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Research Article

Agro- physiological responses of *Carthamus tinctorius* L. to sources of nitrogen fertilizer and organic manure

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Abstract

Excessive consumption of nitrogen (N) to enhance production, may lead to environmental pollution. Meanwhile sustainable production is threatened due to low soil fertility and organic matter. This study was carried out as a factorial experiment in the randomized complete block design and three replications at the research field of the University of Jiroft in 2018-2019. The present study investigated the effects of sources of N fertilizers and cow manure on safflower products in the arid climates of Iran. Two levels of cow manure (0 and 30 t ha^{-1}), and six levels of sources of N fertilizer (control, 130 Kg ha^{-1} N from the sources of urea with a sulfur coating, ammonium nitrate, nano-N, urea, and 1L nitroxin per Kg grain) were used according to the results of soil analysis. Interaction of cow manure and different sources of N fertilizer increased 32-47 % SPAD index at the late vegetative and 37-50 % at the early reproductive stage, 58-81% oil percent and oil yield, 28-81% protein yield, 42-57 % plant height, 52-72 % lateral branches number and 49-78 % grain yield compared to the control. Among the sources of nitrogen fertilizers, nitroxin, nano N, urea with a sulfur coating, ammonium nitrate, and urea had a more positive effect on evaluated characteristics, respectively. There was a significant positive correlation (P≤0.01) between yield components. The best result in this study, were obtained by simultaneous use of cow manure to the soil and inoculating the grains to nitroxin before being planted. Therefore, the simultaneous use of organic and biological fertilizers, in addition to reducing environmental pollution due to the non-use of chemical fertilizers and reducing production costs, can produce healthy products and byproducts of safflower in arid climates.

Keywords: Cow manure, Safflower, Nano N, Nitroxin, Urea, Yield

Introduction

Safflower (*Carthamus tinctorius* L.) is an annual oil plant belonging to the Asteraceae family. Its grains have 20-45% oil and 15-20% protein, suitable as a commercial crop for oil extraction and its byproducts can be used as livestock feed. Its flowers are used for flavoring foods, dyes, and medicinal properties (Iftikhar Hussain *et al*., 2015; Seyed Sharifi, 2017; Delshad *et al*., 2018; Mani *et al*., 2020). All parts of this plant are used to treat various diseases (Nimbkar, 2002). The oil extracted by cold pressing from the seeds of safflower had high antioxidant effects and antimicrobial properties (Khemiri *et al*., 2020). The global safflower sowing area is 1.14 million ha and the average yield is 830 kg ha^{-1} (FAO, 2018). In Iran, the safflower production area is about 6500 ha, its production is approximately 5400 tons, and its yield is approximately 840 kg per ha (MJA, 2017). Low soil organic matter is a challenge in Iranian agriculture in many parts of the country (Naiji and Souri, 2018). Thus, producing safflower in an arid climate does necessarily involve seeking alternatives to obtaining high productivity at a low cost. Using organic sources is a rational way to reduce the environmental impacts of agriculture, and it is suggested that the combined application of long-term organic and inorganic N fertilizers to improve the soil's physicochemical properties and changed the prokaryotic community composition (Lui, 2020). Cow manure involves a good source of nutrients, such as nitrogen (N), phosphorus (P), potassium (K), sulfurous (S), and magnesium (Mg), as well as other trace elements. As a result, when cattle manure (raw or composted) is applied to agricultural lands, it could increase crop productivity and soil fertility for a longer term than chemical fertilizers. (De Mendonca Costa *et al*., 2015). Nitrogen (N) is the key element for the increase of safflower growth, development, and productivity in comparison with other nutritional elements (Seyed Sharifi, 2017; Beeby *et al*., 2020). Chemical fertilizers such as urea are the most important source of nitrate in agriculture. Inorganic N is rapidly released in an environment. The use of excess chemical fertilizers can lead to serious soil and environmental pollutions, while consumption of biological and nano fertilizers can

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increase soil fertility, crop production, and at the same time, prevent environmental contaminations (De Rsoa *et al*., 2010; Souri, 2010; Souri *et al*., 2019). N leaching reduces the efficiency of N fertilizers and, to solve this problem, the use of slow-release fertilizers such as urea with a sulfur coating or the use of fertilizers that increase soluble adsorbent surface such as nono N fertilizers can be effective, which can prevent N wasting (Gentile *et al*., 2009; Alimohamadi *et al*., 2020). The application of bio-fertilizers can reduce the need for chemical fertilizers while preventing adverse environmental effects. They can increase soil organic matter, improve soil properties, and enhance crop yield (Namvar, 2012; Beeby *et al*., 2020). Nitroxin is a biofertilizer that involves N-stabilizing bacteria of Azotobacter and Azosprillium (Parvizi *et al*., 2019). Plants absorb nutrients from fertilizers, but most fertilizers have low nutrient absorption efficiency. Most reports have shown that nano-fertilizers are made to be target-oriented, not easily lost, and easily absorbed by the plants (Elemike, 2019). So far, there has been little investigation about the combined application of organic manure and sources of N fertilizers to the crops. This study was aimed to determine the effect of the combined application of cow manure with sources of N fertilizer (biological, chemical, and nanoforms) on the physiological and agro-ecological characteristics of safflower in the arid climate.

Material and methods

This experiment was performed during 2018-2019 in the research field in dry climate with mild winters and hot summers (De Pauw *et al*., 2008) of southern Kerman, at the University of Jiroft, Iran (35º 28' N, 47º 57' E and altitude of 650 m) (Table 1, 2).

