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Effect of biological phosphate and chemical phosphorus fertilizer on quantity and quality of black cumin (*Nigella sativa* L.)

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Abstract

Nigella sativa is an annual flowering plant, native to Iran and other parts of southwest Asia, belonging to the buttercup family (*Ranunculaceae*). Its grain has several important health-related properties such as anti-parasitic, anti-virus, anti-bacterial, increased milk production, carminative, and anti-diabetic. To determine effects of fertilizer treatments on this plant, this experiment was conducted in randomized complete block design in three replications with six treatments. Fertilizer treatments included biological phosphate (*Pseudomonas putida*) at two levels inoculated and non-inoculated and chemical phosphorus (P_2O_5) at three levels (Zero, 40 and 80 kg.ha^{-1}). The application of fertilizers showed a significant effect (p<0.01) on biological yield, grain yield, harvest index (HI) and essential oil yield. There were no observed significant differences (p<0.01) on the essential oil percentage between chemical fertilizer and biological phosphate. Thus, it can be concluded that there is no preventative for biological fertilizer to produce a good yield of biological product for human health.

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Introduction

Black cumin (*Nigella sativa* L.), is a dicotyledon, an annual plant belonging to the buttercup family (Bafghi *et al.*, 2011). It is an herbaceous plant that grows in Iran especially in Esfahan, Arak and also in other Middle East countries. It has been widely cultivated and used as a condiment in pickles, bread and other foods. It is a common indigenous plant, its seed has major constituents such as nigellin, metarbin, melanthin, anthraquinones, glycosides, saponines, volatile oils, fixed oil, albuminous proteins, tannin, glucose, and mucilage resins. Black cumin has proved itself a forceful tool against many ailments caused by bacteria, viruses, and common allergies (Burits and Bucar., 2000; Goreja., 2003). Black cumin is a potent anti-histamine, anti-tumor, anti-bacterial, and anti-inflammatory agent, and these actions alone provide relief for a multitude of ailments and disorders (Hafeez and Hassan., 2012). Bio fertilizer is most commonly referred as soil micro-organisms to increase the availability and uptake of mineral nutrients by plants. Thus, it is necessary to define the term "bio-fertilizer". Bio-fertilizers are low-cost, renewable sources of plant nutrients which supplement or supplant chemical fertilizers. Biological fertilizers contain micro-organisms that enhance soil fertility, plant growth and yield crop (Hafeez and Hassan., 2012). The global approach in production of medicinal plants is to improve their health quality and quantity of active ingredient. The use of biological fertilizers in agriculture involves the sustainable practices combined with conventional practices and it is quite necessary to improve plant performance and quality by using healthy plants (Navarro *et al.*, 2012; Ganesan and Rhizoremediation., 2012). Bio phosphate is an organic fertilizer with or without antagonistic bacteria. Phosphate solubilizing microbes are among others of the *Pseudomonas*, *Micrococcus*, *Bacillus*, *Flavobacterium*, *Penicillium*, *Sclerotium*, *Fusarium*, and *Aspergillus* genera (Tang *et al.*, 2011). Some micro-organisms such as bacteria, fungi and streptomycetes are known to be able to dissolve P from natural phosphate fertilizers as well as those fixed in the soil. Fungi and bacteria are more able to dissolve P (AlPO4) in aluminum acid soil and Ca3 (PO4)2 in alkaline soil, respectively (Abubakar., 2010; Babalola., 2010). The use of biological fertilizers such as phosphate-solubilizing micro-organisms, mycorrhiza fungi, and vermicompost, is needed to improve the soil biologic condition and provide required nutrients for plants. Recently, the use of bio-fertilizers has been gaining momentum due to the increasing emphasis on maintenance of soil health, minimizing environmental pollution and reducing the use of chemicals in agriculture. Use of bio-fertilizers is one of the most important components of integrated nutrient management, as they are cost-effective and renewable sources of plant nutrients to supplement chemical fertilizers in sustainable agriculture. The objective of this study was to determine the effect of biological phosphate and chemical phosphorus fertilizer on quantity and quality of Black cumin (*Nigella sativa* L.).

Materials and methods

Experimental location condition

The experiment was carried out in 2013 at the research farm Nourabad, Lorestan Iran, located in the longitude 48º and 21' and the latitude 320 and 30' with a height of 1117 m above sea level, with annual precipitation of 524 mm and average annual temperature of 17ºC. The physical and chemical properties of the experimental soil where shown in table 1. The field was prepared in autumn and in March the crop was planted.

