Asian Journal of Mycology 5(2): 1–10 (2022) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/5/2/1

Effects of arbuscular mycorrhizal fungi on growth parameters of *Pisum sativum*

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Manjula, Aishwarya, Payal, Avasthi S, Verma RK, Gautam AK 2022 – Effects of arbuscular mycorrhizal fungi on growth parameters of *Pisum sativum***.** Asian Journal of Mycology 5(2), 1–10, Doi 10.5943/ajom/5/2/1

Abstract

Mycorrhizal fungi are associated in mutualistic symbiosis relationships with fine roots of the plants. These fungi help in enhancing plant health to combat both biotic and abiotic stresses. The present investigation was undertaken to study the effects of arbuscular mycorrhizal fungi (AMF) on the growth performance of pea (*Pisum sativum*). Soils were collected from the experimental fields of Abhilashi University to be used in the pot experiment. The pots were filled with a mixture of sterilized soil and sterilized Farm Yard Manure (FYM). Labelled experimental pots were sown with two pea seeds in each. Experimental pots were inoculated using four AM fungi, namely *Glomus intraradices, G. aggregatum, G. clarum* and *Sclerocystis microcarpa*. The effects of mycorrhizal fungi were examined in terms of plant height, leaf length, total number of leaves and root length. From the results, it was observed that fungi pose a significant effect on plant height, root length and total number of roots of peas. These findings revealed that AMF can significantly contribute to improve some growth aspects of pea.

Key words – AM fungi – inoculation – growth parameters – mutualistic symbiosis – *Pisum sativum*

Introduction

Pea (*Pisum sativum* L.) is a commonly grown leguminous crop for its edible seeds. As a herbaceous annual plant belonging to the family Fabaceae, the pea is placed among one of the oldest cultivated crops. Besides being used as a vegetable, pea seeds are a good source of vitamins C and E, zinc and other antioxidants along with other vitamins like A & B and coumestrol. Equipped with such, peas present themselves with immune and anti-inflammatory properties (Millar et al**.** 2019). The crop is cultivated throughout the world, including India. India occupies the 4^{th} position with respect to area under pea cultivation and 5^{th} in production. Pea is primarily grown as a vegetable in north Indian hills and plains. It is extensively cultivated in Uttar Pradesh, Bihar and Madhya Pradesh (FAO Stat. 2014, Senapati et al. 2019, Mondor 2020).

Mycorrhizal fungi are associated with plants and establish mutualistic symbiosis relationships with fine plant roots (Sieverding 1991). About 90% of terrestrial plants are colonized by mycorrhizal fungi. These fungi grow in plant rhizosphere and help in the increased absorption of immobile or fixed ions, water and nitrogen. The fungus benefited by receiving carbon compounds provided by the host plant (Lin et al. 1991). The increased uptake of useful nutrients helps in the

Submitted 14 April 2022, Accepted 21 July 2022, Published 3 September 2022
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overall performance of plants. Mycorrhizal fungi help in increasing plant health and stress tolerance by enhancing pathogen and drought resistance. In addition, the association of these fungi reduced the irrigation and fertilizers requirements of plants and increased the success rate of transplanting as well. Isolation and identification of these genera and species have already been reported by other researchers from the rhizosphere of different plants like *Pisum sativum, Zea mays, Asparagus* sp.*, Smilax* sp.*, Rhizophagus* sp.*, Withania* sp.*, Claroideoglomus* sp*.* (Stubblefield et al. 1987, Kristek 2005, Thangavelu & Raji 2016, Yaseen et al. 2016, Surayya et al. 2021, Johny et al. 2021, Aishwarya et al. 2022). Keeping these beneficial aspects of mycorrhizal association with plants in view, the aim of this research was to study the effects of mycorrhizal fungi on some of the growth parameters of pea (*Pisum sativum*).

