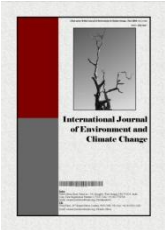

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Impact Assessment of Different Level of Mycorrhiza on the Growth Parameters and Nutrient Content of *Capsicum annum*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Soil microbes play an important role in the biogeochemical cycling of nutrients and their availability to plants, which is important for sustaining soil health and agricultural sustainability. In light of this, vesicular arbuscular mycorrhiza (VAM) plays an important function. Hyphae of VAM can reach far beyond the plant root zone, allowing it to obtain nutrients from a considerably larger region of soil. Mycorrhiza improves plant nutrition by facilitating the uptake of minerals such as phosphorus and immobile trace elements such as zinc, cobalt, magnesium, iron, copper, molybdenum, and others. Mycorrhiza improves plant growth, productivity and yield by increasing the rate of photosynthesis. In the present study *Glomus fasciculatum* was tested on *Capsicum annum* plant with different inoculum levels (100, 150, 200 and 400 chlamydospores/kg soil) and found that Plant height, Root length, Dry weight of root and shoot, NPK content, per cent mycorrhizal colonization and sporocarp number were maximum when 400 spores were used for inoculation and minimum were found in untreated plants. SPAD chlorophyll content was highest at 90 DAT among all the observation periods.

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1. INTRODUCTION

Arbuscular mycorrhizal (AM) fungi can be found in all soils and colonise the roots of a wide range of plant species. These fungi can boost plant growth and reproduction by improving nutrient uptake, particularly minerals that are immobile in soil like phosphorus. Plants can also benefit from AM fungus because they stimulate growth-regulating chemicals, increase photosynthesis, improve osmotic adjustment under drought and salinity conditions, and increase pest resistance [1]. Plant growth and nutrient uptake are the key effects of AM on their host plant [2]. In addition, mycorrhizal inoculation minimises the amount of fertiliser required for inoculated plants [3]. The arbuscular mycorrhizal fungi (AMF) found associated with the majority of land plants including those of the arid areas [4], once it establishedin soil than it increases mineral nutrition uptake, mainly phosphorus and enhance plant growth. VAM not only increase the uptake of phosphorous, but also helps in uptake of zinc, copper, sulphur, potassium and calcium [5]. Additionally, it protect plants against environmental stress such as soil salinity [6], drought [7] and pathogens such as Fusarium wilt [8]. Therefore studies were conducted on association of AM fungi with *Capsicum annum* to observe growth and nutrient content in pot experiment.

2. MATERIALS AND METHODS

The present study entitled “Impact assessment of different level of mycorrhiza on the growth parameters and nutrient content of *Capsicum annum* was executed in screen house and laboratories of Plant Pathology department CCS HAU, Hisar during 2018. The AM fungi *i.e*. *Glomus fasciculatum* was taken from the department of Plant Pathology, Chaudhary Charan Singh Haryana Agricutural University, Hisar. One hundred gram of mycorrhizal inoculum containing about 500 extramatrical chlamydospores and infected root bits were put in upper 5 cm soil layer per pot. Thirty days old seedling of *Capsicum annum* cv. Pusa Jwala was sown in the pots (Four numbers of sets were maintained) and statistical design was CRD. These plants were watered regularly. Observations, number of mycorrhiza spores/100 gram of soil [9] and Percentage mycorrhizal colonization in roots [10] were taken. Plant height, root length, dry weight of root and shoot at 30, 45, 60 and 90 day after transplanting (DAT) and Nutrient content (NPK) of root and shoot at 90 DAT were calculated.

2.1 Statistical Analysis

Statistical analysis of experiment was carried out using opstat software at http://hau.ac.in.

