Interaction between *G. fasciculatum* and *A. chroococcum* for Yield, Nutrients Uptake and Cost Economy of *Lepidium sativum* in Indian Arid Region

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Abstract

The yield, nutrient uptake and cost efficacy of *L. sativum* were studied using dual inoculation with AM fungi (*Glomus fasciculatum*) and *Azotobacter chroococcum*. The experiment was conducted for two consecutive years, with two levels of chemical fertilizers (F1: N-P-K: 24-24-32 kg ha^{-1}) and (F2: N-P-K: 30-30-40) kg ha^{-1}). Inoculation of bioinoculants generated encouraging results; the seed yield (1501 kg ha^{-1}), stover yield (535 kg ha^{-1}), plant height (64.2 cm) and oil content (26.4 %) obtained were highest with treatment T12 (F2 + both bioinoculants) followed by T9 (F1 + both bioinoculants), T11 (F2 + AM fungi) and T10 (F2 + *A. chroococcum*) as compared to T1 (control), T2-T4 (bioinoculants) and T5-T6 (F1 and F2 fertilizer levels). Growth parameters were non significant between F1 and F2 dose of chemical fertilizer when inoculated with dual microorganisms. The nitrogen uptake was more in *Azotobacter* treated plants, while higher P and K uptake was attained in AM fungi inoculated plants. The survival count of inoculated bacteria improved gradually and highest count was observed on 50th d of inoculation. Economic analysis revealed the net profit from tubers was highest in T9 using dual inoculation of microorganisms with 80% of recommended dosage of fertilizer followed by T12 and T11.

Keywords: AM fungi, *A. chroococcum*, *Lepidium sativum*, cost economy, nutrients uptake, yield

Introduction

Garden cress or Chandrasur (*Lepidium sativum*) is a fast-growing, edible herb that is botanically related to watercress and mustard (crucifereae), sharing their peppery, tangy flavor and aroma. In some regions, garden cress is known as garden pepper cress, pepper grass, pepperwort or poor man's pepper. The plant attains a height of 50-60 cm and prefers sandy to sandy loam soil. The leaves are antiscorbutic, diuretic and stimulant.

Recently, garden cress has gained more interest from consumers and producers and can be a good choice for salads with its peppery taste, and health promoting substances such as glucotropaeolin, a glucosinolate compound and the precursor of benzyl isothiocyanate (Kassie et al., 2002, Sharma and Agarwal, 2011), having antioxidant potential (Chand et al., 2010) and sterols (Conforti et al., 2009). Garden Cress seeds are loaded with not just protein, but also linoleic and arachidic fatty acids. Since they contain phytochemicals that mimic estrogen to some extent, intake of these seeds is known to regulate menstruation and stimulate milk production in lactating mothers. That is precisely why women are given foods containing Garden

Cress following childbirth (Kirtikar and Basu, 2006). Since the testae of these seeds contain mucilage, they are invaluable in the management of both dysentery and constipation (Rehman et al., 20011).

Nitrogen plays important role in the growth and development of the plant and its deficiency leads to sharp decline in the yield in semi-arid region of Rajasthan. Hence, an experiment was conducted to optimize the sowing time, row spacing and nitrogen dose for garden cress. In India, cultivation of *L. sativaum* is gaining popularity among farmers due to its profitable commercial value, but the lack of scientific acumen and costly inputs (chemical fertilizers and pesticides) becomes a constraint to produce the high yield to fetch the competitive price and fulfill the market demand nationally and internationally. In the arid and semi arid environments, the crop production is unsustainable and unreliable due to poor soil fertility status, lack of precipitation and uncongenial climatic conditions. The recommended doses of chemical fertilizers in semi arid region in India for different crops vary, but for garden cress, it is NPK (60-40-40 kg ha^{-1}). Application of appropriate technological approach for overcoming unsuitable cultivation practices may result in increased seed yield in the arid soil (Singh and Khanuja, 2003). Cost efficacy is an important aspect of an experiment or any crop; one must know how much investment in the form of inputs and labor has been put in field and what are the net and gross returns, or benefit after the harvest (Kumar et al., 2004, 2009, 2011).

