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Mixed effect of *Rhizobium* and *Azotobacter* as biofertilizer on nodulation and production of chick pea, *Cicer arietinum*

Anis Siddiqui*, Ruchi Shivle*, Nidhi Magodiya* and Kirti Tiwari**

*Department of Zoology, Government Autonomous Holkar Science College, Indore (M.P.)
**Department of Zoology, Government New Science College, Indore (M.P.)

ABSTRACT

In some regions of the world, vast areas of land are highly weathered, very low in soil fertility including macro- and micronutrients, and there is high application of nitrogen and phosphorus. In some other regions, there is low rainfall, high evaporative demand, increase in soil salinity, and increase in soluble salts concentration of irrigation water. In the regions, these issues have been major impediments against agriculture. The concept of mixed nutrient management systems remains very important. Experiments to see the mixed effect of *Rhizobium* and *Azotobacter* nodulation, nitrogen fixation and yield of chick pea (*Cicer aeritinum*) were conducted under pot conditions during Rabi season of 2013. Inoculation of the seed with an effective strain of *Rhizobium species* along with *Azotobacter chroococcum* resulted in significant increase in nodulation, nitrogen content in the root and grain yield over uninoculated controls. The beneficial effect of test microbial inoculant and plant might be attributed to the synthesis of some growth promoting substances.

KEY WORDS: *AZOTOBACTER*, NODULATION, NITROGEN FIXATION, *RHIZOBIUM.*

INTRODUCTION

Biofertilizers are one of the important beneficial microorganisms used in promoting plant growth and productivity. The concept of mixed nutrient management system as proposed by Adesemoye and Kloepper, (2009) relating to the use of biofrtilizers combination to stimulate uptake of nutrients remains very important. *Rhizobium* and *Azotobacter* interact with a wide range of other soil microorganisms in the rhizosphere of plants. These interactions

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*Corresponding Author
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may be either stimulatory or inhibitory. These are stimulatory when they increase the growth response of the host in the presence of other microorganisms and inhibitory when they control soil borne pathogens (Kennedy and Islam, 2001; Nosheen, 2011).

Azotobacter and *Rhizobium* are known to be good nonsymbiotic and symbiotic nitrogen fixers, respectively. Apart from its nitrogen fixing ability, *Azotobacter* is also known to synthesize certain growth promoting substances (Mishustin, 1963; Jones and Greaves, 1943; Brown and Walker, 1970). The idea behind the concept of mixed inoculation is that if *Azotobacter*, with the help of the production of auxins and gibberellins could stimulate the root growth and elongation, it would be presenting more area for rhizobia to infect the root system, thereby bringing about more nodulation, nitrogen fixation and yield of crop (Verma *et al*., 2012 and Parveen *et al*., 2013). Therefore, a study was undertaken to find out the efficiency of Rhizobium and Azotobacter inoculation on nodulation, nitrogen fixation and yield of Pigeon pea (Parveen *et al*., 2010).

MATERIAL AND METHODS

The experiments conducted under laboratory conditions in earthen pots of 21.5 to 22cm. inner diameter size were filled with 10.0 kg of experimental sterilized soil. The soil used in the experiment was collected from Delmi form, Biotech Research Center, district Dhar (M.P.). The characteristics of the soil were pH, 7.40; soluble salt (E.C.) 59 m. mhos/cm., organic carbon, 0.97%, available phosphorus 82.4 kilo gram per hectare and available potash 436 kilo gram per hectare. The soil was sterilized in an autoclave and then it was used for experiment. Pot culture trial was conducted on Chick pea (*Cicer aeritinum*) using high yielding variety. Data are average of five plants grown in three pots with 8 treatments (table-2) replicated four times was carried out in medium black soil. The soil was basal dressed, with urea and superphosphate at 20 kilogram nitrogen and 40 kilo gram Phosphate per hectare respectively (Nakul, 1990). Two strains of Rhizobium species viz., RS.C-111 and RS.C-112 respectively and two strain of *Azotobacter chroococcum* viz., AC.C-111 and AC.C-112 were selected. *Rhizobium species* strains were maintained on yeast extract mannitol agar medium and *Azotobacter chroococcum* strain was maintained on Ashby's nitrogen free agar medium. After an incubation period of 10 days the bacterial inoculants were used for seed treatment. 2 ml of bacterial suspension and 5 mile liter of sticker solution were added to 10 seeds in a beaker and thoroughly shaken. Bacterial treatment was done just before sowing. The seeds of Chick pea (*Cicer aeritinum*) were sown at 10 kilogram in each pot. A few were uprooted for study of nodulation at 45 days after sowing, while at harvest the grain yield was recorded. The data were analyzed statistically and compared at 5% level of significance.

