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RESEARCH ARTICLE

Effect of nitrogen fertilizers on agronomic, essential and enzymatic properties of marigold (*Calendula officinalis* L.) under normal and drought conditions

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Abstract

To investigate the effect of mineral and organic nitrogen fertilizers on some of the quantitative and qualitative reactions of marigold under normal and drought stress, a study was conducted in a split-plot factorial and based on a completely randomized block design with three replications. The first factor included irrigation (normal and water deficit) in the main plot and sub-factor included foliar application of nitrogen fertilizer (control, nitrogen (N), nano-chelate nitrogen (NC) and nitrogen fertilizer+ nano-nitrogen fertilizer (N+NC)) and biofertilizer (control, *Azotobacter* and *Azospirillum*) as factorial was placed in sub-plots. Although water deficit stress reduced plant height, flower dry weight, number of seeds per head, 1000-grain weight, biological yield, grain yield and essential oil yield, N + NC foliar application could moderate the adverse effect of water stress on the grain yield, biological yield and essential oil yield. In addition, inoculation of seeds with *Azospirillum* under water stress conditions could increase the number of seeds per head, 1000-grain weight and essential oil yield compared to the control treatment. NC foliar application and Azotobacter inoculation showed a synergistic effect on the content of proline enzymes and antioxidant activity. Also, simultaneous application of nitrogen + nano-chelate nitrogen foliar application along with seed inoculation with *Azospirillum* could have a positive effect on increasing flower dry weight, the number of seeds per head, 1000-seed weight, biological yield, grain yield, essential oil yield and proline content.

Keywords

Antioxidants, flower dry weight, marigold, water deficit

Introduction

Pot marigold (*Calendula officinalis* L), which belongs to the family of Asteraceae, is an annual perennial plant, It is propagated by sowing seeds in spring. Pot marigold or calendula is known as a medicinal plant and its main essential oil compound is α-cadinol, which has antioxidant, antibacterial, anti-inflammatory, antiviral anti-HIV, anti-tumor, anti-mutagenic and cytotoxic properties (1).

Plants react to adverse environmental conditions by changing their morphological, biochemical and physiological characteristics. One of the most important adverse environmental stresses is water deficit stress; this stress significantly reduces crop production in the world and is the most limiting environmental stress among the environmental stresses (2).

Drought stress reduces the economic yield of medicinal plants by reducing the effectiveness of photosynthetic active radiation, decreasing leaf area and photosynthetic level of the plant and reducing yield by decreasing the harvest index (3). Under water deficit stress, the plant's ability to absorb and transport nutrients is impaired, which affects the content of nutrients (4).

Nitrogen is one of the most important and effective nutrients for all the crop plants. Various sources of nitrogen fertilizer can be used to supply the plant nitrogen requirement. Urea is one of the most widely used sources of nitrogen fertilizer in agricultural fields (5). Nevertheless, urea fertilizers usually lead to problems (such as leaf necrosis and toxicity) that may override their benefits associated with crop productivity (6). Reduction or replacement of chemical fertilizers has attracted great attention in recent years (7). Nano-fertilizers provide less harmful, but more effective, agricultural inputs (8). In addition, biofertilizers are nowadays introduced as an alternative for chemical fertilizers to increase soil fertility (9). The application of biofertilizers improves the uptake of nutrients and water, and promotes growth and resistance to abiotic and biotic stresses in plants (9). *Azotobacter* is a biofertilizer that improves plant growth by increasing mobility and absorption of nutrients and plant growth hormones (10). *Azospirillum* is another nitrogen which stabilizes microorganisms. *Azospirillum* enhances the quality and quantity of growth by improving nitrogen biosynthesis, developing roots, enabling the absorption of water and nutrients and producing growth hormones and some vitamins (11).

In one study, the effect of biofertilizers on yield and yield components of fennel (*Foeniculum vulgare* Mill.) under water deficit conditions was investigated and the results showed that the application of biofertilizer had a positive effect on morphological characteristics, grain yield and yield components and essential oil yield under water stress conditions (12). In the study on sunflower, drought increased the amount of proline and activity of antioxidant enzymes, while inoculation of seeds with *Azotobacter* + *Azospirillum* moderated the destructive effect of drought stress on biological yield, grain yield, oil percentage and oil yield (13). In the study on chickpea, the highest grain yield, oil content and grain protein were obtained in the application of 75 Kg/ha of nitrogen fertilizer along with seed inoculation with biofertilizer (14). It was found that the simultaneous application of potassium fertilizer in nano or chemical forms with nitrogen fertilizer (nitroxin þ 50% urea) could alleviate the adverse effects of water deficit on biochemical and physiological properties of sesame (*Sesame indicum* L.) (15). It was showed that the combination of *Azotobacter* with 100% of the recommended nitrogen fertilizer had the most positive effect on growth and yield indices in strawberries (16).

One of the main centers for the production of medicinal plants in Iran is the western regions of the country. In these areas, most of the rainfall occurs during autumn and plants do not grow during these periods due to low temperatures. Since these plants are exposed to different intensities of water deficit stress during the growing season, it is necessary to identify farming methods that can minimize the adverse effect of this stress. Therefore, the present study was conducted to investigate the effect of mineral and biological sources of nitrogen on the response to drought stress.

Materials and Methods

To investigate the effect of mineral and organic nitrogen fertilizers on some quantitative and qualitative reactions of marigold under normal and drought stress, a study was conducted at Agricultural Research Station of Saat Lo, Urmia, Iran (37.53°N, 45.08°E and 1320 m above sea level) in 2016-2018.

