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Influence Of Manure Application And Nitrogen Fixing Bacteria On Yield And Yield Components Of Black Cumin (*Nigella Sativa* L.)

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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. 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}). 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 affects 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. Combined application of nitrogen fixing bacteria and manure can be helpful in developing of production and yield in *Cicer arietinum*.

Key words: Azotobacter, Azospirillum, Black cumin, Yield.

INTRODUCTION
Current trends in agriculture are centered on reducing the use of inorganic fertilizers by biofertilizers such as vermicompost (Gyaneshwar et al., 2002). The management practices with organic materials influence agricultural sustainability by improving physical, chemical and biological properties of soils (Haj Seyed Hadi et al., 2011). 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 (Patel et al., 1996).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 (Bendahou *et al*., 2008). In vivo, it inhibits fore stomach and fibro sarcoma tumor incidence and multiplicity in mice (Badary et al., 1999). Pharmacological investigations explored the effectiveness thymoquinone and carvone against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications (Ivankovic et al., 2006). 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 (Okon, 1985; Sarig *et al*. 1988; Das et al., 2007; Verma, 2011; Pereyra et al., 2012). Positive effects of inoculation have been demonstrated on various root parameters, including increase in root length, particularly of the root elongation zone (Kolb

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and Martin1985; Sarig *et al*. 1988). 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 (Najm et al., 2012). 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 amino acids spraying on yield of mungbean (*Vigna radiate*).

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 35º 45´ N and 52º 44´ E with the altitude of 1930 m. The soil of the experimental region was loamy-clay with pH 7.6 (Table 1).

Table1. Chemical analysis of vermicompost used in the experiment.

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 10^8 CFU/ml for 15 min. Nitrogen (20 kg ha^{-1}) was applied to the plots before planting as starter. Also, 50 kg ha^{-1} P_2o_5 and K_20 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) (Azizi et al., 2009; Haj Seyed Hadi, 2012). 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 (Abdou et al., 2004; Khalid and Shafei, 2005; Haj Seyed Hadi, 2012).

Statistical analysis

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 (Zar, 1996). 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.

RESULTS AND DISCUSSION

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 2). Among various levels of inoculation, the application *Azotobacter* + *Azospirillum* simultaneously, could result in highest plant height (74.61 cm) (Table 3). 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 3).

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 (Millet and Feldman, 1986; Warembourg et al., 1987; Nadjafi and Rezvani, 2002). Bhaskara and Charyulu (2005) indicated that inoculation of *Setaria italica* with *Azospirillum* resulted in increased plant height. Also, Das et al. (2007) and Haj seyed Hadi et al. (2012) found the same result for maize and black cumin, respectively.

1000 seeds weight

The results presented in Table 2 have demonstrated that 1000 seed weight was not influenced by bacteria inoculation and manure application.

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 2). Among various levels of inoculation, the highest seed number per follicle (75.42) was obtained when *Azotobacter* and *Azospirillum* were used together (Table 3). The present results show that the interaction of manure and biofertilizers has a significant effects 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 3).

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 2). The highest follicle yield (5480.9 kg/ha) was obtained when seeds inoculated with *Azotobacter* and *Azospirillum*, simultaneously (Table 3). 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 effects 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 3).

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 2). Among various levels of inoculation, the application *Azotobacter* + *Azospirillum* simultaneously, could result in highest seed yield (734.15 kg/ha) (Table 3). 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 (Fulchieri and Frioni, 1994). Inoculation of wheat with various strains of *Azospirillum* caused significant increases over controls in grain yield, ranging from 23 to 63% (Caballero-Mellado et al., 1992). Also, Haj Seyed Hadi et al., (2012) indicated that *Azospirillum* could increase black cumin seed yield.

Harvest index

Mean comparison showed significant differences between various levels of seed inoculation with nitrogen fixing bacteria (Table 3). 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 2).

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 NO_3^-, NH_4^+, $P0_4^{2-}$, K^+, Rb^+ and Fe^{++} by *Azospirillum* and *Azotobacter* (Barton *et al*. 1986; Murty and Ladha 1987) was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves.

Table2. Effects of irrigation treatments and *Azospirillum* inoculation on yield, yield components and plant height of black cumin (*N. sativa* L.)

