



ENHANCED GROWTH AND YIELD OF *CAPSICUM ANNUUM* L. WITH TWO ENDOMYCORRHIZAL FUNGI AND OTHER BIOINOCULANTS

ANJU TANWAR*, ASHOK AGGARWAL**,
ALPA YADAV AND NISHA KADIAN

*Mycology and Plant Pathology Laboratory, Department of Botany,
Kurukshetra University, Kurukshetra-136119, Haryana, India*

*E-mail: anjutanwarbotany@gmail.com

**E-mail: aggarwal_vibha@rediffmail.com

A pot experiment with sixteen different combinations of *Glomus mosseae*(G), *Acaulospora laevis*(A), *Trichoderma viride*(T) and *Pseudomonas fluorescens*(P) was carried out to assess the interaction between bioinoculants and their consequent effect on the growth, nutrition and yield of *Capsicum annuum*. Among the single inoculation treatment, only *G. mosseae* increased maximum growth parameters, over the control. Almost all growth parameters i.e., plant height, root length, fresh and dry shoot weight, chlorophyll content were found maximum in the consortium of all the four bioinoculants i.e., *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* but for fresh and dry weight of roots and leaf area which was maximum in triple inoculation series of *G. mosseae* + *T. viride* + *P. fluorescens*. Triple inoculation also caused maximum increase in the mycorrhizal root colonization, spore numbers, plant biomass, N, P uptake and yield. These two treatments proved to be the best for inoculating *C. annuum* in order to get healthy and vigorously growing plants with better yield.

Key Words: *Acaulospora laevis*, *Glomus mosseae*, *Pseudomonas fluorescens*, synergistic response, *Trichoderma viride*, vegetables

A large number of microorganisms are found to be associated with the roots of plants in their natural habitat. Among them the most important and highly evolved association is that formed by arbuscular mycorrhizal fungi (AMF). Arbuscular Mycorrhiza (AM) is the mutualistic symbiotic association between most vascular land plant species and fungi of the phylum Glomeromycota (Smith and Read 2008). These fungi form an important component of the soil microbial mass and regulates several essential biological processes at the plant soil interface. AM fungi improve plant growth by capturing relatively immobile nutrients like P (Soucie *et al.* 2006), other macroelements (Hodge *et al.* 2001) and some microelements (Faber *et al.* 1990) also. Mycorrhizal fungi interact with a wide range of other soil organisms in the root or in the rhizosphere of the soil. Some form a symbiotic association and in turn modify the host physiology (Fitter and Garbaye 1994).

During the last few decades there has been a great emphasis on the use of bacterial and other fungal strains along with AM fungi for enhancing plant growth. The free living microbial inoculants could also stimulate mycorrhizal colonization (Vosátka and Gyndler 1999). The plant growth promoting rhizobacteria (PGPR) are also beneficial for plant growth (Kloepper *et al.* 1989), either by synthesizing plant growth promoting substances or by facilitating the uptake of certain nutrients from soil. Among them, *Pseudomonas fluorescens* is of utmost importance in increasing the plant growth. *Trichoderma* spp. also act as biocontrol agents, either producing antimicrobial compounds or by parasitising fungal plant pathogens. The use of biocontrol agents such as fluorescent *Pseudomonas* and *Trichoderma* requires a particular attention because of the possibilities that these antagonists interact not only with fungal plant pathogens but also with AM fungi

Table-1: Interactive effect of mycorrhizal fungi and other bioinoculants on growth performance of *C. annuum* plants after 120 days of inoculation