A field experiment was carried out in a split-plot factorial based on a completely randomized block design with three replications. The first factor included irrigation including normal (60 mm evaporation from pan class A)) and water deficit (120 mm evaporation from pan class A) in the main plot and foliar application of nitrogen fertilizer (control, nitrogen (N), nano-chelate nitrogen (NC) and nitrogen fertilizer+ nano-nitrogen fertilizer (N+NC) and biofertilizer (control, *Azotobacter* and *Azospirillum*) as factorial was placed in the sub-plots.

The soil physical and chemical properties of the experimental site are shown in Table 1. According to the results of soil analysis, 150 kg ha^{-1} triple superphosphate and 100 kg ha^{-1} potassium sulfate were added to soil at the soil preparation stage. Inoculation with biofertilizers including *Azospirillum brasilence* and *Azotobacter chroococcum* bacteria was done before planting the rootstock for 10 min.

Table 1. Soil physical and chemical characteristics at the experimental site

Salinity dS m^{-1}	Saturation%	Lime%	Clay%	Sand%	Organic matter (%)	Available potassium (ppm)	Available phosphorous (ppm)	Total nitrogen (%)
1.3	49	17	31	25	1.1	335	4.8	0.11

Seeds were planted in 6 rows of 6 m at the distance of 50 cm from each other, with the planting depth of 2 to 3 cm. Planting was done manually on March 16th, 2016-2018 the same date every year. To guarantee uniformity and facilitate seed germination, the first irrigation was done immediately after planting. At the 4-6 leaves stage, the plants were thinned to reach the desired density (50 plants per square meter) (distance between plants was 10 cm). Manual weeding was done in 3 stages; after emergence, at the 4-6 leaves stage and at the same time as canopy closure. Also, 2 sources of nitrogen fertilizers included urea with 2.5 g nitrogen L^{-1} and nano-chelated nitrogen (nano-N) with 6 g nitrogen L^{-1} and their combination was used for foliar application. Nitrogen treatment was used at 2 stages (after thinning and before the start of flowering). A drip irrigation system was implemented. Drought stress was applied after plant establishment (stage 4 or 5 leaves).

The plant height, number of seeds/head, thousand-grain weight, number of flowers/m^2, flower dry yield, biological yield and grain yield were measured. For this purpose, the samples were collected from the two middle rows of each trial plot from the area of 3 m^2 during the growth period. To extract the essential oils, flowers were gathered from each treatment at the flowering stage, air-dried and weighed. The ground samples(25g) were distilled using 300 ml distilled water by Clevenger standard method for 3 hr. Essential oil yield was calculated as per the following formulae:

Essential oil yield (kg ha^{-1}) = Essential oil % × Flower yield (kg ha^{-1})

Proline content of the leaves was estimated spectrophotometrically, following the ninhydrin method (17).

To extract phenol, plant samples were dried and, then, macerated in 80% methanol (HPLC grade). In the next step, the samples were incubated for 24 hr at 4 °C. The incubated samples were centrifuged at 2000 rpm for 15 min and the supernatants of the extracts were collected for further analysis. Finally, another standard method was used to analyze the phenol content (18). To measure the activity of catalase, peroxidase and superoxide dismutase enzymes, the standard methods (19-21).

Statistical analysis: The performed statistical analyses included the Shapiro-Wilk normality test and analysis of variance, carried out in SAS 9.2 and SPSS19 software. Comparison of the mean treatments was conducted with the SNK test at 5% probability level

Results

Based on the results of the combined analysis of variance of the data, the effect of year was only significant on plant height at a 1% probability. There was a significant difference between the levels of irrigation, nitrogen fertilizer and nitrogen biofertilizer in terms of the effect on all studied traits at a probability level of 1%. The interaction effect of irrigation with nitrogen fertilizer on plant height, number of seeds per head, biological yield, grain yield, % of flower essential oil, proline content and activity of catalase, superoxide dismutase and peroxidase was significant at a 1% probability level.

Between interaction of irrigation with nitrogen biofertilizer treatments in terms of the effect on plant height, flower dry weight, proline content, catalase activity and superoxide dismutase at a 1% probability level and in terms of effect number of grains per head, grain yield, and peroxidase activity at the level of 5% probability. The difference was significant. Finally, the difference between the interactions of chemical fertilizer and nitrogen biofertilizer in terms of the effect on flower dry weight, number of seeds per head, 1000-seed weight, biological yield, grain yield, essential oil yield and proline content at a probability level of 1% and in terms of catalase and superoxide dismutase activity at 5% probability level significant differences were observed (Supplementary Table 1).

Plant Height

In the present study, the highest plant height with the average of 34.69 and 35.20 cm respectively, was allocated to nanocholate nitrogen and nitrogen + nanocholate nitrogen foliar application. The lowest plant height with the average of 39.29 cm was allocated to nitrogen foliar application under water stress (Supplementary Table 2).

Among the interactions of biofertilizer and irrigation treatments, the highest plant height with the average of 35.83 cm was the inoculation treatment with *Azotobacter* under normal irrigation conditions. But the difference between this treatment and control treatment (without inoculation) and inoculation with *Azospirillum* was not significant. The lowest plant height among these treatments was related to control and *Azospirillum* inoculation with the average of 29.54 and 30.51 cm respectively (Supplementary Table 4).

