

Evaluation of Biofertilizer and Manure Effects on Quantitative Yield of *Nigella sativa* L.

Mohammad Reza Haj Seyed Hadi, Fereshteh Ghanepasand, Mohammad Taghi Darzi

Abstract—The main objective of this study was to determine the effects of Nitrogen fixing bacteria and manure application on the seed yield and yield components in black cumin (*Nigella sativa* L.). The experiment was carried out at the RAN Research Station in Firouzkouh in 2012. A 4×4 factorial experiment, arranged in a randomized complete blocks designed with three replications. Nitrogen fixing bacteria at 4 levels (control, Azotobacter, Azospirillum and Azotobacter + Azospirillum) and manure application at 4 levels (0, 2.5, 5 and 7.5 ton ha⁻¹) were used at this investigation. The present results have shown that the highest height, 1000 seeds weight, seed number per follicle, follicle yield, seed yield and harvest index were obtained after using Azotobacter and Azospirillum, simultaneously. Manure application only effects on follicle yield and by 5ton manure ha⁻¹ the highest follicle yield obtained. Results of this investigation showed that the maximum seed yield obtained when Aotobacter+Azospirillum inoculated with black cumin seeds and 5 ton manure ha⁻¹ applied. According to the results of this investigation the integrated management of Azotobacter and Azospirillum with manure application is the best treatment for achieving the maximum quantitative characteristics of Black cumin.

Keywords—Azotobacter, azospirillum, black cumin, yield, yield components.

I. INTRODUCTION

CURRENT trends in agriculture are centered on reducing the use of inorganic fertilizers by biofertilizers such as vermicompost [1]. The management practices with organic materials influence agricultural sustainability by improving physical, chemical and biological properties of soils [2].

Black cumin (*Nigella sativa* L.) is an annual species that have originated from arid and semi -arid zones and is used widely in traditional and industrial pharmacology [3]. One of the most important constituents of volatile oil of the *Nigella sativa* seeds are thymoquinone. Thymoquinone belongs to class of compounds known as terpenoids [4]. In vivo, it inhibits fore stomach and fibro sarcoma tumor incidence and multiplicity in mice [5].

Pharmacological investigations explored the effectiveness thymoquinone and carvone against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications [6].

Several types of studies in the last 20 years have shown a beneficial effect on crop plants by inoculation of seeds with Azospirillum and Azotobacter strains [7]-[10]. Positive effects

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of inoculation have been demonstrated on various root parameters, including increase in root length, particularly of the root elongation zone [11].

Manure is the source of N and other nutrients for plants [such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu)] that can make valuable contributions to soil's organic matter, can improve physical fertility, and is a center for biological activities. However, only a small fraction of cattle-manure nutrients are immediately available for plant use; thus, it is required to supply soil with both chemical fertilizers and cattle manure for high plant efficiency and reach the maximum yields [12]. So, integrated use of organic manures and other sources of N fertilizers could be a key factor for Integrated Nutrition Management (INM) in sustainable production systems, especially for medicinal plants farms.

The main objective of this study was to assess the effects of nitrogen fixing bacteria and manure application on yield of Black cumin (*Nigella sativa* L.).

II. MATERIALS AND METHODS

The experiment was conducted during the growing season of 2013 at the research fields of RAN Company in Firouzkouh, Iran. The geographical location of the experimental station was 35° 45' N and 52° 44' E with the altitude of 1930 m. The soil of the experimental region was loamy-clay with pH 7.6 (Table I).

TABLE I
CHEMICAL ANALYSIS OF SOIL EXPERIMENTAL SITE

Texture	pH	ECe ds/m	Total N %	K ppm	P ppm	OC %	Fe mg/kg	Zn mg/kg	Mn mg/kg
Loamy-Clay	7.6	1.55	0.127	720	48	1.86	8	1.1	6.6

A 4×4 factorial experiment, arranged in a randomized complete blocks designed with three replications. The treatments consisted of 4 level of nitrogen fixing bacteria (control, Azotobacter, Azospirillum and Azotobacter + Azospirillum) and 4 level of manure (0, 2.5, 5 and 7.5 ton ha⁻¹). Inoculation was carried out by dipping the black cumin seeds in the cells suspension of 108 CFU/ml for 15 min. Nitrogen (20 kg ha⁻¹) was applied to the plots before planting as starter. Also, 50 kg ha⁻¹ P₂O₅ and K₂O were used according to the soil analysis.

