

## Biological effects of fertilizer treatments on growth, yield and yield components of black cumin

MAHDI FARAVANI<sup>1\*</sup>, SOHEILA KOOREPAZ<sup>2</sup>, BARAT ALI GHOLAMI<sup>1</sup>, YASER ZARE<sup>3</sup>

<sup>1</sup>Khorasan Agricultural Research Center  
P.O.Box 91735-488 Mashhad, Iran

<sup>2</sup>Islamic Azad University  
Jiroft Branch  
Tehran, Iran

<sup>3</sup>MSc student of Islamic Azad University,  
Jiroft Branch

\*corresponding author: e-mail: faravani@yahoo.com

### S u m m a r y

*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 seven treatments. Fertilizer treatments consisted of Bio phosphor (B 2 kg·ha<sup>-1</sup>), Bio sulphur (S 5 kg·ha<sup>-1</sup>), Biological fertilized phosphate BAVAR 2 (F 100 g·ha<sup>-1</sup>), chemical fertilizer (M) according to soil analysis, and combined fertilizer (B+S), (S+F), and (C) treatment. The application of fertilizers showed a significant effect ( $p < 0.01$ ) on plant height, number of capsules per plant, seed weight, grain yield (g/m<sup>2</sup>), number of flowers per plant, and biological yield (g/m<sup>2</sup>). There were no observed significant differences ( $p < 0.01$ ) on the number of seeds per capsule, seed weight and yield between chemical fertilizer and biological BARVAR 2. Thus, it can be concluded that there is no preventative for biological fertilizer to produce a good yield of biological product for human health.

**Key words:** environment, black seed, bio-fertilizers

## INTRODUCTION

Black Seed (*Nigella sativa* L.), is a dicotyledon, an annual plant belonging to the buttercup family [1]. It is a 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 Seed has proved itself a forceful tool against many ailments caused by bacteria, viruses, and common allergies [2, 3]. Black Seed 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 [4].

In this study, the effect of biological fertilizers on growth and black seeds were investigated to determine the possibilities of decreasing use of chemical fertilizer in sustainable agriculture systems. 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 [5]. Nitrogen transformation can be carried out both by biological and non-biological processes. Important processes in the nitrogen cycle include fixation, mineralization, nitrification, and denitrification that micro-organisms carry out to make them available for biological use. They are also a good substitute fertilizer for inorganic compounds [6]. 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 [7, 8].

Plant growth-promoting rhizobacteria of *Azotobacter* species, *Nitrobacter* species, and *Nitrosomonas* species were isolated and identified using standard methods (PGPR). Screening of PGPR showed that they produce phytohormones such as indole acetic acid (IAA) and siderophore to encourage plant growth by a wide variety of mechanisms such as phosphate solubilization, phytohormone production, antifungal activity, etc. Thus, the use of combined bio-fertilizers is advocated for excellent growth performance of plants [9].

### Type of bio-fertilizer

Biophosphate 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 [10]. 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 ( $\text{AlPO}_4$ ) in aluminum acid soil and  $\text{Ca}_3(\text{PO}_4)_2$  in alkaline soil, respectively [11, 12]. Bio Sulphur is one of the most important micronutrients. It is extensively used for the treatment of Sulphur deficiency of crops by helping to convert insoluble Sulphur (S) into the simple sulfate ( $\text{SO}_4$ ) anion, a soluble form easily absorbed by plants [13, 14]. 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 bio-fertilizer on element-availability and the growth and yield of Black Seed Cumin in Mashhad.