Experimental design, treatment structure, and crop management: This experiment was done as a factorial in a randomized complete block design with three replications. Cow manure and sources of N fertilizers were the first and second factors, respectively. The first factor included two levels of cow manure (X1=0 and X2=30 t ha^{-1}) and the second factor included six levels of N fertilizers (control (Y1), urea with a sulfur coating (Y2), ammonium nitrate (Y3), nitroxin (Y4), nano N (Y5) and urea (Y6). To prepare the culture medium, the soil was treated with 0 or 30 t ha^{-1} of rotten cow manure before the cultivation of the grain. To correct soil phosphorus deficiency based on the results of the soil test, triple superphosphate was added to the soil in all plots before planting. The sources of N fertilizers were used based on the results of soil analysis (130 kg ha^{-1}) and nitroxin used 1 L/Kg grain based on the instruction. Urea with a sulfur coating was applied before planting, urea and ammonium nitrate were added to the soil at planting, rosette, and stemming stages. Biological fertilizer, nitroxin, was used as grain inoculation (the grains were inoculated to the nitroxin and dried in shade before planted). Nano-N fertilizer (0.17% N) was applied as leaf spraying three times; at the planting time, after the first weeding, and at the beginning of flowering 50% of floret open (65 code) (Felemmer *et al*., 2014). The "Isfahan local" variety of safflower was used in this study, which suitable for autumn cultivation in tropical regions and was prepared from Karaj Seed and Plant Improvement Institute. The grains were sown at a depth of 3 cm, the spacing of the rows was 30 cm and the spacing of the in-row was 10 cm. There were 6 rows in each plot, the area of each plot was 6 m^2 (2m×3m). The plants were irrigated by a drip irrigation system according to field capacity. Thinning the plants was carried out at two stages (8 and 15 days after sowing) to maintain the optimum plant population in each plot.

Procedure and protocols for physiological and agro-ecological parameters: The chlorophyll contents (SPAD index) were evaluated at the late (60 days after planting) vegetative and the early reproductive (74 days after planting) stages of plant growth by a portable chlorophyll meter (SPAD 502 plus Konica Minolta, Europe) from 10 plants in each plot. When the capitols opened in plants, flowers were harvested in three stages to evaluate flower yield. The plants were harvested at the physiological maturation stage, about 5 months after planting time, above the ground to measure physiological and agro ecological trails. Five plants from each plot were selected randomly for the determination of plant height and lateral branch number, grains number and grains weight per plant, the weight of 100-grain, grain yield, oil yield, and harvest index oil content and protein yield.

Grain yield was determined using the grain weight of each plot divided by the plant number which was numbered in each plot at the harvest time.

Grain yield (Kg ha^{-1}) = grain weight (kg $plot^{-1}$) × 10000 m^2/ plot area (m^2).

The harvest index (HI) was calculated in agronomy trials as the ratio of grain yield (economic yield) to above-ground dry matter yield (total biomass) using the formula (Canavar *et al*., 2010).

HI = (economic yield / total biomass) × 100

To measure the grain oil, ground and dried samples related to each plot were used for oil extraction by using n-hexane as an extraction solvent. The oil content of sample per plot was determined based on dry matter, as percentage (Asghari and Gharibi asl, 2016).

Oil yield = grain yield × oil percentage.

To measure the protein content, the content of N was measured by the Kjeldahl. To do this, 1 g of dried and grounded grains along with 5 g catalyst (copper sulfate, potassium sulfate and silicon oxide) and 20 ml of sulfuric acid were poured into test tubes and placed in a Kjeldahl apparatus. The temperature of the device firstly was set at 250 °C and then at 410 °C and it was taken for 1.5 h. After cooling, 20 ml of distilled water was added to each of them and they were read by Kajdal device with the addition of NaOH. Then, the samples were removed from the Kjeldahl apparatus and titrated with sulfuric acid titrazole. The amount of acid used in

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Table 1. Average temperature, humidity and monthly rainfall of the region during the growing season

Month-Year	Average maximum temperature (ºC)	Average minimum temperature (ºC)	Humidity (%)	Average rainfall (mm)
November-2018	24.9	7.8	51.3	0.2
December-2018	22.4	6.7	55.6	0.1
January-2019	20.9	7	59	2.4
February-2019	22.9	9.1	58.8	1.7
March-2019	28.5	15.3	59	1.7
April-2019	36.3	18.2	39.9	0.2

Table 2. Physical and chemical properties of soil used in this study

Soil texture	pH	EC (ds/m)	SAR	Organic carbon (%)	N (%)	$P_2 0_5$ (ppm)	K^+ (ppm)	Ca^{2+} , Mg^{2+} (meq/l)	Na^+ (meq/l)
loamy-sandy	8.1	0.56	1.01	0.11	0.008	5.6	133.3	8.4	2.08

the titration was given in the below formula and the nitrogen percent was determined.

Nitrogen (%) = [(amount of acid consumed in titration 0.00014)) / sample weight] × 100

Then protein yield was calculated by the method of Jones *et al*. (1999) based on the following formula:

Protein (%) = N (%) × 6.25

Statistical analysis: We initially checked the data for homogeneity of variance and normality. Then the data were analyzed using the SAS software (9.4). The correlation analysis was done for the obtained clear result between assorted treatments. The graphs were drawn using Excel software (2013). Differences of means among the treatments were evaluated with Duncan's multiple range test at a 5% probability level. Correlation between evaluated traits was assessed using Minitab 16 software.

Results and discussion

The interaction between application of cow manure and sources of N fertilizers affected significantly SPAD index, growth parameters, harvest index, yield components, the yield of grain, flowers, oil, and protein, as well as oil percent of safflower "Isfahan local" variety, at 5% probability (Table 3).