2.1.1 Maintenance of *Glomus fasciculatum*

In 20 cm diameter earthern pots the mycorrhizal fungi (*Glomus fasciculatum*) were maintained on wheat (*Triticum aestivum*) and pearl millet (*Pennisetum typhoides*). These pots were filled with 5 kg sterilized river sand. In upper 5 cm soil layer put one hundred g of mycorrhizal inoculum which contain about 450-500 chlamydospore and root bit and then ten seeds of wheat or pearlmiellet were sown and watered regularly. Hoagland’s nutrient solution was applied @ 10 ml/pot after every 30 days of transplanting. After 90 days shoot portion of plant were cut at soil level and left the soil in pots to air dry. The soil was crumbled and cut the rootlets into 1 cm segments. This soil was used as a mycorrhizal inoculum.

2.1.2 Mycorrhizal colonization

Mycorrhizal colonization was calculated by Staining of roots by following procedure given by Phillips and Hayman[10].

2.1.3 Staining of root

Roots were cut into 1 cm segments, heat the roots in 10 per cent KOH at 90^{o}C for one hour, washed these roots with fresh (10 per cent) KOH solution, immersed roots in alkaline hydrogen peroxide (H_2O_2) for 30 minutes. Then rinsed with distilled water to remove the excess of H_2O_2 and acidified with 5 N HCL for 30 minutes. Roots were simmering in trypane blue in lactophenol (0.05%) for 5 min. Finally, roots were put in lactophenol to remove the extra dye and examine the roots under microscope.

$$\textbf{Mycorrhizal colonization (\%) in roots} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of sample assessed} \times \text{Maximum scale}}$$

Chart 1. Hoagland Solution (Plant Nutrition Solution)

Components	Stock Solution	mL stock solution/1L
2M KNO_3	202 g/L	2.5ml/L
2M $Ca(NO_3)_2$•$4H_2O$	236 g/0.5 L	2.5 ml /L
Iron (Sprint 138 iron chelate)	15 g/L	1.5 ml /L
2M $MgSO_4$•$7H_2O$	493 g/L	1 ml /L
NH_4NO_3	80g/L	1 ml /L
Minors:		
H_3BO_3	2.86 g/L	1 ml /L
$MnCl_2$•$4H_2O$	1.81 g/L	1 ml /L
$ZnSO_4$•$7H_2O$	0.22 g/L	1 ml /L
$CuSO_4$•$5H_2O$	0.051g/L	1 ml /L
H_2MoO_4•H_2O or	0.09 g/L	1 ml /L
Na_2MoO_4•$2H_2O$	0.12 g/L	1 ml /L
1M KH_2PO_4 (pH)	136 g/L	1 ml /L

2.1.4 Estimation of sporocarp in soil

Estimation of sporocarp in soil was done by Wet Sieving and Decantation Techanique given by Gerdemann and Nicolson [9]. Firstly, the soil sample was mixed well and then 100 g soil was suspended in a pan A added one liter of water and mix it well. Wait for 30 seconds. Suspension was passed through 20 mesh sieve and filtrate was collected into a pan B. Material of pan A was discarded. Suspension of B pan was stirred with hand and allows it for few second to settled down then passed through 60 mesh sieve. Filtrate was collected in pan C. Suspension of pan C was passed through the 100 mesh sieve. Maximum mature sporocarps were collected on 100 mesh sieve. One hundred mesh sieve residue was collected into a beaker after washing in order to remove the excess soil and other particles. 1ml of this solution was taken in counting dish and examined under stereomicroscope microscope and count the sporocarp population in soil.

2.1.5 Estimation of Nitrogen content, phosphorous content and potassium content

For the estimation of nitrogen content, The Lindner method (1944) was adopted. For the estimation of phosphorous content, Vanadomolybdophosphoric yellow color method given by Koenig and Johnson, (1942) was adopted. Potassium was determined in the acid digest of plant samples by using flame photometer (Elico CL 361, India) by direct reading.

2.1.6 Chlorophyll content

Chlorophyll content of the plant was calculated by using SPAD chlorophyll meter.