Materials & Methods

Experimental Material

The soil used in the pots was collected from the agriculture field of Abhilashi University. After examining the general characteristics (colour, texture, structure, etc.), the soil was sterilized by autoclaving in the laboratory, followed by air drying to remove the excess moisture. Sterilized Farm Yard Manure (FYM) was also added to air-dried soil in the ratio of 3:1. This mixture was then filled in labelled pots. Two seeds were placed in each pot at a depth of 2-3 cm. A total of nine pots for each treatment and control were maintained for every experiment.

Isolation and inoculation of experimental fungi

For isolation of AM fungi, 25 g of soil samples collected from the rhizosphere of pea plants were mixed in 100 ml of water to obtain a uniform suspension and left for five minutes so that debris floated on the top. The suspension was passed through a series of sieves of different sizes (240 µm, 120 µm, 100 µm, 63 µm, and 30 µm). The final decanted suspension of sieving was passed through Whatman filter paper. The filter papers were examined under the stereo-microscope for the detection of spores. The spores observed under the microscope were picked up with the help of a needle and transferred onto a clean glass slide for identification (Gerdeman & Nicolson 1963). The standard literature was used to identify the spores of AM fungi (Phillips & Hayman 1970). Four AM fungi, namely *Glomus intraradices*, *Glomus aggregatum*, *Glomus clarum* and *Sclerocystis microcarpa* were identified and selected to be used in this experiment.

The experimental pots were inoculated with the selected fungi at the time of sowing and repeated after 10 days. The pots with inoculated mycorrhizal fungi and the non-inoculated (untreated) served as treatments. A total of nine pots with AM fungi (*G. intraradices, G. aggregatum, G. clarum* and *S. microcarpa*) for each treatment and control were maintained. The plants were analyzed at three time points, i.e., 30 days post inoculation (DPI), 60 DPI and 90 DPI.

Analysis of morphological properties

To determine the effect of AM fungi (*G. intraradices, G. intraradices, G. aggregatum, G. clarum* and *S. microcarpa*) on plant height, leaf length, the total number of roots & leaves and root length of pea plants were examined. The plant height and length of leaves and roots were given in centimeters. The number of leaves and roots were counted by visual analysis.

Statistical analysis

The means were calculated for each treatment and control, and the data obtained after analyses of each morphological characteristic were analyzed with the help of student's t-test for comparison with the control.

Results

Results of the present study were expressed in terms of microscopic characteristics of the four AM fungi used. Variations in morphological characteristics, viz. plant height, leaf length, the total number of roots & leaves and root length of pea plants were observed when inoculated with AM fungi. It was observed that these fungi have significant effects on plant height, root length and total number of roots, while no significant effects were observed on leaf length and the total number of leaves.

Microscopic characteristics of AM fungi

The identification of AM fungi was carried out based on diameter, wall width, colour and presence or absence of hypha. Microscopic observations revealed that the spores of *G. intraradices* were dark black with hypha, whereas spores of *G. aggregatum* were dark brown to yellow without hypha. Similarly, light brown spores were observed in the case of *G. clarum* and dark brown to yellow spores in *S. microcarpa.* The detailed microscopic characters of AM fungi are provided in Fig. 1 and Table 1.

