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Full Length Research

Investigation of biofertilizers influence on quantity and quality characteristics in *Nigella sativa* L.

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To reduce farmers' dependency on mineral fertilizers, to increase water use efficiency, and to increase households' incomes, participatory on-farm research trials to test the beneficial impact of biological fertilizers application on black cumin (*Nigella sativa* L.) some yield characters were investigated. Our objectives in this study were the interactive effects of biofertilizer application on quality and quantity yields at Iran in 2010. In this respect, the experimental unit had been designed by the achieved treatments in factorial on the basis of completely randomized block design with three replicates. Certain factors including four levels of animal manure (0, 10, 20 and 30 ton/ha respectively) and two levels of azotobacter (non-application and application) were studied. The final statistical analysis indicated that in the 20 ton/ha animal manure and azotobacter application, yield components were significantly higher. Those such as: biological yield, seed yield, essential oil yield, stem dry weight, capsule dry weight, leaf dry weight and plant height were higher in unites by 20 ton/ha animal manure and azotobacter application. The finding may give applicable advice to farmers for management and concern on fertilizer strategy and carefully estimate chemical fertilizer supply by biofertilizer application.

Key words: Azotobacter, animal manure, essential oil yield, *Nigella sativa* L.

INTRODUCTION

Nigella sativa is an annual flowering plant, native to southwest Asia. It grows to 20 to 30 cm (7.9 to 12 inch) tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually coloured pale blue and white, with 5 to 10 petals. The fruit is a large and inflated capsule composed of 3 to 7 united follicles, each containing numerous seeds. The seed is used as a spice. Origian Black cumin is rarely available so *N. sativa* is widely used instead, in India *Carum carvi* is the substitute. {cumins are from Apiaceae or Umbelliferae (both names are allowed by the ICBN) family but *N. sativa* is from Ranunculaceae family} Black cumin (not *N. sativa*) seeds come as paired or separate carpels, and are 3 to 4 mm long. They have a striped pattern of nine ridges and oil canals, and are fragrant (Ayurveda says"Kaala jaaji sugandhaa cha" =Black cumin seed is fragrant itself), blackish in colour, boat-shaped, tapering at each extremity, with tiny stalks attached; has been used for medicinal purposes for centuries, both as a herb and pressed into oil, in Asia, Middle East, and Africa. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support, as analgesic, anti-inflammatory, antiallergic, antioxidants, anticancer, antiviral and for general well-being. *N. sativa* oil (not Black cumin seed oil) contains nigellone, which protects guinea pigs from histamine-induced bronchial spasms (Oxford Uni, 2000). Azotobacter is a genus of usually motile, oval or spherical bacteria that form thick-walled cysts, and may produce large quantities of capsular slime. Azotobacter is an aerobic, free-living soil microbe which fixes nitrogen from the atmosphere. Beyond Azotobacter's use as a model organism it has biotechnological applications. Examples are its use for alginate production and for nitrogen production in batch fermentations. Polyhydroxybutyrate is produced under certain conditions. Azotobacter is Gram-

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Table 1. The results of soil analysis.

Soil texture	Sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (mg/kg)	N (mg/kg)	Na (Ds/m)	EC (1: 2.5)	pH	Depth of sampling(cm)
Ca.L	35	30	35	142.2	5.2	38.7	0.05	0.18	7.9	0-30