The use of cow manure with sources of N fertilizers increased the SPAD index values ranging from 54 to 71 at the late vegetative stage (Figure 1a) and from 46 to 59 at the early reproductive stage (Figure 1b). In general, organic and sources of N fertilizers showed differences in SPAD index between the late vegetative and early reproductive stages. The application of cow manure with nitroxin (X2Y4), nano-N (X2Y5), urea with a sulfur coating (X2Y2), ammonium nitrate (X2Y3) and or urea (X2Y6) increased the SPAD index by 47, 40, 37, 34, and 32% at the late vegetative stage and by 50, 42, 40, 39, and 37% at the early reproductive stage, compared to the control (X1Y1) respectively (Figure 1). The reason for the most increase in SPAD index value is due to the use of which nitroxin can be considered to increase the absorption of N and improve photosynthesis in the plants. Because, nitroxin includes N-fixing bacteria, rhizobacteria, Azosprillum, and Azotobacter (Parvizi *et al*., 2019). Nitrogen is a structural component (necessary to the formation of pyrrole ring) of chlorophyll pigment and has a major role in the biosynthesis of these pigments, participating in the chlorophyll structure and increasing SPAD index. Also, nano-fertilizers increase the availability of nutrients to the plants, longer and by suitable release in line with plant growth that increases the formation of chlorophyll, the rate of photosynthesis, consequently, the overall plant growth. Our results were in agreement with the finding by Al-juthery and Al-Maamouri (2020) who reported of nano-N had a positive effect on the SPAD index in potato compared to urea fertilizer. Zandonadi *et al*. (2016) reported a positive linear correlation between chlorophyll content or SPAD index and N content in phonological stages of sorghum.

Nitroxin biofertilizer and then nano N had more positive effects on the growth parameters in the presence and or absence of cow manure. The plant height by 57, 54, 48, 45, and 42%, and also the number of lateral branches increased by 72, 67, 63, 54, and 52%, through the application of cow manure and nitroxin (X2Y4), nano-N (X2Y5), urea with a sulfur coating (X2Y2), ammonium nitrate (X2Y3) and or urea (X2Y6) compared to the control (X1Y1), respectively (Figure 2-3). An increase in chlorophyll content can be due to an increase in growth parameters. In this study, there was a significant and positive correlation ($P \leq 0.01$) between plant height and yield component (table 4). This increase in height of plants by N can be attributed to the role of N in the structure of the amino acid (tryptophan) which is the precursor of indole acetic acid (IAA) biosynthesis and is necessary for cell elongation, therefore, increase the plant height (Velasquez *et al*., 2016). Ferreira Santos *et al*. (2018) reported similar heights and number of lateral branches in safflower by using N fertilizer. Under the same environmental conditions, the preparation of nutrients, especially N for the plant is very important in increasing plant height by affecting cell division and growth. The reason for the increase in height which is due to the use of nitroxin can be considered plant-symbiosis microorganisms, on the nitroxin provide more and better plant growth promote through plant growth (IAA) production, N fixation, and help with the avoidance of various stressors, pesticide degradation and heavy metal metabolism (Sumbul *et al*., 2020; Dahham, 2021). Rhizobium species on the

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Table 3. Analysis of variance for experimented factors' effect on SPAD index, growth parameters, harvest index, yield components, the yield of grain, flower, biological, oil and protein, and oil percent.

Sources of variation	Degrees freedom	SPAD index at late vegetative stage	SPAD index at early reproductive stage	Plant height	Lateral branches number	Harvest index	Filled grain number	Grain weight
Replication	2	8.3^{ns}	2.32^{ns}	45.41^{ns}	8^{ns}	1.75^{ns}	968.5^{ns}	0.27^{ns}
Cow manure	1	1437.67^{**}	1814.05^{**}	2690.84^{**}	206.25^{**}	2.15^{ns}	20884.1^{**}	140.18^{**}
N Fertilizers	5	326.62^{**}	191.1^{**}	2046.96^{**}	61.36^{**}	126.8^{**}	24886.3^{**}	35.47^{**}
Cow manure×N Fertilizers	5	16.5^{*}	54.18^{*}	54.31^{*}	5.32^{**}	6.97^{ns}	1099.7^{**}	1.27^{*}
Error	22	6.09	15.79	14.6	1.09	5.97	86.2	0.46
C.V.	-	4.1	7.4	4.06	8.6	7.9	5.7	5.4

ns, * and **, non-significant and significant at 5% and 1% level of probability

Continu of table 3.

Sources of variation	Degrees freedom	Weight of 100- grain	Grain yield	Flower yield	Biological yield	Oil yield	Protein yield	Oil percent
Replication	2	0.28^{ns}	47313.7^{ns}	19.67^{ns}	482528.7^{ns}	4174.9^{ns}	989.6^{ns}	0.78^{ns}
Cow manure	1	27.94^{**}	1721870.5^{**}	40.83^{**}	14162918.6^{**}	266675.5^{**}	174080.8^{**}	43.93^{**}
N Fertilizers	5	4.41^{**}	2426395.5^{**}	8.62^{**}	12132946^{**}	286842.5^{**}	157979.4^{**}	9.81^{**}
Cow manure×N Fertilizers	5	0.406^{*}	109977^{**}	0.68^{**}	586139.3^{*}	12488^{**}	28103.9^{**}	7.94^{**}
Error	22	0.118	15349.5	0.134	211419.4	1772	1422.4	0.52
C.V.	-	7.5	8	7.3	9.4	8.4	13.3	2.2