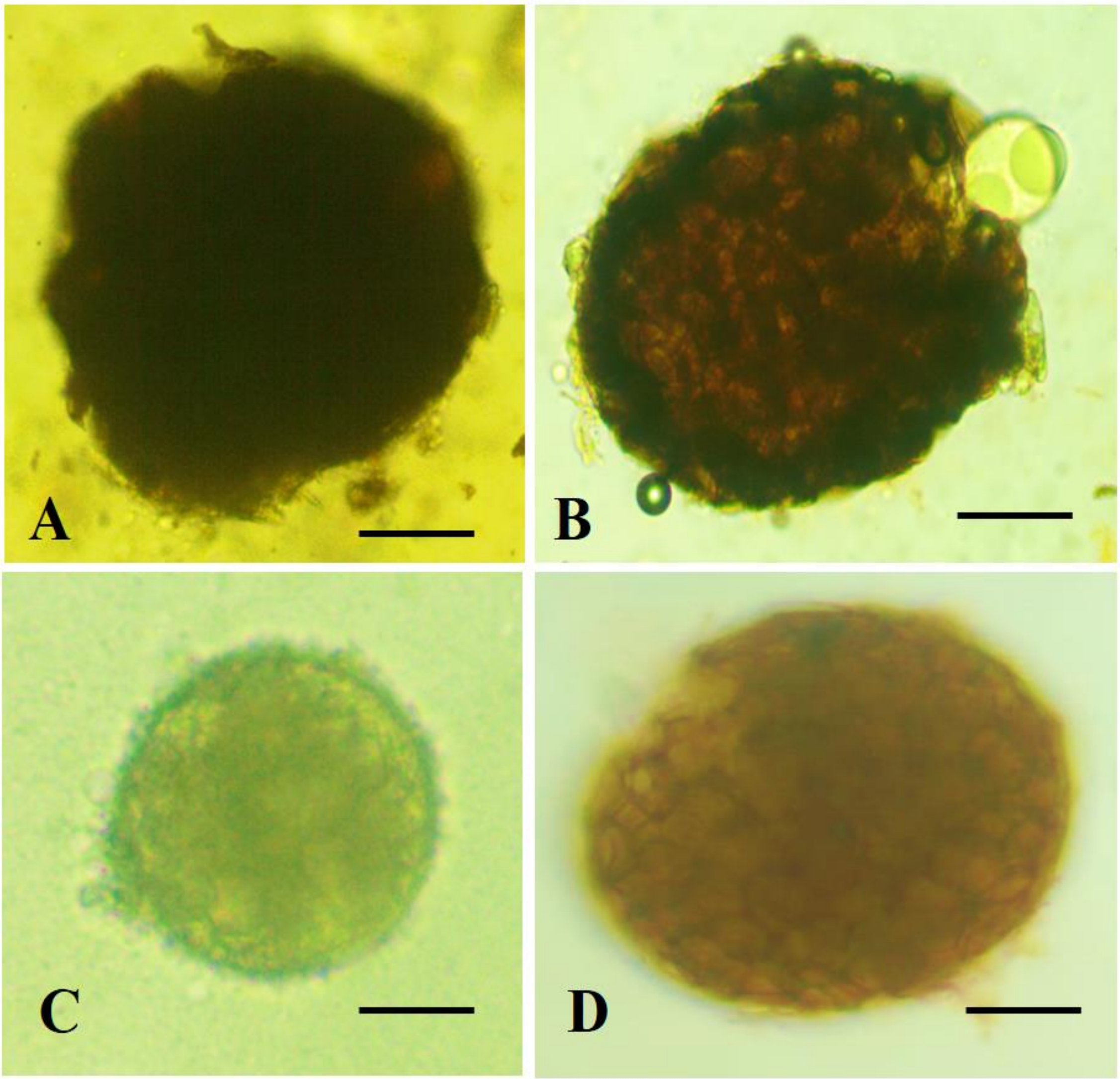

Fig. 1 – Species of AM fungi used for inoculation with pea plant experimentation. A *G. intraradices*, B *G. aggregatum*, C *G. clarum*, D *S. microcarpa.* Scale bar*:* A–D = 50 µm.

Table 1 Microscopic characteristics of AM fungi isolated from pea rhizosphere.

AM fungi	Identification parameters				Reference
	Diameter	**Wall width**	**Color**	**Hypha**	
G. intraradices	181.5 × 214.5 µm	16 µm	Dark black	Present	Schenck & Smith (1982)
G. aggregatum	132 × 165 µm	8 µm	Dark brown to yellow	Absent	Walker (1983)
G. clarum	297 × 198 µm	10 µm	Light brown	Absent	Nicolson & Schenck (1979)
S. microcarpa	264 × 231 µm	10 µm	Dark brown to yellow	Absent	Iqbal & Bushra (1980)

Variations in morphological parameters of pea after inoculation with AMF

Effects of AM fungi on growth characteristics of pea were observed on the 30th, 60th and 90th day after sowing. Analyses of results showed that these fungi have significant effects on plant height, root length and numbers, while their effect on leaf length and the total number of leaves were not significant (Tables 2-5).