Flower dry weight

According to the results of the present study, under normal conditions, the highest dry flower weight was allocated to control and seed inoculation treatments with *Azospirillum* and *Azotobacter* with the average of 5.73, 5.77 and 5.91 g / m^2 respectively; the difference between them was not significant (Supplementary Table 2). The lowest value of the mentioned trait with the average of 4.53 g was allocated to the control treatment under drought stress conditions. In this study, seed inoculation with *Azospirillum* significantly increased flower dry weight compared to the control treatment.

Results of mean comparisons showed that nitrogen + nano-nitrogen fertilizer foliar application along with with *Azospirillum* seed inoculation had the highest dry flower weight with the average of 5.81 g/m^2, while the lowest value of the mentioned trait with the average of 4.89 g/m^2 was allocated to the nitrogen fertilizer foliar application and non-inoculation of the seeds with biofertilizer (Fig. 1).

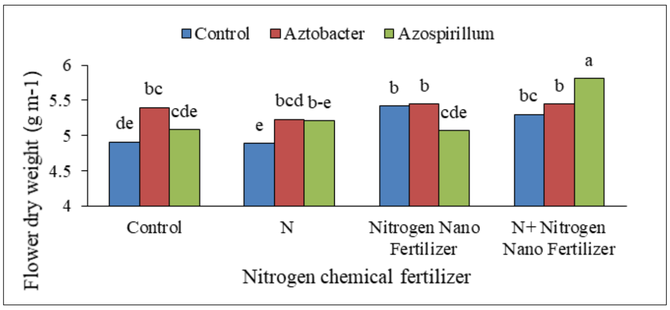

Fig. 1. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on flower dry weight SE = 0.163.

Number of seeds per head

The results showed that nitrogen + nano-chelate nitrogen foliar application treatment under normal irrigation conditions with the average of 23.45 grains had the highest, and nitrogen foliar application treatment under drought stress conditions with the average of 18.22 grains had the lowest number of grains per head. In this study, in both environmental conditions, nitrogen + nano-chelate nitrogen foliar application significantly increased the number of grains per head compared to the control treatment (Supplementary Table 3).

The results revealed that under normal irrigation conditions, seed inoculation with *Azospirillum* and *Azotobacter* showed the highest number of seeds with the average of 23.11 and 23.72 seeds respectively. The lowest number of seeds was recorded with the average of 18.23 and 18.58 for control treatment and seed inoculation with *Azotobacter*; in both environmental conditions, seed inoculation with *Azospirillum* was able to significantly increase the number of seeds per head compared to the control treatment (Supplementary Table 4).

Comparison of the mean of interaction treatments showed that the highest number of grains per head was recorded for nitrogen + nano-chelate nitrogen treatment with seed inoculation with *Azospirillum* and the average of 27.7 grains. It should be noted that no significant difference was found between the mentioned treatment and control treatment of chemical fertilizer along with the inoculation of seeds with *Azotobacter*, nano-chelate nitrogen treatment along with seed inoculation with *Azotobacte* and nitrogen + nano-chelate nitrogen treatment along with inoculation of seeds with *Azotobacter* and *Azospirillum*. The lowest number of seeds with the average of 19.59 seeds was allocated to the control treatment of biofertilizer and nitrogen foliar application (Fig. 2).

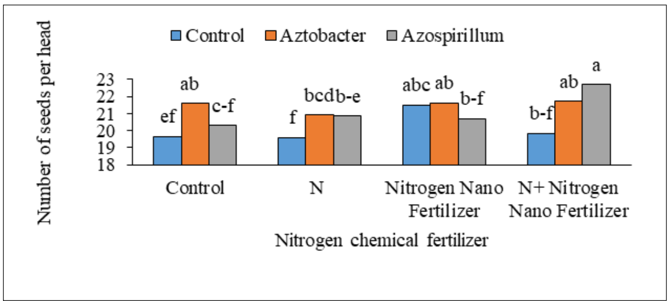

Fig. 2. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on number of grains per head. SE = 0.633.

Thousand kernel weight

Mean comparison of the interaction treatments in terms of thousand kernel weight showed that nitrogen foliar application with seed inoculation with nitrogen with the average of 14.49 g and nitrogen + nano-chelate nitrogen treatment with seed inoculation with *Azospirillum* with the average of 14.69 g had the highest thousand kernel weight.

The lowest value of this trait with the average of 11.14 g was allocated to the control treatment of both treatments. In this study, in the control treatment of chemical fertilizer and nitrogen + nano-chelate nitrogen, seed inoculation with *Azotobacter* and *Azospirillum* and the bano-chelate nitrogen treatment, seed inoculation with *Azotobacter* significantly increased thousand kernel weight compared to the control treatment (Fig. 3).

Grain yield

In the present study, application of nitrogen + nano-chelate nitrogen under normal irrigation conditions had the highest grain yield with the average of 2361.9 kg/ha.

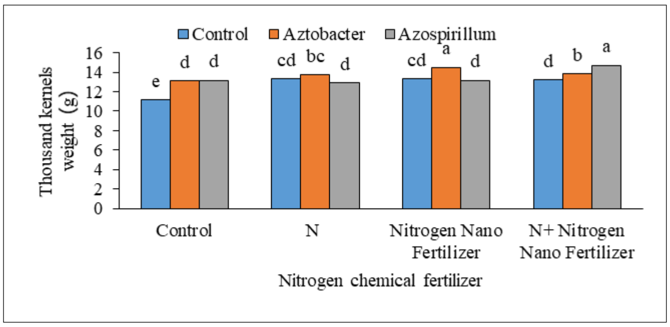

Fig. 3. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on thousand kernel weight SE = 0.244.