ns, * and **, non-significant and significant at 5% and 1% level of probability

Table 4. Pearson correlation coefficient between some of evaluated parameters

	Plant height	Number of lateral branches	Filled grain number per plant	Grain weight per plant	Weight of 100-grain	Biological yield	Grain yield	Harvest index	Oil percent	Oil yield
Number of lateral branches	0.895^{**}									
Filled grain number per plant	0.941^{**}	0.925^{**}								
Grain weight per plant	0.933^{**}	0.947^{**}	0.916^{**}							
Weight of 100-grain	0.853^{**}	0.932^{**}	0.837^{**}	0.928^{**}						
Biological yield	0.913^{**}	0.929^{*}	0.866^{**}	0.939^{**}	0.933^{**}					
Grain yield	0.896^{**}	0.955^{**}	0.958^{**}	0.931^{**}	0.922^{**}	0.901^{**}				
Harvest index	0.902^{**}	0.932^{**}	0.972^{**}	0.913^{**}	0.895^{**}	0.845^{**}	0.978^{**}			
Oil percent	0.899^{**}	0.916^{**}	0.884^{**}	0.953^{**}	0.904^{**}	0.957^{**}	0.913^{**}	0.865^{**}		
Oil yield	0.874^{**}	0.943^{**}	0.938^{**}	0.918^{**}	0.909^{**}	0.894^{**}	0.995^{**}	0.958^{**}	0.916^{**}	
Protein yield	0.879^{**}	0.910^{**}	0.928^{**}	0.902^{**}	0.865^{**}	0.879^{**}	0.930^{**}	0.920^{**}	0.893^{**}	

****, *, and ^{ns} show significant in P≤ 0.01%, P≤ 0.05% and no significant respectively**

nitroxin fertilizer improve the growth of non-legumes via inducing changes in root morphology and growth physiology and can improve crop growth by increasing plant height, seed germination, leaf chlorophyll, and N content (Nosheen *et al*., 2021). The superiority of biofertilizer application of nitroxin over other treatments can be attributed to the positive effects of N-bacteria and mycorrhizal fungi, which in addition to providing sufficient N, cause plant growth and development through the synthesis and secretion of growth stimulants (Tilak *et al*., 2005). Using rhizobacteria as plant growth-promoting in agriculture is offered as an effective material to replace the application of fertilizers and pesticides as well (Vejan *et al*., 2016).

The simultaneous use of cow manure and nitroxin (X2Y4), nano-N (X2Y5), urea with a sulfur coating (X2Y2), ammonium nitrate (X2Y3), and or urea (X2Y6) increased the grain weight per capitula by 60, 57, 51, 49, and 44%, and increased filled grains number per plant by 80, 77, 67, 65, and 57%. Also, they increased the weight of 100-grain by 65, 55, 56, 52, and 47%, and the grain yield by 78, 73, 67, 63, and 49%, compared to the control (X1Y1) respectively (Figure 4). There was a positive correlation (r = 0.916**) between grain number and grains weight per plant (table 4). The grain yields of safflower were 615 Kg ha^{-1} in the control condition, and 2050 Kg ha^{-1} when nitroxin was used alone. However, it was 2840 Kg ha^{-1} when cow manure was used with nitroxin. Therefore, using the N fertilizer can be a key factor in determining safflower yield. In this study the grain yield that was improved was due to the increase in yield components such as the weight of filled grains number of grains per plant, lateral branches, and of the plant. Ferreira Santos *et al*. (2018)

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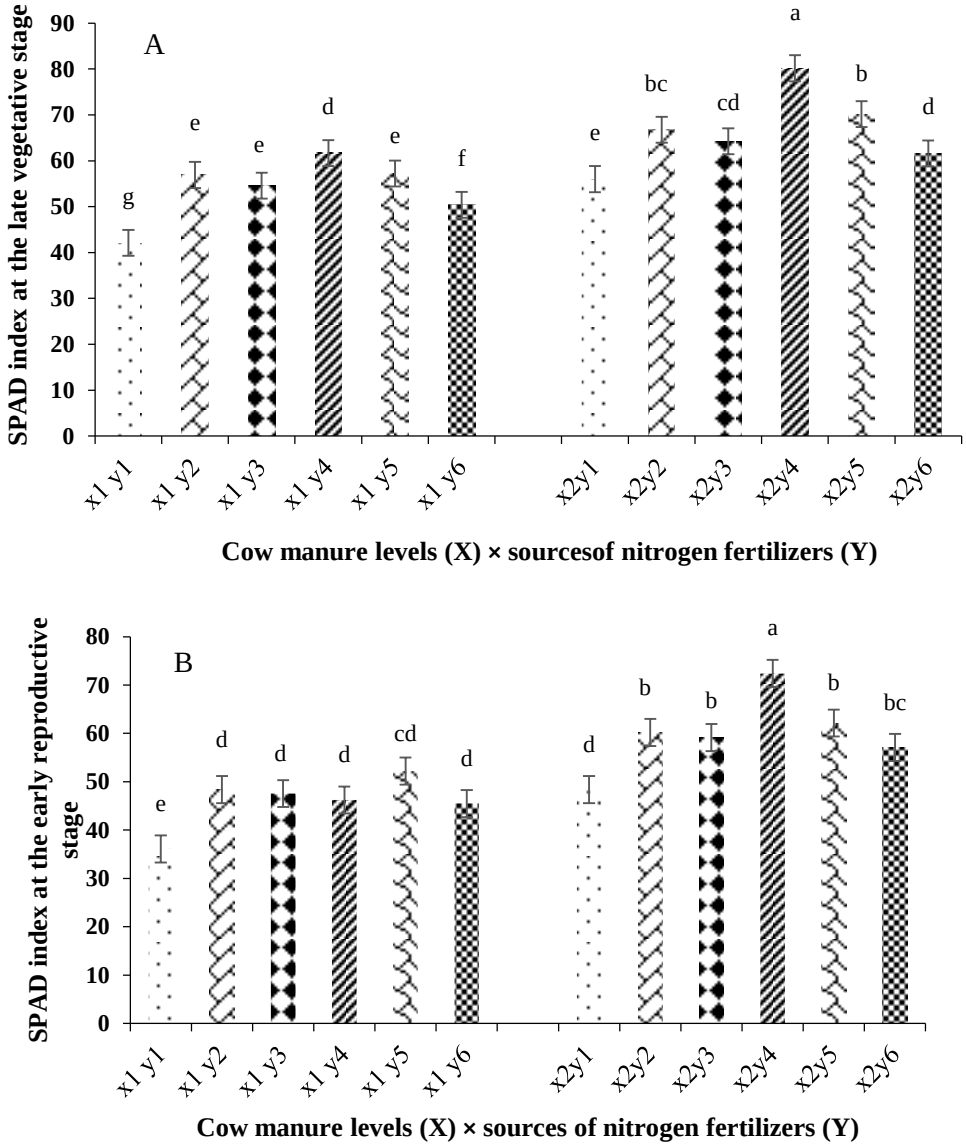