Azotobacter chroococcum, a free living diazotroph, fixes atmospheric nitrogen, produces growth hormones and solubilizes phosphate (Kumar and Narula, 1999) has been used as a favorable bioinoculant for crops like wheat (Kumar et al., 2001b), cotton (Narula et al., 2005), sorghum (Kumar et al., 1999), sunflower (Kumar et al., 2000) and herbal crop *Withania somnifera* (Kumar et al., 2009). Since the literature on the dual inoculation of AM fungi and *A. chroococcum* on *L. sativum* is scanty, therefore, the purpose of this study was to understand the co-inoculative effect of AM fungi and *A. chroococcum* on yield, nutrients uptake and cost economics of the *L. sativum* in Indian arid region.

Materials and Methods

The study was done in November - January (20010-2011 and 2011-2012) on *L. sativum* var. MDH Bio-13 in a farmer's field in District Udiapur (24.58°N 73.68°E), Rajasthan (India). The crop was grown in a sandy clay loam soil with good drainage having 45% sand, 25% clay, 30% silt, pH 7.4, organic carbon 0.25 %, available nitrogen 145 kg ha^{-1}, phosphorus 12.31 kg ha^{-1} and potash 149.7 kg ha^{-1}. In the experiment, authors have selected 80% of recommended dose of chemical fertilizer in addition to recommended dose (60-40-40 kg N-P-K ha^{-1}). This chemical fertilizer level has been recommended by agricultural department, Government of Rajasthan, India). Generally, the use of biofertilizers can save up to 15-25% of chemical fertilizers (Pandey and Kumar, 1998), therefore, a gap of 20% less fertilizer was kept to be fulfilled by using biofertilizers. Treatments consisted of

T1	Control (No Inoculation)
T2	*A. chroococcum*
T3	AM fungi (*Glomus fasciculatum*)
T4	*A. chroococcum*+AM fungi
T5	F1: 80% of recommended rate as equal to 48-32-32 kg N-P-K ha^{-1}
T6	F2: recommended rate as equal to 60-40-40 kg N-P-K ha^{-1}
T7	F1+*A. chroococcum*
T8	F1+AM fungi
T9	F1+*A. chroococcum*+AM fungi
T10	F2+*A. chroococcum*
T11	F2+AM fungi
T12	F2+*A. chroococcum*+AM fungi

Microbial Treatment of Seeds and Soil

Azotobacter chroococcum nitrogen fixing strain (nitrogenase activity 79.6 n mol C_2H_2 h^{-1} mg^{-1} protein) was obtained from the culture collection of Dr. Shivesh Sharma and AM fungi (*Glomus fasciculatum*) used in the experiment was obtained from the local market, produced and marketed by a government certified company. The seeds of *L. sativum* were sprinkled with 20% sugar solution (it could be normal sugar or Jagerry) and then coated with charcoal powder containing *A. chroococcum* cells (10^8 g^{-1}), the seeds were dried for 30 min in shade (ambient temperature 20°C) and sown

immediately in experimental plots of 4×3 m^2. AM fungi inoculation was done by layering method (Jackson et al. 1972), the AM fungi granules (at the rate of 4 kg ha^{-1}) were spread in beds before sowing the seeds. Seeds of *L. sativum* were sown at the rate of 4 kg ha^{-1} in experimental plots with a row spacing of 40 cm. The data was analyzed using ANOVA, where the means of studied treatments were compared using LSD at $P = 0.05$ significant level. MSTAT-C software was used for computing the data.

Survival Studies of *A. chroococcum*

The survival count of bacteria was carried at regular intervals of 25, 50, 75 and 90 d of inoculation. The selected plants were carefully uprooted and rhizospheric soil adhering intimately to the roots was separated by gentle tapping and composite samples were prepared. Soil samples were air dried at room temperature (25°C) and *A. chroococcum* counts were determined using dilution technique (Kumar, 2011) on *Azotobacter* agar (Hi Media, Mumbai, India) at 30°C. The *A. chroococcum* strain was marked with antibiotic erythromycin. During inoculated bacterial survival count studies, the antibiotic erythromycin was added in the *Azotobacter* agar medium, which resulted in colony formation of inoculated strain only and not the colonies of non indigenous bacteria (NIB), because NIB were antibiotic sensitive and did not grow on the agar plates.