The lowest grain yield with the average of 1329.3 kg/ha was allocated to the control chemical treatment under drought stress conditions (Supplementary Table 3).

Results of mean comparison of interaction treatments showed that seed inoculation with *Azotobacter* and *Azospirillum* under normal irrigation conditions with the average of 2169.5 and 2063.6 kg/ha respectively, had the highest grain yield. Biofertilizer control treatment under drought stress conditions with the average of 1297.5 kg/ha showed the lowest grain yield (Supplementary Table 4).

In the present study, nitrogen + nano-chelate nitrogen foliar application along with seed inoculation with *Azotobacter* and *Azospirillum* with the average of 2206 and 2005.3 kg/ha respectively produced the highest grain yield. The lowest grain yield was recorded with the average of 1465.9 kg/ha for foliar application of nitrogen fertilizer and control treatment of biofertilizer (Fig. 4).

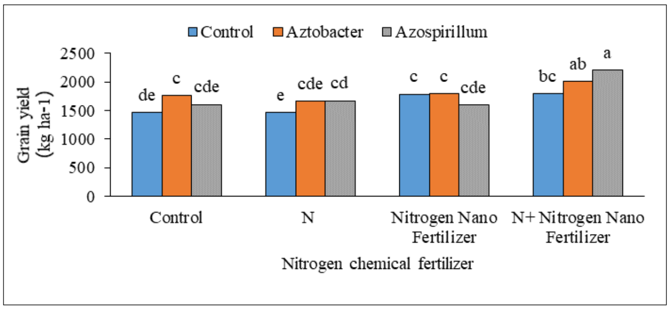

Fig. 4. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on grain yield. SE = 101.15.

Biological Yield

Results showed that nano-chelate nitrogen and nano-chelate nitrogen + nitrogen spraying under normal irrigation conditions with the average of 17855 and 18006 kg/ha respectively had the highest biological yield. The lowest biological yield was assigned to chemical control treatment under drought stress conditions with the average of 10733 kg/ha (Supplementary Table 3).

In the present study, nitrogen + nano-chelate nitrogen foliar application with seed inoculation with the average of 17450 kg / ha had the highest biological yield. While the control treatment of both treatments (no foliar application and no seed inoculation) with the average of 11164 kg / ha had the lowest biological yield, based on the results of the present study in the control treatment of chemical fertilizer and nitrogen foliar application seed inoculation with *Azospirillum* and in nitrogen + nano-chelate nitrogen treatment, seed inoculation with *Azotobacter* and *Azospirillum* was able to increase biological yield compared to the control treatment significantly (Fig. 5).

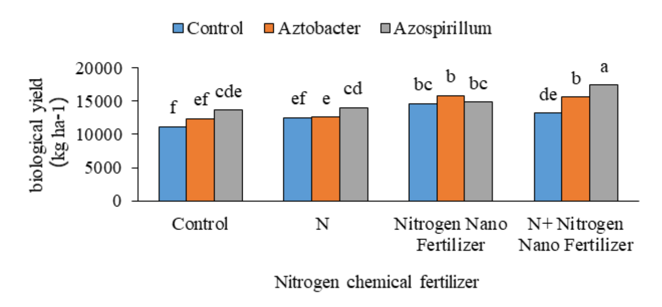

Fig. 5. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on biological yield. SE = 7.04.

Flower essence percentage and essence yield

The results showed that the highest flower essence percent with the average of 0.247 and 0.251% belonged to the control treatment and foliar application with nano-chelate nitrogen under drought stress conditions. Furthermore, the application of nano-chelate nitrogen and nano-chelate nitrogen + nitrogen under normal irrigation conditions with the average of 0.167 and 0.163% respectively had the lowest flower essence percent (Supplementary Table 3).

In the present study, foliar application of nitrogen + nano-chelate nitrogen and nitrogen and nano-chelate nitrogen under normal irrigation conditions with the average of 14.92, 14.30 and 14.44 kg/ha respectively showed the highest essential oil yield. The lowest essential oil yield with the average of 8.59 kg/ha was assigned to the control treatment of chemical fertilizer under drought stress conditions (Supplementary Table 3).

Among the interactions of chemical fertilizer and inoculation with biofertilizer treatments, the highest essential oil yield with the average of 14.2 kg/ha was allocated to nitrogen foliar application with seed inoculation with *Azotobacter*. There was no significant difference between the mentioned treatment with nitrogen + nano-chelate nitrogen treatment with seed inoculation with *Azospirillum*. In this study, chemical fertilizer and biofertilizer control treatments with the average of 9.26 kg/ha had the lowest essential oil yield (Fig. 6).

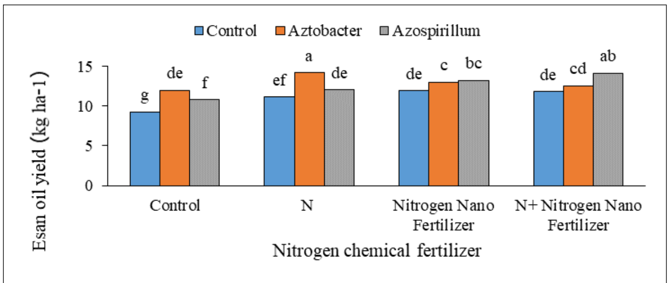

Fig. 6. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on essential oil yield. SE = 0.452.