Figure 1. The interaction effects of cow manure levels and sources of N fertilizers on SPAD index at the late vegetative stage (A) and SPAD index at the early reproductive stage (B). Means with the same letter are not significantly different at P≤ 0.01 according to Duncan's multiple range test. X1: Control and X2: Consumption of cow manure; Y1: Control, Y2: Urea with a sulfur coating, Y3: Ammonium nitrate, Y4: Nitroxin, Y5: Nano N, and Y6: Urea.

reported a maximum weight of 100-grain and grain yield in safflower when applying 150 kg ha^{-1} N under irrigation conditions. The positive effects of nitroxin on grain yield can be attributed to the special role of Azotobacter and Azospirillium in root development, increasing capillary roots in physiologically active areas for water and nutrient uptake, increasing plant growth and development, and finally improving growth and photosynthesis. Because Azotobacter is a non-symbiotic microbe, its maximum potential for increasing plant productivity can be eliminated by co-inoculation with some other biofertilizers compared to a single application (Sumbul *et al*., 2020). Azotobacters within nitroxin improve the rate of seed germinating, help in root proliferation, accelerate plant growth, initiate early flowering and maturation, and increase crop yield up to 10-15 percent (Mishra *et al*., 2021). Therefore, using cow manure in soil by increasing the organic matter and providing moisture in the soil was more suitable for plant nutrition and had created more favorable conditions for the production of more lateral branches, filled grain number, and grain weight per plant. The results of the present study were consistent with the results of the previous researches who reported that consumption of composted manure and Nitroxin increased significantly the grain and oil yield in safflower (Moradi Telavat *et al*., 2019), and the consumption of urea and Nitroxin increased the grain and oil yield in safflower (Jorfi *et al*., 2017). Bayer and Mielniczur (2008) reported that organic matter has been a key component of the productive capacity of soils, due to various effects on nutrient availability, the cation

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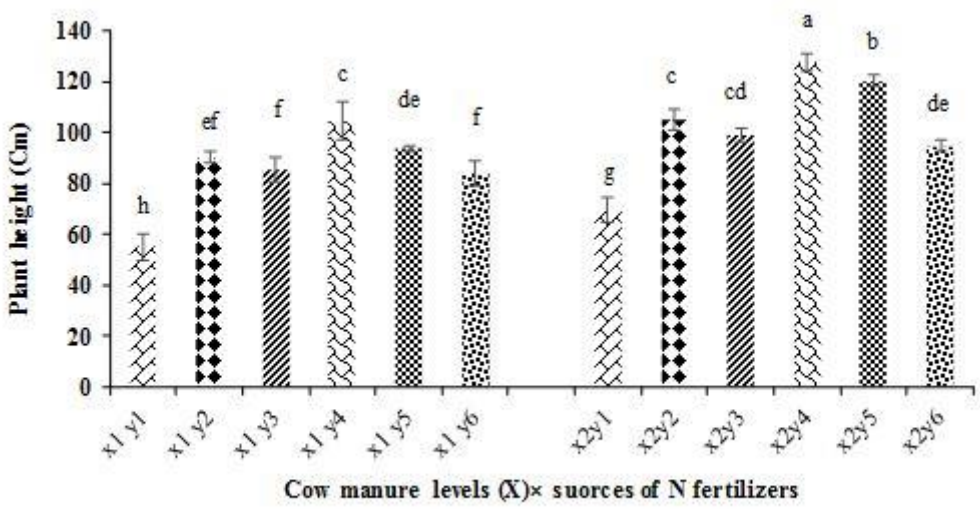

Figure 2. The interaction effects of cow manure levels and sources of N fertilizers on plant height. Means with the same letter are not significantly different at P≤ 0.05 according to Duncan's multiple range test. X1: Control and X2: Consumption of cow manure; Y1: Control, Y2: Urea with a sulfur coating, Y3: Ammonium nitrate, Y4: Nitroxin, Y5: Nano N, and Y6: Urea.

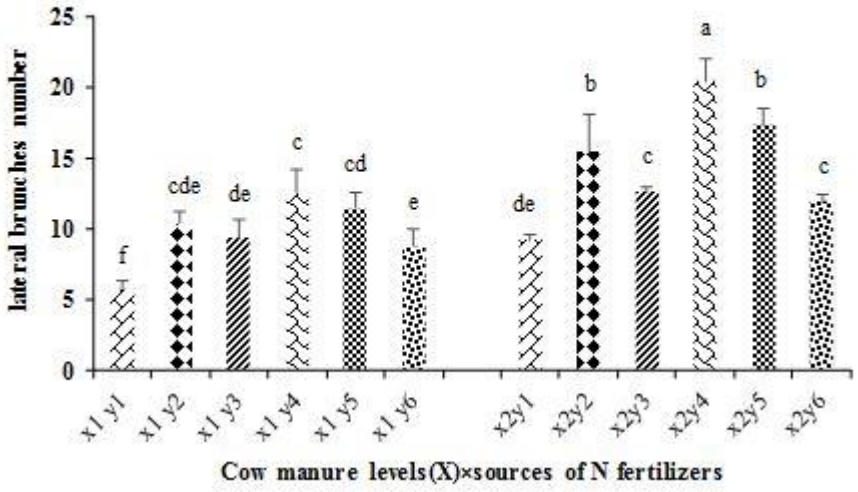

Figure 3. The interaction effects of cow manure levels and sources of N fertilizers on lateral branches number. Means with the same letter are not significantly at P≤ 0.01 according to Duncan's multiple range test. X1: Control and X2: Consumption of cow manure; Y1: Control, Y2: Urea with a sulfur coating, Y3: Ammonium nitrate, Y4: Nitroxin, Y5: Nano N, and Y6: Urea.