S.E. ±	7.6682	7.4238	7.6712
C.D. at 5%	24.8046	24.0206	28.0275

RESULTS AND DISCUSSION

The data on nodulation, total dry weight and grain yield presented in (Table-2) It was found to be statistically significant. The data reflect that the increase in nodulation, , dry weight and grain yield among the different test strain under study ranged from 49.2% to 14.5% test strain ranged AC.C-112+RS.C-112 (49.2%), AC.C-111+RS.C-112 (45.8%), AC.C-112+RS.C-111 (40.0%), AC.C-111+RS.C-111 (35.0%), RS.C-112 (30.8%), RS.C-111 (25.6%), AC.C-112 (20.0%) and AC.C-111 (14.5%) showed maximum increase in nodulation, dry weight and grain yield. Co-inoculation can be exploited as biofertilizer for better yield (Siddiqui *et al*., 2001 and Elkoca *et al*., 2007).

TABLE 1: *Rhizobium* and *Azotobacter* strains employed for inoculant production.

S. No.	Strain	Medium	Gram reaction	Nodulation test	N2 -fixation in mg.	Growth on Congo red YEMA	Growth on Jenson medium
1	RS.C-111	YEMA	-ve	3	-	White translucent	-
2	RS.C-112	YEMA	-ve	3	-	Growth	-
3	AC.C-111	Ashby's nitrogen free agar	-ve	-	17.20 mg.	White translucent	White translucent Growth
4	AC.C-112	Ashby's nitrogen free agar	-ve	-	22.10 mg.	Growth	White translucent Growth

TABLE 2: Influence of seed inoculation with a mixed inoculum of *Rhizobium species* and *Azotobacter chroococcum* on nodulation, nitrogen of shoot and grain yield of Chick pea (*Cicer aeritinum*.).

Treatment	No. of Nodules/ plant (45 days)	Nodule dry weight (mg.)	Grain yield (kg/pot)	% increase over control
Control	-	-	15.26.	-
AC.C-111	62	150	17.47	14.5
AC.C-112	83	165	18.31	20.0
RS.C-111	106	172	19.16	25.6
RS.C-112	119	199	19.96	30.8
AC.C-111 + RS.C-111	122	237	20.60	35.0
AC.C-112 + RS.C-111	129	261	21.36	40.0
AC.C-111 + RS.C-112	137	300	22.24	45.8
AC.C-112 + RS.C-112	142	317	22.76	49.2

Azotobacter chroococcum (AC.C-112) along with *Rhizobium species* strain (RS.C-112) increased nodulation, dry weight and grain yield (49.2 %) significantly over control, indicating beneficial effect of the mixed inoculum of *Azotobacter chroococcum* with *Rhizobium species* (AC.C-112+RS.C-112). The beneficial effect of *Azotobacter chroococcum* on *Rhizobium species* and plant might be attributed to the synthesis of growth promoting substances like auxine, indole 1-3 acetic acid and gibberrelin by *Azotobacter chroococcum* (Sarig *et al*., 1986; Brown and Walkar, 1970) possibly helpful in the nodulation process. Increase in nitrogen content is due to increased population of *Rhizobium* by *Azotobacter* (Wigeleva, 1954 and Arora, 1971).

The benifites of mixed nutrient management system to different cropping system have been further discussed by other authors (Maeshwari *et al*., 2011). Maximizing the impacts of beneficial microbes towards enhancing the response of plant to environmental stress (Egamberdieva, 2011) is also very important.

CONCLUSION

Azotobacter chroococcum (AC.C-112) along with *Rhizobium species* strain (RS.C-112) increased nodulation, dry weight and grain yield (49.2 %) significantly over control, indicating beneficial effect of the mixed inoculum of *Azotobacter chroococcum* with *Rhizobium species* (AC.C-112+RS.C-112).

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