Proline content

In the present study, drought stress increased the activity of proline Also, foliar application of nitrogen under these conditions increased the content of these enzyme and the highest activity of proline was obtained from nitrogen foliar application under drought conditions (Supplementary Table 3).

Among the biological fertilizer and irrigation treatments, seed inoculation with *Azospirillum* under drought stress showed the highest activity of proline. The lowest amount of leaf proline was allocated to chemical fertilizer control and biofertilizer control under normal irrigation conditions (Supplementary Table 4).

In the present study, the highest leaf proline content was obtained in nano-chelate nitrogen + nitrogen foliar application with seed inoculation with *Azospirillum* and nitrogen foliar application with seed inoculation with *Azotobacter*. These treatments increased leaf proline content compared to the control treatment and the simultaneous application of chemical and biological fertilizers significantly (Fig. 7).

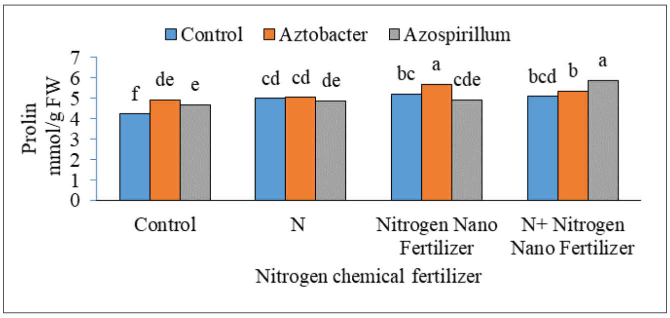

Fig. 7. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on proline. SE = 0.081.

Antioxidant properties

Catalase

In the present study, seed inoculation with *Azotobacter* in chemical fertilizer control treatment and seed inoculation with *Azospirillum* in nitrogen foliar application treatment could significantly increase catalase content in comparison with chemical fertilizer and biofertilizer control treatment (Fig. 8).

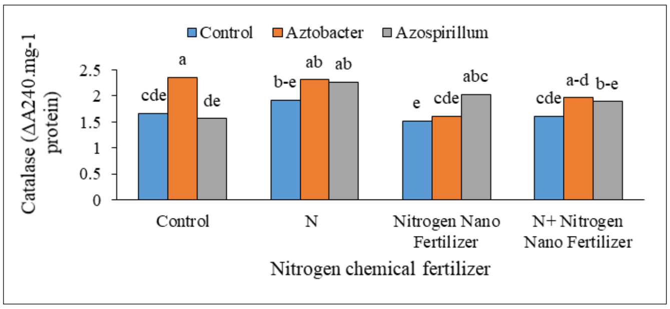

Fig. 8. Mean comparison of chemical fertilizer with nitrogen biofertilizer interaction in terms of the effect on catalase. SE = 0.216.

In this study, the highest activity of catalase, superoxide dismutase and peroxidase enzymes were obtained in response to seed inoculation treatment with *Azospirillum*. The lowest activity of these enzymes was recorded under normal irrigation conditions and biofertilizer control treatment (Supplementary Table 4).

Discussion

The results of the present study showed that, although drought stress reduced plant height, nano-chelate nitrogen foliar application was able to moderate the adverse effect of drought stress on plant height. Nano-chelate nitrogen foliar application in both environmental conditions showed a positive effect on increasing plant height. Water deficit can reduce plant growth and plant height by reducing the adequate moisture in the rhizosphere, nutrient uptake, vitality and cellular extension as well as photosynthesis (22). Under normal irrigation conditions, inoculation of seeds with biofertilizers could not increase plant height compared to control treatment; but under drought stress, inoculation with *Azotobacter* can counteract the adverse effect of drought on plant height.

The application of biofertilizers has a positive effect on improving plant nutritional conditions and nutrient uptake under drought stress. The ability of *Azotobacter* to increase the solubility of phosphorus from inorganic insoluble compounds has been proven; this ability of *Azotobacter* can be used to increase solubility, transport and absorption of nutrients. *Azotobacter* can instate the proper rate between nitrogen and phosphorus by producing the suitable hormones, reducing ethylene synthesis and, finally, increasing vegetative growth and plant height. In the present study, nitrogen fertilizer in both forms increased plant height. It has been noted that nitrogen deficiency can limit plant height by inhibiting parenchyma and sclerenchyma development, but nitrogen adequacy can improve plant height by raising the meristem cells division and turgidity. In another study, it was showed that water deficit and nitrogen deficiency reduced plant height in marigold (23).

The results showed that the flower dry weight under normal irrigation conditions was not affected by biofertilizer treatments, while under drought conditions, seed inoculation with *Azospirillum* was able to significantly increase the flower dry weight compared to the control treatment. Also, inoculation of seeds with *Azospirillum* along with foliar application of nitrogen + nano-nitrogen fertilizer had a synergistic effect on increasing flower dry weight. In the study on strawberry, it was revealed that the combination of *Azotobacter* with 100% of the recommended nitrogen fertilizer increased growth and yield indices (16). Biofertilizers have a significant role in nitrogen uptake and effective use of this element in the photosynthetic process and production of green surface (24). The effect of drought stress on flower dry weight reduction can be related to reduced carbon storage and dry matter reduction due to reduced absorption, transport and consumption of nutrients (25). A study on marigold revealed that water shortage stress significantly reduced the flower dry weight (23).

