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ORIGINAL ARTICLE

Effect of Phosphate and Nitrogene Bio-fertilizers on yield, yield components, oil and protein in sunflower (*Helianthus annus* L.)

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ABSTRACT
This experiment was laid out in order to study on effect of P and N Bio fertilizers on yield, yield components, oil and protein of sunflower (Helianthus annus L.) at Islamic Azad University, Boroujerd branch, Iran (Experimental farm: Songhor, Kermanshah province, Iran) at 2014. The experiment was laid out in a factorial design based on randomized block design with three replications. Treatments were Nitrogen bio-fertilizers in four levels (N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin and N_4=control) and phosphor biofertilizer in four levels (P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control) with control for them. Analysis of variance results showed that effect of P and N-biofertilizer yield and all yield components and seed oil and protein were significant at 1% and interaction between them on all treat were significant except plant height and seed protein percent. Nitrokara biofertilizer treatment had the highest plant height. Also, combined application of Nitroxin and Biozar had the highest 1000 grain weight and HI. This study shows that application of Nitroxin other P biofartilizers compared to non application of Nbiofertilizers had a better effect on biomass, seed yield, oil and protein. In addition, our results indicated that the combination of integrated fertilizers, using N and P fertilizer, improved the growth and quality of sunflower plants.
Key words: N and P Bio-fertilizer, sunflower, quality and Yield

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INTRODUCTION

Sunflower (*Helianthus annuus* L.), belonging to the family Compositae, is a major oilseed, used for the production of edible oil, with an annual production of about 25 million tons is one of the most important oil seeds in arid and semi-arid regions [12]. This oily plant is one of the main suggested oil crops to solve edible vegetable oil shortage in the world that seeds contain 24-49% of oil and the cake contains 25-35% of protein [13].

Bio-fertilizers play vital role for increasing the number of microorganisms and accelerate certain microbial process in plants which can change the available form of many nutrients for crops [1, 8, 19].Although nitrogen is the key element in increasing productivity but large rates of fertilizer N loss to the environment could cause a serious environmental problem such as groundwater contamination. Nitrogen fertilizer application and seed biopriming with PGPR can increase quantitative and qualitative yield of Safflower [23].

Bio-fertilizers have a positive effect on growth, yield and yield component of many crops. Fertilizer management is one of the most important factors in successful cultivation of crops affecting yield quality and quantity [26]. Overuse of different chemical fertilizers is one of the causes for the degradation of environment and soil. Bio fertilizers are the newest and most technically advanced way of supplying mineral nutrients to crops. Compared to chemical fertilizers, their supply nutrient for plant needs, minimizes leaching, and therefore improves fertilizer use efficiency [23]. Soleimani [25] reported that application of nitrogen fertilizer increased grain yield of safflower.

Tilak et al [28] reported improving grain yield of maize due to seed inoculation with Azotobacter and Azospirillum. Azimi *et al* [5] found that application of Super nitroplass biofertilizer with Phosphate barvar2 treatment has the highest seed yield (7.6 ton/ha) and non-application of biofertilizers treatment has the Pishtaz cultivar has the lowest seed yield (6.3 ton/ha). They told that for gave the highest seed

yield we should apply both nitrogen and phosphate biofertilizers. Although most agricultural soils have large amounts of inorganic and organic P, these are immobilized and mostly unavailable. Therefore, only a very low concentration of P is available to plants and many soils are actually P deficient [2]. Hence, alternative strategies to increasing P availability and crop yield are required. The application of soil microorganisms is regarded to be a promising approach in this context. Among the whole microbial population in soil, bacteria are also more effective in phosphorus solubilization [15].