Fig. 1. Estimation of chlorophyll content of *Capsicum annum* using SPAD meter

3. RESULTS

The present study was done with the objective of best dose of mycorrhization in *Capsicum annum* plants. A pot experiment was conducted in screen house of Plant Pathology, CCS HAU, Hisar, to see the effect of *Glomus fasciculatum* with different doses. Mycorrhizal colonization in roots and number of sporocarps in soil was calculated at 30, 45, 60 and 90 days after transplanting. Plant height, root length, dry weight of root and shoot and SPAD chlorophyll content at 30, 45, 60 and 90 days after transplanting were recorded. Nutrient content (NPK) at 90 days after transplanting was calculated.

Effect of soil application with different doses of AM fungi on plant height and root length of *Capsicum annum* (Table 1) was observed and found that application of the mycorrhizal species *Glomus fasciculatum* with different doses

significantly increases in plant height of *Capsicum annum* at 30, 45, 60 and 90 days after transplanting as compared to control. The maximum plant height (29.23 cm) was observed, when 400 chlamydospores/kg soil were used for inoculation followed by 200 chlamydospores/kg soil (25.27 cm) among the all the inoculum levels and minimum plant height was recorded in control (19.77 cm). Irrespective of inoculum level maximum plant height was observed at 90 DAT. Highest root length (14.60 cm) was observed at 90 DAT, when 400 chlamydospores/kg soil were used followed by 200 chlamydospores/kg soil (12.80 cm) among the all the inoculum levels and the lowest root length was recorded in control (9.93 cm). Among all the inoculum levels maximum plant height and root length was observed, when 400 chlamydospores/kg soil were used for inoculation as compare to control.

The effect of mycorrhizal inoculation on dry shoot weight and dry root weight of *Capsicum annum* was observed (Table 2) and found that maximum dry shoot weight was observed, when 400 spores were inoculated (2.17 g), followed by 200 spores (2.08 g), 150 spores (1.91 g), 100 spores (1.84 g) and minimum was found in control (1.09 g). Irrespective of the inoculum level the maximum dry root length was observed, when *Glomus fasciculatum* was inoculated (90 DAT). Among all the four observations period (30 DAT, 45 DAT, 60 DAT and 90 DAT) the maximum dry shoot weight was observed at 90 DAT. The maximum dry root weight was recorded, when 400 spores of *Glomus fasciculatum* (1.79 g) were inoculated and minimum was recorded in control (1.11 g) at 90 DAT.

Mycorrhizal root colonization (%) and sporocarp number were calculated at 30, 45, 60 and 90 days after transplanting (Table 3). The maximum root colonization (77.33 %) and sporocarp number (89.3) were found when 400 chlamydospores/kg soil were used and minimum root colonization (60 %) and sporocarp number (51) were calculated when 100 chlamydospores /kg soil were used.

Application of *Glomus fasciculatum* with different doses (100, 150, 200 and 400 sporocarp/kg soil) significantly increase the chlorophyll content of *Capsicum annum* (Table 4). Data was statistically analysed and found that the chlorophyll content varied significantly at 30, 45, 60 and 90 days after transplanting. The maximum chlorophyll was recorded, when 400 chlamydospores/kg soil were inoculated (32.63) and minimum was recorded in control (20.67) at 90 DAT among all the inoculum levels. The maximum NPK content was recorded, when 400 spores of *Glomus fasciculatum* were inoculated (1.19 per cent N, 0.56 per cent P and 1.84 per cent K at 90 DAT at 90 DAT and minimum NPK content was recorded in control (1.12 per cent N, 0.38 per cent P and 1.43 per cent K). Irrespective of the days and species the maximum NPK content was observed when 400 sporocarp/kg soil was inoculated. Among all the four observation period *i.e.* 30 DAT, 45 DAT, 60 DAT and 90 DAT, the maximum NPK content was observed at 90 DAT.