The results showed that drought stress significantly reduced the number of grains per head and grain yield. But nitrogen + nano-chelate nitrogen foliar application as well as seed inoculation with *Azospirillum* could increase the number of seeds per head and grain yield in drought stress conditions compared to the control treatment. It should be noted that the highest number of grains per head and grain yield were obtained in nitrogen + nano-chelate nitrogen, foliar application as well as seed inoculation with *Azotobacter* and *Azospirillum* under normal conditions.

In this study, the highest yield and grain yield components were obtained in nitrogen + nano-chelate nitrogen foliar application along with seed inoculation with *Azospirillum* and *Azotobacter*.

The results also showed that under both environmental conditions, foliar application of chemical fertilizers significantly increased biological yield compared to the control treatment. Also, the highest biological yield was obtained from nitrogen + nano-chelate application and seed inoculation with *Azotobacter* and *Azospirillum*, which showed the synergistic effect of the two treatments in improving biological yield. Therefore, the combination of nitrogen chemical fertilizer with biological fertilizer had a beneficial effect on increasing grain yield and its components in marigolds. We assumed that the solubilization capacity of *Azotobacter* and *Azospirillum* may have improved N availability in the soil and the plants could absorb the necessary amount of nutrients.

Decreased grain yield under water limitation stress can be associated with reduced production of photosynthetic material for grain filling or reduced absorb photoassimilates of the sink for photosynthetic material and reduced grain filling period. Also, it has been stated that a decline in grain yield is probably due to a premature cessation of sucrose activity, a decline after anthesis photosynthesis and remobilizable assimilate (26) and has been suggested to be the major reason for below drought stress situations (27). The positive effect of nitrogen fertilizer application on 1000-seed weight and grain yield in marigold was also reported (28). Reports ar on the increased grain yield and yield components fennel (*Foeniculum vulgare* Mill.) under water deficit conditions (12). Reports are also on the highest biomass and grain yield in chickpeas was recorded for bio-inoculation + N fertilization treatment (14). In another study on maize, the highest green fodder yield was reported in the application of 100% of the recommended chemical fertilizer along with seed inoculation with AM + *Azospirillum* (29). In a study, the highest grain yield in sesame was recorded in the combination of 50% urea application, seed inoculation with nitroxin and KO_2 application under normal irrigation conditions (15).

The results of the present study showed that nano-chelate nitrogen foliar application decreased the essential oil content under normal irrigation conditions and increased the mentioned trait under water stress conditions. The accumulation amount of secondary metabolites in the plant increased in response to environmental stresses such as drought (30). It was reported that drought stress increased the essential oil percentage in Valerian (31).

Results revealed that drought stress reduced the essential yield. However, foliar application of all three fertilizer treatments increased the essential yield in both environmental conditions compared to the control treatment. The positive effect of nitrogen application on oil yield can be attributed to the role of these treatments in increasing the flower dry weight component of essential yield. Given that there is a direct relationship between oil yield and flower dry weight, such results were not unexpected. The most important reasons for increasing essential oil yield in medicinal and aromatic plants due to nitrogen application are increasing photosynthesis produced during photosynthesis, increasing the content of photosynthetic pigments, increasing the activity of Rubisco enzyme and increasing the photosynthetic surface of plants and biomass (32, 33). In a study on fennel (*Foeniculum vulgare* Mill.). It was found under water deficit conditions application of biofertilizer had a positive effect on essential oil and essential oil yield (12). In the study on chickpea, application of 75 Kg/ha of nitrogen fertilizer along with seed inoculation with biofertilizer recorded the highest grain yield (14).

Observations are on the highest essential oil yield was assigned to treatment of 100 and 75% nitrogen fertilizer with inoculation and non-inoculation treatments with biofertilizer (34). The application of *Azotobacter* biofertilizer significantly increased the essential oil content (35). The positive effect of inoculation with biofertilizers on increasing the yield of essential oil has been also reported on *Cuminum cyminum* L., *Coriandrum sativum* L. and valerian (*Valeriana officinalis* L.) respectively (31, 36, 37).

As shown, drought stress and foliar application of nitrogen and nano-chelate nitrogen, as well as inoculation of seeds with *Azospirillum* and *Azotobacter* induced proline synthesis in the leaves .One of the most important osmoprotectants in plants is proline. Proline synthesis is induced in response to water limitations and improves the ability of water holding in plants by regulating the osmotic potential (38).

In the study on Valerian, it was found that drought stress and application of biofertilizers increased leaf proline content simultaneously (31).

In the present study, the highest activity of 2 enzymes, catalase and superoxide dismutase, was recorded in response to drought and nitrogen foliar application. Also, under drought stress, inoculation of seeds with *Azotobacter* significantly increased the activity of catalase, superoxide dismutase and peroxidase. An increase in the content of the antioxidant enzymes under water deficit conditions could indicate high production of ROS and build-up a protective mechanism to reduce oxidative damage triggered by stress imposed in plants (39). It seems that inoculation with biofertilizers by increasing the activity of antioxidant enzymes in the plant has been able to increase plant protection against environmental stresses (39). In a study on sunflower, the activity of SOD, CAT and GPX enzymes showed a significant increase under water limitation and inoculation with *Azotobacter* and *Azospiril* biofertilizers (13). In another study on sesame, the highest activity of antioxidant enzymes (Catalase and Peroxides) was reported in the treatment of 50% urea fertilizer + seed inoculation with nitroxin (15). In a study on olive, foliar application of urea and nano-chelate nitrogen significantly increased the activity of antioxidant enzymes (40).

