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Influence of plant growth promoting rhizobacteria and plant growth regulators on growth and yield of black cumin (*Nigella sativa* L.) VAR. NS-44

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Abstract
Black cumin (*Nigella sativa* L.) belongs to the family Ranunculaceae is an annual, herbaceous spice crop. It is also used as a food additive and as a flavourant in many countries. The major chemical constituent found in black cumin seed is thymoquinone which has immense medicinal and preservative qualities. The other constituents found in the black cumin seed are glucosides *viz.*, melanthin and melanthingenin, essential oil (0.5 to 1.6 %), fixed oil, resins and tannins. The modern and intensive agriculture necessitates heavy dependence on fertilizers and chemicals, which cause the pollution and environmental hazards and heavy residual toxicity in produce. In many areas, health and productivity of soil have declined to the extent that they cannot sustain profitable farming any more. To avoid the above mentioned problems, emphasis is now focused on judicious usage of bio-fertilizers and plant growth regulators, to increase growth and yield, to achieve maximum profit per unit area besides a step forward for eco-friendly farming system. A field experiment was conducted during *Rabi*, 2017-2018 at College of Horticulture, UHS Campus, Bengaluru, to study the influence of plant growth promoting rhizobacteria and plant growth regulators on growth and yield of black cumin (*Nigella sativa* L.) var. NS-44. Three different PGPR (*Azospirillum*, *Bacillus megaterium*, *Pseudomonas fluorescence*) and three different plant growth regulators (GA_3 50ppm, NAA 25ppm, BA 25ppm) were applied singly and in combination and compared with the control. Among different treatments, maximum plant height (90.93cm) and minimum days to 50% flowering (41.33 days) was found with foliar application of GA_3 50ppm whereas, foliar spray of BA 25ppm recorded the maximum number of branches (13.80) per plant. Among yield and yield attributing characters, seed treatment with *Azospirillum* + PSB + *Pseudomonas fluorescence* recorded maximum number of capsules per plant (74.60), number of seeds per capsule (101.33), fresh weight per plant (102.20g), dry weight per plant (88.22g), seed yield per plant (10.66g) and seed yield per hectare (1.193 ton ha^{-1}).

Keywords: Black cumin, PGPR, plant growth regulators, growth, yield

Introduction
Black cumin (*Nigella sativa* L.) is an annual herbaceous plant belongs to the family Ranunculaceae is an important seed spice crop native to Mediterranean region and later spread to West Asia and then to North India. It is widely cultivated throughout South Europe, Syria, Egypt, Saudi Arabia, Iran, Pakistan, India and
Turkey (Riaz *et al.*, 1996) [13]. In India, it is cultivated commercially in Rajasthan, Punjab, Jharkand, Himachal Pradesh, Bihar and Assam. It has been used as herbal medicine for more than 2000 years. It is also used as a food additive and as a flavourant in many countries. The major chemical constituent found in black cumin seed is nigellone which has immense medicinal and preservative qualities. The other constituents found in the black cumin seed are glucosides *viz.*, melanthin and melanthingenin, essential oil (0.5 to 1.6 %), fixed oil, resins and tannins. The amino acids present in seeds are cystine, lysine, aspartic acid, glutamic acid, alanine and tryptophan (Lindley, 1981) [8].
The modern and intensive agriculture necessitates heavy dependence on fertilizers and chemicals, which cause soil pollution, environmental hazards and residual toxicity in produce. In many areas, health and productivity of soil have declined to the extent that they cannot sustain profitable farming any more. To avoid the above mentioned problems, emphasis is now