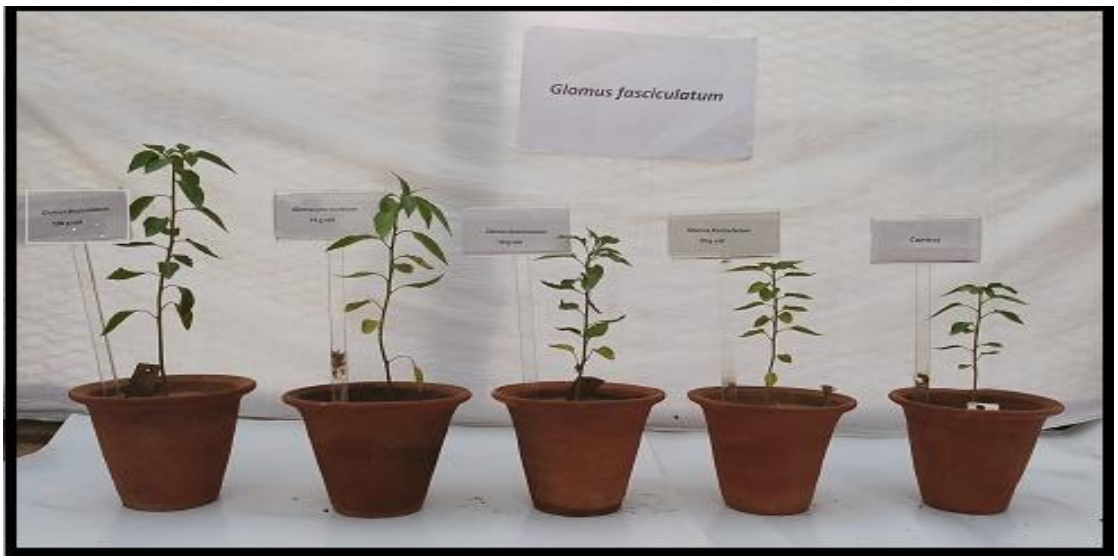

Fig. 2. Effect of different doses of mycorrhiza on plant height

Table 1. Effect of mycorrhization on plant height and root length of *Capsicum annum* plant at 30, 45, 60 and 90days after transplanting

Plant height (cm)						**Root length (cm)**				
Inoculum levels (Sporocarp/Kg soil)	**Days After Transplanting (DAT)**					**Days After Transplanting (DAT)**				
	30	**45**	**60**	**90**	**Mean B**	**30**	**45**	**60**	**90**	**Mean B**
100	10.10	11.67	17.13	20.03	14.73	7.43	8.80	9.30	10.04	8.89
150	12.13	12.50	18.53	20.87	16.01	7.80	9.24	11.23	11.87	10.03
200	12.87	15.80	19.67	25.27	18.40	8.53	11.83	12.13	12.80	11.33
400	15.60	19.33	21.40	29.23	21.39	8.77	13.40	13.60	14.60	12.59
Control	9.57	11.43	14.93	19.77	13.93	7.23	8.40	9.20	9.93	8.69
Mean A	12.05	14.15	18.33	23.03		7.95	10.33	11.09	11.85	
CD at 5 % level	**DAT =** 0.46					**Inoculum level =** 0.31				
	Inoculum level = 0.52					**DAT =** 0.28				
	DAT× Inoculum level= 1.03					**DAT× Inoculum level =** 0.62				

Table 2. Effect of different doses of mycorrhiza on dry shoot weight and dry root weight of *Capsicum annum* plant at different inoculum levels