## MATERIALS AND METHODS

In order to evaluate the effect of bio-fertilizer and soil fertilizing treatment on yield, yield components and oil content, an experiment using experimental plots ( $3 \times 2$  m) was conducted in a randomized complete block design with three replications seven treatments at Torogh station in Khorasan Agricultural and Natural Resources Research Institute, Mashhad, Iran, with the geographic coordinates of East latitude 16.38 North and longitude E 59.36 at an elevation of 985 meters above sea level in 2011. The soil texture and soil content of nutrients of Fe, P, and N in *Nigella sativa* L. are presented in table 1. The recommended dosage NPK ratio was 90-60-30 for black cumin. The land was fallow in the last year prior to this study. The soil was prepared by deep plowing in the fall and following this cultural operation simultaneously with disking. In this study three types of bio-fertilizer were applied:

1. Bio-phosphor in a series of phosphate-solubilizing bacteria of the genera *Pseudomonas* and *Bacillus*.

2. Bio-sulphur, with the major sulphur oxidizing bacterium of *Thiobacillus ferrooxidans*.

3. BARVAR-2 contains two types of highly efficient phosphate-solubilising bacteria (PSB) which secrete organic acids and phosphatase enzymes which hydrolyze insoluble inorganic and organic phosphate compounds into soluble phosphate ions around plant roots. The number of viable cells per gram of bio-phosphate to give the bacterial population as CFU (colony-forming unit) was 107 living cells per milliliter of each bacterial genera. The seeds were incubated with bacteria as thio-bacillus 1 g for 50 kg seed, adding sugar and water. They were air-dried in shade.

Table 1.

Soil characteristics of field soil used for the black cumin study

C (%)	EC (dS/m)	(pH)	N (%)	P (ppm)	K (ppm)	Mn (ppm)	Fe (ppm)	Soil texture		
								Clay	Silt	Sand
0.45	1.63	8	0.092	10	219	5.40	3.76	23	36	41

For bio-phosphate fertilizer: 200 grams of seed of black cumin were mixed well with 2 grams of bio-phosphate fertilizer (BARVAR 2) after adding a small amount of water. Then, seeds were air-dried in shade. In late April 2011, after sowing in row spacing of 50 cm and 5 cm apart, they were immediately watered. Plant thinning was performed after germination to get a plant population density of 400,000 plants per hectare. Treatments consisted of control (c), bio-phosphor 2 kg.ha<sup>-1</sup> (B), bio sulphur 5 kg.ha<sup>-1</sup> (S), BARVAR-2 (F 100 kg.ha<sup>-1</sup>), S (2 kg.ha<sup>-1</sup>) + B(5 kg.ha<sup>-1</sup>), B (5 kg.ha<sup>-1</sup>) + F (100 kg.ha<sup>-1</sup>) and NPK (M) according to the results of soil chemical analysis. After counting the seeds, they were mixed with fertilizer and air-dried in shade for 5 minutes, then planted in the farm. Different growth and yield characters were plant height at flowering time, number of flowers per plant, number of capsules per plant, number of seeds per capsule, seed weight, grain yield (g/m<sup>2</sup>), and biological yield (g/m<sup>2</sup>). Analysis of Variance (ANOVA) and Duncan multiple range test were used to compare the several treatments.

## RESULTS AND DISCUSSION

In general, the fertilizer treatments significantly ( $p < 0.01$ ) influenced in all measured characters including plant height, number of capsules per plant, seed weight, grain yield (g/m<sup>2</sup>), number of flowers per plant, and biological yield (tab. 1).

Table 2.

Analysis of variance of measured traits in response to fertilization of black cumin

Characters	D.F	HI	1000 K	Oil percentage	Biomass (g.m <sup>-2</sup> )	No. flowers/plant	Seed yield (g.m <sup>-2</sup> )	Seed weight/plant	No. seed/capsule	Capsule/plant	Plant height
Blocks	2	3.76	0.009	0.302	12.10	0.449	0.808	0.0005	0.292	0.335	0.20
Treatment	6	18.20**	0.010**	22.85**	305.54**	3.60**	111.07**	0.0693**	2.36**	28.34**	18.59**
Residual	12	1.121	0.002	0.25	11.38	0.277	0.525	0.0003	0.149	0.432	0.6281
C.V		2.2	2.04	2.06	5.6	7.08	2.5	2.54	5.6	1.46	3.37

\*\* show the significant level  $p < 0.01$

## Plant height

Fertilizer treatments significantly ( $p < 0.01$ ) affected plant height. There was no difference in plant height among the fertilizer treatments. Maximum plant height of 28.5 cm and lowest of 19.31 cm were observed in M and C treatments, respectively (fig. 1).