Sowing was done manually, 0.5 cm depth and in rows with 35 cm apart. Three weeks after sowing, the seedlings were thinned up to 140-plant m⁻². Irrigation furrows with uniform

slopes were constructed in each experimental plot. A one-time irrigation was applied immediately after sowing for uniform emergence.

Each experimental plot was 2.5 m long and 2 m wide. There was a space of 50 cm between the plots and 3 meters between replications. Sowing was done manually, 0.5 cm depth and in rows with 35 cm apart. Three weeks after sowing, the seedlings were thinned up to 140-plant m⁻². T-Tape irrigation was constructed in experimental plots. A one-time irrigation was applied immediately after sowing and the second irrigation done at 2-3 days later for uniform emergence. After that, plots were irrigated each 5-7 days intervals. There was no incidence of pest or disease on black cumin during the experiment. Weeding was done manually. All necessary cultural practices were followed uniformly for all the plots during the entire period of experimentation.

Data were recorded for the plant height, 1000 seeds weight, seed number per follicle, follicle yield, seed yield and harvest index. Ten plants were randomly selected from each plot and the observations were recorded. At the flowering stage, the plant height, from plant base to the tip of plant, was measured for each plot using a ruler (± 0.1 cm) [2], [15]. Seed number per follicle was recorded at the end of growth season. In addition, in order to determine seed yield, the plots were manually harvested following the air-drying of seeds at 23-29°C and then the seeds were removed from plants by hand [2].

All the data were subjected to statistical analysis (one-way ANOVA) using SAS software (SAS Institute, version 8, 2001). Means of comparisons between the treatments were performed by Duncan's Multiple Range Test (DMRT) at 5%

probability level. Data were transformed when necessary before analysis to satisfy the assumptions of normality and to assure that the residuals had normal distribution [2]. However, any values mentioned in this section refer to the original data of present experiment. Regression models were fitted to the responses of variables to the manure application.

III. RESULTS AND DISCUSSION

A. Plant Height

The present results have indicated that plant heights were affected by the inoculation of seeds by Azotobacter and Azospirillum. But, manure application and interaction of treatments did not caused significant differences on plant height (Table II). Among various levels of inoculation, the application Azotobacter + Azospirillum simultaneously, could result in highest plant height (74.61 cm) (Table III). The present results show that the interaction of manure and biofertilizers was significant. The highest plant height (78.11 cm) was obtained after the integrated application of 7.5 ton manure per hectare and integrated inoculation of seeds by Azotobacter + Azospirillum (Table III).

The aboveground plant responses to Azotobacter and Azospirillum inoculation in cereals and non-cereal species were often reported as greater plant height and leaf size [13]-[15]. Bhaskara and Charyulu [16] indicated that inoculation of *Setaria italica* with Azospirillum resulted in increased plant height. The same result has reported for maize and black cumin [8], [17].

TABLE II
EFFECTS OF BIOFERTILIZER AND MANURE ON YIELD OF BLACK CUMIN

S.O.V	Follicle yield	1000-seed weight	Seed numbers per follicle	Seed yield	Harvest index
N	40518623.6**	0.01182431 ^{ns}	388.398208 ^{ns}	235894.776 ^{ns}	0.04018056
M	69159750.2**	0.00987431 ^{ns}	3250.878730**	1731436.821**	0.11258611
N × M	3492470.1 ^{ns}	0.01753542*	443.048802 ^{ns}	127303.973 ^{ns}	0.05843241
Error	4474014.0	0.00678181	611.256160	127365.950	0.04728819
CV%	15.60	4.73	8.32	17.43	15.23