Studied Parameters

Observations on seed yield (t ha^{-1}), stover yield (t ha^{-1}), plant height (cm), Number of branches $plant^{-1}$, oil content (%) were recorded at the time of harvest (150 d after sowing) according to the methods follow by Apkan et al., (2006). For nutrients analysis in the plants, the plant samples (stem and leaves) were dried in an oven at 65°C for 48h. The dried samples were powdered and N, P, K was determined using the method described by Cottenie et al., (1982). The cost efficacy of the experiment was calculated using benefit cost (B:C) ratio as mentioned by Gittinger (1984).

Results and Discussion

The cultivation data in tables are shown as mean of two years for brevity and clarity. In general, all quantitative plant traits showed significant increase which was almost linear in response to bioinoculants and chemical fertilizer treatments. Inoculation of seeds and soil with bioinoculants in treatments T2 and T3, significantly increased plant growth parameters compared to control (Table 1). Dual inoculation of bioinoculants (T4) performed better than control and individual microbial inoculation. The treatment T4 exhibited a significant increase in seed yield (39.4%), number of tubers $branch^{-1}$ (40.4%) and tuber length 48.7 %) over control. Inoculation of *A. chroococcum* (T2) produced 24.8% and 30.2 % more fresh tuber yield and number of tubers $branch^{-1}$ more than control, while AM fungi (T3) increased 30.6% and 28.6 % fresh tuber yield and number of tubers $branch^{-1}$ over control. The combined effect of both bioinoculants (*A. chroococcum* and AM fungi) and chemical fertilizer (F2), (T12) showed highest fresh tuber yield (52.13 q ha^{-1}), number of tubers $branch^{-1}$ (13.29), tuber length (9.69 cm) and tuber diameter (6.68 mm) followed by T9, but the treatments were non significant with each other. Similarly, inoculation of *Azotobacter* alone and with AM fungi in combination with chemical fertilizer increased plant growth parameters; this increment was significantly higher over chemical fertilizer treatments (T5 and T6).

It was observed that N uptake in plant was highest in T12 followed by T10 and T11, P uptake was highest in T12 followed by T11 and T10, while the K uptake was also highest in T12 followed by T11 and T10, all these treatments were significantly higher over control. The N, P and K uptake of all the treatments increased with increase in fertility levels which was further complimented by *A. chroococcum* and AM fungi inoculation. (Figure 1). Application of *A. chroococcum* resulted in more uptake of N compared to P and K in crop, while the AM fungi inoculation produced more P and K uptake compared to N. The higher N uptake might

Table 1 Effect of dual inoculation of *A. chroococcum* and AM fungi on growth yield parameters of *L. sativum*.

Treatment	Seed yield	Stover yield	Plant height	Branches	Oil content
	(---------- kg ha^{-1} ----------)		(cm)	(No. plant^{-1})	(%)
T1	802	262	32.7	12.1	11.8
T2	910	309	39.8	12.3	12.1
T3	918	318	41.6	12.4	12.4
T4	924	337	45.3	12.8	15.4
T5	1078	398	52.8	12.8	17.8
T6	1157	436	54.1	13.6	19.4
T7	1309	482	60.3	15.1	22.7
T8	1380	501	61.5	16.3	24.1
T9	1523	542	63.5	17.2	26.9
T10	1452	505	62.6	16.8	24.8
T11	1427	519	63.0	17.0	25.1
T12	1501	535	64.2	17.1	26.4
LSD (*P*=0.05)	32.67	17.97	2.86	1.09	0.965