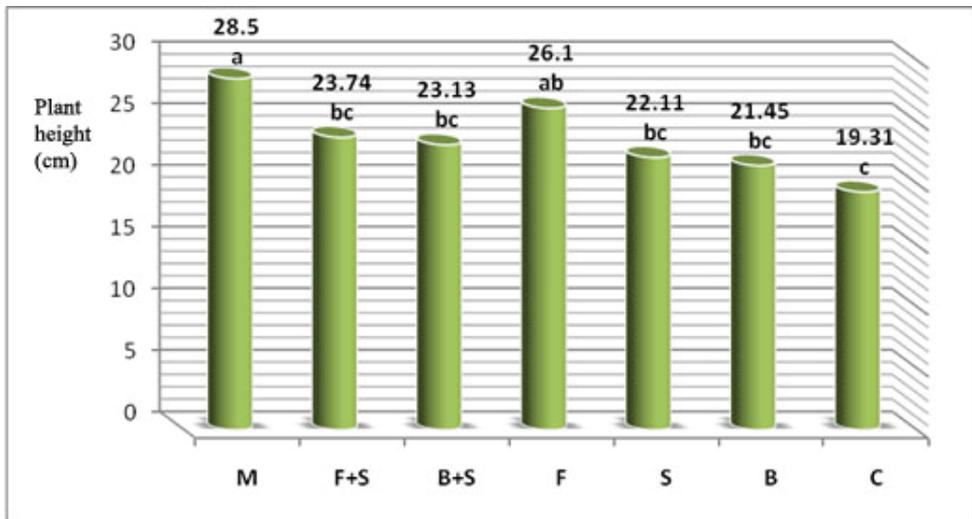


Figure 1.

The effect of fertilizer treatments on plant height, compared by Duncan's multiple range tests at 5% level

The overall results showed that the highest plant growth occurred in treatments M fertilizer which was a macro/micro nutrient mix. Among bio-fertilizers, BARVAR-2 caused an increase in plant height. Effective micro-organisms (EM), organic and bio-fertilizers as well as their combinations increased the supply of minerals through the dissolved nutrient solutions that plants could take in through their roots. The macro-nutrients taken in by plant roots in greater amounts, included nitrogen, phosphorus and potassium, the main ingredients of store-bought, non-organic, conventional, commercial, and artificial fertilizers. Positive effects of bio-fertilizer on improving crop growth were due to increase in nitrogenase activity by vesicular arbuscular mycorrhizae and synthesis of growth-promoting substances by phosphate solubilizing bacteria. Phosphorus-solubilizing bacteria play a strong role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilization and mineralization [15] It is the reason why adding bio-fertilizer BARVAR 2 to the soil increases plant height and other characteristics

of plant growth. Bio-fertilizer as a growth regulator is responsible for rapid cell multiplication resulting in vigorous growth by increasing plant height and branch production [16].

The efficiency of biological bacteria (PSB) is to increase P uptake by plants from the soil solution and result in higher plant biomass and plant height [17].

### Number of seeds per capsule

Although fertilizer treatments had no statistically significant effect on the number of seed per capsule ( $p < 0.01$ ), Duncan's multiple range test showed that the greatest number of seeds per capsule of 50.66 was achieved by the treatment of M and followed by other biological fertilizer which produced 48 in F and combined treatment FS with 33.44 had the next highest number of seeds per capsule. The total number of seeds was lowest in the control treatment (fig. 2). In fact, the number of seeds per capsule is the reservoir capacity of the plant. Plants keep photosynthetic material and ultimately lead to increase in biomass. Number of capsules per plant, number of seeds per capsule and biomass are good indicators of seed yield per plant. There was a positive correlation between the number of seeds per capsule and seed yield, with  $r = 0.95$ .