*N: Nitrogen fixing bacteria; M: Manure

TABLE III
MEAN COMPARISON OF THE MEASURED TRAITS FOR BLACK CUMIN AT VARIOUS LEVELS OF TREATMENTS

Treatments	Follicle Yield (Kg/ha)	1000-Seed Weight (g)	Seed Numbers per follicle	Seed Yield (Kg/ha)	Harvest Index (%)
Biofertilizer					
Control	2785.5 b	1.76 a	53.42 a	300.25 c	26.71 b
Azotobacter	3541.5 b	1.78 a	61.25 a	419.05 bc	30.63 ab
Azospirillum	4714.2 a	1.72 a	56.75 a	552.65 b	28.38 ab
Azotobacter+Azospirillum	5480.9a	1.74 a	75.42 a	734.15 a	37.71 a
Manure					
0 ton/ha	4107.1 b	1.72 a	52.91 a	464.25 a	27.37 a
2.5 ton/ha	4303.5 ab	1.74 a	48.18 a	547.50 a	34.41 a
5 ton/ha	5170.5 a	1.75 a	55.27 a	576.11 a	30.41 a
7.5 ton/ha	2940.5 c	1.79 a	61.85 a	426.15 a	32.21

B. 1000 Seeds Weight

The results presented in Table II have demonstrated that 1000 seed weight was not influenced by bacteria inoculation and manure application.

C. Seed Number per Follicle

The results showed that seed number per follicle was affected by the inoculation of seeds by Azotobacter and Azospirillum. But, manure application and interaction of treatments did not caused significant differences on seed number per follicle (Table II). Among various levels of inoculation, the highest seed number per follicle (75.42) was obtained when Azotobacter and Azospirillum were used together (Table III). The present results show that the interaction of manure and biofertilizers has a significant effect on this trait. The highest seed number per follicle (95.38) was obtained after the integrated application of 7.5 ton manure per

hectare and integrated inoculation of seeds by Azotobacter + Azospirillum (Table III).

D. Follicle Yield

Seed follicle yield was affected by the inoculation of seeds by Azotobacter and Azospirillum. Also, manure application caused significant differences on follicle yield (Table II). The highest follicle yield (5480.9 kg/ha) was obtained when seeds inoculated with Azotobacter and Azospirillum, simultaneously (Table III). Among various levels of manure application, 5 ton manure per hectare could increase follicle yield, significantly. The present results show that the interaction of manure and biofertilizers has a significant effect on follicle yield. The highest follicle yield (6645.23 kg/ha) was obtained after the integrated application of 5 ton manure per hectare and integrated inoculation of seeds by Azotobacter + Azospirillum (Table III).

E. Seed Yield

The present results have indicated that seed yield was affected by the inoculation of seeds by Azotobacter and Azospirillum. But, manure application and interaction of treatments did not caused significant differences on plant height (Table II). Among various levels of inoculation, the application Azotobacter + Azospirillum simultaneously, could result in highest seed yield (734.15 kg/ha) (Table III). The present results show that the interaction of manure and biofertilizers was significant. The seed yield (876.12 kg/ha) was obtained after the integrated application of 5 ton manure per hectare and integrated inoculation of seeds by Azotobacter + Azospirillum (Fig. 1).

In field experiments in Argentina, corn inoculated with *A. lipoferum* showed double the seeds per ear and increased seed yield [18]. Inoculation of wheat with various strains of Azospirillum caused significant increases over controls in grain yield, ranging from 23 to 63% [19]. Also, it has reported that Azospirillum could increase black cumin seed yield [17].