focused on use of bio-fertilizer in crop production. Rhizobacteria that exerts beneficial effect on plant growth and development are referred to as plant growth promoting rhizobacteria (PGPR), their application is often associated with increased rate of plant growth, yield and quality. The direct promotion of plant growth by PGPR may include the production and release of secondary metabolites such as plant growth regulators or facilitate the uptake of certain nutrients from the root environment (Shankar, 2009) [15]. Application of PGPR strains can provide an effective, economical and practical way of plant protection *via* disease suppression, P-solubilization, phytoharmone production *etc*. The PGPR strains mixture often show synergistic action in plant protection and growth promotion involving many mechanisms (Zahir and Arshad, 2004) [19]. *Azospirillum* is nitrogen fixing bacteria and PGPR, effective for all non-leguminous crops. This microorganism fixes the atmospheric nitrogen and makes it available to plants in asymbiotic manner and increases the crop production in large scale. Phosphate solubilizing bacteria (*Bacillus megaterium*) are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble forms (Chen *et al.,* 2006) [2]. In addition to PGPR activities phosphate solubilization ability of rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition (Mohammed *et al.,* 2009)[10]. *Pseudomonas fluorescens* get certain nutrients and environmental protection from the plants they reside near and in-turn, aid the plant in several ways. They destroy certain toxins and pollutants and also protect the plants from infection by pathogens by producing secondary metabolites which kill other bacteria and fungi.

Plant growth regulators have great potential in increasing crop production and helps in removing many of the barriers imposed by genetics and environment (Yugandhar *et al*., 2014) [18]. Gibberllins (GA_3) are a class of endogenous plant growth substances actively involved in stem elongation, flower and fruit development (Huttly and Phillips, 1995) [7], alters membrane permeability to ions (Maneul and Guardia, 1980 [9]; Gilory and Jones, 1992 [4]), enhance the translocation potential from source to sink (Pereto and Bertlan, 1987) [12]. Cytokinins promotes cell division, delays senescence and helps the leaves to remain green much longer by decreasing the breakdown of chlorophyll, protein and nucleic acids and also promotes the growth of lateral buds. NAA is a synthetic auxin helps in root formation, increases fruit set.

Materials and Methods

A field experiment was conducted during *Rabi*, 2017-2018 at the Department of Plantation Spice Medicinal and Aromatics, College of Horticulture, UHS Campus, Bengaluru. The experiment was conducted by randomized complete block design (RCBD) with twelve treatments replicated thrice as follows. T_1: Control (RDF 40:20:20 N:P:K and 5 tonns FYM per ha), T_2:RDF + *Azospirillum* (Seed treatment), T_3 : RDF + Phosphate solubilizing bacteria (Seed treatment), T_4 : RDF + *Pseudomonas fluorescence* (Seed treatment), T_5 : RDF + *Azospirillum* + Phosphate solubilizing bacteria (Seed treatment), T_6 : RDF + *Azospirillum* + *Pseudomonas fluorescence* (Seed treatment), T_7 : RDF + Phosphate solubilizing bacteria + *Pseudomonas fluorescence* (Seed treatment), T_8 : RDF + *Azospirillum* + Phosphate solubilizing bacteria + *Pseudomonas fluorescence* (Seed treatment), T_9 : Foliar spray of GA_3 50 ppm (30 DAS), T_{10}: Foliar spray of BA 25 ppm (30 DAS), T_{11}: Foliar spray of NAA 25 ppm (30 DAS), T_{12}: GA_3 50 ppm + BA 25 ppm + NAA 25 ppm (30 DAS). The NS-44 variety of black cumin seeds are were procured from NRC Seed spices, Ajmer, Rajasthan. The seeds of black cumin were inoculated with bio-fertilizers (*Azospirillum*, *Bacillus megaterium* and *Pseudomonas fluorescence*) according to the treatments at 20 g kg^{-1} of seeds using jaggery solution and the seeds were kept in shade overnight before sowing. The seeds were sown in 30 cm between rows and 10 cm between the plants at one cm depth. Farm yard manure was incorporated into the individual plots at 5 t ha^{-1} and was mixed thoroughly with the soil. Fertilizers were applied at the rate of 40 kg N, 20 kg P_2O_5 and 20 kg K_2O per hectare. Full dose of phosphatic and potassic fertilizers and half the dose of nitrogenous fertilizers were applied in the form of Urea, SSP and Murate of Potash (MOP) at the time of sowing. The furrows were covered properly and the plots were watered immediately after sowing at alternate days till germination, thereafter the irrigation was given through drip with 16 mm inline laterals laid in alternate rows. The remaining 50 per cent of nitrogenous fertilizer was given in two equal splits at 30 (25%) and 45 (25%) days after sowing. The seeds germinated in 10 days after sowing. Excess seedlings were thinned out manually 21 days after sowing and two healthy seedlings were retained per hill. A second thinning was done on 28th day after sowing, to retain only one seedling. Spraying of plant growth regulators (NAA 25 ppm, BA 25 ppm, GA_3 50 ppm) was done according to the treatment at 30 days after sowing. The plots were kept weed free by regular hand weeding. The first weeding was done at 30 days after sowing and later at 40, 60 and 90 days after sowing. Wilt/collar rot was noticed in the experimental plots at 40 days after sowing and was controlled by drenching with Redomil M-Z® and Kavach® 2gm per litre at 40 and 50 days after sowing. Aphids infestation was managed by spraying 3ml per litre Azardaracthin at evening hours. The crop was harvested when the whole plants as well as the capsules (pods) turned yellow in color and the capsules discharged deep black coloured seeds with pleasant characteristic aroma. The whole plants were cut and dried under shade. The seeds were separated by threshing and were cleaned by winnowing. The data on growth and yield parameters were statistically analysed with WASP 2.0 developed by ICAR Research complex, Goa.