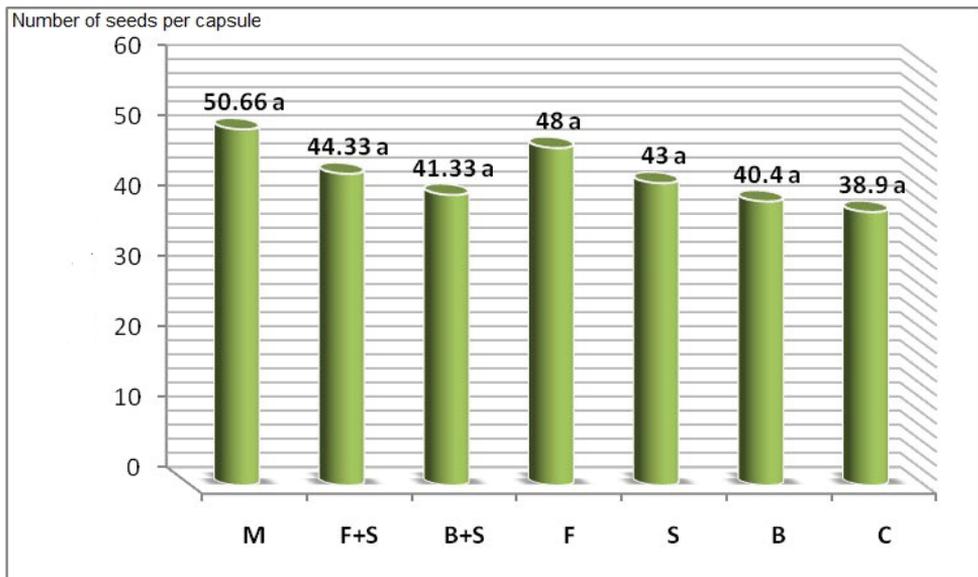


Figure 2.

Duncan's multiple range test comparing the average number of seed per capsule

## Number of capsules per plant

The number of capsules per plant was affected significantly ( $p < 0.01$ ) by fertilizer treatments. Maximum number of capsules was in M treatments with 7.85, 7.73 in F, and 7.28 in F + S which were significantly different from control treatment (fig. 3).

Application of bio-fertilizer showed a significant increase in plant height and number of branches/plant compared to those obtained without bio-fertilizer treatment (control) [16]. Black cumin symbiosis with micro-organisms increased the vegetative growth and consequently the number of lateral branches and capsules per plant. It caused the production growth-stimulating hormones by the co-existence relation in plants.

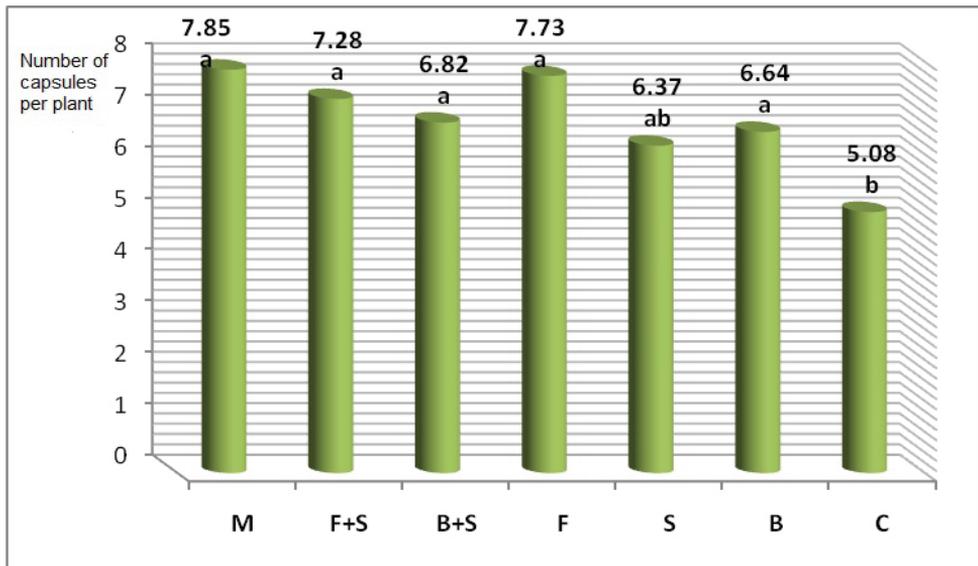


Figure 3.