Experimental design and field preparing

The experiment was a factorial with two factors arranged in a randomized complete block design with three replications. The first factor was biological phosphate (bacterial strain *Pseudomonas putida*) (for preparation of biological phosphate solution, 100 g *Pseudomonas putida* was added to 2000 ml water and was sprayed on seeds) at two levels; inoculated (+) and non-inoculated (-) and second factor was three levels of chemical phosphorus fertilizer (P_2O_5) P1= Zero, P2= 40 and P3= 80 kg.ha^{-1}. Each experimental plot was three meters long and two meters wide with the spacing of 50 cm between the rows and a distance of 20 cm between plants in the rows. There was a space of one meter between the plots and two meters between replications. The Black cumin seeds were planted distance were two cm apart, covered with wet sand and about a centimeter thick and after emerging from the soil, thinning operation to set the desired density was performed. Three-quarters of fertilizer was applied at planting seeds and the rest was applied to plant at shooting. Black cumin seeds were directly sown by hand. There was no incidence of pest or disease on Black cumin during the experiment. Basin irrigation until harvest was done depending on weather conditions and weeds were controlled. In order to measurement of characteristics of effective on yield components and substance effective, after removing the marginal effects of each plot, 10 plants from each plot were harvested randomly. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. Dried seeds (50 g) of each plot were separated and powdered. The powder subjected to hydro distillation (400 ml distilled water), using a Clevenger-type apparatus for 2.5 hours and its essential oil was separated. Collected essential oil value was expressed regarding seed weight as essential oil yield.

Studied factors

In this experiment biological yield, grain yield, harvest index (HI), essential oil percentage and essential oil yield were studied. Fifteen plants were randomly selected from each plot and the observations were recorded. The present investigation was done in order to evaluate the effect of different treatments methods of biological phosphate fertilizer and chemical phosphorus fertilizer on quantity and quality of Black cumin (*Nigella sativa* L.).

Experimental Analysis

Data analysis was done by using software SAS. The ANOVA test was used to determine significant ($p \leq 0.01$ or $p \leq 0.05$) treatment effect and Duncan Multiple Range Test to determine significant difference between individual means.

Results and discussion

Grain yield

The results presented in Table 2 have revealed that different levels of treatments had significant effects on the grain yield ($P \leq 0.01$). Mean comparison table showed that the maximum (475 kg.ha^{-1}) and minimum (377 kg.ha^{-1}) grain yield were obtained by a treatment of biological phosphate + chemical phosphorus (40 kg.ha^{-1} P_2O_5) and control (non-fertilizer application), respectively (Fig. 1). Increase of grain yield under the influence of phosphorus fertilizers, can be attributed to the ability of phosphorus solution bacteria in fertilizer in increasing phosphorus liberalization of insoluble phosphorus sources. In another study Rokhzadi *et al.*, (2004) reported that grain yield of chickpea increased by utilization of biological fertilizer.

Table 1. several physical and chemical properties of the experimental soil.

Ec mmos/cm	pH	Cu ppm	Zn ppm	Mn ppm	Fe ppm	N ppm	K ppm	P ppm	O.C (%)	Deep (cm)
0.61	7.7	0.68	0.8	6.6	7.6	80	230	8	0.79	0-30

Biological yield

Effect of by all treatments on Black cumin biological yield was significant at 1% probability level (Table 2). The mean comparison of data in different treatments showed that the highest biological yield (1917 kg.ha^{-1}) was achieved by biological phosphate + chemical phosphorus (40 kg.ha^{-1} P_2O_5). The lowest biological yield (1347 kg.ha^{-1}) was obtained in control (non-fertilizer application) (Fig. 2). Plants treated by phosphorous because of positive effects of microorganisms at better availability of plant to phosphorous had an increasing trend in plant growth. Results of Migahed *et al.*, (2004) who investigate effects of both individualy or in combination inoculation of the *lipoferum*, *Azospirillum*, *Azotobacter*, *chroococcum* and *megaterium Bacillus* on wild celery reported that inoculation treatment let to production of plant growth promoting components and this increased plant growth, yield and essential oil compared to non-inoculated plants. In *Vallisneria spiralis* application of biological fertilizer including *Bacillus spp*. and *Pseudomonas rubiacearum* with combination of compost organic fertilizer caused to 34% increasing of plant dry weight compared to treatments which treated only by organic fertilizers. More this increased phosphorous solublzing bacteria and nitrogen fixation bacteria in root rhizospher of treated plants (Lewis, 1995).

Table 2. Analysis of variance for effects of biological and chemical fertilizers phosphorus on quantity and quality of Black cumin (*Nigella sativa* L).

