Steer et al. (1986), also reported an increscent in N uptake and dry matter production by enhancing nitrogen fertilizer levels, as the application of low amounts of nitrogen fertilizer reduced leaf expansion and also the accumulation of dry matter in sunflower. We also obtained the higher dry matter of leaf, root, and head amounts in pinched plants in positive relation with urea levels, while there was an optimum level at 300 kg ha^{-1} in non-pinched plants (Table 6).

Leaf area, SLA, and LAR traits were the highest in 400 kg ha^{-1} of urea fertilizer application with pinching (Table 3). Leaf area is a critical index for plant growth as it is associated with important criteria including light interception, photosynthesis, transpiration, and evapotranspiration rates (Goudriaan and Van Laar, 1994; Zahoor et al., 2010). Leaf growth in earlier stages needs more nitrogen amounts (Evans, 1989; Johnson et al., 2010) and leaf area is limited when nitrogen is deficient by affecting cell division and enlargement (Roggatz et al., 1999). Pinching also had a positive effect on leaf area expansion in chrysanthemum 'Snowball' (Ona et al., 2015). An increase in leaf number after pinching is reported by others on different herbaceous plants (Sehrawat et al., 2003; Tomar et al., 2004; Sudarshan, 2004; Salyh, 2013; and Ona et al., 2015). It seems that it might be related to the fact that pinching alters the direction of growth from upward to lateral parts of the plant (Salyh, 2013).

The findings of this study indicated that pinching and urea application extended vegetative and flowering stages up to eight more days (Table 4. B). The number of days to flowering increased up to 70 days in pinched ornamental sunflowers, while non-pinched plants started to flower after 63 days (Wien et al., 2016). The same results were reported by Ona et al. (2015) for chrysanthemum 'Snowball' and other species (Ahmad et al., 2007; Ryagi et al., 2007; Salyh, 2013). The pinching effect on delayed flowering is due to delay in flower initiation and bud physiological maturity (Naresh and Singh, 2012) because the growth rate in axillary buds is slower than apical buds.

In pinched plants, flower number was affected by urea levels in a positive trend, while head diameter indicated a negative trend. Flower diameter was independently by cultivar decreased by pinching intensity (Burnett, 2017; Cheema, 2018). Other studies also confirmed these results (Ryagi et al., 2007; Habiba, 2012; Salyh, 2013; Ona et al., 2015). The removal of the shoot apex leads to the activation of dormant axillary buds below it to form branches Naresh and Singh (2012). Flower disk diameter of sunflower (Wien, 2016b) and the flower size of chrysanthemum (Ona et al. 2015) were reduced by pinching due the competition between branches and flowers. They have revealed that the number of the branches in a unit area has a negative linear relationship with head size in sunflower (Majid and Schneiter, 1987; Robinson et al., 1980; Wien, 2016b).

5 CONCLUSION

This study demonstrated that adding urea in the soil and pinching improved photosynthetic traits by increasing leaf area and number, SLA, LAR, and total chlorophyll content. The findings also revealed that the

interaction of pinching × urea application at 400 kg ha^{-1} is the best combination of investigated variation sources for the cultivation of sunflower 'Teddy Bear'. The suitable amount of dry matter production (219.04 g), number of flowers (77.7), head diameter (110.04 cm), and plant height (147.3 cm) are the important reasons for this recommendation.

6 AUTHOR CONTRIBUTION STATEMENT

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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8 CONFLICT OF INTEREST

The authors certify the following:

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue;

- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

9 DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

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