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Effect of biofertilizers with various levels of inorganic nutrients on growth and yield of black cumin (*Nigella sativa* L.) var. Azad Kalonji

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Abstract

The objective of the study is to reduce the amount of inorganic fertilizers by using biofertilizers with the aim of sustainable agriculture. The field experiment was conducted at the HRS, Mondouri, BCKV, West Bengal, India during *rabi* season of 2019-20. The experiment consisted of 14 treatments replicated thrice, comprising of three levels inorganic fertilizers (50, 75 and 100%) of recommended NPK and three biofertilizers *viz. Azospirillum lipoferum* (N fixer), *Bacillus megaterium* (potash mobilizer) and *Fraturia aurantia* (K mobilizer) as soil application. The results indicated that the maximum plant height (86.06 cm) at 100 DAS, number of primary (10.66) and secondary branches (17.33), number of capsules plant^{-1} (25.33), number of seeds capsule^{-1} (97.66), test weight (3.54 g) and projected yield (538.50 kg ha^{-1}) were recorded in 100% RDF + *Azospirillum* + PSB + KS. The yield was at par with 75% RDF + *Azospirillum* + PSB + KS (536.74 kg ha^{-1}). Considering the economics, maximum net return (Rs. 1,08,083.74 ha^{-1}) and B: C ratio (4.14: 1) was obtained from 75% RDF + *Azospirillum* + PSB + KS. These results suggested that combination of *Azospirillum* + PSB + KS with 75% RDF was best for optimum production of black cumin without loss in yield and reduction of 25% of inorganic fertilizers through application of biofertilizers and environmental pollution to some extent.

Key words: ***Azospirillum, Bacillus, Biofertilizers, Black cumin, Economics, Fraturia, Inorganic***

Introduction

Black cumin (*Nigella sativa* L.) is an annual flowering species of the family *Ranunculaceae*. It is an important seed spices crop native to Mediterranean region and widely grown across Middle East, Europe and Asia. The crop is cultivated in different parts of the world (Aggarwal *et al*., 2008; Al-Sman *et al*., 2017). The leading producers are India, Sri Lanka, Bangladesh, Pakistan, Afghanistan, Egypt, Iraq, Iran, Turkey, Syria and Ethiopia. Among the countries, India, Syria, Ethiopia and Turkey are the major exporter (Sultana *et al*., 2018). It is widely grown for its flavorful seeds and leaves, used as culinary spices in Indian and Middle Eastern cuisines. They are used for flavouring of foods, especially cheese and bakery products and in preparation of black cumin pastry. The seeds are also associated with various medicinal properties and are widely used in Ayurveda, Unani, Siddha and other ethnomedicine systems all over the world (Padmaa, 2010). The extracts of seeds have numerous medicinal properties like antihelmintic, antibacterial, antiviral, antipyretic, galactagogue, carminative and antidiabetic effects (Salem, 2005; Darakhshan *et al*., 2015). In recent years, there is increased use of biofertilizers as a supplement to nutrient supply in agricultural system due to increasing cost of chemical fertilizers and global concern for environmental pollution. Feeding is one of the key aspects affecting the quantity and quality of medicinal plants (Arun, 2002). The most critical constraint limiting crop yield in developing countries globally and especially among the poor farmers, is soil infertility (Khosro and Yousef, 2012). So, maintaining soil fertility and supplying optimum level of nutrients from all feasible sources in an integrated way is necessary to sustain the desired productivity of crop (Chundawat, 2001). We can increase the efficiency of nutrient inputs with proper nutrition of plants, while preserving environment, biodiversity and reducing erosion. Besides, avoiding the unnecessary utilization and consumption of nutrients, we will be able to reduce