Results and discussion

The vegetative growth parameters differed significantly among the different treatments at all the stages of crop growth (Table 1). Foliar spray of GA_3 50 ppm resulted with maximum plant height (90.93cm) which differed significantly with rest of the treatments at harvest, this is due to effect of GA_3 in increasing the cell elongation and cell division (Huttly and Phillips, 1995) [7]. Whereas, the maximum number of branches (13.80) at harvest was recorded with foliar spray of BA 25 ppm, which differed significantly with rest of the treatments, this might be due to that suppression of apical dominance, promotion of cell division and lateral bud formation by BA. These findings are line with the findings of Shah *et al.* (2006) [14] in black cumin, Gour *et al.* (2009) [5] in fenugreek and Parari *et al.* (2012) [11] in black cumin.

Minimum number of days taken to 50% flowering (41.33 days) (Table 1) and maturity (110.33 days) (Table 2) was observed with foliar spray of GA_3 50 ppm, which indicated GA_3 involvement in transition of vegetative apices to floral apices. Singh *et al*. (2012) [16] and Yugandhar *et al*. (2014) [18] also observed similar findings in coriander.

The yield and yield attributing characters such as weight of

capsule per plant, number of capsule per plant, seeds per capsule, fresh weight of plant and dry weight of plant were also showed significant variation among the different treatments (Table 2). Seed treatment of *Azospirillum* + PSB + *Pseudomonas fluorescence* found to be the best for various yield attributing characters such as weight of capsule per plant (15.23), number of capsule per plant (74.60), number of seeds per capsule (101.33), seed test weight (0.318g), fresh weight of plant (102.20g), dry weight of plant (88.22g). According to the analysis, the increase in yield attributing parameters might be due to the combined effect of PGPR, *Azospirillum* as a nitrogen fixing organism, fixes free nitrogen and increases nitrogen uptake to plants, *Bacillus megaterium* as a PSB increases phosphorous availability and *Pseudomonas fluorescence* as biocontrol agent against pathogens it decreases severity of biological stress. These finding were also supported by Ali and Hassan (2014) [1] and Valadabadi1 and Farahani (2011) [17] in black cumin.