Conclusion

The purpose of planting marigold is to extract the essential oil and the most important trait of this plant is essential oil yield. In this study, foliar application of nitrogen in both environmental conditions increased the essential oil yield. Also, inoculation of seeds with *Azotobacter* and *Azospirillum* under normal irrigation conditions and inoculation of seeds with *Azospirillum* under drought conditions could increase the essential oil yield in marigold. In addition, the simultaneous use of nitrogen + nano-chelate nitrogen treatment with seed inoculation with *Azospirillum* had the highest essential oil yield. It can be concluded that the separate and simultaneous application of nitrogen fertilizers by improving vegetative characteristics and regulating the activity of antioxidant enzymes could have a positive effect on increasing the economic yield of crops. Therefore, the use of mineral and biological resources of nitrogen can be a way to improve the economic yield of the medicinal plant marigold, which faces different periods of water shortage stress during growth.

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Authors contributions

AP, SS and TMM carried out the experiment. AP and TMM wrote the manuscript with support from SYS and TMM.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Supplementary Table 1. Combine analysis of variance of irrigation regime and nitrogen levels on studied traits in medicinal *Calendula officinalis*

Supplementary Table 2. Mean comparison of main effects of the year, irrigation, nitrogen fertilizer sources on studied traits of *Calendula officinalis*

Supplementary Table 3. Mean comparison of the interactive effects of irrigation and nitrogen levels on studied traits of *Calendula officinalis*

Supplementary Table 4 .Mean comparison of the interactive effects of irrigation and bio- fertilizer sources on studied traits of *Calendula officinalis*

References

1. Amirghofran Z, Azadbakht M, Karimi MH. Evaluation of the immunomodula tory effects of five herbal plants. J Ethnopharmacol. 2000;72:167-72. https://doi.org/10.1016/S0378-8741(00)00234-8

2. Lambers H, Chapin FS, Pons T L. Plant Physiological Ecology. New York: 2008; Springer. https://doi.org/10.1007/978-0-387-78341-3

3. Earl H, Davis RF. Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. J Agron. 2003;95 (3):688-96 https://doi.org/10.2134/agronj2003.0688

4. Lauer J. What happens within the corn plant when drought occurs? University of Wisconsin Extension, 2003; http://corn.agronomy.wisc.edu/WCM/W137.aspx

5. Etehadnejad F, Aboutalebi A. Evaluating the effects of foliar application of nitrogen and zinc on yield increasing and quality improvement of apple cv.'golab kohanz'. Indian Journal of Fundamental and Applied Life Sciences. 2014;4:125-29.

6. Wijesinghe WPSL, Weerasinghe AMCP. Development of nano fertilizers as slow release fertilizers. Sciscitator. 2015;2:28-29.

7. Vishwakarma K, Upadhyay N, Kumar N, Tripathi D.K, Chauhan DK, Sharma Sh, Sahi S. Potential applications and avenues of nanotechnology in sustainable agriculture. In: Tripathi DK, Ahmad P, Sharma S, Chauhan DK, Dubey NK. (Eds). Nanomaterials in Plants, Algae and Microorganisms (concepts and controversies Vol. 1). 2018; Elsevier. https://doi.org/10.1016/B978-0-12-811487-2.00021-9

8. Wu H, Shabala L, Shabala S, Giraldo JP. Hydroxyl radical scavenging by cerium oxide nanoparticles improves Arabidopsis salinity tolerance by enhancing leaf mesophyll potassium retention. J Environ Sci. 2018;(5):1567-83. https://doi.org/10.1039/C8EN00323H

9. Itelima JU, Bang WJ, Onyimba IA, Sila MD, Egbere OJ. Biofertilizers as key player in enhancing soil fertility and crop productivity: (A Review). Direct Res J Agric and Food Sci. 2018;6 (3):73-83.

10. Mrkovacki N, Milic V. Use of *Azotobacter chroococcum* as potential useful in Agricultural. Application Ann Microbiol. 2001;51:145-58.

11. Sturz AV, Christie BR. Beneficial microbial alleloplathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Res. 2003;72(2):107-23. https://doi.org/10.1016/S0167- 1987(03)00082-5

12. Azizi KH. Biofertilizers and drought stress effects on yield and yield components of Fennel (*Foeniculum vulgare* Mill). Journal of Medicinal Plants and by-Products. 2017;1:17-25.

13. Khademiana R, Ghassemib S, Asgharic B. Bio-fertilizer improves physio-biochemical characteristics and grain yield of safflower (*Carthamus tinctorius* L.) under drought stress. Russ Agric Sci. 2019;45(5):458-63. https://doi.org/10.3103/S1068367419050124

14. Khaitov B, Abdiev A. Performance of chickpea (*Cicerarietinum* L.) to bio-fertilizer and nitrogen application in arid condition. J Plant Nutr. 2018;41(15):1980-87. https://doi.org/10.1080/01904167.2018.1484134

15. Mahdavi Khorami A, Masoud Sinaki J, Amini Dehaghi M, Rezvan S, Damavandi A. Sesame (*Sesame indicum* L.) biochemical and physiological responses as affected by applying chemical, biological and nano-fertilizers in field water stress conditions. J Plant Nutr. 2020;43(3): 456-75. https://doi.org/10.1080/01904167.2019.1683189