Beyranvand et al [10] reviled that application nitrogen and phosphate biofertilizers increased yield and yield components of maize under Boroujerd environmental condition (10). They suggested that effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in plant height, ear weight, and number of grain per cob, grain yield and biomass yield. Also Azimi et al (2013b) found that that application nitrogen and phosphate biofertilizers increased yield and yield components of barley under Boroujerd environmental condition. They suggested that Grain yield and biomass yield increasing was reported with the biofertilizer application which account important benefit, causing decreasing in the inputs of production because of economizing much money to chemical fertilizers and increasing in yield and biological yield [6].

Therefore the aim of this study is evaluation of effects of N and P Bio fertilizers on yield and yield components of sunflower in Kermanshah province, Iran.

MATEREALS AND MTHODS

Field material and Experimental design

This experiment was laid out in order to study on effect of P and N Bio fertilizers on yield, yield components, oil and protein of sunflower (*Helianthus annus* L.) at Islamic Azad University, Boroujerd branch, Iran (Experimental farm: Songhor, Kermanshah province, Iran) at 2014. The experiment was laid out in order to evaluate the effects of nitrogen and phosphate bio fertilizers on yield components, oil and protein of sunflower. The Songhor region has a continental semi-arid climate with annual precipitation of 450 mm.

Treatments

The experiment was laid out in a factorial design based on randomized block design with three replications. Treatments were Nitrogen bio-fertilizers in four levels (N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin and N_4=control) and phosphor biofertilizer in four levels (P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control) with control for them.

Yield and yield components determination

There were 4 rows in each from 16 plot with 0.5 m row spacing. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle 1 m^2 of the tow central rows were harvested. Then yield components were calculated as standard methods with using 8 plant. To determine grain yield, biomass yield and harvest index, we removed and cleaned all the seeds produced within two central rows in the field. Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare. Replicated samples of clean seed (separated grain and foreign material removed) were sampled randomly and 1000-grain were counted and weighed. The harvest index was accounted with follow:

HI = (Economical yield / Biological yield)*100

Seeds oil was determined by Sucsilate machine .Then oil yield was determined by following formula:

Oil yield= % oil × seed yield

Seed nitrogen determined and total protein obtained by following formula:

Protein= %nitrogen×0.54

Protein yield was determined by following formula:

Protein yield= % protein × seed yield

Statistical analysis

The statistical analyses to determine the individual and interactive effects of time cultivation and weeds control methods were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis [22].

RESULTS

Plant height: The results of analysis of variance showed that, the effect of N bio-fertilizers on the plant height was significant only (table 1). The comparison of the mean values for N bio-fertilizers on plant height for sunflower showed that Nitrokarabiofertilizer had the highest (211.75 cm).

Table 1. Analysis of variance (mean squares) for yield, yield components, oil and protein of sunflower under application of N and P bio-fertilizer

S.O.V	df	Plant height	1000 grain weight	biomass	grain yield	HI	Oil	Oil yield	protein	Protein yield
R	2	130.9	2.58	2161	1350	1210059	0.06	0.38	0.56	26.78
N biofertilizer	3	514.1**	531**	83234**	1154315**	1957534	2.55*	1472**	5.27**	1164**
P biofertilizer	3	51.8	236**	27586**	432347**	12712085**	6.61**	971**	4.01*	537.7**
N * P	9	611	100**	7428**	411500**	23818090**	5.95**	924**	1.56	287.5**
Error	30	34.6	13	2102	27775	2049764	0.69	61.8	0.96	46.13
CV		2.85	2.02	2.49	2.62	4.14	2.9	4.31	5.17	5.63

ns: Non-significant, * and **:Significant at 5 and 1% probability levels, respectively.

1000 grain weight: The results showed that, the effect of N and P bio-fertilizers, and interaction between them on 1000 grain weight was significant at 1% (table 1). The comparison of the mean values of the 1000 grain weight showed that combined application of Nitroxin and Biozar had the highest (200 g) and the control treatment had the lowest 1000 grain weight (173 g) and difference between them and was significant (figure 1).