S.no.	Treatments	Change in height (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	AM spore number /10 g of soil	AM root colonization (%)
1.	Control	21.28±0.94 ^c	9.26±0.33 ^c	3.65±0.17 ^c	1.66±0.08 ^c	0.72±0.05 ^c	0.276±0.02 ^c	11.20±2.33 ^c	15.77±3.38 ^c
2.	<i>G. mosseae</i>	68.64±0.58 ^b	18.36±0.26 ^{bc}	13.7±0.58 ^{bc}	2.82±0.06 ^{cd}	5.03±0.35 ^{ab}	0.78±0.24 ^b	70.8±6.1 ^{ab}	88.22±7.01 ^{ab}
3.	<i>A. laevis</i>	51.24±0.78 ^c	15.44±0.41 ^{cd}	10.31±0.3 ^c	2.73±0.08 ^d	4.3±0.36 ^b	0.58±0.01 ^c	65.4±5.64 ^{ab}	86.71±4.61 ^{ab}
4.	<i>T. viride</i>	48.34±0.58 ^c	17.64±0.61 ^c	16.18±0.62 ^b	3.77±0.07 ^{ab}	3.55±0.17 ^{bc}	0.86±0.021 ^{ab}	9.00±1.8 ^e	18.02±6.40 ^d
5.	<i>P. fluorescens</i>	34.95±1.12 ^d	11.52±0.53 ^{dc}	9±0.4 ^{cd}	2.18±0.03 ^{dc}	2.47±0.24 ^d	0.47±0.014 ^d	10.20±2.3 ^e	10.65±2.61 ^e
6.	<i>G. mosseae</i> + <i>A. laevis</i>	82.14±1.3 ^a	21.18±0.33 ^b	18.3±0.60 ^{ab}	3.45±0.04 ^b	6.53±0.21 ^{ab}	0.84±0.02 ^{ab}	74±4.24 ^{ab}	95.87±3.81 ^a
7.	<i>G. mosseae</i> + <i>T. viride</i>	72.6±0.66 ^{ab}	19.6±0.61 ^b	7.11±0.18 ^{cd}	2.1±0.03 ^{de}	3.6±0.4 ^{bc}	0.67±0.05 ^b	57.6±5.32 ^b	84.83±3.3 ^{ab}
8.	<i>G. mosseae</i> + <i>P. fluorescens</i>	67.24±0.53 ^b	17.12±0.28 ^c	7.36±0.21 ^{cd}	2.6±0.05 ^d	3.2±0.33 ^b	0.32±0.02 ^c	52.8±4.6 ^{bc}	74.91±7.91 ^b
9.	<i>A. laevis</i> + <i>T. viride</i>	35.34±1.05 ^d	17.82±0.33 ^c	10.1±0.19 ^c	2.9±0.05 ^c	3.01±0.2 ^b	0.65±0.024 ^{bc}	47.2±3.83 ^c	66.14±6.46 ^{bc}
10.	<i>A. laevis</i> + <i>P. fluorescens</i>	34.12±0.71 ^d	20±0.48 ^b	8.3±0.25 ^{cd}	2.7±0.05 ^d	3.6±0.25 ^{bc}	0.74±0.02 ^b	67.6±3.64 ^{ab}	74.98±4.97 ^b
11.	<i>T. viride</i> + <i>P. fluorescens</i>	31.16±0.84 ^d	15.84±0.63 ^{cd}	6.0±0.18 ^d	2.22±0.02 ^{de}	2.73±0.13 ^d	0.64±0.02 ^{bc}	39.60±3.71 ^d	19.44±4.93 ^d
12.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>T. viride</i>	63.92±0.63 ^b	21.18±0.47 ^b	17.6±0.33 ^b	3.85±0.04 ^{ab}	4.4±0.21 ^b	0.93±0.02 ^a	82.8±6.46 ^a	87.15±4.12 ^{ab}
13.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>P. fluorescens</i>	45.9±0.86 ^c	12.28±0.59 ^d	13.71±0.33 ^{bc}	2.8±0.13 ^{cd}	1.87±0.09 ^{de}	0.45±0.03 ^d	69.7±4.77 ^{ab}	74.27±3 ^b
14.	<i>G. mosseae</i> + <i>T. viride</i> + <i>P. fluorescens</i>	75.46±0.85 ^{ab}	22.36±0.66 ^{ab}	19.37±0.56 ^a	3.2±0.12 ^{bc}	8.58±0.45 ^a	0.91±0.05 ^a	60.8±4.15 ^{ab}	71.55±3.14 ^b
15.	<i>A. laevis</i> + <i>T. viride</i> + <i>P. fluorescens</i>	47.16±1.04 ^c	17.46±0.61 ^c	17.24±0.54 ^b	3.81±0.08 ^{ab}	2.52±0.18 ^d	0.53±0.02 ^{cd}	47.4±3 ^c	55.74±5.46 ^c
16.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>T. viride</i> + <i>P. fluorescens</i>	82.66±0.61 ^a	25.28±0.92 ^a	25.1±0.8 ^a	4.19±0.07 ^a	8.14±0.42 ^a	0.88±0.04 ^{ab}	60±6.67 ^b	98.57±3.2 ^a
	LSD ($P \leq 0.05$)	1.0493	0.6902	0.5497	0.0886	0.3496	0.0447	6.541	17.13
	ANOVA ($F_{15; 32}$)=	2782.919	287.134	918.812	526.667	301.903	172.469	122.011	174.489

*Each value is a mean of five replicates

±: standard deviation

Mean value followed by different alphabet/s within a column do not differ significantly over one another at $P \leq 0.05$ lead by Duncan's Multiple Range Test.

and thus directly or indirectly enhance plant growth.