Resource changes	df	Grain yield	Biological yield	Harvest index (HI)	Essential oil percentage	Essential oil yield
Repetition	2	10004.667 ns	74332.00 ns	1.584 ns	0.013 ns	0.001 ns
Biological phosphate (A)	1	39200.000 **	161880.50 **	14.580 **	0.013 ns	0.001 **
Chemical phosphorus (B)	2	34838.000 **	166458.50 **	21.369 **	0.014 ns	0.010 **
(A) × (B)	2	19478.000 **	197163.50 **	64.027 **	0.045 ns	1.112 **
Error	10	310.867	2950.00	5.643	0.039	0.690
CV (%)	-	2.83	3.65	5.67	9.40	6.52

**: Significant at= 1%, ns: Not significant.

Harvest index (HI)

Analysis of variance (ANOVA) showed that the effect of all treatments on the harvest index (HI) was significant at 1% probability level (Table 2). The mean comparison of data in different treatments showed that the highest harvest index (44.93%) was determined by application of biological phosphate + chemical phosphorus (40 kg.ha^{-1} P_2O_5). The lowest highest harvest (36.53 %) was obtained in control (without application of fertilizer) (Fig. 3). Biological fertilizer increased harvest index due to increasing economic performance. Results were in agreement with finding most of the workers like (Aslam-khan *et al*., 2005).

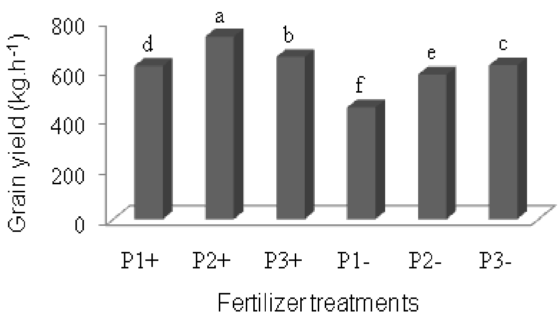

Fig. 1. Effect of fertilizer treatments on grain yield.

Essential oil percentage

The results of ANOVA showed that the effect of all treatments on essential oil percentage was not significant (Table 2).

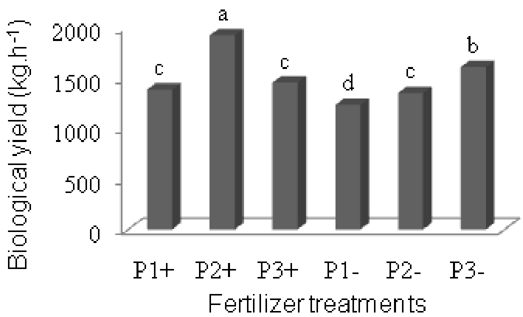

Fig. 2. Effect of fertilizer treatments on biological yield.

Essential oil yield

The results of the analysis variance showed that the essential oil yield was significantly affected by all treatments ($P \leq 0.01$) in this experiment (Table 2). Mean comparison table showed that the highest (1.796 kg.ha^{-1}) and lowest (0.823 kg.ha^{-1}) essential oil yield were obtained by a treatment of biological phosphate + chemical phosphorus (40 kg.ha^{-1} P_2O_5) and control (non-fertilizer application), respectively (Fig. 4). Although the effective elements of plants are produced by genetic processes but their production is affected by different factors such as: yield loss, wrong management and particularly nutrients deficit (Malakouti, 2009). The results of the present study confirm with the results of Azizi (2000) reporting the effect of biological fertilizer on the essence yield in anis plant. Shalaby and Razin (1992) reported that application of 105 $kg.ha^{-1}$ of phosphorus increased essence and *thymul* in *Thymus* plant.

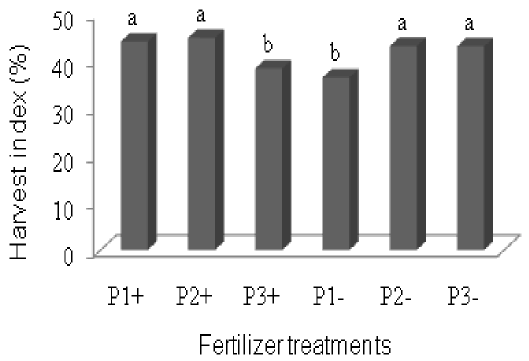

Fig. 3. Effect of fertilizer treatments on harvest index.

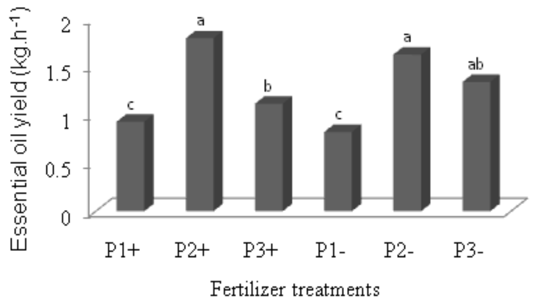

Fig. 4. Effect of fertilizer treatments on essential oil yield.

Conclusion

Biological fertilize are widely applied in crop production and they are proper substitutions for chemical fertilizers. Application of biological fertilizes significantly improved quality and quantity in Black cumin. Maximum of biological yield, grain yield, harvest index (HI), essential oil percentage and essential oil yield was obtained in treatment of biological phosphate + chemical phosphorus (40 $kg.ha^{-1}$ P_2O_5). Totally, the obtained results revealed that using biological fertilizes combined with chemical fertilizer significantly improved the quantity and quality components compared to control.

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