Dry shoot weight (g)						**Dry root weight (g)**				
Inoculum levels (Sporocarp/Kg soil)	**Days After Transplanting (DAT)**					**Days After Transplanting (DAT)**				
	30	**45**	**60**	**90**	**Mean B**	**30**	**45**	**60**	**90**	**Mean B**
100	0.44	0.92	0.93	1.84	1.03	0.83	0.93	1.05	1.17	1.00
150	0.62	1.00	1.06	1.91	1.15	0.94	1.09	1.24	1.39	1.16
200	0.84	1.12	1.33	2.08	1.34	1.16	1.23	1.38	1.56	1.33
400	0.97	1.27	1.48	2.17	1.47	1.23	1.47	1.63	1.79	1.53
Control	0.47	0.96	1.22	1.70	1.09	0.48	0.80	1.03	1.11	0.86
Mean A	0.67	1.05	1.20	1.94		0.93	1.10	1.27	1.40	
CD at 5 % level	**DAT =** 1.18					**DAT =** 1.65				
	Inoculum level = 1.18					**Inoculum level =** 1.65				
	DAT× Inoculum level= 2.36					**DAT× Inoculum level=** 3.30				

Table 3. Impact of soil application with different doses of AM fungi on Mycorrhizal colonization (%) and Sporocarp/ 100g soils

Mycorrhizal colonization (%)						Sporocarp number (per 100g soil)				
Inoculum levels (Sporocarp/Kg soil)	**Days After Transplanting (DAT)**					**Days After Transplanting (DAT)**				
	30	**45**	**60**	**90**	**Mean B**	**30**	**45**	**60**	**90**	**Mean B**
100	23.33	35.00	57.33	60.00	43.92	23.0	35.0	44.7	51.0	38.4
150	28.00	40.00	59.00	63.67	47.67	24.3	42.7	55.3	64.7	46.8
200	31.33	46.67	67.33	72.00	54.33	34.7	55.3	66.7	75.0	57.9
400	35.00	51.33	72.33	77.33	59.00	40.3	69.0	81.0	89.3	69.9
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean A	29.42	43.25	64.00	68.25		30.6	50.5	61.9	70.0	
CD at 5 % level	**DAT =** 1.18 **Inoculum level =** 1.18 **DAT× Inoculum level=** 2.36					**DAT =** 1.65 **Inoculum level =** 1.65 **DAT× Inoculum level=** 3.30				

Table 4. Effect of mycorrhization on chlorophyll content and NPK content of *Capsicum annum*

	Chlorophyll content							
Treatments	**Days after transplanting (DAT)**					**NPK content**		
	30	**45**	**60**	**90**	**Mean B**	**N**	**P**	**K**
100	17.33	19.73	20.70	21.43	19.80	1.14	0.38	1.58
150	20.00	21.50	22.87	25.07	22.36	1.16	0.4	1.67
200	24.03	24.87	25.57	28.87	25.83	1.18	0.48	1.77
400	26.57	27.23	29.50	32.63	28.98	1.19	0.56	1.84
C	16.77	17.80	19.50	20.67	18.68	1.12	0.38	1.43
Mean A	20.94	22.23	23.63	25.73				
CD at 5 % level	**DAT=** 0.83 **Inoculum level =** 0.93 **DAT×inoculum level = NS**					**NS**	**0.03**	**0.09**