Plant height

The effect of AM fungi on plant height was significantly higher compared to the control. The plant height on the 30th day after sowing was found to be highest in pea plants inoculated with *G. clarum* (33.83±0.06 cm), followed by *G. aggregatum* (31.73±2.57 cm), *G. intraradices* (29.66±1.78 cm) and *S. microcarpa* (16.60±2.46 cm). On the 60th day, plant heights were 79.60±3.16 cm, 75.50±6.84 cm, 71.70±1.52 cm and 71.20±1.59 cm for plants inoculated with *G. intraradices*, *G. aggregatum*, *S. microcarpa* and *G. clarum,* respectively. Similarly, the plant heights on the 90th day after sowing were observed to be highest in pea plants inoculated with *G. intraradices* (88.36±2.82 cm), followed by *S. microcarpa* (81.15±2.70 cm), *G. aggregatum* (77.03±4.83 cm) and *G. clarum* (76.20±1.18 cm). These results revealed a positive effect of AM fungi on the plant height of pea plants (Tables 2-5).

Leaf length

The results suggested that pea plants inoculated with AM fungi have significant effects on leaf length as compared to the control. The leaf length on the 30th day after sowing was highest in pea plants inoculated with *G. aggregatum* (2.66±0.10 cm), followed by *G. clarum* (2.40±0.11 cm), *G. intraradices* (2.20±0.07 cm) and *S. microcarpa* (2.05±0.06 cm). On 60th day after sowing, leaf length was found highest in pea plants inoculated with *S. microcarpa* (3.45±0.06 cm), which was followed by *G. aggregatum* (3.36±0.06 cm), *G. intraradices* (3.20±0.06 cm) and *G. clarum* (2.83±0.12 cm). On 90th day after sowing, the leaf length was found to be highest in pea plants inoculated with *G. aggregatum* (3.73±0.06 cm), followed by *G. intraradices* (3.66±0.09 cm), *S. microcarpa* (3.35±0.20 cm) and *G. clarum* (3.23±0.16 cm) (Tables 2-5).

Total number of leaves

The results revealed that inoculation of pea plants with AM fungi has no significant effect on the total number of leaves. However, a favourable variation in leaf number was observed between the control and AM fungi-treated plants. The total number of leaves was found to be highest in pea plants inoculated with *G. clarum* (on the 30th and 90th day after sowing), while it was highest in pea plants inoculated with *G. aggregatum* (on the 60th day after sowing) (Tables 2-5).

Root length

The inoculation with AM fungi showed variations in root length between inoculated and the control pea plants. All four fungi did not show similar effects. This effect was found positive in pea plants inoculated with *G. aggregatum* and *G. intraradices*. The root length was observed to be 11.80± 0.93 cm, 18.30± 1.43 cm and 22.43± 1.17 cm in pea plants inoculated with *G. aggregatum* on the 30th, 60th and 90th day after sowing, respectively. Likewise, pea plants inoculated with *G. intraradices* showed root lengths 11.20± 0.06 cm, 41. 40± 5.0 cm and 44.0± 4.5 cm on the 30th, 60th and 90th days after sowing, respectively. No effect on root length was observed in pea plants inoculated with *G. clarum* and *S. microcarpa* (Tables 2-5).