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costs of production to minimum which could be taken into consideration as a step to sustainable agriculture (Safaei *et al*., 2014). Application of chemical fertilizers and pesticides increases soil acidity and poses the risk of polluting ground water and the atmosphere. It also impairs the roots of plants decreasing the ability for proper absorption of water and nutrients thereby making them susceptible to undesirable diseases (Chun-Li *et al*., 2014). In this view, biofertilizers has been identified as an alternative form to chemical fertilizers to improve soil fertility and crop production. Biofertilizers are cultures of special bacteria and fungi and unlike inorganic and organic nutrients they do not supply any nutrient directly to the plants. Biofertilizers comprises of symbiotic nitrogen fixers *Rhizobium spp*., asymbiotic nitrogen fixers (*Azospirillum*, *Azotobacter*, etc.), blue green algae, *Azolla*, phosphate solubilizing bacteria and potash mobilizing *mycorrhizae* (Goel *et al*., 1999). The application of biofertilizers is known to promote plant growth through the supply of plant nutrients and may support to sustain environmental health and productivity of the soil (O' Connell, 1992). Karlidag *et al*., 2007 demonstrated that biofertilizer could minimize the chemical fertilizer requirement and reduce its adverse impact on the environment. Fayez *et al.* 1985 reported that *Azotobacter chroococcum* and *Azospirillum lipoferum* not only have the potential to fix nitrogen but also release phytohormones similar to gibberellic acid and indole acetic acid, which could enhance nutrients absorption, plant growth and photosynthesis. Phosphate solubilizing bacteria promotes plant growth through the synthesis of antibiotics, phytohormones and few enzymes such as ACC (1-aminocyclopropane-1-carboxylate) deaminase that adjusts the level of plant hormones and makes phosphorous available to the plants by solubilization and mineralization of inorganic and organic phosphate (Rodriguez and Fraga, 1999). Potassium solubilizing bacteria solubilise minerals directly such as orthoclase, illite and micas which bears potassium by dissolving rock potassium by secreting organic acids or release potassium into solution by chelating silicon ions (Barker *et al*., 1998). Plant nutrition is one of the main elements that influence the growth and yield of crop plants and biofertilizers offers an efficient and ecologically sustainable way of minimizing external inorganic inputs and maintaining natural land resources. In general, only 10% to 40% of the total applied fertilizers are taken up by plants and remaining 60% to 90% is lost. Therefore, biofertilizers can be an effective component in integrated nutrient management systems for sustaining agricultural productivity and a healthy environment (Adesemoye and Kloepper, 2009). So, considering the importance of biofertilizers on optimization of nutrients, the present experiment was designed to find out the effect of biofertilizers with different grades of inorganic fertilizer on growth and yield of black cumin.

Materials and Methods

Experimental site: The field experiment was carried out during *rabi* season (November to March) of 2019-2020 at Horticultural Research Station, Mondouri of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India. The area is in new alluvial zone which is situated between 22^o N latitude and 88^o E longitude with an altitude of 13 m above sea level. The soil of experimental field had 6.06 pH, 0.72% organic carbon, 237.5 kg ha^{-1} available nitrogen, 14.3 kg ha^{-1} available phosphorus and 226.4 kg ha^{-1} available potassium.

Experimental design and details: The field experiment was laid out in randomized complete block design (RCBD), replicated thrice comprising of 14 treatments. Nitrogen fixing bacteria (*Azospirillum lipoferum*), phosphate solubilizing bacteria (*Bacillus megaterium*) and potassium solubilizer (*Fraturia aurantia*) with a concentration of 2 × 10^7 cfu g^{-1} were applied with three levels of recommended dose of NPK *i.e*., 100%, 75% and 50%. The treatment combinations were T_1- 100 % RDF + *Azospirillum*, T_2- 100 % RDF + PSB, T_3- 100 % RDF + KS, T_4- 100 % RDF + *Azospirillum* + PSB + KS, T_5- 75 % RDF + *Azospirillum*, T_6- 75 % RDF + PSB, T_7- 75 % RDF + KS, T_8- 75 % RDF + *Azospirillum* + PSB + KS, T_9-50 % RDF + *Azospirillum*, T_{10}- 50 % RDF + PSB, T_{11}- 50 % RDF + KS, T_{12}- 50 % RDF + *Azospirillum* + PSB + KS, T_{13}- *Azospirillum* + PSB + KS and T_{14} (control) - 100 % RDF ($N_{40}P_{40}K_{45}$).