Bell pepper (*Capsicum annuum* L. var. California Wonder) belongs to the family Solanaceae. In India it is grouped under non-traditional category of vegetables. Bell pepper has attained a status of high value crop in India in recent years and occupies a pride of place among vegetables in Indian cuisine because of its delicacy and pleasant flavour coupled with rich content of ascorbic acid, vitamins A and C and other minerals. However, its supply is inadequate due to the low productivity of the crop (Muthukrishnan *et al.* 1986) especially in Haryana. Yield and quality improvement are

the two main aims of farmers to achieve. The poor establishment and lower yield are the two problems for bell pepper growers. Since AM symbiosis can benefit plant growth and nutrition uptake, there is an increasing interest in their application. Understanding the role of mycorrhizae and their interaction with other bioinoculants, will further improve the manipulation of inoculation techniques and planning to maximize the benefits derived from mycorrhizal association.

The purpose of this study was to examine the influence of bioinoculants (*G. mosseae*, *A. laevis*, *T. viride* and *P. fluorescens*) alone as well as in different combinations on the

Table-2: Effect of mycorrhizal fungi and other bioinoculants on the yield and physiological parameters of *C. annuum*

S.no.	Treatments	Yield per plant (g)	Chlorophyll a	Chlorophyll b	Chlorophyll total	Leaf area (sq cm)	Stomatal conductivity (mmol ⁻² s ⁻²)	
							L	U
1.	Control	-	0.030±0 ^d	0.30±0.017 ^c	0.33±0.02 ^c	10.54±0.46 ^c	L	121.2±2.4 ^c
							U	51.4±2.8 ^c
2.	<i>G. mosseae</i>	4.8±0.4 ^c	0.049±0 ^b	0.52±0.008 ^{bc}	0.57±0.005 ^{bc}	13.38±0.40 ^{de}	L	250.8±4.34 ^b
							U	132.4±5.9 ^{ab}
3.	<i>A. laevis</i>	2.2±0.46 ^d	0.052±0.004 ^b	0.53±0.007 ^{bc}	0.58±0.005 ^{bc}	14.5±0.27 ^{de}	L	124.8±3.1 ^c
							U	63.4±3.8 ^d
4.	<i>T. viride</i>	4.26±0.58 ^c	0.036±0.004 ^c	0.34±0.012 ^d	0.38±0.013 ^d	18.5±0.2 ^{bc}	L	148.4±6 ^d
							U	57.2±1.64 ^d
5.	<i>P. fluorescens</i>	2.7±0.56 ^d	0.048±0.004 ^b	0.54±0.005 ^{bc}	0.60±0.01 ^{bc}	17.4±0.31 ^c	L	212.2±3.7 ^c
							U	94.2±3.7 ^b
6.	<i>G. mosseae</i> + <i>A. laevis</i>	13.4±0.80 ^b	0.044±0.01 ^{bc}	0.56±0.024 ^b	0.59±0.017 ^{bc}	15.6±0.28 ^d	L	238.6±3 ^{bc}
							U	76.6±1.14 ^c
7.	<i>G. mosseae</i> + <i>T. viride</i>	12.9±0.58 ^b	0.047±0.004 ^b	0.53±0.004 ^{bc}	0.58±0.007 ^{bc}	18.5±0.52 ^{bc}	L	148.4±3 ^d
							U	70.2±4.15 ^c
8.	<i>G. mosseae</i> + <i>P. fluorescens</i>	18.44±0.71 ^{ab}	0.058±0.004 ^{ab}	0.61±0.006 ^{ab}	0.66±0.006 ^b	20.7±0.40 ^b	L	252.8±3.84 ^b
							U	108.2±2.2 ^b
9.	<i>A. laevis</i> + <i>T. viride</i>	9.5±0.71 ^{bc}	0.044±0.01 ^b	0.52±0.01 ^{bc}	0.56±0.01 ^{bc}	20.1±0.64 ^b	L	223±1.87 ^{bc}
							U	89±1.58 ^b
10.	<i>A. laevis</i> + <i>P. fluorescens</i>	4.2±0.87 ^c	0.047±0.004 ^b	0.52±0 ^{bc}	0.57±0 ^{bc}	19.1±0.16 ^b	L	268±2.55 ^{ab}
							U	134.2±2.9 ^{ab}
11.	<i>T. viride</i> + <i>P. fluorescens</i>	3.32±0.74 ^d	0.043±0.01 ^b	0.52±0.01 ^{bc}	0.57±0.004 ^{bc}	19.3±0.16 ^b	L	284.4±3.8 ^{ab}
							U	75.4±3.4 ^c
12.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>T. viride</i>	18.1±0.61 ^{ab}	0.048±0.004 ^b	0.54±0.01 ^{bc}	0.59±0.01 ^{bc}	17.3±0.28 ^c	L	301±2.4 ^{ab}
							U	126.8±2.4 ^{ab}
13.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>P. fluorescens</i>	11.9±0.60 ^{bc}	0.039±0.004 ^c	0.44±0.01 ^c	0.47±0.01 ^c	15.3±0.31 ^d	L	199±3.4 ^c
							U	85±1.82 ^b
14.	<i>G. mosseae</i> + <i>T. viride</i> + <i>P. fluorescens</i>	25.86±0.91 ^a	0.066±0.004 ^a	0.8±0.01 ^a	0.86±0.01 ^a	23.3±0.43 ^{ab}	L	341±3.7 ^a
							U	142.4±1.7 ^a
15.	<i>A. laevis</i> + <i>T. viride</i> + <i>P. fluorescens</i>	12.75±0.54 ^b	0.050±0.01 ^b	0.53±0.015 ^{bc}	0.56±0.01 ^{bc}	16.1±0.23 ^{cd}	L	212.4±2.8 ^c
							U	72.6±2.8 ^c
16.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>T. viride</i> + <i>P. fluorescens</i>	19.94±1.1 ^{ab}	0.049±0.004 ^b	0.56±0.006 ^b	0.62±0.004 ^b	27.6±0.54 ^a	L	372.8±3.2 ^a
							U	171.6±4.2 ^{ab}
LSD ($P \leq 0.05$)		0.8668	0.00752	0.0136	0.01205	0.4454	L-4.6013 U-3.9842	
ANOVA (F _{17, 36})=		575.847	12.918	515.799	758.844	658.784	L-2035.722 U-619.525	