negative bacteria (Dixon and Kahn, 2004). Biofertilizers are products containing living cells of different types of microorganisms (Vessey, 2003; Chen, 2006) that have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003) and are known to help with expansion of the root system and better seed germination. Biofertilizers differ from chemical and organic fertilizers in that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Some microorganisms have positive effects on plant growth promotion, including the plant growth promoting rhizobacteria (PGPR) such as *Azospirillum*, *Azotobacter*, *Pseudomonas fluorescens*, and several gram positive *Bacillus* spp. (Chen, 2006). The diazotrophic rhizobiocoenosis is an important biological process that plays a major role in satisfying the nutritional requirements of the commercial medicinal plants (Deka et al., 1992). The strong and rapidly stimulating effect of fungal elicitor on plant secondary metabolism in main crops has attracted considerable attention and research efforts (Zhao et al., 2005). *Azotobacter* and *Azospirillum* are free-living N_2-fixing bacteria that in the rhizospheric zone have the ability to synthesize and secret some biologically active substances that enhance root growth. They also increase germination and vigour in young plants, leading to improved crop stands (Chen, 2006). Various *Pseudomonas* species have shown to be effective in controlling pathogenic fungi and stimulating plant growth by a variety of mechanisms, including production of siderophores, synthesis of antibiotics, production of phytohormones, enhancement of phosphate uptake by the plant, nitrogen fixation, and synthesis of enzymes that regulate plant ethylene levels (Abdul-Jaleel et al., 2007). Good soil fertility management ensures adequate nutrient availability to plants and increases yields. High above-ground biomass yield is obviously accompanied by an active root system, which releases an array of organic compounds into the rhizosphere (Mandal et al., 2007). It is well known that a considerable number of bacterial and fungal species possess a functional relationship and constitute a holistic system with plants. They are able to exert beneficial effects on plant growth (Vessey, 2003) and also enhance plant resistance to adverse environmental stresses, such as water and nutrient deficiency and heavy metal contamination (Wu et al., 2005). An experiment was conducted to evaluating the effects of *Azotobacter* inoculant on the yield of wheat (cv. Kanchan). The treatments were T_0 (control), T_1, T_2, T_3, T_4 and T_5. Except 1000 grain weight, all the yield components of wheat viz. plant height, filled spikelets spike^{-1}, spike length and the number of grain spike^{-1} were influenced significantly by the treatments. The highest grain yield of 780 mg plant^{-1}, that is, 84% increase over the control (425 mg plant^{-1}) was obtained due to the treatment of T_5 which did not differ significantly from the yield obtained (687, 732 mg and 715 mg plant^{-1}) with the application of T_1, T_3 and T_4, respectively. There was 18% increase in grain yields due to using *Azotobacter* inoculant only over the control, which was not statistically significant. The straw yields showed a similar pattern. *Azotobacter* inoculation also influenced the root growth significantly. Total N uptake in grain, straw and root increased significantly due to different treatments. The highest N uptake (23.17 mg plant^{-1}) was recorded with the treatment T_5 and the lowest with the T_0 (control), (11.03 mg plant^{-1}). The total N uptake was increased by 89, 36, 101, 88 and 109% over the control due to T_1, T_2, T_3, T_4 and T_5, respectively. *Azotobacter* either alone or in combination with urea N had some beneficial effects on the yield of wheat, which amounted to saving about 20% of urea N (Kader et al., 2002). The experiment of Sudhakar et al. (2000) was conducted in Berhampore, India, from 1993 to 1996 to evaluate *Azotobacter* as a biofertilizer under graded levels of inorganic nitrogen fertilizer to reduce the cost of mulberry leaf production. *Azotobacter* was applied as biofertilizer (farmyard manure plus inoculum with more than 10^8 cells/g carrier). This application of biofertilizer with 150 kg N/ha per year as inorganic nitrogen gave as much leaf as the recommended dose (300 kg N/ha per year) of inorganic nitrogen fertilizer alone. Further reduction in inorganic nitrogen fertilizer, however, reduced leaf yield and quality. Application of *Azotobacter* with 50% of the recommended inorganic nitrogen had no effect on rearing and cocoon characters of silkworm. Therefore, the objective of this study was to evaluate the biofertilizers influence on quantity and quality characteristics of black cumin (*N.sativa* L.).

MATERIALS AND METHODS

This study was conducted on experimental field of Islamic Azad University, Shahr-e-Qods Branch at Iran (27°38' N, 40°21' E; 1417 m above sea level) during 2010, with clay loam soil (Table 1), mean annual temperature (31°C) and rainfall in the study area is distributed with an annual mean of 215 mm. The experimental unit had designed by achieved treatments in factorial on the basis completely randomized block design with three replicates. Certain factors including four levels of animal manure (0, 10, 20 and 30

Table 2. Analysis of variance.

Sources of variation	df	Mean square					
		Biological yield (kg/ha)	Seed Yield (kg/ha)	Capsule dry weight(kg/ha)	Essential oil yield(kg/ha)	Leaf dry weight(kg/ha)	Plant height(cm)
Replication	2	0.007	15.931 **	189143.313	0.005	5.414 **	0.084 **
Azotobacter	1	1.423 **	10.087 **	1520201.243**	0.071 **	41.633 **	0.041 **
Animal manure	3	1.515 **	11.797 **	1089514.354**	0.058 **	67.903 **	0.022 **
Azotobacter× animal manure	3	0.027	0.008	211820.317 *	0.056	0.267	0.004
Error	18	0.009	0.032	86199.201	0.003	1.541	0.002
CV(%)		6.92	4.54	8.22	2.77	9.64	8.08

* and ** : Significant at 5% and 1% levels respectively

ton/ha respectively) and two levels of azotobacter (non-application and application) were studied. The soil consisted of 25% clay, 30% silt and 45% sand (Table 1) and further the field was prepared in a 15 m^2 area (5 × 3 m).