Nutrient application and sowing: Biofertilizers were applied as sole or in combination along with each level of inorganic fertilizers. Biofertilizers were mixed thoroughly with farm yard manure (FYM) and applied directly to the soil @ 2.5 kg ha^{-1} each during field preparation .The total amount of inorganic fertilizer was applied at three split doses. $1/3^{rd}$ of N along with full dose of P and K were applied 7 days before sowing and rest $2/3^{rd}$ of N was applied in two splits during later stage at 40 and 60 days after sowing. Urea, single super phosphate and muriate of potash were used as inorganic source of N, P_2O_5 and K_2O.

Prior to sowing, the seeds of black cumin variety Azad kalonji were soaked in water overnight before sowing to promote early germination and treated with *Trichoderma viride* @ 2g kg^{-1} seed. The seeds were sown in a plot size of 2.1 x 1.5 m in a row of 30 cm spacing and the seedlings were thinned out at 30 days after sowing at a distance of 15 cm.

Statistical analysis: During the experimentation period, five random plants from each treatment and replications were selected for observations. The experimental data recorded were subjected to statistical analysis by employing RCBD at 5% level of significance for determination of critical difference (CD). The data were analyzed using a window based computer package OPSTAT (Sheoran, 2004).

Results and Discussion

Plant height and number of branches: The results obtained from the present experiment are presented in the Table 1. The plant height (86.06 cm) at 100 days after sowing (DAS), average number of primary branches (10.66) and secondary branches (17.33) of plant during harvest were significantly higher under T_4, where 100% RDF + *Azospirillum* + PSB + KS were used. The treatment comprising of *Azospirillum* + PSB + KSB without chemical fertilizers T_{13} recorded the least number of primary (5.80) and secondary (5.21) branches and lowest plant height (50.14 cm). The results are in agreement with study carried out in onion, where maximum plant height was recorded under the application of 100% NPK and biofertilizers (Das *et al*., 2020). Valadabadi and Farahani, 2013 reported similar finding in black cumin, where maximum plant height (84 cm) was recorded under the treatment of *Azotobacter* and animal manure. Ali and Hassan, 2014 reported that the maximum plant height (54.72 cm) and average number of branches (11.47) were recorded under the application NFB (mixture of *Azotobacter chroococcum* and *Azospirillum brasilense*) and PSB (mixture of *Bacillus megatherium* var. *phosphaticum* and *Bacillus polymyxa*) along with cattle manure in black cumin. Sen *et al*., 2019 also recorded highest number of branches in black cumin under 100% RDF chemical fertilizers, FYM and biofertilizer treatment. In celery, height of the plants has increased with the application of biofertilizers which enhanced the content of nitrogen and photosynthesis rate (Migahed *et al*., 2004). Biofertilizers produces phytohormones that provide favourable effect on active root rhizosphere by causing faster multiplication and elongation of cells that helped in proliferation of roots and improves nutrient uptake resulting in better vegetative growth including plant height, number of primary and secondary branches. Increase in growth attributes can be correlated to the positive impact of biofertilizers on uptake of nutrients by plants (Gad, 2001). Increased plant growth could also be associated to suitable combination of inorganic nutrients and biofertilizers which provide immediate availability of nutrients at initial stage through inorganic fertilizers and at steady and constant rate as long term availability through the process of biofertilizers resulting in higher plant height and number of branches. Combined applications of *Azospirillum,* PSB and KS have significant effect than their sole application along with inorganic fertilizers. The application of *Azospirillum* helped in increasing nitrogen availability through atmospheric nitrogen fixing which reflected on better production of growth and yield attributes. PSB effects the production of organic acids, vitamins, amino acids, IAA and GA which helped in obtaining better growth as well as yield and yield attributes of the plants. KS solubilise potassium bearing minerals and convert to soluble form from insoluble K and make them available to the plants. Silicate minerals can be dissolved by KSB and potassium is released through the production of inorganic and organic acids (Prasad *et al*., 2018).