Mean value followed by different alphabet/s within a column do not differ significantly over one another at $P \leq 0.05$ lead by Duncan's Multiple Range Test.

*Each value is a mean of five replicates

±: standard deviation

L: Lower surface

U: Upper surface

mycorrhizal status, growth, nutrition and yield of bell pepper.

MATERIALS AND METHODS

Experimental Site

The experiment was set up in poly house, Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India. The experiment was performed during July, 2010 to September, 2010. The soil used in the experiment had sand-64.2%, silt-21.81%, clay-

3.90%, pH-8.08, total N-0.042%, available P-0.017%.

Inoculum Preparation

Dominant AM spores i.e. *Acaulospora laevis* and *Glomus mosseae* isolated from rhizosphere of *C. annuum* by 'Wet sieving and Decanting technique' of Gerdemann and Nicolson (1963), were mass produced by Funnel Technique (Menge and Timmer 1982) using lemon grass as host for three months. *T. viride* was isolated

Table-3: Phosphorus and nitrogen content in root and shoot after 120 days of inoculation

S. no.	Treatments	P% in shoot	P% in root	Total N% in plant
1.	Control	0.23±0.008 ^d	0.30±0.008 ^c	3.7±0.085 ^c
2.	<i>G. mosseae</i>	0.33±0.008 ^c	0.52±0.005 ^b	4.25±0.16 ^c
3.	<i>A. laevis</i>	0.31±0.008 ^c	0.44±0.001 ^c	4.06±0.16 ^c
4.	<i>T. viride</i>	0.31±0.008 ^c	0.36±0.008 ^d	3.82±0.23 ^d
5.	<i>P. fluorescens</i>	0.31±0.004 ^c	0.41±0.007 ^c	4.18±0.06 ^c
6.	<i>G. mosseae</i> + <i>A. laevis</i>	0.38±0.008 ^{bc}	0.55±0.008 ^b	4.7±0.06 ^b
7.	<i>G. mosseae</i> + <i>T. viride</i>	0.37±0.052 ^{bc}	0.53±0.058 ^b	4.64±0.11 ^b
8.	<i>G. mosseae</i> + <i>P. fluorescens</i>	0.41±0.008 ^b	0.64±0.013 ^a	4.52±0.22 ^b
9.	<i>A. laevis</i> + <i>T. viride</i>	0.30±0.008 ^c	0.44±0.008 ^c	4.13±0.25 ^c
10.	<i>A. laevis</i> + <i>P. fluorescens</i>	0.40±0.01 ^b	0.51±0.01 ^b	4.34±0.11 ^{bc}
11.	<i>T. viride</i> + <i>P. fluorescens</i>	0.32±0.005 ^c	0.55±0.008 ^b	4.2±0.07 ^c
12.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>T. viride</i>	0.49±0.008 ^{ab}	0.70±0.004 ^a	5.8±0.07 ^{ab}
13.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>P. fluorescens</i>	0.47±0.008 ^{ab}	0.60±0.015 ^{ab}	4.9±0.23 ^{ab}
14.	<i>G. mosseae</i> + <i>T. viride</i> + <i>P. fluorescens</i>	0.60±0.008 ^a	0.70±0.008 ^a	7.03±0.17 ^a
15.	<i>A. laevis</i> + <i>T. viride</i> + <i>P. fluorescens</i>	0.44±0.008 ^b	0.56±0.008 ^b	4.5±0.13 ^b
16.	<i>G. mosseae</i> + <i>A. laevis</i> + <i>T. viride</i> + <i>P. fluorescens</i>	0.52±0.019 ^a b	0.67±0.015 ^a	5.11±0.2 ^{ab}
LSD ($P \leq 0.05$)		0.0202	0.0128	0.202
ANOVA (F_{15, 32})=		195.787	661.242	113.120

*Each value is a mean of five replicates

±: standard deviation

P: phosphorus

N: nitrogen

Mean value followed by different alphabet/s within a column do not differ significantly over one another at $P \leq 0.05$ lead by Duncan's Multiple Range Test.

from the soil and then further mass produced in the medium of wheat bran, saw dust and distilled water prepared in the ratio of 3:1:4. *P. fluorescens* (MTCC No. B103) was procured from IMTECH, Chandigarh, India and

multiplied in nutrient broth medium.