exchange capacity, water retention, and infiltration. Therefore the presence of microorganisms due to the use of nitroxin fertilizer in the root environment increased the availability of N for the plant more than the N fertilizer sources and can have a positive effect on plant growth which has led to an increase in the number of capitula in safflower. Oil production and oil yield rates ranged from 29.4 - 35.3% in 180.9-972.1 Kg ha^{-1}, respectively, and the lowest belongs to the control (X1Y1). The highest value was observed by the application of cow manure + nitroxin (X2Y4).

The simultaneous application of cow manure with nitroxin (X_2Y_4), nano-N (X_2Y_5), urea with a sulfur coating (X_2Y_2), ammonium nitrate (X_2Y_3), and or urea (X_2Y_6) increased flower yield per ha by 72, 69, 66, 64, and 60%, compared to the control (X_1Y_1), respectively (Figure 5).

The sources of N fertilizers and cow manure increased significantly the biological yield and oil yield compared with the control and the application of the N fertilizers alone. The use of cow manure and nitroxin (X2Y4), nano-N (X2Y5), urea with a sulfur coating

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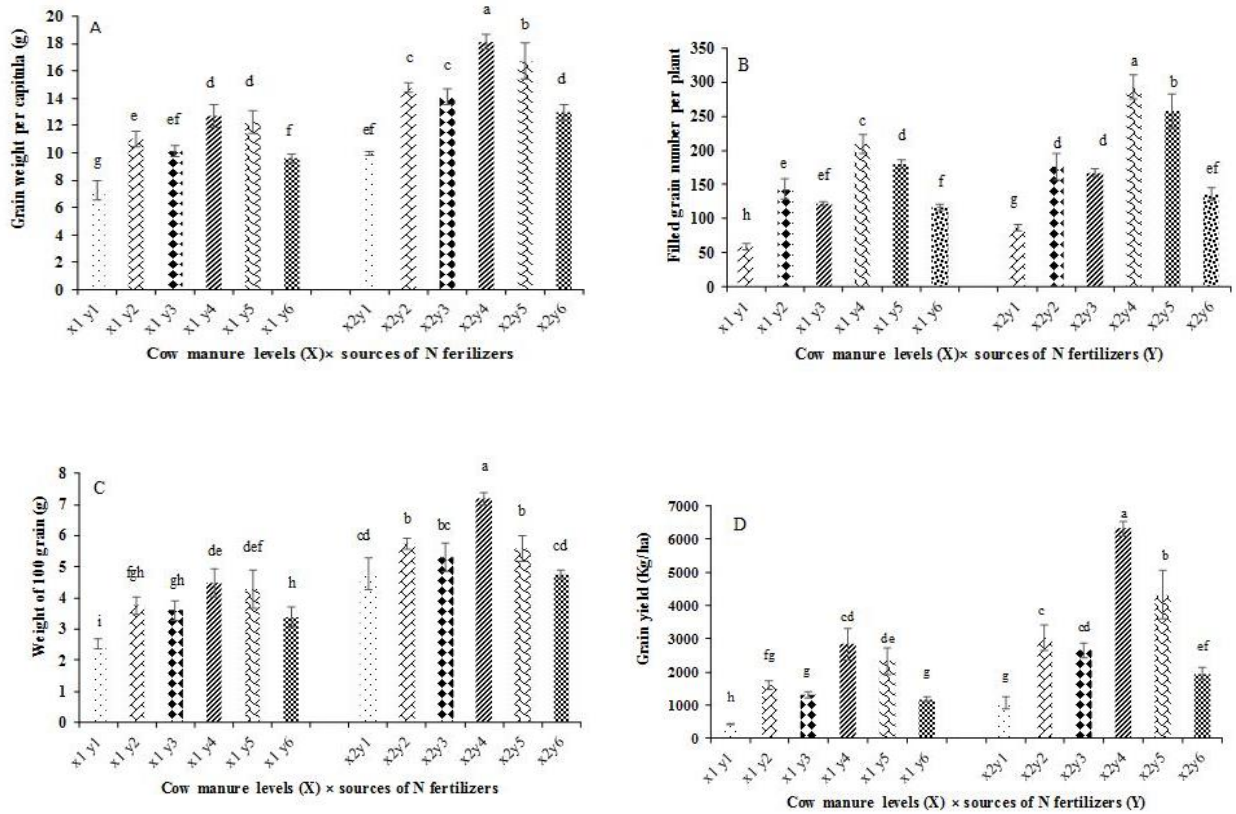

Figure 4. The interaction effects of cow manure levels and sources of N fertilizers on grain weight per capitula (A), filled grain number per plant (B), the weight of 100-grain (C) and grain yield (D). Means with the same letter are not significantly different at P≤0.01 according to Duncan's multiple range test. X1: Control and X2: Consumption of cow manure; Y1: Control, Y2: Urea with a sulfur coating, Y3: Ammonium nitrate, Y4: Nitroxin, Y5: Nano N, and Y6: Urea.