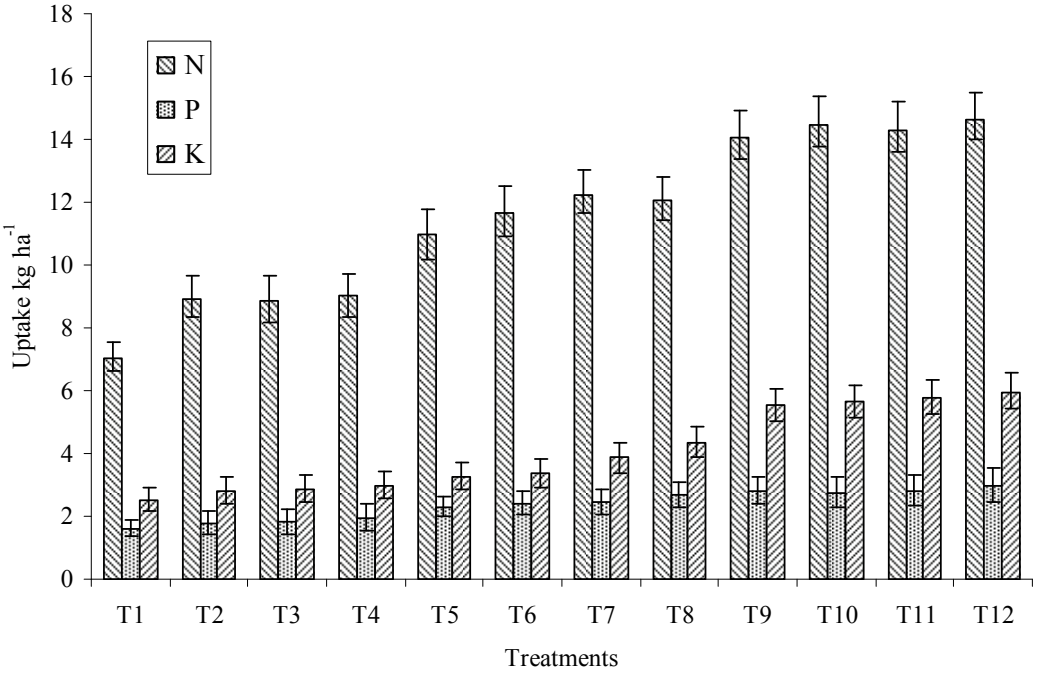

Figure1 Effect of dual inoculation of *A. chroococcum* and AM fungi on nutrients uptake (kg ha^{-1}) by *L. sativum*.

be an effect of N_2 fixation by *A. chroococcum* which has to be proved in further experiments. The survival count of the inoculated bacteria was found to increase gradually and highest count was reached at the time of harvest i.e. 150th d (Figure 2).

The economic analysis of the control treatment was calculated as follows: total cost in US Dollar (USD) (USD 224.7), gross return (USD 475.3), and net return (USD 250.6). The benefit cost (B:C) ratio was determined to be 2.11 (Figures 3 and 4). The highest net return was with treatment T9, (USD 2223.1 and a B:C ratio of 2.94), followed by treatment T12 for which the net return and B:C ratio were USD 2213.2 and 2.85, respectively. Treatment T4 (both bioinoculants only) was also good and the net return and B:C ratio were 1022.3 and 2.29, respectively (Figures 3 and 4).

The response of a crop to inorganic fertilizers is well understood; however, a combination of microbial inoculants and fertilizer has shown

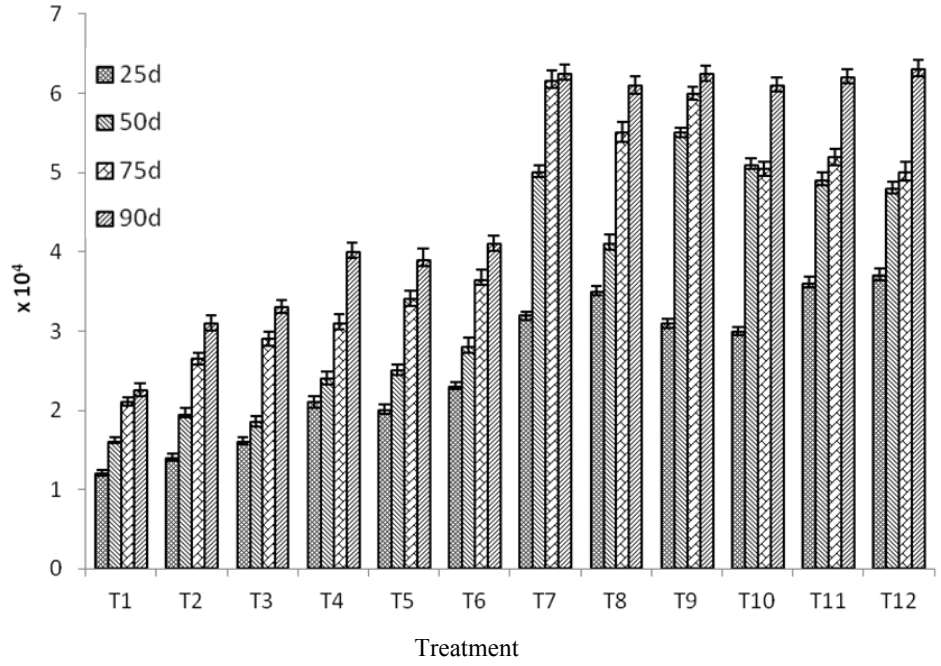

Figure 2 Survival count (Colony forming units) of *A. chroococcum* at different intervals of time.