Yield and yield attributes: Plants supplied with 100% RDF + *Azospirillum* + PSB + KS (T_4) was recorded with maximum number of capsules $plant^{-1}$ (25.33), number of seeds $capsule^{-1}$ (97.66), test weight (3.54 g) and projected yield (538.50 kg ha^{-1})

Table 1: Effect of biofertilizers and inorganic fertilizers on growth, yield and yield attributes of black cumin var. Azad Kalonji

Treatment	Plant height			Number of branches		No. of Capsule plant^{-1}	No. of seeds capsule^{-1}	1000 seed wt. (g)	Projected yield (kg ha^{-1})
	60 DAS	80 DAS	100 DAS	Primary	Secondary				
T_1	29.90	57.16	80.83	9.33	15.33	23.33	96.66	3.32	494.78
T_2	27.83	53.66	78.83	8.66	14.66	22.66	96.50	3.25	491.75
T_3	24.73	45.16	78.40	8.00	14.33	21.45	96.33	3.19	490.84
T_4	31.33	58.43	86.06	10.66	17.33	25.33	97.66	3.54	538.50
T_5	28.02	57.00	82.33	8.66	13.33	20.33	94.36	2.98	489.79
T_6	27.76	51.66	72.26	7.68	12.66	19.50	94.00	2.90	483.96
T_7	23.13	44.50	69.13	7.33	11.33	19.33	93.50	2.85	477.01
T_8	30.83	58.16	84.50	9.66	16.86	24.66	97.33	3.47	536.74
T_9	27.90	54.23	73.66	6.68	8.66	16.66	89.33	2.79	392.33
T_{10}	25.76	46.73	60.36	6.45	6.83	15.90	87.50	2.75	388.81
T_{11}	22.50	43.24	58.16	6.20	6.33	15.50	87.10	2.70	383.97
T_{12}	30.73	57.13	64.86	7.10	9.33	18.20	86.86	2.72	399.64
T_{13}	21.26	40.16	50.14	5.80	5.21	13.33	84.33	2.56	298.91
T_{14}	27.24	57.00	78.60	9.00	14.00	21.83	94.22	3.06	499.94
SEm (±)	0.400	0.755	1.091	0.125	0.205	0.310	1.310	0.045	6.853
CD at 5%	1.169	2.208	3.188	0.364	0.598	0.907	3.830	0.131	20.032

Note: T_1- 100 % RDF + *Azospirillum*, T_2- 100 % RDF + PSB, T_3- 100 % RDF + KS, T_4- 100 % RDF + *Azospirillum* + PSB + KS, T_5- 75 % RDF + *Azospirillum*, T_6- 75 % RDF + PSB, T_7- 75 % RDF + KS, T_8- 75 % RDF + *Azospirillum* + PSB + KS, T_9-50 % RDF + *Azospirillum*, T_{10}- 50 % RDF + PSB, T_{11}- 50 % RDF + KS, T_{12}- 50 % RDF + *Azospirillum* + PSB + KS, T_{13}- *Azospirillum* + PSB + K and T_{14} (Control) - 100 % RDF ($N_{40}P_{40}K_{45}$).

Table 2: Gross return, Net return and B: C ratio of black cumin as influenced by biofertilizers and various levels of inorganic fertilizers

Treatments	Total cost of cultivation (Rs.)	Gross return (Rs.)	Net return (Rs.)	B:C ratio
T_1 100 % RDF + *Azospirillum*	26,952.00	1,23,695.00	96,743.00	3.58
T_2 100 % RDF + PSB	26,952.00	1,22,937.50	95,985.50	3.56
T_3 100 % RDF + KS	26,952.00	1,22,710.00	95,759.00	3.55
T_4 100 % RDF + *Azospirillum* + PSB + KS	27,232.00	1,34,625.00	1,07,393.00	3.94
T_5 75 % RDF + *Azospirillum*	25,821.26	1,22,447.50	96,626.24	3.74
T_6 75 % RDF + PSB	25,821.26	1,20,990.00	95,168.74	3.68
T_7 75 % RDF + KS	25,821.26	1,19,252.50	93,431.24	3.61
T_8 75 % RDF + *Azospirillum* + PSB + KS	26,101.26	1,34,185.00	1,08,083.74	4.14
T_9 50 % RDF + *Azospirillum*	24,690.88	98,082.50	73,391.62	2.97
T_{10} 50 % RDF + PSB	24,690.88	97,202.50	72,511.62	2.93
T_{11} 50 % RDF + KS	24,690.88	95,992.50	71,301.62	2.88
T_{12} 50 % RDF + *Azospirillum* + PSB + KS	24,970.88	99,910.00	74,939.12	3.00
T_{13} *Azospirillum* + PSB + K	22,710.00	74,727.50	52,017.50	2.29
T_{14} 100 % RDF ($N_{40}P_{40}K_{45}$)	26,812.00	1,24,985.00	98,173.00	3.66