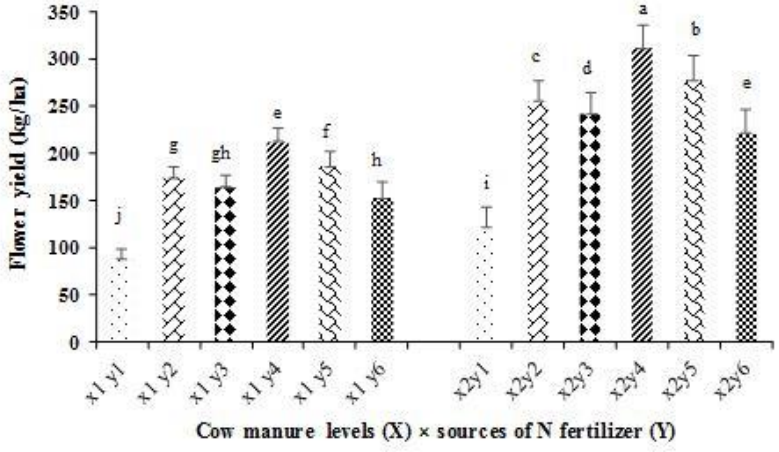

Figure 5. The interaction effects of cow manure levels and sources of N fertilizers on flower yield per ha. Means with the same letter are not significantly different at P≤ 0.01 according to Duncan's multiple range test. X1: Control and X2: Consumption of cow manure; Y1: Control, Y2: Urea with a sulfur coating, Y3: Ammonium nitrate, Y4: Nitroxin, Y5: Nano N, and Y6: Urea.

(X2Y2), ammonium nitrate (X2Y3), and urea (X2Y6) increased the biological yield by 63, 62, 57, 52, and 34% and the oil yield by 81, 75, 72, 66, and 58%, compared to the control (X1Y1) respectively (Figure 6). The biological yield had a positive correlation with grain yield.

Using the sources of N fertilizers and the cow manure increased significantly the harvest index, oil content, as well as protein yield, compared with the control and the application of N fertilizers alone. The

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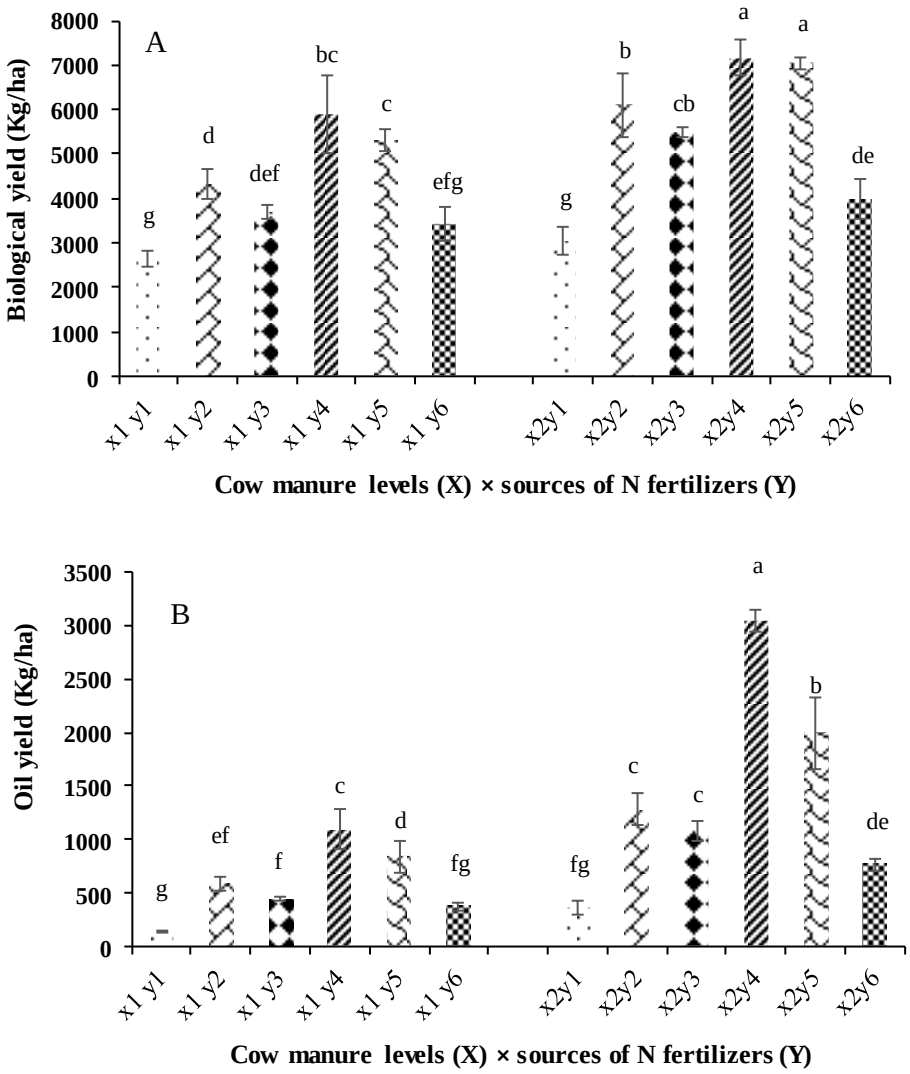

Figure 6. The interaction effects of cow manure levels and sources of N fertilizers on biological yield (A) and oil yield (B). Means with the same letter are not significantly different at P≤ 0.01 according to Duncan's multiple range test. X1: Control and X2: Consumption of cow manure; Y1: Control, Y2: Urea with a sulfur coating, Y3: Ammonium nitrate, Y4: Nitroxin, Y5: Nano N, and Y6: Urea.

cow manure and nitroxin (X2Y4), nano-N (X2Y5), urea with a sulfur coating (X2Y2), ammonium nitrate (X2Y3), and urea (X2Y6) increased the oil percent by 81, 75, 72, 66, and 58%, and the protein yield by 81, 74, 63, 47, and 28%, compared to the control (X1Y1) respectively (Figure 7). Applying the cow manure with nitroxin increased harvest index by 85%, oil percent by 36%, and protein yield by 81%, compared with the control (X1Y1). There was a significant and positive correlation between oil percent and biological yield, grain yield and harvest index (table 4). The result of oil yield was in agreement with the results of Moradi Telavat *et al*. (2019) who found that the application of composted manure fertilizer and nitroxin increased oil yield (1440 kg ha^{-1}), compared with the control (743 kg ha^{-1}), and differences may be related to the differences in climatic conditions, genotypes, irrigation management, and fertilizer management. Oz (2016) found that the oil contents of the grains ranged from 23.05 - 31.99% in the varieties of safflower. Ferreira Santos *et al*. 2018 reported that the maximum oil percentage (29.8%) in safflower achieved by 400 kg ha^{-1} N under irrigation conditions. In this study application of cow manure to the soil improved soil properties and enhanced grain yield, thereby leading to the total increase in oil yield.