variable results, partly the promotive effect is attributed to the favorable influences exerted by root exudates which contain organic acids, sugars, phytohormones etc. It is evident from Table 1 that the treatments with single or dual microorganisms, along with fertilizer resulted in higher tuber yield parameters compared to control and fertilizer alone treatments (T5 and T6). In terms of percentage increase the fresh tuber yield in treatments T12 using dual microorganisms resulted in 22.8% higher than T6 and T9 was 24.2% more than T5, similarly the number of tubers branch^{-1} was 29.6% and 23.1% higher than fertilizer alone treatments, also the tuber length was 5.52-5.98% higher than fertilizer alone treatments. This increase in plant growth yields in treatments T7 to T12 over T5 and T6 was due to the application of beneficial microorganisms, as the fertility level was same in the treatments (T7-T12). Treatments T10 to T12 and T7 to T9 were higher than T6 and T5 respectively. Although treatment T12 was superior to all the treatments but non significant to second best treatment T9, therefore, the application of microorganisms saved the 20% chemical fertilizer.

One of the best described events in *Azospirillum* and *A. chroococcum* root colonization is the impact on the root development in terms of increase in root length, number of lateral roots and root hair (Martin et al., 1989; Kumar et al., 2001a). Enhanced proliferation of the root system is believed to promote increased minerals uptake (NPK) and consequently to increase the plant growth yield (Narula et al., 2000). Inoculation of *A. chroococcum* resulted in more uptake of N compared to P and K. There was 1.25% higher N uptake in treatment T10 over T11; this could be due to nitrogen fixation by bacteria. The AM fungi inoculation also resulted in increased uptake of P (2.54% higher in treatment T11 over T10), similarly, K uptake was 2.48% more in T11 compared to T10, this might be due to increasing surface area of roots by AM fungi to absorb and transport nutrients to the host. Increased in nutrient uptake and productivity of field crops with mycorrhizal fungi inoculation have been reported in basil (Gupta et al., 2000), tomato (Gao et al., 2001), forage legumes (Sockley et al., 2004), potato (Davies et al., 2005) and spring safflower (Mirzakhani et al., 2009).

The stimulatory effect of chemical fertilizer on the survival of *A. chroococcum* may be exerted directly through their effect on the growth and proliferation of bacteria thereby creating a favorable habitat for the growth and survival of inoculated bacteria (Kumar et al., 2001a). The *A. chroococcum* count increased till the day of

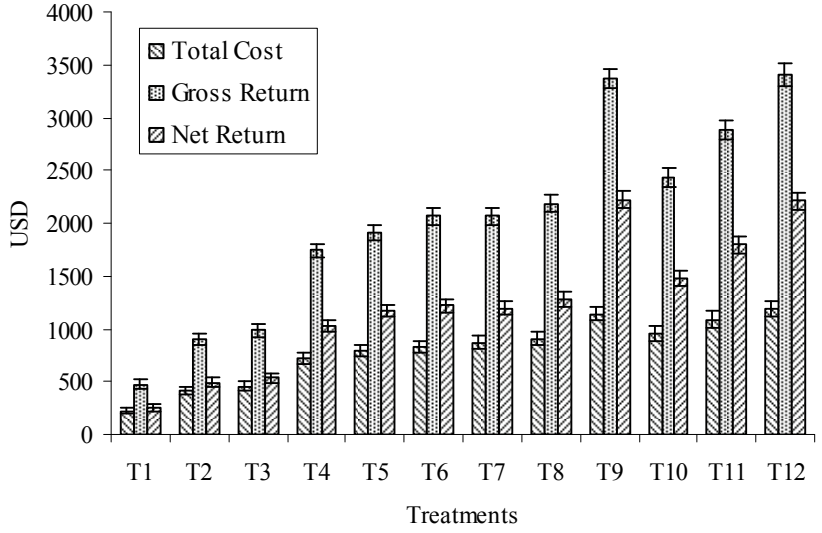

Figure 3 Economic analysis of different treatments.