At the end of growth stage we collected 10 plants from each plot randomly for determination of plant characteristics and selected 100 g seed from each plot for determination of essential oil percentage by Clevenger. Finally, essential oil yield was determined by the following formula (Farahani et al., 2008).

Essential oil yield = Essential oil percentage × Root yield

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System [SAS, 1988] and followed by Duncan's multiple range tests. Terms were considered significant at $P < 0.05$.

RESULTS

Final results of plants values showed that azotobacter significantly affected biological yield, seed yield, essential oil yield, stem dry weight, capsule dry weight, leaf dry weight and plant height in $P \leq 0.01$ (Table 2) which indicated the highest biological yield (9206 kg/ha), seed yield (1815 kg/ha), stem dry weight (3728 kg/ha), capsule dry weight (305.2 kg/ha), leaf dry weight (3476 kg/ha), essential oil yield (17.5 kg/ha) and plant height (84 cm) were obtained by azotobacter application (Table 3). Also, animal manure significantly affected biological yield, seed yield, essential oil yield, stem dry weight, capsule dry weight, leaf dry weight and plant height in $P \leq 0.01$ (Table 2). The highest biological yield (10080 kg/ha), seed yield (2279 kg/ha), stem dry weight (4231 kg/ha), capsule dry weight (363.5 kg/ha), essential oil yield (20.1 kg/ha), leaf dry weight (1714 kg/ha) and plant height (82 cm) were achieved by application of 30 ton/ha animal manure (Table 3). Interaction of the azotobacter and animal manure had significant effect on capsule dry weight in $P<0.05$ (Table 2) and highest biological yield (9643 kg/ha), seed yield (2047 kg/ha), stem dry weight (3979 kg/ha), capsule dry weight (334.3 kg/ha), essential oil yield (18.8 kg/ha), leaf dry weight (1595 kg/ha) and plant height (103 cm) were obtained under application of azotobacter and 20 ton/ha animal mature (Table 4).

DISCUSSION

In this study, increases in agronomic criteria were observed following inoculation with azotobacter. This may be due to better utilization of nutrients in the soil through inoculation of efficient microorganisms. A positive effect of azotobacter on yield and yield components has been reported in the literature (Migahed et al., 2004). In addition, higher dry matter production by the inoculated plant might be because of the augmented uptake of N which in turn was a consequence of the root proliferation. Also, the increased growth parameters in hyssop might be due to the production of growth hormones by the bacteria (Ratti et al*.,* 2001). The results showed that application of azotobacter and 20 ton/ha animal mature increased yield and yield components of black cumin, because **N** is a primary constituent of proteins, is extremely susceptible to loss when considering that average recovery rates fall in the range of 20 to 50% for dry matter production systems in plants. N generally because deficiency of potassium increased carbohydrate storage and reduced proteins, alteration in amino acid balance and consequently change in the quality of proteins and are a main element in chlorophyll production. Toxic concentrations of nitrogen fertilizers cause characteristic symptoms of nitrite or nitrate toxicity in plants, particularly in the leaves. Although pre plant fertilizer applications decrease the potential for nutrient deficiencies in early stages of growth, presence of residual soil NO_3–N (plant-available mineral N from the previous season) may pose a risk to the environment. The water of soil be salt by inordinate N application and increase its potential. Finally, the plant use high energy for absorb of salt water that be causes dry matter reduces in this condition. For example the experiment of Manjunatha et al. (2002) was conducted to study the effect of bio-fertilizers on growth, yield and essential oil content in Patchouli (*Pogostemon cablin* Pellet.) at the Medicinal and Aromatic Crops Section, Department of Horticulture, University of Agricultural Sciences, Bangalore during 1999 to 2000. The cultivar 'Johore' was used for the experiment. The fifteen treatments included three levels

Table 3. Means comparison.