Conclusion

Among the biological, nano, and chemical sources of N fertilizers, the highest to lowest effectiveness was attributed to nitroxin, nano-N, urea with a sulfur coating, ammonium nitrate, and urea respectively. These had the most effective influence on plant height, biological, oil, grain and flower yield, oil percent, and

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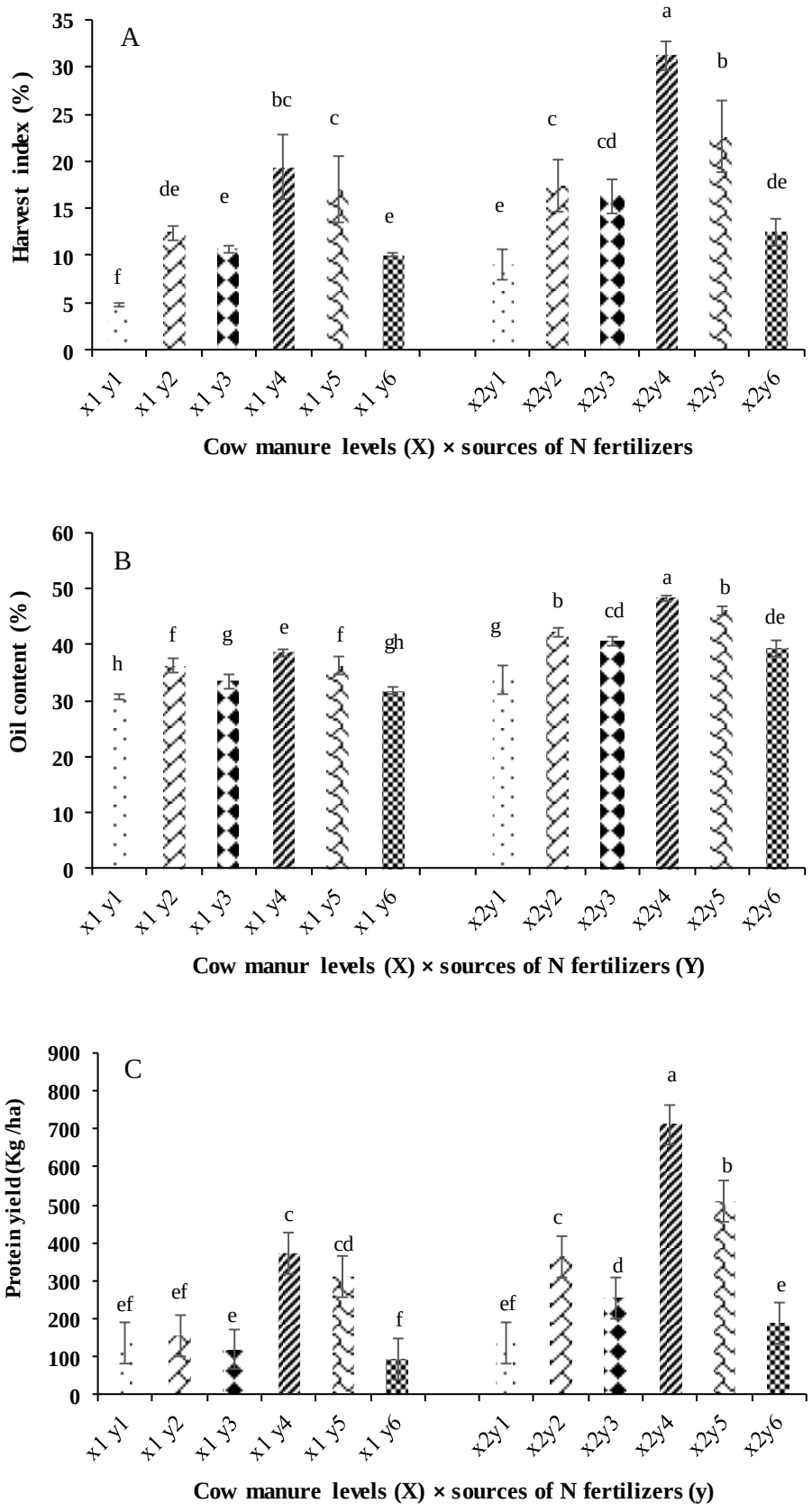

Figure 7. The interaction effects of cow manure levels and sources of N fertilizers on harvest index (A), oil content (B), and protein yield (C). Means with the same letter are not significantly different at P≤ 0.01 according to Duncan's multiple range test.

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harvest index, respectively, especially in the presence of cow manure. The best result for yield and yield components was achieved by the interaction between nitroxin biofertilizer and cow manure. In fact, the combination of integrated fertilizers, using N fertilizer, improved the growth and yield of safflower plants, with a reduction of the chemical fertilizer consumption was the cheapest and the most economical source among the N fertilizers. Moreover, it had better compatibility with the environment and poses less risk of pollution. Therefore, it can be suggested that the combined application of nitroxin and cow manure could be used for safflower cultivation in arid climates.

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