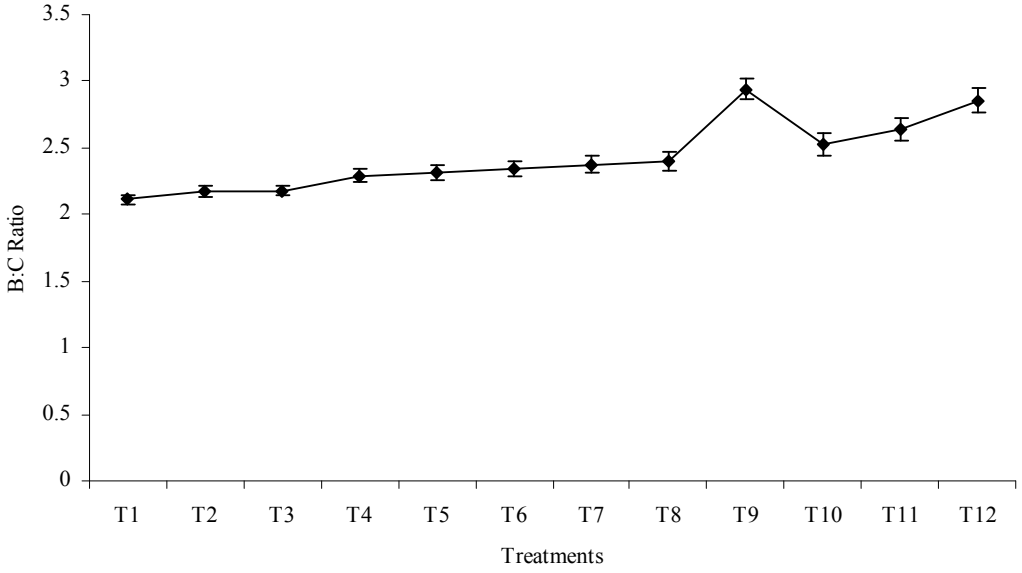

Figure 4 Benefit cost (B:C) ratio of the treatments.

harvesting sowing in all treatments, which may have been attributable to more root exudates, supporting better plant-AM fungi-*A. chroococcum* interaction and nutrient mobilization of fungal (Hetrick et al., 1992) and bacterial symbionts (Behl et al., 2007). The productiveness of the rhizosphere for AM fungi may be attributed to favorable influence exerted by root exudates (Bais et al., 2006), which contain amino acids, carbohydrates, organic acids and growth promoting substances and phytohormones produced by *A. chroococcum*.

Economic analysis revealed higher profitability in the presence of both bacteria with F1 level of fertility. It is evident from Figures 3 and 4 that treatment T9, followed by treatment T12 was more economically profitable, as net return was higher compared to the other treatments. Our results are in corroboration with Kumar *et al*. (2004), who also calculated the cost economics of three phosphate responsive wheat varieties using phosphate solubilizing *A. chroococcum* mutants and parent strains, they calculated a gain of Rs. 2.87 to 31.28 ha^{-1} by investing one Indian Rupee ha^{-1} (1 USD = 55 Indian Rupee). Our study suggested that microbial inoculants can be used as an economic input to increase crop productivity and maintaining sustainability of soil and harvesting more nutrients.

Conclusions

Inoculation of *A. chroococcum* and AM fungi under the soil and climatic conditions of arid region, characterized by fluctuations in precipitation, could result in increased plant yield. This study exhibited that dual inoculation of *A. chroococcum* and AM fungi with 80% of recommended fertilizer dose in *L. sativum* in arid region could be profitably used to maximize tuber production. Our study suggested that microbial inoculants can be used as an economic input to increase crop productivity and maintaining sustainability of soil by harvesting more nutrients. The economics of the experiment also advocates that application of favorably coinoculated bioinoculants can provide partial substitution to chemical fertilizers, thus saving the chemical fertilizers to great extent and exchequer by being costeffective.

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