Biomass yield: The effect of N and P bio-fertilizers, and interaction between them on biomass were significant at 1% (table 1). The comparison of the mean values of the biomass yield showed that combined application of Nitroxin and Superplas had the highest (2 kg/m^2) compared to control (figure 2).

Grain yield: The results showed that, the effect of N and P bio-fertilizers, and interaction between them on grain yield was significant at 1% (table 1). The comparison of the mean values of grain yield for interaction between P and N bio-fertilizer showed that application of Nitroxin with other P biofertilizers had the highest (6900 kg/ha) and differences between this treatment with other combined application of biofertilizers were significant as well (figure 3).

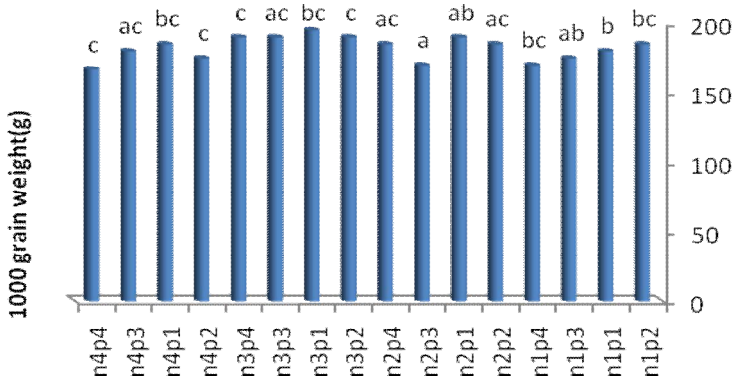

Figure 1.Interaction effect of N and P bio-fertilizers on 1000 grain weight in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

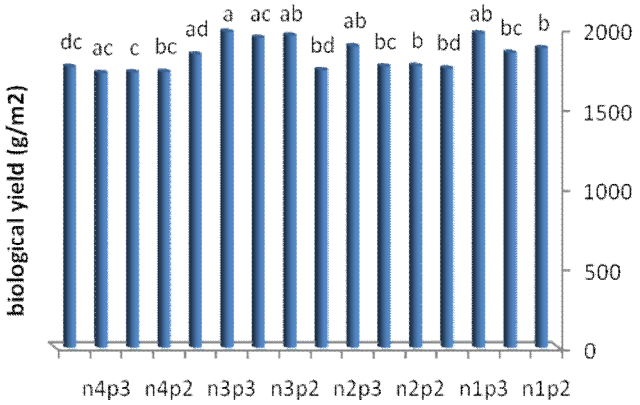

Figure 2.Interaction effect of N and P bio-fertilizers on biomass yield in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).

(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

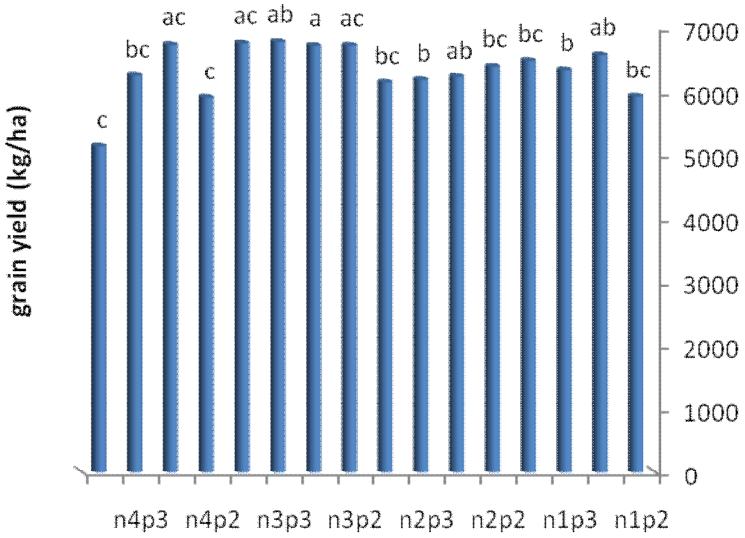

Figure 3.Interaction effect of N and P bio-fertilizers on grain yield in soybean.
Means by the uncommon letter in each column are significantly different ($p<0.05$).
(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

Harvest index (HI): The results showed that, the effect of N and P bio-fertilizers, and interaction between them on HI were significant at 1% (table 1). The comparison of the mean values of HI for P bio-fertilizer showed that single application of Biozar had the highest (40%) and the control treatment had the lowest HI (33%)(figure 4).