S.O.V	Plant height	Follicle yield	1000-seed weight	Seed numbers per follicle	Seed yield	Harvest index
Bacteria inoculation	26.839158^{ns}	40518623.6**	0.01182431^{ns}	388.398208^{ns}	235894.776^{ns}	0.04018056
Manure application	426.815824**	69159750.2**	0.00987431^{ns}	3250.878730**	1731436.821**	0.11258611
B × M	17.708159^{ns}	3492470.1^{ns}	0.01753542*	443.048802^{ns}	127303.973^{ns}	0.05843241
Error mean square	22.074267	4474014.0	0.00678181	611.256160	127365.950	0.04728819
CV%	7.09	15.60	4.73	8.32	17.43	15.23

Table3. Mean comparison of the measured traits for black cumin at various levels of Nitrogen fixing bacteria inoculation and manure application.

Treatments	Plant height(cm)	Follicle yield(Kg/ha)	1000-seed weight(g)	Seed numbers per follicle	Seed yield(Kg/ha)	Harvest Index (%)
Nitrogen Fixing Bacteria						
Control	60.76 c	2785.5 b	1.76 a	53.42 a	300.25 c	26.71 b
Azotobacter	63.69 bc	3541.5 b	1.78 a	61.25 a	419.05 bc	30.63 ab
Azospirillum	65.88 b	4714.2 a	1.72 a	56.75 a	552.65 b	28.38 ab
Azotobacter+Azospirillum	74.61 a	5480.9a	1.74 a	75.42 a	734.15 a	37.71 a
Manure application						
0 ton/ha	64.97a	4107.1 b	1.72 a	52.91 a	464.25 a	27.37 a
2.5 ton/ha	64.94a	4303.5 ab	1.74 a	48.18 a	547.50 a	34.41 a
5 ton/ha	67.23 a	5170.5 a	1.75 a	55.27 a	576.11 a	30.41 a
7.5 ton/ha	67.80 a	2940.5 c	1.79 a	61.85 a	426.15 a	32.21 a

Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

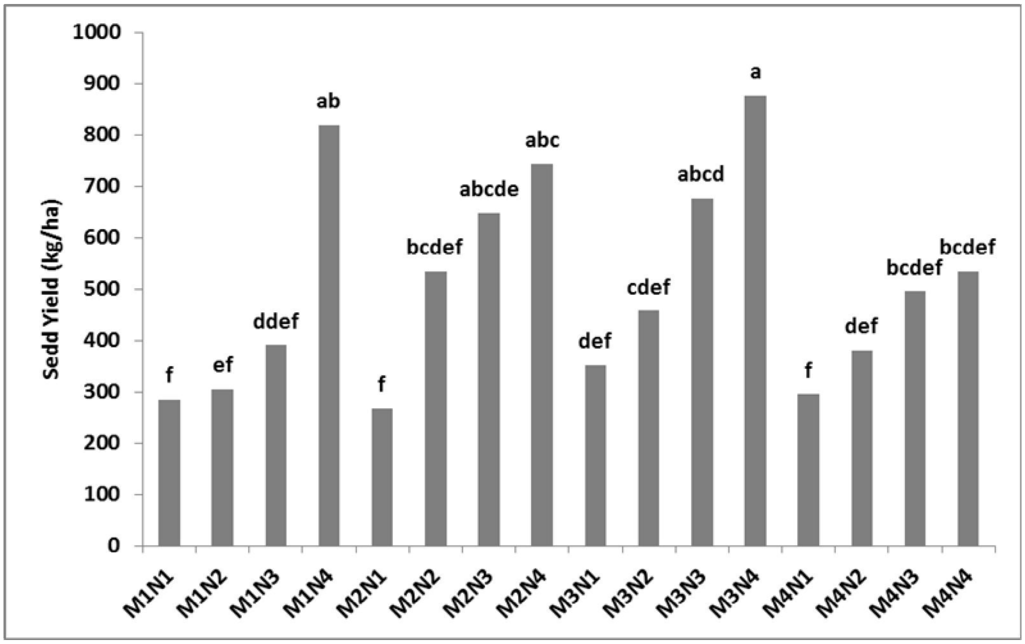

Figure1**.** Effects of interaction between various levels of nitrogen fixing bacteria and manure application on seed yield. (M1 = control, M2 = 2.5 ton ha^{-1}, M3= 5 ton ha^{-1} and M4 = 7.5 ton ha^{-1}; N1 = control, N2 = *Azotobacter*, N3 = *Azospirillum* and N4: *Azotobacter+Azospirillum*)

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 and quality of tuber.

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