Experimental Setup

An experiment was designed to determine the effect of two mycorrhizal fungi (*G. mosseae* and *A. laevis*), *T. viride* and *P.*

fluorescens alone and in different combinations on growth, yield and nutrient uptake of bell pepper. Soil from experimental site was sieved through 2mm sieve, mixed with sand: soil (1:3) and autoclaved for 20 minutes for two consecutive days. Earthen pots (25.4 × 25 cm) were selected having capacity of 2 kg soil. To each pot 10 percent inoculum of AM fungi (approx. 865 spores), *T. viride* and *P. fluorescens* alone and in combinations were added. The experiment was set with single inoculation (*G. mosseae*, *A. laevis*, *T. viride*, *P. fluorescens*), double inoculations (*G. mosseae* + *A. laevis*, *G. mosseae* + *T. viride*, *G. mosseae* + *P. fluorescens*, *A. laevis* + *T. viride*, *A. laevis* + *P. fluorescens*, *T. viride* + *P. fluorescens*), triple inoculations (*G. mosseae* + *A. laevis* + *T. viride*, *G. mosseae* + *A. laevis* + *P. fluorescens*, *G. mosseae* + *T. viride* + *P. fluorescens*, *A. laevis* + *T. viride* + *P. fluorescens*) and lastly consortium of all the bioinoculants together i.e., (*G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens*). In control set no inoculum was added. Mycorrhizal inoculum of each fungus consists of AM spores, mycelium and infected root pieces obtained from pot culture of lemon grass. In each pot single seedling of *C. annuum* was planted and placed in poly house conditions. Plants were watered regularly. Hoagland's nutrient solution without phosphorus (100ml/pot) was added to each plant after regular intervals of 15 days. Each treatment was replicated five times.

Measurement And Harvest

The effects of different inoculations were recorded after 120 days of inoculation on various growth parameters. Some parameters were first measured in the standing plant i.e., plant height (cm), leaf area, by using leaf area meter (Systronics 211), stomatal conductance by using porometer (AP4-Delta T devices, cambridge, UK) and chlorophyll content by using Arnon's method (1949). After plant harvest, roots and shoots were weighted

separately for their fresh weight (g) and dry weight (g). Similarly, all the fruits were harvested and weighed together from each plant for yield/plant (g). Percentage mycorrhizal root colonization (%) was measured by 'Rapid clearing and Staining technique' by microscopic observation of fungal colonization after clearing roots in KOH (10%) and staining with trypan blue (0.5%), by the method of Phillips and Hayman (1970). AM spores were isolated by 'Wet sieving and Decanting technique' of Gerdemann and Nicolson (1963). The phosphorus content in shoot and root was determined by Vanado-molybdo-phosphoric acid yellow colour method, outlined by Jackson (1973). Total nitrogen was calculated by Kjeldahl method (Kelplus nitrogen estimation system, supra-LX).

Statistical Analysis

All results were analyzed using Analysis of Variance (ANOVA), followed by post hoc test through computer software SPSS 11.5 version. Means were than ranked at $P \leq 0.05$ level of significance using Duncan's Multiple Range Test for comparison.

RESULTS

The effect of inoculation with *P. fluorescens*, *T. viride* and two AM fungi (*G. mosseae* and *A. laevis*) on growth and yield of *C. annuum* differed significantly (Table 1- 3). Change in plant height was significant in all the plants and maximum plant height was observed in mixed consortium of *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* treated plants (82.66±0.61) and dual inoculation of *G. mosseae* + *A. laevis* (82.14±1.3), which was four times higher than the uninoculated plants (Table 1). The addition of *G. mosseae* to a combination of *A. laevis*, *T. viride* and *P. fluorescens* resulted in the highest increase in root length, fresh shoot weight, fresh root weight and dry root weight of *C. annuum* plants. As stated above maximum root length

was found in consortium of *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* and least in uninoculated control. The increase in plant height and root length is accompanied by an increase in fresh shoot as well as dry shoot weight which was again found maximum in the mixed consortium of *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (fresh-25.1±0.8, dry-4.19±0.07), which was eight times more than the uninoculated control one (fresh-3.65±0.17, dry-1.66±0.08). Similarly, fresh root weight and dry root weight was found maximum in *G. mosseae* + *A. laevis* + *T. viride* (fresh-8.58±0.45, dry-0.91±0.05).