Total Number of roots

It was observed that only two AM fungi, namely *G. intraradices* and *G. aggregatum* were found to be effective in the present study. The total number of roots in pea plants inoculated with *G. intraradices* was observed to be 41±5, 145±7.72 and 152±8.18 on 30th, 60th and 90th days after sowing, respectively. Similarly, the total number of roots in pea plants was observed to be 35± 2.99, 153± 11.39 and 162± 11.55 on the 30th, 60th and 90th days after sowing, respectively when inoculated with *G. aggregatum*. No effect on the total number of roots was observed in pea plants inoculated with *G. clarum* and *S. microcarpa* (Tables 2-5).

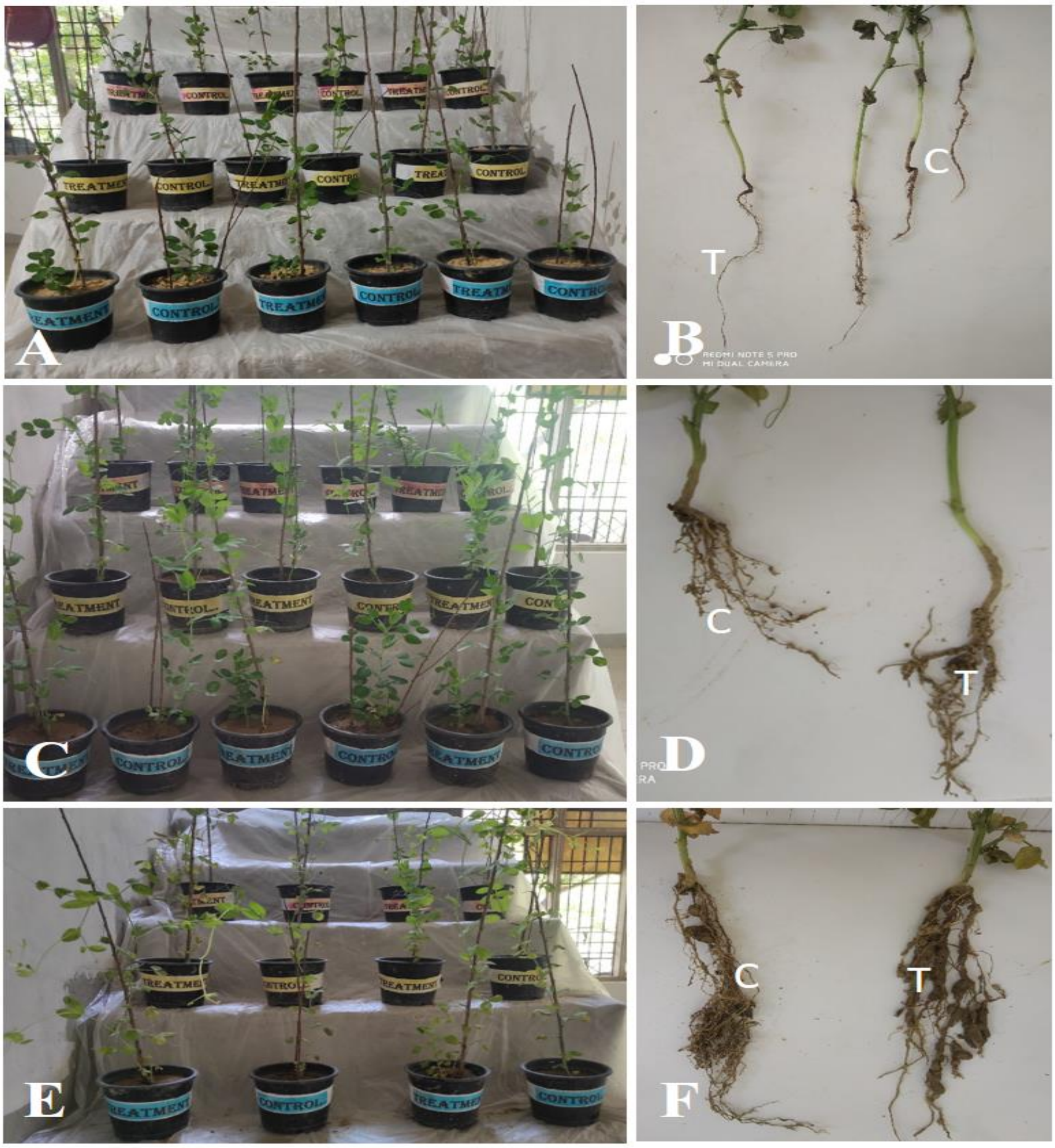

Fig. 2 – Effect of AM fungi inoculation on morphological attributes of pea. A–B Pot experiment on 30th day after AM inoculation. C–D 60th day after AM inoculation. E–F 90th day after AM inoculation. (C = control; T = treatment)

Table 2 Effect of *Glomus intraradices* on different characteristics of pea plant.

Parameters	*G. intraradices*					
	Treatment			Control		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Plant height (cm)	29.66±1.78***	79.6±3.16**	88.36±2.82*	22.36±1.22	73.5±2.26	84.23±1.78
Leaf length (cm)	2.2±0.07	3.2±0.06	3.66±0.09***	2.3±0.06	2.83±0.15	3.26±0.1
Total no. of leaves	31±1.78	68±2.09**	74±1.79**	31±1.22	57±6.04	61±5.8
Root length (cm)	11.2±0.06**	41.4±5.0***	44±4.5***	8.93±1.07	23±1.58	28.33±1.9
Total no. of roots	41±5.0***	145±7.72**	152±8.18	23±1.5	102±25.99	142±12.9