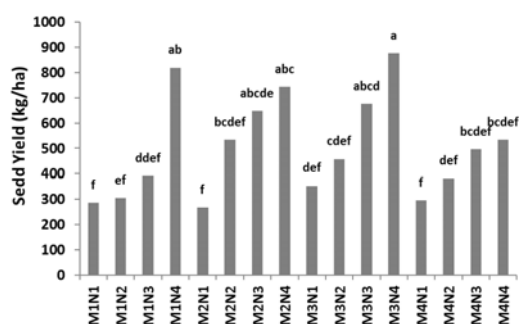


Fig. 1 Effects of interaction between various levels of nitrogen fixing bacteria and manure application on seed yield. (M1 = control, M2 = 2.5 ton ha⁻¹, M3= 5 ton ha⁻¹ and M4 = 7.5 ton ha⁻¹; N1 = control, N2 = azotobacter, N3 = azospirillum and N4: azotobacter+azospirillum)

F. Harvest Index

Mean comparison showed significant differences between various levels of seed inoculation with nitrogen fixing bacteria (Table III). The highest harvest index (37.71%) was recorded when seeds inoculated by Azotobacter and Azospirillum

together. But, there were not significant differences between various levels of manure application on this trait (Table II).

According to the interaction effects between various levels of treatments, application of 2.5 ton manure per hectare + seed inoculation with Azotobacter and Azospirillum could result the highest harvest index (39.50 %) for black cumin.

Azotobacter+azospirillum inoculation could enhance growth and production of black cumin in comparison with control. These changes were directly attributed to positive bacterial effects on mineral uptake by the plant. Enhancement in uptake of NO₃⁻, NH₄⁺, P₀₄₂⁻, K⁺, Rb⁺ and Fe⁺⁺ by Azospirillum and Azotobacter [20]. Also is proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves by nitrogen fixing bacteria inoculation with seeds [21].

IV. CONCLUSION

The present result was derived from the improvement of nitrogen fixing bacteria activities in soil at Azotobacter+Azospirillum treatment. Nitrogen fixing bacteria increase the growth rate which leads to the biological yield improvement. This finding is in accordance with the previous observations. Manure application successfully manipulates the growth of black cumin, resulting in beneficial changes in yield and yield components. Combined application of nitrogen fixing bacteria and manure can be helpful in developing of production and yield in black cumin.

It can be concluded from the present study that the separate application of nitrogen fixing bacteria and manure has a positive effect on the measured traits but their combined use has more beneficial effect on these parameters. Thus, the integrated use of cattle manure and nitrogen fixing bacteria could be a suitable solution for achieving maximum yield of Black cumin.

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REFERENCES

- [1] Gyaneshwar, P., Naresh Kumar, G., Parekh, L.J., Poole, P.S., 2002. Role of Soil Microorganisms in Improving P Nutrition of Plants. *Plant. Soil.* 245: 83-93.
- [2] Haj Seyed Hadi, M.R., Darzi, M.T., Riazii, G.H., Ghandehari, Z., 2011. Effects of Vermicompost and Amino Acids on the Flower Yield and Essential Oil Production from *Matricaria Chamomile L.* *J. Med. Plants Res.* 5(23):5611-5617.
- [3] Patel, B.S., Patel, J.C., Sadaria, S.G., 1996. Response of Blond Psyllium (*Plantago ovata*) to Irrigation and Phosphorus. *Indian J. Agron.* 41: 311314.
- [4] Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J.M., Bernardini, A.F., Costa, J., 2008. Antimicrobial Activity and Chemical Composition of *Origanum glandulosum* Desf. Essential Oil and Extract Obtained by Microwave Extraction: comparison with Hydrodistillation. *Food Chem.* 106: 132-139.
- [5] Badary, O.A., Al-Shabanah, O.A., Nagi, M.N., Al-Rikabi, A.C., Elmazar, M.M., 1999. Inhibition of Benzo(a)Pyrene-Induced