Seed oil: The results showed that, the effect of N and P bio-fertilizers, and interaction between them on oil percent and oil yield were significant at 1% (table 1). The comparison of the mean showed that combined application of Nitroxin and Phosphate barvar$_2$ had the highest oil percent (33%) and oil yield (230 g/m^2) and the control treatment had the lowest of them (figure 5 and 6).

Seed protein: The results showed that, the effect of N and P bio-fertilizers on seed protein percent, N and P bio-fertilizers and interaction between them on protein yield were significant at 1% (table 1). The comparison of the mean showed that single application of Nitroxin and single application of Biozar had the highest protein yield (140 g/m^2) and the control treatment had the lowest (86 g/m^2) (figure 7).

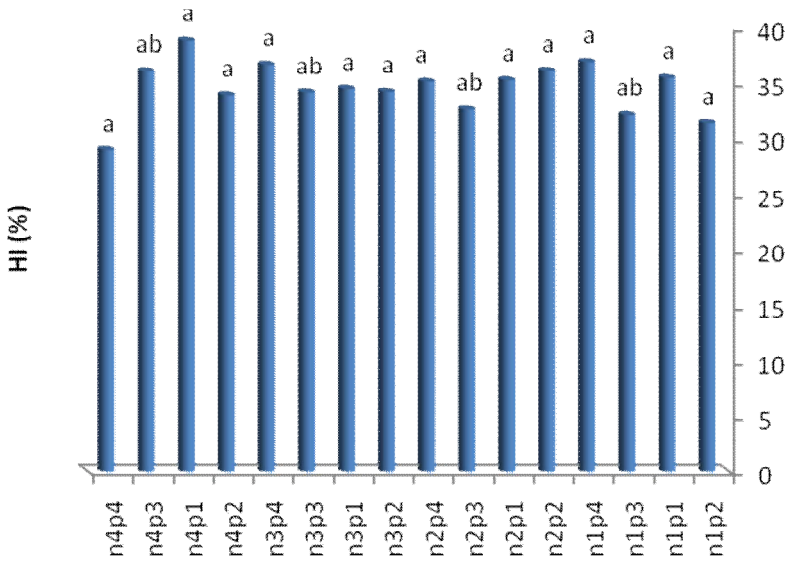

Figure 4.Interaction effect of N and P bio-fertilizers on HI in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

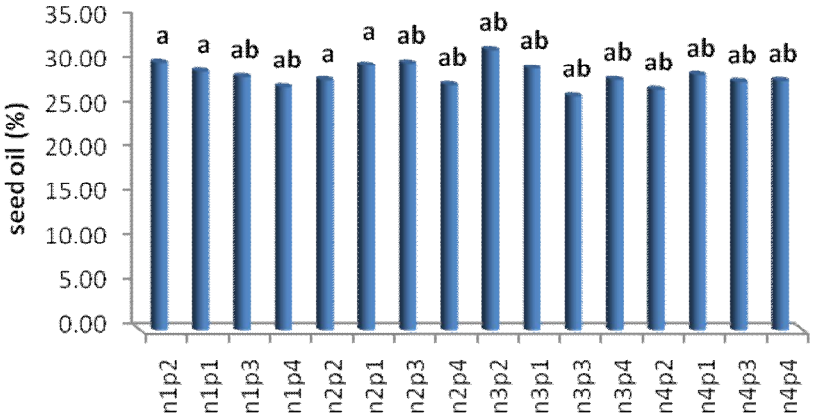

Figure 5.Interaction effect of N and P bio-fertilizers on seed oil in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