*Mean ± standard deviation; **Significant ($p \leq 0.05$), ***Highly significant ($p \leq 0.01$)*

Table 3 Effect of *Glomus aggregatum* on different characteristics of pea plant.

Parameters	*G. aggregatum*					
	Treatment			Control		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Plant height (cm)	31.73±2.57	75.5±6.84	77.03±4.83***	33.33±0.26	85.93±2.06	91.46±2.05
Leaf length (cm)	2.66±0.1***	3.36±0.06	3.73±0.06**	2.16±0.04	3.3±0.06	3.53±0.07
Total no. of leaves	31±2.57**	78±1.58***	82±1.14***	35±0.26	65±1.2	77±0.96
Root length (cm)	11.8±0.93***	18.03±1.43***	22.43±1.17***	8.2±0.46	11.5±0.49	16.03±1.08
Total no. of roots	35±2.99**	153±11.39***	162±11.55**	26±3.98	100±20.73	142±1.82

*Mean ± standard deviation; **Significant ($p \leq 0.05$), ***Highly significant ($p \leq 0.01$)*

Table 4 Effect of *Glomus clarum* on different characteristics of pea plant.

Parameters	*G. clarum*					
	Treatment			**Control**		
	30 Days	**60 Days**	**90 Days**	**30 Days**	**60 Days**	**90 Days**
Plant height (cm)	33.83±0.06***	71.2±1.59	76.2±1.18	23.86±2.29	66.86±3.19	76±2.14
Leaf length (cm)	2.4±0.11**	2.83±0.12	3.23±0.16	2.2±0.06	2.9±0.11	3.2±0.14
Total no. of leaves	34±0.06	77±3.56	90±2.15***	36±2.29	72±2.38	78±2.7
Root length (cm)	11.3±0.33	16.46±0.78	18.53±0.24***	12.86±1.15	16.83±1.31	21.33±1.12
Total no. of roots	32±3.48	162±10.78	173±1.74	32±3.82	153±11	161±10.8

*Mean ± standard deviation; **Significant ($p \leq 0.05$), ***Highly significant ($p \leq 0.01$)*

Table 5 Effect of *Sclerocystis microcarpa* on different characteristics of pea plant.