Mycorrhizal spore numbers in the root zone soil ranges between 82.8±6.46 to 9.00±1.8. Highest AM spore number was observed in plants inoculated with *G. mosseae* + *A. laevis* + *T. viride* (82.8±6.46) compared to the plants treated only with *G. mosseae* (70.8±6.1) or *A. laevis* (65.4±5.64) alone as shown in Table 1. There was no such significant correlation between percent root colonization and mycorrhizal spore numbers in the root zone soil as maximum root colonization was observed in plants inoculated with *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (98.57±3.2) followed by *G. mosseae* + *A. laevis* (95.87±3.81), *G. mosseae* (88.22±7.01), *A. laevis* (86.71±4.61) and least in *P. fluorescens* (10.65±2.61) and uninoculated control (15.77±3.38). Meanwhile, the highest value of leaf area was registered in those plants which were treated with *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (27.6±0.54), followed by *G. mosseae* + *A. laevis* + *T. viride* (23.3±0.43), which showed significant difference from control (10.54±0.46), but there was no significant difference among single inoculated plants.

Chlorophyll content was also found to be increased in all the inoculated plants over control (Table 2). Chlorophyll a (0.066±0.004), chlorophyll b (0.8±0.01) and total chlorophyll (0.86±0.01) were maximum in plants treated

with *G. mosseae* + *T. viride* + *P. fluorescens* and minimum in control (a-0.030±0, b-0.30±0.017, total-0.33±0.02) plants after 120 days of inoculation. With regard to stomatal conductance, AM fungi were found to be stimulatory in combination with other bioinoculants and was more in the lower surface than upper surface of leaf. It is clear from Table 2, that stomatal conductance was maximum in *G. mosseae* + *T. viride* + *P. fluorescens* (341±3.7) in lower surface of leaf as compared to upper surface (142.4±1.7), followed by *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (lower-372.8±3.2, upper-171.6±4.2) whereas in control it was (lower-121.2±2.4, upper-51.4±2.8).

A trend similar to those observed for total plant growth was observed for phosphorus content of root and shoot as well as for total nitrogen percent of plant (Table 3). The increase in the P content of root was higher over shoot and was found significantly higher in plants with triple inoculation of *G. mosseae* + *T. viride* + *P. fluorescens* (shoot P-0.60±0.008, root P-0.70±0.008), whereas in control it was (shoot P-0.23±0.008, root P-0.30±0.008). Similarly, total N% in plant was also found maximum in the same triple inoculation (7.03±0.17) as compared to control one (3.7±0.085).

Regarding fruit yield, it was measured in total weight of all the fruits appeared on each plant which ranges from 2.7±0.56 to 25.86±0.91. Highest fruit yield was observed in the combination of *G. mosseae* + *T. viride* + *P. fluorescens* (25.86±0.91) and least was observed in single inoculation of *P. fluorescens* (2.7±0.56). Fruiting appeared in all the treatments but no fruiting was observed in control plants till 120th day of experimentation.

DISCUSSION

In general, the response of *C. annuum* to different treatments for different characters were better as compared to control. In this experiment, none of the single inoculation

treatments had significant effects on all the experimental parameters or showing the consistent plant growth promoting effects as observed in dual, triple and mixed inoculation. In this investigation all the observed parameters were found maximum in triple as well as four combination treatment i.e., *G. mosseae* + *T. viride* + *P. fluorescens* and *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* for inoculating *C. annuum* plants. The enhanced growth is because AM fungi are known to possess the ability to increase nutrient uptake of plants by developing an association with roots (Schreiner *et al.* 1997) and sometimes also promotes the growth of other rhizospheric microorganisms and thus enhances plant growth (Johansson *et al.* 2004). The other reason can be that, once host roots are colonized by the AM fungi, it changes the root exudates released and produces phosphatase enzyme in the rhizosphere. These phosphatases produced by extraradical hyphae of AM fungi could hydrolyze extracellular phosphate ester bonds and ultimately made P available to the plants (Joner *et al.* 2000). Among both the AM fungi studied, *G. mosseae* was found to be much compatible strain for *C. annuum* than *A. laevis* by increasing the capability of the root systems to absorb and translocate nutrients through extensive mycelia.

The possible outcome of improved plant growth indicates the improvement in fresh shoot and root and hence better biomass accumulation and this in turn improves P uptake. As phosphorus is essential for the process of nitrogen fixation, in this experiment also, triple inoculation might have influenced the plants with both P and N uptake. This may be the cause for enhanced leaf area, chlorophyll content and hence yield of *C. annuum* seedlings inoculated with *G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens*. The hyphae of AMF have the tendency to extract nitrogen and transport it from the soil to plants. They contain enzymes that breakdown organic nitrogen and

contain nitrogen reductase which alters the forms of nitrogen in the soil. Higher values of growth and other physiological parameters indicate higher quality of the seedlings and hence better establishment in the soil.

