Original Research Article

Study of nitrogen rates and nano urea effects on physiological parameters and yield of Safflower (*Carthamus tinctorius* L.)

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is an important oil seed crop, essential as vegetable oil for humans and feed for livestock. The experiment was conducted in a split plot design to study the effects of foliar application of nano urea and urea on safflower growth and development. The results showed that application of 100% nitrogen in the form of urea topdressing at vegetative stage and foliar spray of nano urea twice at flowering and seed filling stages significantly improved the physiological parameters *viz*. plant height (cm), primary branches of safflower.The CSI, RWC, Seed yield (kg/ha), 100 seed weight (g)and hull content (%) of safflower also significantly improved with 100% N and foliar spray of nano urea twice at flowering and seed filling stages. The results from the study suggests that application of urea as split doses and foliar spray of nano urea at reproductive stage is beneficial for the crop and will improve seed yield and oil quality of safflower. Foliar application of nano urea improves the nutrient uptake by the crop through its slow and controlled release of nutrients.It also helps in reducing environmental pollution due to leaching of fertilizers.

Keywords: *Nano urea, Safflower, Foliar spray, Chlorophyll stability index, Seed Yield*

1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an important*rabi* oil seed crop belonging to the family Asteraceae. Safflower is a strongly tap rooted, highly branched and thistle like herbwith the ability to withstand drought, salinity and cold stress.The oil content of the seed ranges from 24-36 per cent.Safflower oil is preferred for its higher poly unsaturated fatty acid (78% linoleic acid) and it is nutritionally similar to sunflower oil, which is very useful for reducing blood cholesterol content (1,2).Oleic safflower oil is highly preferred in the food, pharmaceutical, biofuel production, paints, lubricants and cosmetics industries (3).

Plant nutrition is vital for agricultural production and fertilizer application accounts for 40% to 60% of global food production. Fertilizing with nitrogen is important as it determines yield due to its multi-dimensional effects on the growth and development of safflower (4). Nitrogen is required for the manufacture of several growth-promoting enzymes, proteins and so plays an important role in plant growth regulation, particularly during the vegetative phase (5). Excessive fertilizer applications lead to economic losses and discharge of excessive amount of nitrogen in the nitrate form through leaching (6). Agricultural use of inorganic fertilizers in 2019 was about 190 million tonnes of nutrients, of which 57% was nitrogen (7). Farmers were forced to increase the amounts of applied nitrogen fertilizers in order to achieve better crop production due to the high N loss and low use efficiency, which increased the costs of farming while also increasing the environmental implications (8, 9, 10). As a result, there is a compelling need to increase nitrogen availability for plants while avoiding adverse environmental impacts. Some studies proposed the use of nanoparticle fertilizers, particularly nanoparticle urea (NPU), to avoid the issues associated with the application of bulk urea while not depriving the plant of its benefits (8, 9,11). When compared to traditional fertilizers, nano fertilizers improve growth parameters (plant height, leaf area, number of leaves per plant), dry matter production, chlorophyll production and photosynthesis rate, resulting in increased photosynthesis and translocation to different parts of the plant (12, 13). Nano urea when applied to foliage, easily enters through stomata and distributed through phloem. Unutilized nitrogen is stored in plant vacuole and is slowly released for proper growth and development of plant (14). The lack of synchronisation between nutrient release from the fertilizer and plant need is the fundamental cause behind low nitrogen use efficiency of bulk chemical fertilizer's (10). The controlled release of nutrients provided by nanostructured fertilizers will allow for a longer effective duration of nutrient supply to the plant, (15) ensuring without any negative environmental consequences (16). Nitrogen fertilizer application to the soil may result in nutrient losses, particularly through leaching and denitrification; however, foliar spraying may reduce such losses to provide nutrients and can mitigate nutrient deficiencies(17). Nano Urea is an important tool in agriculture to improve crop efficiency, yield and quality parameters with increase nutrient use efficiency, reduce wastage of fertilizers and cost of cultivation (14).

2. MATERIALS AND METHODS

The experiment was carried out at the research farm of All India Coordinated Research Project (AICRP) on Safflower, VNMKV, Parbhani, Maharashtra during *rabi* season of 2021-22 on medium black soil. The experiment was conducted on PBNS 86 variety of safflower in a split plot design with three replications.