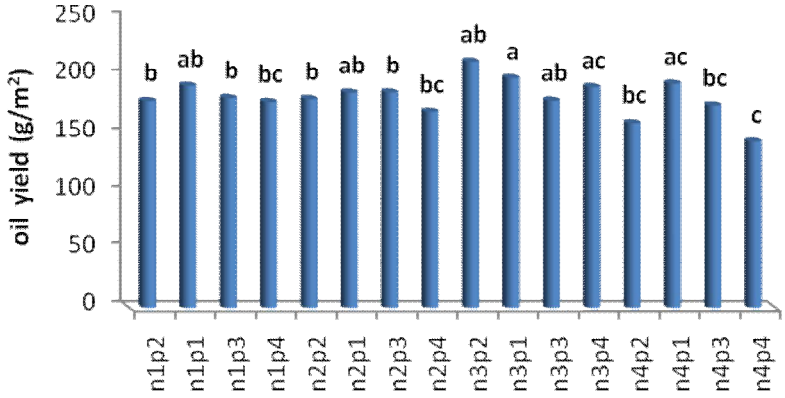

Figure 6.Interaction effect of N and P bio-fertilizers on oil yield in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

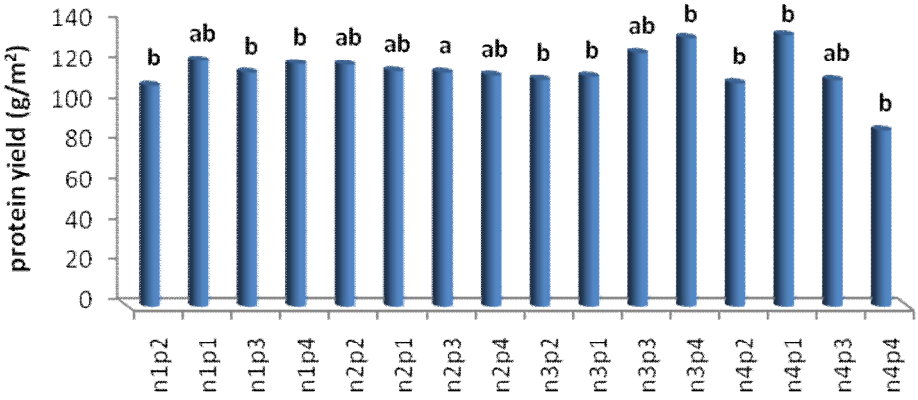

Figure 7.Interaction effect of N and P bio-fertilizers on protein yield in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(N_1=Supernitroplas, N_2=Nitrokara, N_3=Nitroxin, N_4=control, P_1=Bio-Zar, P_2=Phosphate Barbar 2, P_3=Superplas and P_4=control)

DISCUSSION

The results also showed that the weight of one thousand seeds with combined application of biofertilizers (Nitroxin and Biozar) 13% higher than that of non-application (figure 1). Biofertilizers are good tools to reduce environmental damages and enhance the yield [18]. Application of biofertilizers can increase the production efficiency of many plants and reduce the production costs. Nazarli et al [20] showed there was a significant difference between seed inoculation by Azotobacter and non-inoculation treatment concerning the weight of one thousand seeds. The results of this study showed that combined application of N and P biofartilizers had a synergistic effect on 1000 grain weight of sunflower and increased that compared to control or single application of them. Rahimi and Mazaheri [21] reported that lower weight of 1000 grain weight of sunflower in low fertilizer treatments was due to competition between seeds in gaining nutrients and reduction in plants reserved carbohydrates.