Parameters	*S. microcarpa*					
	Treatment			**Control**		
	30 Days	**60 Days**	**90 Days**	**30 Days**	**60 Days**	**90 Days**
Plant height (cm)	16.6±2.46	71.7±1.52	81.15±2.7	18.05±1.36	71.75±0.96	81.95±1.05
Leaf length (cm)	2.05±0.06***	3.45±0.06	3.35±0.2	1.7±0.08	3.15±0.11	3.1±0.04
Total no. of leaves	20±2.46	68±0.89	82±1.79***	18±1.36	66±2.46	73±2.46
Root length (cm)	10.35±0.2***	15.35±0.38***	18.45±0.55	14.05±0.46	17.45±0.29	18.7±0.4
Total no. of roots	28±0.89	159±4.02	164±2.46***	40±0.67	169±2.09	177±1.79

*Mean ± standard deviation; **Significant ($p \leq 0.05$), ***Highly significant ($p \leq 0.01$)*

Discussion

The present study reveals that the inoculation of plants with different AM fungi posed significant effects on the growth characteristics of peas. Four fungi, namely *G. aggregatum*, *G. clarum*, *G. intraradices*, and ***S.** microcarpa* used in the present study were isolated as major AM fungi from different plant hosts, including pea. It was observed that the maximum number of AM fungi were isolated during the autumn season, i.e., in November. These results are in accordance with the previous study carried out by Camilla et al. (2003), where they reported that *G intraradices* showed the potential to affect microbial activity in the rhizosphere of pea plants. In a similar study, variable percentages of AM fungi were associated with roots and plant rhizosphere. This variability in the occurrence of these fungi may be due to the growing season, plant growth stage, soil properties and the availability of water (Sanchez 1990, Nelson 1997, Xavier & Germida 2003, Cong et al. 2010, Hunanj 2011).

All the test AM fungi, namely *G. aggregatum*, *G. clarum*, *G. intraradices*, and *S. microcarpa* have the potential to cause positive effects on one or more growth parameters. The variations in growth parameters in inoculated plants compared to control are perhaps due to the alteration of physiological and biochemical properties (Grant 2012, Abdelaal et al. 2020). The presence of AM fungi is reported to break complex nutrients into simpler ones and may enhance their absorption, which is otherwise not available to pea plants because they are in complex forms (Creus et al. 2004). This might be the possible cause to obtain significant increase in plant height and root length. A similar study on the influence of different AMF inocula (*G. mossae*, *G. etunicatum* and *Acaulospora kentinensis*) on cowpea roots colonization and soil improvement was carried out by Surayya et al. (2021). A study carried out by Wu & Xia (2004) reported that arbuscular mycorrhizal fungi inoculation could increase plant growth, such as plant height, stem diameter, leaf area, shoot dry weight, root dry weight and plant dry weight. Similarly, Begum et al. (2019) reviewed up-to-date available knowledge on AMF and their influence on host plants at various growth stages. The major findings depicted that plants inoculated with AMF can effectively fight against various environmental conditions, like alkali stress, cold stress, drought, extreme temperatures, nutrient stress and salinity. The use of AM fungi as plant growth regulators is a well-known and emerging technology in crop production. The effects of *G. aggregatum* on the leaf length of chickpea was also reported by Singh et al. (2004). A similar study on the effect of *G. intraradices* on the plant height of common bean (*Phaseolus vulgaris*) was carried out by Olivera et al. (2004). Inoculation of *Lolium perenne* with *Glomus* sp. significantly increased root vigor and chlorophyll content along with increased photosynthetic carbon assimilation capacity (Zhang et al. 2018). The outcomes of the present study revealed that mycorrhizal fungi have the potential as biofertilizers. Despite such useful properties, the potential of these fungi is still untapped as a viable biofertilizer for sustainable agricultural production. Encouragement of the use of AM fungi is very useful for the present day agricultural systems for their consistent sustainability.

Acknowledgements

The authors are thankful to Abhilashi University for providing laboratory facilities to carry out this research work successfully. Respective organizations of each author are also gratefully acknowledged for their support towards this research directly and indirectly. We also thank the Anonymous reviewers for their helpful comments and suggestions.

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