Each replication consists of three main plots which include F1 with 100% N (60kg N/ha), F2 with 75% N (45kg N/ha) and F3 with 50% N (30kg N/ha) and each main plot is divided into five sub plots, which include N0 (No spray of nano urea), N1 (nano urea spray at flowering stage), N2 (nano urea spray at flowering and seed filling stages), N3 (2% urea spray at flowering stage) and N4 (2% urea spray at flowering and seed filling stages) thus, each replication has total fifteen treatment combinations.

Table 1. Initial physico-chemical properties of experimental soil

FC%	33.00%	EC at 0-15 cm	0.53 ds/m
BD	1.28 Mg/m^3	EC at 15-30 cm	0.64 ds/m
Soil texture	Clayey	Available N (kg/ha)	157.86
Soil pH	7.71	Available P (kg/ha)	14.17
Organic carbon	0.73%	Available K (kg/ha)	619.81

The net plot size was 5.4 x 6.0m. The spacing is 45 X 20cm. One third of nitrogen was applied at the time of sowing and remaining 2/3rd in 2 equal splits at 30 DAS and 60 DAS as urea top dressing. The nano urea @ 3ml/litre water and 2% urea @ 20g of urea/litre water were applied as foliar spray at flowering and seed filling stages.

Biometric observations were recorded from five randomly selected plants from each plot periodically. The data for studying plant height, number of primary branches was recorded from each plot at 30 DAS, 60 DAS and 90 DAS.

2.1 Leaf Chlorophyll content and Chlorophyll stability index (CSI)

Fresh leaves from each treatment were collected randomly for chlorophyll studies. Shoaf and Lium (1976) devised an improved method of extraction of chlorophyll by dimethyl sulphoxide (DMSO) (18). Weigh 100mg of fresh leaf and put in a test tube. Incubate the leaf in 7.0 ml of DMSO at 65^0 C for 30 minutes. After 30 minutes, decant the supernatant into a test tube and discard the leaf tissue. The final volume was made up to 10 ml with DMSO. The absorbance of the extract was measured at 645 and 663 nm in spectrophotometer using DMSO as blank.

$$\text{Total chlorophyll} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W \times a}$$

Where,

A = Absorbance at specific wavelength (645 and 663 nm)

V = Final volume of the chlorophyll extract (ml)

W = Fresh weight of the sample (g)

a = path length of light (1cm)

Chlorophyll stability index was computed by using the method given by Dhopte and Livera (1989). The same procedure was followed for stressed leaf but in this case the leaf will be subjected to high temperature stress by placing the leaf tissue in a test tube containing 10 ml of distilled water. Place it in hot water bath and subject it to high temperature of 65^0 C for 1 hour. After 1 hour drain out the water, add 7.0 ml of DMSO and incubate it. Likewise, the unheated sample, same procedure is followed after water bath and then record spectrophotometer readings for both leaf samples. The difference in two readings on absorbance (reading without heating and reading after heating of sample at 65^0 C control) is defined as chlorophyll stability index.

CSI (%) = (Chlorophyll content of treated sample/Chlorophyll content of control) X100

2.2 Relative Water Content(%)

Barrs and Weatherley (1962) given the method for estimation of relative water content. A physiologically functional leaf i.e., third from top was selected for RWC estimation. The fresh weight of leaf samples was immediately recorded. These leaf samples were kept floating on water for 4-6 hrs. depending upon the degree of imbibitions to get turgid weight. Finally, the same leaf samples were kept in an oven at 75^{O}C for assessing dry weight and the values of RWC were calculated by using following formula.

$$RWC = \frac{[\text{Fresh weight (Wf) - dry weight (Wd)}]}{[\text{turgid weight (Wt) - dry weight (Wd)}]} X\ 100$$

2.3 Hull content (%)

Weigh five grams seeds and soak them in water for 24 hours. Drain the water and separate the hull from seed by hand. Collect all the hulls and dry them in oven at 40^{0}C for 8 hours or dry them in sun for 2 days. Find the weight of oven/sun dried hulls. To find the hull content the following formula is used:

$$\text{Hull content (\%)} = \frac{\text{Weight of oven/sun dried hulls (g)}}{5} x\ 100$$

The observations forDays to 50% flowering, Chlorophyll stability index, Relative water content were taken once during crop development stage. Yield parameters *viz*. 100 seed weight(g),seed yield per plant (g), seed yield(kg/ha), oil contentand hull contentwere recorded at the time of harvest.