Duncan's multiple range test comparing the average number of capsules per plant

## Plant seed

Treatments significantly increased ( $p < 0.01$ ) per-plant seed yield of *Nigella sativa*. The highest seed weight per plant of 1.06 g was observed in the complete fertilizer treatment (M), followed by treatments C (0.48 g) and B (0.51 g) which had the lowest seed weight with no statistically significant difference

(fig. 4). Although all nutrients are necessary, NPK are most responsible for the growth of above- and below-ground plant parts, and for budding, flowering and fruiting.

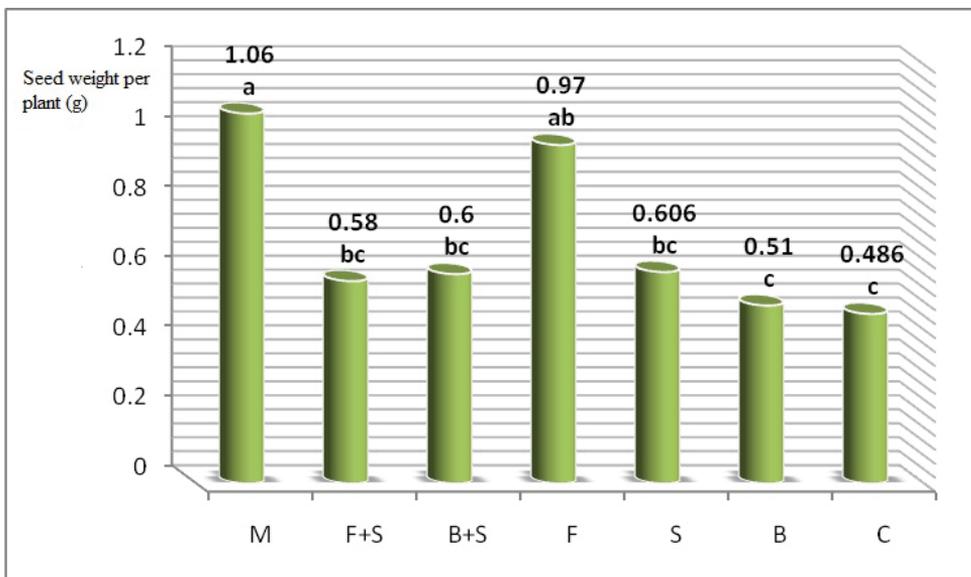


Figure 4.

Duncan's multiple range test comparing the seed weight per plant

When seeds are inoculated with biological fertilizers (BARVAR 2), it may increase the motility and the absorptive capability of the root system to improve nutrient absorption of phosphor, resulting in increased photosynthesis and seed yield per plant.

### Seed yield

Effect of fertilizers on grain yield ( $g/m^2$ ) was statistically significant at  $p < 0.5$ . Maximum yield was achieved in the complete fertilizer treatment M and bio-fertilizer F with 41.82 and 37.79  $g/m^2$ , respectively. There was no significant difference in seed yield for the application of M & F. The lowest seed yield (18.98  $g/m^2$ ) was control treatment (fig. 5).

These treatments were statistically significant at  $p < 0.5$  on 1000 K weight. The maximum 1000 K weight of 2.42 g was in the application of chemical treatment (M).

Despite the highest yield per unit area occurring in complete chemical fertilizer M due to high nutrient intake (NPK), bio-fertilizer has more advantages for plant

feeding to improve the root system and create deeper and more abundant roots. Phosphorus-solubilizing bacteria increase the available nitrogen and phosphorus in the soil, which could enhance crop production.