which was at par under T_8 (75% RDF + *Azospirillum* + PSB + KS) (24.66, 97.33, 3.47 g and 536.74 kg ha^{-1} respectively), indicating that there is a chance of reducing of 25% recommended RDF through biofertilizers without any reduction in yield (Table 1). These results are in agreement with the findings of Sahu *et al.*, 2014 in coriander. Hellal *et al.*, 2011 recorded that the maximum yield was obtained under full RD of N + biofertilizer during first year and 2/3 of full RD of N + biofertilizer in second year in dill. Das *et al.*, 2020 also reported that the application of biofertilizers can save 25 % inorganic fertilization in garlic. The least number of capsules plant^{-1} (13.33), number of seeds capsule^{-1}

(84.33), test weight (2.56 g) and projected yield (298.91 kg ha^{-1}) were recorded under T_{13} (*Azospirillum* + PSB + KS). Kusuma *et al*., 2019 recorded that the maximum number of yield attributes like umbels plant^{-1}, umbellets umbel^{-1}, seeds umbellet^{-1} and projected yield was obtained with the application of 75% RDN + RDPK + FYM + *Azospirillum* + PSB in fennel. Abdel-Azieza *et al*., 2013 reported that the productivity and quality of black cumin was improved when *Azotobacter* was applied along with half dose of nitrogen fertilizers. The combined application inorganic fertilizers and biofertilizers were significantly higher in overall improvement in yield and yield attributes. This might be due to the influence of biofertilizers by increasing photosynthetic efficiency and translocation of photosynthates towards flowers and seeds development. And also the activities of microorganisms to mobilize the soil bound minerals to the plants in soluble form during its growing period resulting in better nourishment of crop, which led to production of higher yield.

Economics: The economics of the experiment under different treatment was calculated with respect to gross return, net return and benefit cost ratio (B:C ratio). The data is presented in Table 2. The maximum gross return (Rs. 1,34,625.00 ha^{-1}) was recorded under T_4 (100 % RDF + *Azospirillum* + PSB + KS) which was at par with T_8 (75 % RDF + *Azospirillum* + PSB + KS) (Rs. 1,34,185.00 ha^{-1}). But the net return (Rs. 1,08,083.74 ha^{-1}) and B: C ratio (4.14) was recorded higher under T_8. This was due to 25 % reduction of inorganic fertilizers. Roy and Hore, 2010 also reported higher plant growth, yield and B: C ratio with NPK (75%) + *Azospirillium* + VAM in turmeric, which indicate that 25% of inorganic fertilizer can be reduced. The lowest gross return (Rs. 74,727.50 ha^{-1}), net return (Rs. 52,017.50 ha^{-1}) and B: C ratio (2.29) was observed under T_{13} (*Azospirillum* + PSB + KS). Singh *et al*., 2012 reported that the combined application of 75% RDF + *Rhizobium* + PSB was suitable in terms yield and net return, resulting in saving 25% RDF in kasuri methi. It can be concluded that the application of biofertilizers along with inorganic fertilizers can significantly increase the profit in black cumin production.

Conclusion

The application of *Azospirillum,* PSB and KS can significantly improve the productivity by reducing the amount of inorganic fertilizer requirement. The improvement of growth and yield attributes of black cumin may be associated to growth promoting substances that acted on plant development process. On the basis of results, it may be concluded that combined application of 75 % RDF + *Azospirillum* + PSB + KS were found to be effective for higher yield, net return and B: C ratio and resulted in reduction of 25 % RDF due to constant and steady availability and supply of mineralized nutrients to the plants. Thus, the application of 75 % RDF + *Azospirillum* + PSB + KS can be recommended for production of black cumin in new alluvial zone of West Bengal to get higher economic yield and return thereby reducing environmental pollution to some extent.

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