Maximum seed yield per plant (10.66g) and per hectare (1.19 ton ha^{-1}) was found to be with seed treatment of *Azospirillum* + *Bacillus megaterium* + *Pseudomonas fluorescence*. The increase in seed yield per hectare might be due to increase in yield attributes such as weight of capsule per plant, number of capsule per plant, seeds per capsule, fresh weight of plant, dry weight of plant and seed yield per plant. These finding were also supported by Hadi *et al.* (2015) [6] in black cumin and Garg (2007) [3] in fennel.

Conclusion

The foliar spray of GA_3 50 ppm increased the plant height and shown early flowering whereas, BA 25ppm showed maximum number of branches. The seed treatment of combined application of *Azospirillum*, PSB and *Pseudomonas fluorescence* increased the yield and yield attributing characters in black cumin.

Table 1: Influence of PGPR and PGRs on plant height and number of branches in Nigella

Treatment	Plant height (cm)			Number of branches per plant			Days to 50% flowering
	60 DAS	90 DAS	At harvest	60 DAS	90 DAS	At harvest	
RDF (Control)	40.20 ^{bc}	58.33 ^{ef}	64.60^{cd}	5.53 ^{bc}	7.33 ^{e}	9.80^{de}	46.33 ^{c}
RDF + *Azospirillum*	42.20 ^{bc}	69.27 ^{b}	76.73^{b}	4.80 ^{bc}	8.47 ^{cd}	10.80^{cd}	47.66 ^{bc}
RDF + PSB	40.87 ^{bc}	58.40 ^{e}	65.06^{c}	4.40^{c}	6.47 ^{f}	8.93^{e}	47.33 ^{bc}
RDF + *Pseu. flo.*	40.53 ^{bc}	64.66^{cd}	71.80^{c}	5.20 ^{bc}	8.53 ^{cd}	10.53^{cd}	47.33 ^{bc}
RDF + *Azo.*+ PSB	45.20 ^{ab}	64.86 ^{cd}	73.07^{bc}	5.33^{bc}	8.26 ^{d}	10.00^{d}	48.66 ^{bc}
RDF + *Azo.* + *Pseu.flo.*	40.00 ^{bc}	63.33 ^{d}	72.73^{bc}	4.87^{bc}	7.07 ^{ef}	9.40^{de}	47.67 ^{bc}
RDF + PSB + *Pseu. flo.*	41.00 ^{bc}	65.13^{cd}	73.33^{bc}	4.67 ^{bc}	8.73 ^{cd}	11.00^{cd}	45.00 ^{cd}
RDF + *Azo* + PSB + *Pseu. flo.*	42.87 ^{b}	66.73 ^{c}	75.73^{bc}	6.20 ^{b}	10.13 ^{b}	12.33^{b}	46.00 ^{cd}
Foliar spray of GA_3 50ppm	48.22 ^{a}	80.80 ^{a}	90.93^{a}	4.80 ^{bc}	8.47 ^{cd}	10.80^{cd}	41.33 ^{d}
Foliar spray of BA 25ppm	39.80 ^{c}	55.20 ^{f}	62.60^{d}	7.87 ^{a}	11.07 ^{a}	13.80^{a}	50.00 ^{b}
Foliar spray of NAA 25ppm	41.93 ^{bc}	61.60^{de}	69.60^{cd}	4.80 ^{bc}	9.53 ^{bc}	11.53^{bc}	53.33 ^{a}
$GA_3$50ppm + BA 25ppm + NAA 25ppm	42.00 ^{bc}	62.20^{de}	70.13^{bc}	4.73 ^{bc}	9.13 ^{c}	11.13^{c}	44.00 ^{cd}
SEm±	2.21	1.21	2.19	0.80	0.39	0.46	1.44
CD @ 5%	4.57	2.51	4.54	1.65	0.81	0.96	2.99

Table 2: Influence of PGPR and PGRs on yield and yield attributing parameters of Nigella