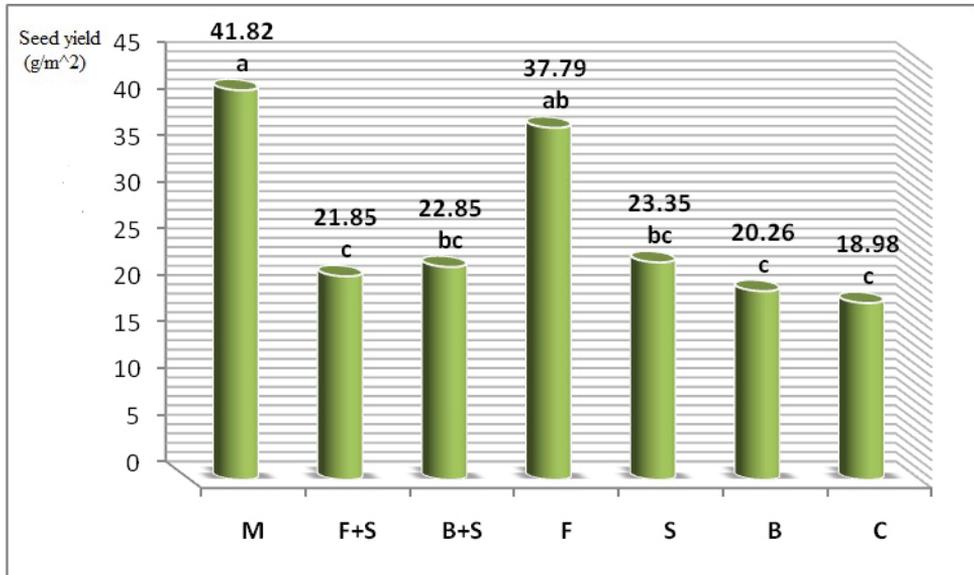


Figure 5.

Duncan's multiple range test comparing seed yield (g/m<sup>2</sup>)

Application of bacteria in the absence of any chemical phosphorus fertilizer had an appropriate performance and could increase biomass production to an acceptable level, so it could be considered as a suitable substitute for chemical phosphorous fertilizer in organic agricultural systems [18].

### Number of flowers per plant

Fertilizer treatments had a significant effect ( $p < 0.01$ ) on the number of flowers per plant. The maximum and the lowest number of flowers per plant were from treatments F (53.9) and C (6.06). These differences were not statistically significant for M – FS with 8.13-7.61 and S-C with 6.55-6.06, respectively (fig. 6).

Seed inoculated with biological fertilizers (BARVAR 2) and F+S increased both the number of flowers per inflorescence and inflorescence height and thus the number of flowers per plant.

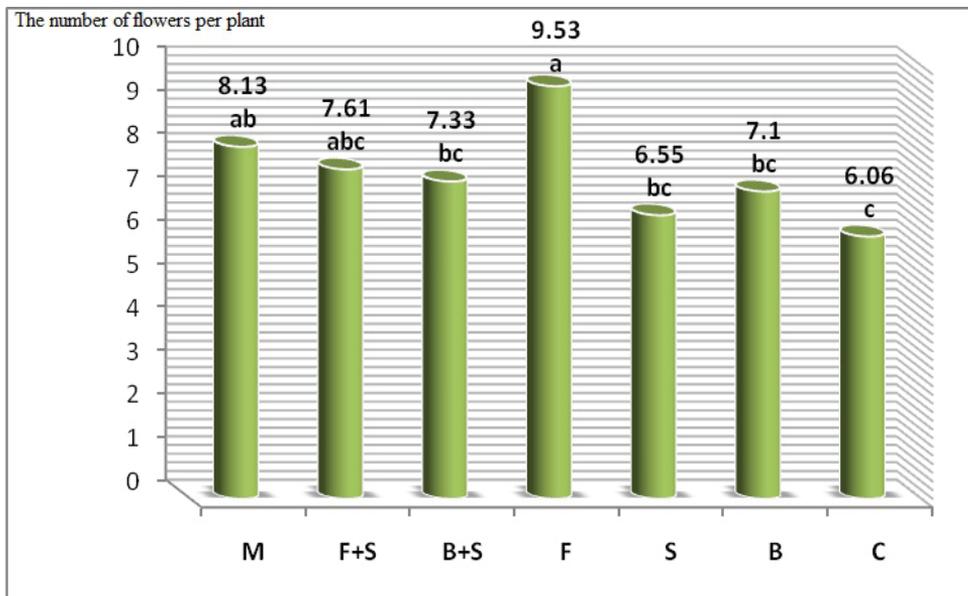


Figure 6.