Treatments		Biological yield(kg/ha)	Seed yield (kg/ha)	Stem dry weight (kg/ha)	Capsule dry weight (kg/ha)	Essential oil yield(kg/ha)	Leaf dry weight (kg/ha)	Plant height(cm)
Azotobacter	Application	7433 b	1352 b	3225 b	247 b	14.8 b	1238 b	92 a
	Non- application	10080 a	2279 a	4231 a	363.5 a	20.1 a	1714 a	58 b
Animal manure	Non- application	7803 b	1433 b	3491 ab	260.5 b	16.5 b	1207 b	82 a
	10 ton/ha	7473 b	1303 b	3330 b	246.2 b	16.6 b	1181 b	78 a
	20 ton/ha	9206 a	3815 a	3728 a	305.2 a	17.5 a	1476 a	84 a
	30 ton/ha	5555 c	673 c	2608 c	166 c	12.6 d	1787 c	63 b

Means within the same column and rows and factors, followed by the same letter are not significantly difference (P<0.05)

Table 4. Means comparison of interaction.

Survey instance qualification		Biological yield(kg/ha)	Seed yield (kg/ha)	Stem dry weight(kg/ha)	Capsule dry weight (kg/ha)	Essential oil yield(kg/ha)	Leaf dry weight(kg/ha)	Plant height(cm)
Application of azotobacter	Non -application	7618 d	1392 cd	3358 bcd	253.7 c	15.6 c	1222 c	100 a
	10 ton/ha	7453 d	1327 d	3277 cd	264.6 c	15.7 c	1209 c	95 a
	20 ton/ha	8319 c	1583 ab	3476 abcd	276.1 c	16.1 c	1357 b	103 a
	30 ton/ha	7325 d	1298 d	3293 d	242.4 c	15.7 d	1107 c	82 b
		8941 b	1856 ab	3861 ab	312 ab	18.3 b	1460 b	63 de
Non – application of azotobacter	Non -application	8776 bc	1791 abc	3780 abcd	304.8 ab	18.2 b	1447 b	59 def
	10 ton/ha	9643 a	2047 a	3997 a	334.3 a	18.8 a	1595 a	66 cd
	20 ton/ha	7718 d	1476 d	3419 d	264.7 c	16.3 c	1250 c	49 f
	30 ton/ha	7618 d	1392 cd	3358 bcd	253.7 c	15.6 c	1222 c	100 a

Means within the same column and rows and factors, followed by the same letter are not significantly difference (P<0.05).

each of nitrogen (N) and phosphate (P_2O_5) [520, 75 and 100% of recommended dose] with Potash (K_2O) at constant level of 50 kg/ha along with different biofertilizers (Azotobacter, Azospirillum, Phosphorus Solubilising Bacteria (PSB) and Vesicular Arbuscular Mycorrhizal Fungi (VAM) in combination. The results revealed that the treatments differed significantly; among the treatments, 75% NP + 100 K + Azotobacter + Azospirillum + VAM recorded significantly superior values for plant height (80.14 cm), number of leaves (357.75), number of branches (22.04), plant spread (76.12 cm), leaf area (4075.66 cm^2), yield of fresh herbage [10.733 t/ha against 7.277 t/ha in control] and essential oil yield[71.74 l/ha as compared 47.5 l/ha in control] which was followed by 50% NP + 100 K + Azotobacter + PSB + VAM and 50% P + NK + PSB + VAM. On the whole, the treatment with 75% NP + 100 K + Azotobacter + Azospirillum + VAM emerged as one of the best treatments and effected the saving of fertilizers to the extent of 25%. Also, an investigation was carried out under Madurai conditions of Tamil Nadu, India to study the influence of nitrogen, application of nitrogen 93.75 kg ha^{-1} and phosphorus 93.75 kg ha^{-1} along with *Azospirillum* gave the highest plant height, number of laterals, fresh and dry weight of shoot, dry matter production, fresh herbage yield and essential oil yield. The maximum fresh and dry weight of root was obtained by application of nitrogen 93.75 kg ha^{-1} and phosphorus 93.75 kg ha^{-1} along with VAM. Application of inorganic nutrients and biofertilizers had no significant effect on essential oil constituents of davana. Therefore, dry matter reduced under application of chemical fertilizer application because injured roots and was reduced the absorption. Our results were similar to the findings of Stark and Tindall (1992); Wuest and Cassman (1992) and Knowles et al (1994).

Conclusion

In general it appears that, as expected, application of azotobacter improved yield and other plant criteria. Therefore, it appears that application of azotobacter could be promising in production of black cumin by reduction of chemical fertilizer application. Our finding may give applicable advice to farmers for management and concern on fertilizer strategy and carefully estimate chemical fertilizer supply by azotobacter application.

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