Treatment	Days to maturity	Weight of capsule per plant (g)	Number of capsules per plant	Number of seeds per capsule	100 seed Test weight (g)	Fresh weight per plant (g)	Dry weight per plant (g)	Seed yield per plant (g)	Seed yield per hectare (ton ha^{-1})
RDF (Control)	115.67 ^{bc}	9.37^{c}	33.33^{c}	66.20^{d}	0.238	51.07^{cd}	22.33^{cd}	6.39^{c}	0.85^{c}
RDF + *Azospirillum*	114.33^{bc}	9.95^{bc}	48.43^{bc}	88.17^{b}	0.237	52.90^{cd}	25.46^{cd}	8.33^{ab}	1.03^{b}
RDF + PSB	116.33^{b}	14.63^{ab}	54.53^{bc}	97.03^{ab}	0.262	58.80^{c}	32.16^{bc}	9.40^{ab}	1.01^{bc}
RDF + *Pseu. flo.*	120.67^{ab}	12.40^{ab}	48.86^{bc}	87.53^{bc}	0.232	70.63^{bc}	31.67^{bc}	7.72^{bc}	1.06^{ab}
RDF + *Azo.*+ PSB	115.33^{bc}	11.25^{b}	48.40^{bc}	83.23^{d}	0.221	73.00^{bc}	25.82^{c}	6.80^{bc}	1.04^{ab}
RDF + *Azo.* + *Pseu. flo.*	118.33^{ab}	9.87^{bc}	44.73^{bc}	76.97^{bc}	0.293	49.73^{d}	22.28^{d}	6.60^{bc}	0.94^{bc}
RDF + PSB + *Pseu. flo.*	115.67^{bc}	14.47^{ab}	53.43^{bc}	68.83^{c}	0.246	64.47^{bc}	27.28^{bc}	8.00^{b}	1.14^{ab}
RDF + *Azo.* + PSB + *Pseu. flo.*	113.33^{bc}	15.23^{a}	74.60^{a}	101.33^{a}	0.318	102.20^{a}	88.22^{a}	10.66^{a}	1.19^{a}
Foliar spray of GA_3 50ppm	110.33^{c}	14.45^{ab}	57.67^{b}	87.90^{bc}	0.270	61.66^{bc}	27.08^{bc}	9.09^{ab}	1.07^{ab}
Foliar spray of BA 25ppm	113.33^{bc}	14.52^{ab}	61.87^{ab}	96.73^{ab}	0.307	61.83^{bc}	31.93^{bc}	8.85^{ab}	0.98^{bc}
Foliar spray of NAA 25ppm	122.33^{a}	13.10^{ab}	56.90^{bc}	84.40^{bc}	0.209	65.10^{bc}	28.25^{bc}	9.57^{ab}	0.99^{bc}
GA_3 50ppm + BA 25ppm + NAA 25ppm	117.67^{ab}	14.87^{ab}	71.13^{ab}	98.27^{ab}	0.278	79.40^{b}	34.56^{b}	10.40^{ab}	1.02^{bc}
SEm±	2.77	1.91	7.55	6.21	0.08	8.81	3.67	1.16	0.08
CD @ 5%	5.75	3.97	15.66	12.88	NS	18.27	7.61	2.41	0.16

Fig 1: General view of experimental plot at 60 DAS

Fig 2: General view of experimental plot at harvest

Fig 3: T_8: *Azospirillum* + PSB + *Pseudomonas flouroscence*

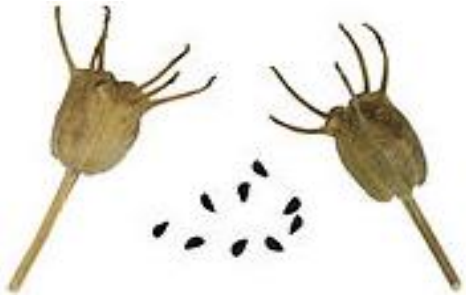

Fig 4: Capsules and seeds of Nigella

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