16. Chandramohan Reddy G, Goyal RK. Growth, yield and quality of strawberry as affected by fertilizer N rate and biofertilizers inoculation under greenhouse conditions. J Plant Nutr. 2020:1-13. https://doi.org/10.1080/01904167.2020.1806301

17. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-07. https://doi.org/10.1007/BF00018060

18. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51:609-14. https://doi.org/10.1021/jf020782a

19. Aebi H. Catalase *in vitro*. Methods in Enzymology. Academic Press. 1984;105:121-26. https://doi.org/10.1016/S0076-6879(84)05016-3

20. Chance B, Maehly AC. Assay of catalase and peroxidase. Methods in Enzymology. 1995;2:764-75. http://dx.doi.org/10.1016/S0076-6879(55)02300-8

21. Beyer WF, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem. 1984;161:559-66. https://doi.org/10.1016/0003-2697(87)90489-1

22. Cregg B. Improving drought tolerance of trees: theoretical and practical considerations. XXVI International Horticultural Congress: Nursery Crops; Development, Evaluation, Production and Use 630, Toronto, Canada, January 14, 2002; pp. 147-58. https://doi.org/10.17660/ActaHortic.2004.630.18

23. Moosavi SG, Seghatoleslami MJ, Fazeli-Rostampoor M, Jouyban Z. Response of marigold flower yield and yield components to water deficit stress and nitrogen fertilizer. J Ornam Hortic. 2014;4(3):153-62.

24. Kader M.A. Effects of Azotobacter inoculants on the yield nitrogen uptake by wheat. J Biol Sci. 2002;2:259-61. https://doi.org/10.3923/jbs.2002.259.261

25. Hu Y. Schmidhalter U. Drought and salinity: A comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sci. 2005;168:541-49. https://doi.org/10.1002/jpln.200420516

26. Azhand M, Saeidi M, Abdoli M, Khas-Amiri M. The impact of source limitations on yield formation, storage capacity and contribution of stem reserves to the growing grains of modern barley cultivars under post-anthesis water deficiency. Plant Knowledge Journal. 2015;4(1):13-24.

27. Saeidi M, Moradi F, Abdoli M. Impact of drought stress on yield, photosynthesis rate and sugar alcohols contents in wheat after anthesis in semiarid region of Iran. Arid Land Res Manag. 2017;31(2):204-18. https://doi.org/10.1080/15324982.2016.1260073

28. Jevdovic R, Todorovic G, Kostic M, Protic R, Lekic S, Zivanovic T, Secanski M. The effects of location and the application of different mineral fertilizers on seed yield and quality of pot marigold (*Calendula officinalis* L.). Turkish J Field Crop. 2013;18(1):1-7.

29. Chandra Sharma R, Banik P. Arbuscular mycorrhiza, azospirillum and chemical fertilizers application to baby corn (*Zea mays* L.): effects on productivity, nutrients use efficiency, economic feasibility and soil fertility. J Plant Nutr. 2014;37(2):209-23. https://doi.org/10.1080/01904167.2013.859692

30. Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav. 2011;6(11):1720-31. https://doi.org/10.4161/psb.6.11.17613

31. Javan Gholilooa M, Yarniab M, Hassanzadeh Ghorttapehc A, Farahvashd F, Mohammad Daneshiane A. Evaluating effects of drought stress and bio-fertilizer on quantitative and qualitative traits of valerian (*Valeriana officinalis* L.) J Plant Nutr. 2019;42 (13):1417-29. https://doi.org/10.1080/01904167.2019.1628972

32. Sifola MI, Barbieri G. Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Sci Hortic. 2006;108:408-13. https://doi.org/10.1016/j.scienta.2006.02.002

33. Sekeroglu N, Ozguven M. Effects of different nitrogen doses and plant densities on yield and quality of *Oenothera biennis* L. grown in irrigated lowland and un-irrigated dryland conditions. Turk J Agric For. 2006;30:125-35.

34. Yousefzadeh S, Mohammad Modarres Sanavy SA, Govahi M, Sadat Khatamian Oskooie O. Effect of organic and chemical fertilizer on soil characteristics and essential oil yield in dragon-

head, J Plant Nutr. 2015;38(12):1862-76. https://doi.org/10.1080/01904167.2015.1061548

35. Leithy S, El-Meseiry TA, Abdallah EF. Effect of biofertilizers, cell stabilizer and irrigation regime on rosemary herbage oil yield and quality. Res J Appl Sci. 2006;2:773-79.

36. Rebey IB, Jabri-Karoui I, Hamrouni-Sellami I, Bourgou S, Limam F, Marzouk B. Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Ind Crops Prod. 2012;36:238-45. https://doi.org/10.1016/j.indcrop.2011.09.013

37. Hassan FAS, Ali EF. Impact of different water regimes based on class- A pan on growth, yield and oil content of *Coriandrum sativum* L. Plant. J Saudi Soc Agric Sci. 2014;13(2):155-61 https://doi.org/10.1016/j.jssas.2013.05.001

38. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: Effects, mechanisms and management. Agron Sustain Dev. 2009;29:185-212. https://doi.org/10.1051/agro:2008021

39. Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang YP, Guo JH. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One. 2012;7:52565. https://doi.org/10.1371/journal.pone.0052565

40. Rohi Vishekaii Z, Soleimani A, Hasani A, Ghasemnezhad M, Rezaei K, Kalanaky S. Nano-chelated nitrogen fertilizer as a new replacement for urea to improve olive oil quality. Int J Hortic Sci Technol. 2021;8(2):191-201.

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