In general, with all treatments, the contents of chlorophylls a and b in mycorrhizal plants were significantly greater than those of non-mycorrhizal ones at all stages of plant growth. The total photosynthetic pigments increased due to mycorrhizal colonization which was more than double the concentration found in uninoculated control. Phosphorus (P) has an important role as energy carrier during photosynthesis and stomatal conductance can also be influenced by P starvation. AM fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots thus providing a stimulus for greater photosynthetic activity (Bevege *et al.* 1975). AM symbiosis needs carbon source from symbiotic partner synthesized by the process of photosynthesis and it was found that upto 20% of the total photoassimilates substances can be transferred to the fungal partner (Graham 2000).

Koide (2000) suggested that the increased stomatal conductance and transpiration rate in AM plants could be due to P-mediated improvement in photosynthetic capacity. Phosphorous concentrations in leaves may affect stomatal response to environmental perturbations, perhaps by affecting the energetic processes involved in guard cell osmotic potential or wall stiffening governing stomatal movements (Weyers and Meidner 1990).

The P and N contents of the plants showed the same trend, which resembled the earlier works performed on other crops (Akhtar and Siddiqui 2010). Content of P was observed more in roots than shoots. Present findings also indicated that AM inoculated plants along with *T. viride* and *P. fluorescens* had a higher phosphorus content than control. Mutualistic

association thus may improve the phosphorus mineralization and its acquisition by plants. This may be due to synergistic interaction between both the AM fungi and other plant growth promoting bioinoculants. It has also been found that PGPR possess a wide variety of other direct mechanisms to support mycorrhizal symbiosis. They solubilize the bound phosphorus from the soil and release P into the soil, which is then taken up by AM colonized roots. Their interaction with AM fungi therefore occasionally produces positive effects by enhancing plant growth and protection (Xavier and Germida 2003).

P. fluorescens had no significant effect on plant growth when applied alone i.e., without any AM fungi, but significantly increases growth when applied along with other bioinoculants. In our study, combined inoculation of AM fungi and *P. fluorescence* enhances almost all the parameters including phosphorus uptake, which is in accordance with the findings of Kremer (2006). Another possibility is that the *P. fluorescens* promotes germination of AM fungal spores and can increase the rate and extent of mycorrhizal root colonization (Johansson *et al.* 2004). Synergistic effect of inoculation of AM along with *T. viride* was found to be beneficial for plant growth by other workers (Srinath *et al.* 2003, Arpana and Bagyaraj 2007, Parkash and Aggarwal 2009, Bhromsiri and Bhromsiri 2010). There are several studies which have focused on mycoparasitic nature of *Trichoderma* species and hence its contribution to plant health (Chet 1987, Egberongbe *et al.* 2010, Allay and Chakraborty 2010).

Conclusion

Based on the response of different characters like plant growth, P and N content and yield, it can be concluded that the triple (*G. mosseae* + *T. viride* + *P. fluorescens*) as well as four combination treatment (*G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens*) are the best consortia of microorganisms for inoculating *C.*

annuum plants. Inoculation with such a microbial consortium may result in healthy, vigorously growing *C. annuum* seedlings. This technology, being simple and ecofriendly, can be adopted easily by any nurserymen for inoculating *C. annuum* seedlings in the nursery. Therefore, this study recommends farmers of Kurukshetra district, Haryana, and also other regions with similar soil and other environmental conditions to add arbuscular mycorrhizal fungi i.e., *G. mosseae* and *A. laevis* in consortium with *T. harzianum* and *P. fluorescens* in their farms at the transplanting stage to have better establishment of crops along with greater yield and nutrients.

The author Anju Tanwar is grateful to Kurukshetra University, Kurukshetra for providing laboratory facilities and financial assistance.

REFERENCES

- Akhtar MS & Siddiqui ZA 2010 Effect of AM fungi on the plant growth and root-rot diseases of Chickpea. *American-Eurasian J Agric & Environ Sci* **8**(5) 544-549.
- Allay S & Chakraborty B 2010 Activation of defense response of Mandarin plants against *Fusarium* root rot disease using *Glomus mosseae* and *Trichoderma hamatum*. *J Mycol Pl Pathol* **40**(4) 499-511.
- Arpana J & Bagyaraj DJ 2007 Response of Kalmegh to an arbuscular mycorrhizal fungus and plant growth promoting rhizomicroorganism at two levels of phosphorus fertilizer. *American-Eurasian J Agric and Environ Sci* **2**(1) 33-38.
- Bevege DI, Bowen GD & Skinner MF 1975 Comparative carbohydrate physiology of ecto and endomycorrhizas. In: *Endomycorrhizas*, eds. Sanders FE, Mosse B and Tinker PB Academic Press, New York. Pp 149-175.
- Bhromsiri C & Bhromsiri A 2010 The effect of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi on the growth, development and nutrient uptake of different vetiver ecotypes. *Thai Agricul Sci* **43**(4) 239-249.
- Chet I 1987 *Trichoderma*-application, mode action and potential as a biocontrol agent of soil borne plant pathogenic fungi. In: *Innovative Approaches to Plant Disease Control*, John Willey and Sons, New York. Pp 137-160.
- Egberongbe HO, Akintokun AK, Babalola OO & Banole MO 2010 The effect of *Glomus mosseae* and