3. Results and Discussion

The results showed that application of nitrogen as two split doses of urea had significantly increased the physiological parameters. Plant height was recorded maximum with 100% N application at all growth stages *i.e.,* 30, 60, 90 DAS and at harvest. Similarly, number of primary branches/plants also showed a decrease with decreasing the dose of nitrogen up to50% N andmaximum were observed with 100% N application at 60 DAS, 90 DAS and at harvest (Table 3). The plants applied with N2 showed maximum plant height followed by N1 and N4 whereas, the plants treated with no foliar spray (N0) recorded minimum plant height. Highest number of primary branches were recorded in N2treatment and lowest was recorded in N0 treatment (Table 3). Nitrogen fertilization helps in preventing premature death of leaves due to nutrient deficiencies (21).

3.1 Effect of nitrogen on Chlorophyll content :

The results showed that split application of nitrogen in the form of urea had significant effect on the chlorophyll a, chlorophyll b and total chlorophyll content of safflower. Among the given nitrogen doses, 100% N (F1) application showed the highest (2.21 mg/g) chlorophyll content of leaves as compared to 50% N (F3) application (1.49 mg/g) as presented in table 2.

Similarly, maximum chlorophyll stability index of safflower was recorded with 100% N (F1) application followed by 75% N (F2) and 50% N (F3) application respectively as presented in table 2.

The maximum leaf chlorophyll content and CSI was recorded with foliar spray of nano urea twice at flowering and seed filling stages (N2) while, the lowest leaf chlorophyll content (1.48 mg/g) and CSI was recorded with no foliar spray of nano urea (N0). However, other treatments showed the effect lower than N2, but were higher when compared to the control. Application of urea as split doses and foliar spray of nano urea at reproductive stage showed higher chlorophyll content as compared to the control where only soil applied nitrogen was given which may be due to the decrease in applied dose of nitrogen as it is important for protein and carbohydrate metabolism and can enhance the plant height, production of photo-assimilates and distribution of assimilates to the reproductive organs (22) and ultimately greater production of dry matter (23) indicating a direct relation of nitrogen content with dry matter production.Nitrogen is essential for synthesizing the amino acids, nucleic acids and chlorophyll. The results from the current study also indicates a positive relationship between chlorophyll content and nitrogen.

3**.2 Effect of nitrogen on Relative water content (%) :**

The effect of split application of nitrogen was significant on relative water content of safflower. Maximum RWC was observed with 100% N (F1) followed by 75% N (F2) and 50% N (F3) respectively as presented in table 2. Foliar application of nano urea and 2% urea at reproductive stage of crop significantly affected the relative water content of leaf. Among the given treatments, foliar spray of nano urea twice at flowering and seed filling stages (N2) recorded maximum RWC and lowest was recorded for control (N0). However, the other treatments recorded comparatively lower values than N2 but higher than control (N0).

With increase in nitrogen content, RWC also increased. Osmatic adjustment (OA) is a powerful mechanism of conserving cellular hydration under drought stress and RWC expresses the effect of OA in this respect. Hence, RWC is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and OA (20).

Table 2: Mean data for Chlorophyll content, Chlorophyll Stability Index (%), Relative Water content (%) and Hull content (%).