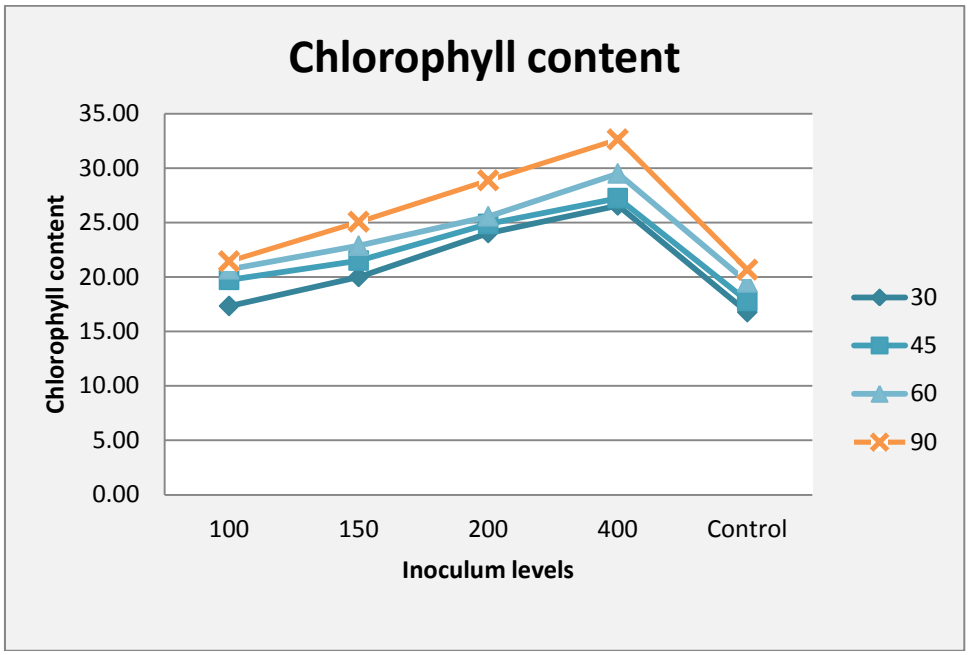

Plate 1. Effect of different inoculum levels of mycorrhiza on chlorophyll content of *Capsicum annum*

4. DISCUSSION

In the present investigation effect of *Glomus fasciculatim* with different doses (100, 150, 200 and 400 sporocarps/kg soil) were observed on growth parameters, mycorrhizal per cent colonization and sporocarp number, NPK content and chlorophyll content of *Capsicum annum* plant and found that with increase in inoculum level of mycorrhiza corresponding increase in plant height, root length, dry weight of shoot and root, chlorophyll content and NPK content of *Capsicum annum* plants was obtained. Maximum plant height and growth parameters were found in inoculated plants and minimum was observed in uninoculated (control). Similarly, Thilagar and Bagyaraj [11] studied on different species of arbuscular mycorrhizal fungi (*Acaulospora laevis, Gigaspora margarita, Glomus bagyarajii, G. etunicatum, G. fasciculatum, G. intraradices, G. leptotichum, G. macrocarpum, G. monosporum, G. mosseae* and *Scutellospora calospora*) and found that all different species of mycorrhiza significantly increase the plant growth parameters. Ibrahim *et al*. [12] conducted an experiment to evaluate the effect of five arbuscular mycorrhizal (AM) fungi *viz., Glomus mosseae, G. clarum, G. caledonium, G. intraradices* and *G. etunicatum* and their mixture were taken on maize and found that inoculated plants with different mycorrhizal fungi, increased in shoot, root dry weight, P, Zn and seedling quality content, seedlings flowered earlier as compared to control plants Similar results were observed by [13-15]. The mutualistic association between roots of higher plants and soil borne fungi was reported by Menendez *et al*. [16]. Mycorrhiza fungi benefit the host plant by translocate phosphorus through a wide network of external hyphae and maximize the capability of the root system to absorb phosphorus. Colonization of AMF greatly increased chlorophyll content and root activity. The macro- and micro nutrient contents *i.e*. N, P, K, S, Ca, Cu, Fe, Mn, Mg, and Zn was also improved in roots [17] similarly application of compost and AMF significantly improved plant growth, stomatal conductance and chlorophyll fluorescence compared to infected and non-infected controls [18].

5. CONCLUSION

The present experiment showed that all the inoculum levels (100, 150, 200 and 400 sporocarps/kg soil) of *Glomus fasciculatim* have growth stimulating effects on *Capsicum annum*. Our results clearly suggest that in *Capsicum annum* var. Pusa Jwala, inoculation of AM fungi (100, 150 and 200 sporocarps/kg soil) showed better vegetative growth of all the plants while 400 sporocarps/kg soil inoculation showed the most promising and synergistic effects on vegetative growth of *Capsicum annum*.

Colonization of AMF greatly increased chlorophyll content and NPK content of *Capsicum annum* at different observation periods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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