- Trichoderma harzianum* on proximate analysis of soybean (*Glycine max* (L.) Merrill.) seed grown in sterilized and unsterilized soil. *Agric Exten Rural Develop* **2**(4) 54-58.
- Faber BA, Zasoski RJ, Burau RG & Uriu K 1990 Zinc uptake by corns as affected by vesicular-arbuscular mycorrhizae. *Plant Soil* **129** 121-130.
- Fitter AH & Garbaye J 1994 Interactions between mycorrhizal fungi and other soil organisms. In: *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*, eds. Robson AD, Abbott AK & Malazczuk Kluwer Academic Publications, Netherlands. Pp 123-244.
- Gerdemann JW & Nicolson YH 1963 Spores of mycorrhiza *Endogone* species extracted from soil by wet sieving and decanting. *Trans Brit Mycol Soc* **46** 235-244.
- Graham JH 2000 Assessing cost of arbuscular mycorrhizal symbiosis in agrosystems. In: *Current Advances in Mycorrhizae Research*, eds. Podila GK and Donds DD APS Press, St Paul. Pp 127-140.
- Hodge A, Campbell CD & Fitter AH 2001 An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413** 297-299.
- Jackson ML 1973 *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi.
- Jayanthi Srinath, Bagyaraj DJ & Satyanarayana BN 2003 Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. *World J Microbiol Biotechnol* **19** 69-72.
- Johnson JF, Paul LR & Finley RD 2004 Microbial interaction in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol Ecol* **48** 1-13.
- Joner EJ, Briones R & Leyval C 2000 Metal binding capacity of arbuscular mycorrhizal fungi. *Plant Soil* **226** 227-234.
- Kloepper JW, Lifshitz R & Zablotowitz RM 1989 Free living bacteria inocula for enhancing crop productivity. *Trends Biotechnol* **7** 39-43.
- Koide RT 2000 Mycorrhizal symbiosis and plant reproduction. In: *Arbuscular Mycorrhizas: Physiology and Function*, eds Kapulnik Y & Douds DD Kluwer Academic Publishers, Dordrecht. Pp 1946.
- Kremer RJ 2006 Deleterious rhizobacteria. In: *Plant-Association Bacteria*, ed. Gnanamanickam SS Springer, Netherlands. Pp 335-357.
- Menge JA & Timmer LW 1982 Procedure for inoculation of plants with VAM in the laboratory greenhouse and field. In: *Methods and Principles of Mycorrhizal Research*, ed. Schenck NC American Phytopathology Society, St. Pauls, USA. Pp 59.
- Muthukrishnan CR, Thangaraj T & Chatterjee R 1986 *Chilli and Capsicum*. In: *Vegetable Crops in India*, eds. Bose TK & Som MG. Pp. 343-384.
- Parkash V & Aggarwal A 2009 Diversity of endomycorrhizal fungi and their synergistic effect on the growth of *Acacia catechu* Willd. *Forest Sci* **55** (10): 461468.
- Phillips JM & Hayman DS 1970 Improved produces for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. *Trans Brit Mycol Soc* **55** 158-161.
- Schreiner RP, Mihara KL, McDaniel H & Bethlenfavay GJ 1997 Mycorrhizal fungi improves plant and soil functions and interaction. *Plant Soil* **188** 199-207.
- Smith S & Read D 2008 *Mycorrhizal Symbiosis*. 3rd edn. New York Academic Press.
- Souchie EL, Azcón R, Barea JM, Saggin-júnior OJ & Ribeiro da Silva EM 2006 Phosphate solubilizing and arbuscular mycorrhizal fungi. *Pesq Agropec Bras* **41**(9)1405-1411.
- Vosátka M & Gryndler M 1999 Treatment with culture fraction from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. *Appl Soil Ecol* **11** 245251.
- Weyers JDB & Meidner H 1990 *Methods in Research*. Longman, London. Pp 912.
- Xavier LJC & Germida JJ 2003 Bacterial association with *Glomus clarum* spores influence mycorrhizal activity. *Soil Biol Biochem* **35** 471-478.