TREATMENTS	Chlorophyll content (mg/g)	CSI (%)	RWC (%)	Hull content (%)
F1	2.21	85.00	89.49	41.58
F2	1.87	84.48	87.63	40.63
F3	1.49	81.69	82.81	40.36
SE ±	0.02	0.44	0.14	0.16
CD at 5%	0.09	1.73	0.55	0.62
FOLIAR SPRAY				
N0	1.48	74.21	78.42	39.27
N1	2.06	87.73	89.38	41.51
N2	2.17	91.39	91.22	42.35
N3	1.65	80.98	86.34	40.21
N4	1.93	84.32	87.86	40.94
SE ±	0.03	0.58	0.31	0.19
CD at 5%	0.07	1.68	0.89	0.56
INTERACTION				
SE ±	0.04	1.00	0.53	0.33
CD at 5%	0.13	NS	1.55	NS
G. MEAN	1.86	83.73	86.64	40.85

3.3 Effect of nitrogen on hull content (%) :

Application of different doses of nitrogen in the form of urea as split doses at vegetative stage showed significant effects on hull content of safflower seeds. The data obtained from the present study showed that maximum hull content was recorded with 100% N (F1) while lowest was recorded with 50% N (F3) application as shown in table 2. Similarly, foliar spray of nano urea twice at flowering and seed filling stages (N2) recorded maximum hull content while, the lowest was recorded for no foliar spray of nano urea (N0). However, the other treatments recorded comparatively lower values than N2 but higher than control (N0).

3.4 Effect of nitrogen on Seed yield

The results showed that enhanced yield of a crop is often associated with optimum nutrient uptake particularly nitrogen.Among the given treatments, the plants applied with 100% N at vegetative stage showed significantly maximum growth followed by 75% N and 50% N application.

Table 3: Mean data for 100 seed weight (g), Seed yield/plant and per plot, oil yield (kg/ha) and hull content (%).

TREATMENTS	100 seed weight (g)	Seed yield/plant (g)	Seed yield(kg/ha)	Oil yield (Kg/ha)
Nitrogen Fertilizer				
F1	6.42	86.47	14.69126	478
F2	6.03	81.27	10.45673	343
F3	5.82	78.60	9.89911	324
SE ±	0.11	0.73	0.622	23.60
CD at 5%	0.44	2.86	2.44	92.50
Foliar spray				
N0	4.88	73.33	9.458097	309
N1	6.56	84.56	11.8552	397
N2	7.10	91.00	16.11444	520
N3	5.76	79.67	10.54176	333
N4	6.14	82.00	10.44233	350
SE ±	0.09	0.69	0.372	15.60
CD at 5%	0.27	2.00	1.086	45.50
Interaction				
SE ±	0.16	1.19	0.644	27.00
CD at 5%	NS	NS	NS	NS
G.Mean	6.09	82.11	11.68237	382

Foliar application of nano urea at the time of flowering and seed filling stages of safflower had increased the returns by promoting maximum yield through its slow and controlled release of nitrogen which is very beneficial for the plant in utilizing the nutrients and improving the yield attributes. Maximum mean values were recorded with N2(Table 3) for the 100 seed weight. Seed yield (kg/ha) had significantly increased with N2over N0. Some studies reported that higher yield in safflower is related to increase in number of heads/plant and number of seeds/head in safflower (24). It has been reported that split application of nitrogen can improve the seed yield by maximising the number of capitula/plant (25) and the responding level of plant to urea fertilizer can be attributed to the needed nitrogen in soil (26).

It is evident that applying nanonitrogen fertilizers in conjunction with reduced dosses of conventional nitrogen fertilizers can boost the productivity of several cereal crops (6, 27) by their smart nutrient delivery system.Applying nitrogen more than required may reduce crop yields and is toxic to plants. So, providing optimum doses of nitrogen is essential for obtaining maximum yields. Through foliar application, nutrients can be supplied as per plant demand and can reduce fertilizer losses.

CONCLUSION

Application of 100% nitrogen fertilizer improved the growth and yield of safflower. When the nitrogen supply is reduced to half there is reduced growth and yield of safflower.The supply of nitrogen through foliar application of nano urea and 2% urea at reproductive stage provided the plant with required nutrients and enhanced the yield attributes and seed yield of safflower. Foliar application of nutrients is an effective way to mitigate nutrient deficiencies when plants are unable to absorb the nutrients directly from soil. Due to lack of synchronization between nutrient release from soil applied urea and its demand by the plant, resulted in less nutrient use efficiency by the crop. This can be managed by applying the nano urea which will release the nutrients in a slow and control manner to meet the plant’s nutrient needs. Application of nano urea will reduce the cost of urea for crop production and will minimize pollution caused by conventional fertilizers.

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