Application of Nitroxin with other P biofertilizer was more useful for yield and biomass, although application of Superplas with Nitroxin had a better effect (figure 2 and 3). Bahamin et al [7] showed that when seeds were in inculcation by Nitroxin biologic fertilizer seed yield reached 3840kg per hectare, showing 28% increase compared to non-inculcation treatment. Also Ahmad et al [3] showed that higher yield under the effects of biologic fertilizers might be because of the increase in metabolic activities of biologic fertilizers and production of growth stimulating hormones by bacteria. In the present study non application of biofertilizers had the lowest biomass and grain yield (figure 2 and 3). Nitroxinbiofertilizerconsists the most effective species of nitrogen stabilizing bacteria including the genus Azotobacter, Azospirillum and phosphate solubilizing bacteria including the genus Pseudomonas has been recommended for potato as well as other crops by producing company [26]. In higher quantities of nitrogen, investing photosynthesis materials in part of leaf and stem increased and thereby concentrated materials in seeds tends to rise [14].

The protein and oil content of sunflower are very important for human duty and animal feed. In the present study combined application of Nitroxin and Phosphate barvar had the highest oil percent (33%) and oil yield (230 g/m2) and the control treatment had the lowest of them (figure 5 and 6). Kumar [17] has found that the oil yield increased by 10 to 40% by applying various Azotobacter strains to soybean plants [17]. Also comparison of the mean showed that single application of Nitroxin and single application of Biozar had the highest protein yield (140 g/m2) and the control treatment had the lowest (86 g/m2). Basu et al. [9] have also reported that the highest protein content for integrated treatments was found with chemical and organic fertilizer. In the present study, the protein content was improved by the inoculation of N fertilizers (Nitroxin) with all other P biofertilizers, as compared to the control treatment (figure 7). Also Akbari et al [4] suggested that application of plant growth promoting rhizobacteria (PGPR) increased oil and protein content of sunflower seeds. Fallahi et al [11] founded that Nitroxin biofertilizer had significant effects on main shoot, number of flower per plant, diameter of flower, fresh flower yield, dry flower yield, seed yield, essential oil and kamauzolen yield in Chamomile. They concluded that this biofertilizer can be considered as a replacement for chemical fertilizers in Chamomile medicinal plant production. Teran and Singh [27] reported that seed yield in cowpea with pods number in plant and seed number has a significantly positive correlation while negative correlation with seed weight.

Positive effect of biofertilizer may resulted from its ability to increase the availability of phosphorus and other nutrients especially under the specialty of the calcareous nature of the soil which cause decreasing on the nutrients availability, results agree with [16]. Sharaf [24] showed that inoculation with a mixture of Azotobacter and Azospirillum with full doses of rock phosphate and inorganic N-fertilizer, in combination with inoculation with vascular arbuscularmycorrhiza, improved growth of both datura (*Datura stramonium*) and ammi (*Ammivisnaga*: Fam. *Umbelliferae*) plants.

Amounts of some macro and micro-nutrients in the environment of root were relatively abundant and soil alkalinity, can be prevent exposure these elements through the soil. However, spraying could have a positive impact on yield and yield components in unsuitable soil pH (14). According to the results, it can be concluded that by application of N and P biofertilizers specially Nitroxin and 3azotobacter yield and it components and quality increased rather than other treatments, because of synergistic effect of them. This effect of nanofertilizer might have helped seed to stay healthier for longer time and subsequently produced more vigorous plants. To the best of our knowledge, effects of nanofertilizer and N- biofertilizer on the efficiency of red bean production have not been evaluated in the field before. However, our results showed that yield and yield components of red bean increased with application of N bio-fertilizer and K nano-fertilizer.

CONCLUSION

This study shows that application of Nitroxin other P biofertilizers compared to non-application of N biofertilizers had a better effect on biomass, seed yield, oil and protein that, was observed in inoculation of seeds by Nitroxin. In addition, our results indicated that the combination of integrated fertilizers, using N and P fertilizer, improved the growth and quality of sunflower plants, with a reduction of the chemical fertilizer consumption to help preserve the environment.

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