Duncan's multiple range test for number of flowers per plant

## Biological yield

The effect of fertilizers on biological yield ( $\text{g/m}^2$ ) was statistically significant at  $p < 0.5$ . Maximum yield was achieved in the complete fertilizer treatment M, BARVAR 2 (F) with 74.82 and 71.3  $\text{g/m}^2$ , respectively. There was no significant difference in biological yield for the application of M&F. The lowest biological yield ( $46.4 \text{ g/m}^2$ ) was found in the control treatment (fig. 7). Harvest index was changed significantly ( $p < 0.01$ ) and the highest and lowest HI were found in M (56.16), F(52.86) and C (35.06), respectively.

The same result was observed in a study on the effects of application of biological fertilizer on biological yield and growth indices of black cumin in 2008 [19]. Results showed 22.81% partitioning of photosynthate was appropriated for grain and the rest for straw. The grain and the vegetative plant and improvements in harvest index emphasize the importance of carbon allocation in grain production. However, increasing grain yield and crop harvest index with high nitrogen grain requires a concomitant increase in crop nitrogen accumulation [20].

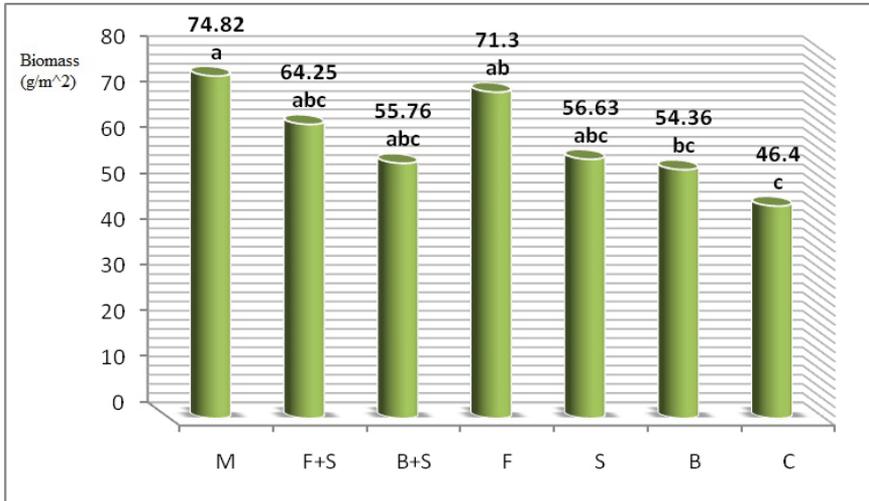


Figure 7.

Duncan's multiple range tests comparing seed yield (g/m<sup>2</sup>)

## Oil percentage

The trend and statistically significant ( $p < 0.01$ ) difference of oil percentage was the same as biological and seed yield with maximum percentage in M 28.62 % and F 25.34% treatments. There was no difference between C, F+S, B, B+S and S treatments (fig. 8).