- Forestomach Carcinogenesis in Mice by Thymoquinone. Eur. J. Cancer. Prev. 8: 435-40.
- [6] Ivankovic, S., Stojkovic, R., Jukic, M., Milos, M., Jurin, M., 2000. The Antitumor Activity of Thymoquinone and Thymohydroquinone *in vitro* and *in vivo*. Exp. Oncol. 28 (3): 220-224.
- [7] Sarig, S., Blum, A., Okon, Y., 1988. Improvement of the Water Status and Yield of Field-Grown Grain Sorghum (*Sorghum Bicolor*) by Inoculation with *Azospirillum Brasilense*. J. Agric. Sci. 110:271-277.
- [8] Das, K., Dang, R., Shivananda, T.N., Sekeroglu, N., 2007. Influence of Biofertilizers on the Biomass Yield and Nutrient Content in *Stevia Rebaudiana* Bert. Grown in Indian Subtropics. J. Med. Plants Res. 1 (1):5-8.
- [9] Verma, N.K., 2011. Integrated Nutrient Management in Winter Maize (*Zea mays* L.) Sown at Different Dates. Afr. J. Plant Breed. Crop Sci. 3(8):161-167.
- [10] Pereyra, M.A., Garcia, P., Colabelli, M.N., Barassi, C.A., Creus, C.M., 2012. A Better Water Status in Wheat Seedlings Induced by *Azospirillum* under Osmotic Stress is Related to Morphological Changes in Xylem Vessels of the Coleoptile. Appl. Soil Ecol. 53:94-97.
- [11] Kolb, W., Martin, P., 1985. Response of Plant Roots to Inoculation with *Azospirillum Brasilense* and to Application of Indole Acetic Acid. In *Azospirillum*. III. Genetics, Physiology, Ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 215-221.
- [12] Najm, A.A., Haj Seyed Hadi, M.R., Darzi, M.T., Rahi, A.R., Fazeli, F., 2012. Effects of Integrated Management of Nitrogen Fertilizer and Cattle Manure on the Leaf Chlorophyll, Yield and Tuber Glycoalkaloids of *Agria* Potato. Commun. Soil. Sci. Plant Anal. 43: 912-923.
- [13] Millet, E., Feldman, M., 1986. Yield Response of a Common Spring Wheat Cultivar to Inoculation with *Azospirillum Brasilense* at Various Levels of Nitrogen Fertilization. Plant Soil, 80:255-259.
- [14] Warembourg, F.R., Dreessen, R., Vlassak, K., Lafont, F., 1987. Peculiar Effect of *Azospirillum* Inoculation on Growth and Nitrogen Balance of Winter Wheat (*Triticum aestivum*). Biol. Fert. Soils 4:55-59.
- [15] Nadjafi, F., Rezvani, P., 2002. Effects of Irrigation Regimes and Plant Densities on Yield and Agronomic Characteristics of *Isabgol* (*Plantago ovata*). Agric. Sci. Technol. 2:59-65.
- [16] Bhaskara, R.K.V., Charyulu, P.B.B.N., 2005. Evaluation of effect of Inoculation of *Azospirillum* on the Yield of *Setaria Italica* (L.). Afr. J. Biotechnol. 4(9):989-995.
- [17] Haj Seyed Hadi, M.R., Darzi, M.T., Ghandehari, Z., 2012. Effects of Irrigation Treatment and *Azospirillum* Inoculation on Yield and Yield Component of Black Cumin (*Nigella sativa* L.). J. Med. Plants Res. 6(30): 4553-4561.
- [18] Fulchieri M, Frioni L (1994). *Azospirillum* Inoculation on Maize (*Zea mays*): Effect on Yield in a Field Experiment in Central Argentina. Soil. Biol. Chem. 26:921-923.
- [19] Caballero-Mellado, J.M., Carcano, M.M.G., Mascarua, E.M.A., 1992. Field Inoculation of Wheat (*Triticum aestivum*) with *Azospirillum Brasilense* under Temperate Climate. Symbiosis. 13:243-253.
- [20] Barton, I.L., Johnson, G.V., Orbock, M.S., 1986. The Effect of *Azospirillum Brasilense* on Iron Absorption and Translocation by Sorghum. J. Plant nut. 9: 557-565.
- [21] Murty, M.G., Ladha, J.K., 1987. Differential Colonization of *Azospirillum Lipoferum* on Roots of Two Varieties of Rice (*oryza sativa*). Biol. Fert. Soils. 4: 3-7.