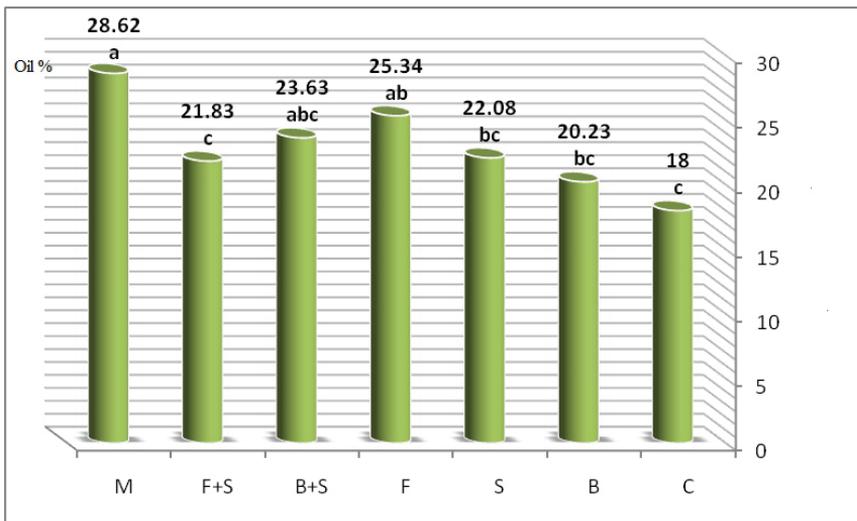


Figure 8.

Duncan's multiple range tests comparing oil percentages

## CONCLUSION

In general, based on analysis of obtained results from this research, application of bio-fertilizers significantly improved plant height, number of flowers per plant, and number of capsules per plant. It tended to increase seed yield, biological yield and oil percentage over those obtained from plants without fertilizer treatment. Thus, fertilization of black cumin crops with bio-fertilizer can be recommended to obtain higher yield of seed.

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#### WPŁYW NAWOŻENIA NA WZROST, PLON I SKŁADNIKI PLONU CZARNUSZKI SIEWNEJ

MAHDI FARAVANI<sup>1\*</sup>, SOHEILA KOOREPAZ<sup>2</sup>, BARAT ALI GHOLAMI<sup>1</sup>, YASER ZARE<sup>3</sup>

<sup>1</sup>Khorasan Agricultural Research Center  
P.O.Box 91735-488 Mashhad, Iran

<sup>2</sup>Islamic Azad University  
Jiroft Branch  
Tehran, Iran

<sup>3</sup>MSc student of Islamic Azad University,  
Jiroft Branch

\*autor, do którego należy kierować korespondencję: e-mail: faravani@yahoo.com

#### Streszczenie

*Nigella sativa* (czarnuszka siewna) jest jednoroczną rośliną kwitnącą występującą w Iranie i innych częściach Azji południowo-zachodniej. Należy do rodziny jaskrowatych (*Ranunculaceae*). Jej nasiona mają wiele właściwości prozdrowotnych: działają przeciw pasożytniczo, przeciwwirusowo, przeciwbakteryjnie, zwiększają produkcję mleka, działają wiatropędnie i przeciw cukrzycowo. By określić efekty nawożenia tej rośliny, przeprowadzono doświadczenie w czterech randomizowanych blokach, w trzech powtórzeniach przy użyciu siedmiu mieszanek nawozów. W skład zastosowanych mieszanek wchodziły: biofosfor (B 2 kg·ha<sup>-1</sup>), biosiarka (S 5 kg·ha<sup>-1</sup>), nawóz biologiczny fosfat BAVAR 2 (F 100 g·ha<sup>-1</sup>), nawóz

chemiczny (M) odpowiednio do analizy gleby i nawóz mieszany (B+S), (S+F), oraz kontrola (C). Wynikiem zastosowania nawozów było wyraźne zwiększenie wysokości roślin ( $p < 0.01$ ), zwiększenie liczby torebek nasiennych w roślinie i plonu ( $\text{g/m}^2$ ). Nie zaobserwowano wyraźnych różnic w liczbie nasion w torebce nasiennej, wadze nasion i plonie pomiędzy roślinami nawożonymi chemicznie i za pomocą nawozu biologicznego BARVAR 2. Zatem można stwierdzić, że nie ma przeciwwskazań do stosowania nawozu biologicznego przy produkcji surowca dla potrzeb zdrowotnych.

**Słowa kluczowe:** *środowisko naturalne, czarnuszka, nawóz biologiczny*