

A STUDY ON ROOT COLONIZATION OF WILD HERBACEOUS PLANTS OF DESHBANDHU COLLEGE WITH MYCORRHIZAL FUNGI

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ABSTRACT

With major advancements in science and technology, excessive usage of chemicals fertilizers and pesticides have drastically effected soil fertility thereby reducing the biodiversity of microorganisms in the soil ecosystem. In recent times, global awareness about the beneficial effects of practicing organic farming has changed the mindset of the farmers encouraging them to use eco-friendly methods for agriculture on a long-term sustainable basis. One such sustainable technology that is being widely explored presently is the usage of ubiquitous, oldest and the most popular symbiotic association, Arbuscular mycorrhizal fungi (AMF) with terrestrial plants in an ecosystem. AMF, being the most popularly used biofertilizers, plays an important role in the soil ecosystem by maintaining the fertility of the soil and sustainability of beneficial microorganisms in the vicinity of the rhizosphere of different plants in a region. AMF technology has several large-scale applications in the agriculture sector, horticulture and forestry. AMF mainly stimulates the uptake of nutrients like phosphorus (P) uptake in plants, soil aggregation, soil moisture, plant biodiversity, resistance against diseases and pests, tolerance to abiotic stresses like drought, salinity, increased crop yield and quality thereby leading to successful plant growth and development. Owing to the multiple beneficial effects of AMF in an ecosystem, more focus should be given to promote AMF-mediated crop yield and productivity in agriculture. The present study gives a comparative account of a few screened wild plants for the presence of AMF in the roots by microscopic examination and their impact on the wild vegetation of the campus. This study would be further helpful to decide the type of wild plants that can be allowed to spread on the campus for increasing the soil fertility and plants diversity by this fruitful AMF association.

Keywords: Agriculture, AMF, Biofertilizers, Ecosystem, Soil, Sustainable

INTRODUCTION

The word ‘Mycorrhizae’ is derived from the two Greek words, ‘mycos’ and ‘rhizos’, meaning fungus and root, respectively. Mycorrhizal association forms a very dynamic and beneficial biological relationship that exists between arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota, and roots of higher plants (vascular plants) ((Panneerselvam *et al.*, 2017). AMF belonging to Glomeromycota phylum requires a host for completing its life cycle. Mycorrhizal associations between a fungus and roots of higher plants are widespread in the natural environment. AMF are perhaps the most abundant fungi found in agricultural soils, making up between 5 and 50% of the total soil microbial biomass (Olsson, 1999), although their actual diversity is low (Helgason *et al.*, 1999). The association can be categorised into one of seven types (Arbuscular, ectomycorrhizal, ectendomycorrhizal, ericoid, arbutoid, orchid and monotropoid) based upon the involvement of fungus, and their resulting structures generated in the host root by the fungus-plant relation. Of all these different types of associations known, the most common is that of arbuscular mycorrhiza (AM), which involves a relatively small number of fungi, but around two-thirds of all terrestrial plant species (Hodge, 2003). More than 80% of terrestrial plants form this AM association except some

plants belonging to non-mycorrhizal families like Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Polygonaceae, Urticaceae, and Juncaceae (Lambers & Teste, 2013) (Kumar *et al.*, 2016). Apart from its acknowledged role in Phosphorus (P) uptake by the host plant, uptake of other micronutrients like Cu, Zn, Al, Mn, Mg, and Fe has also been demonstrated (Kucey & Janzen, 1987); (Purakayastha & Chhonkar, 2001) (Hajiboland *et al.*, 2009) (Panneerselvam *et al.*, 2013). In addition, AMF plays a vital role in wide range of functions from stress reduction to bioremediation in soils polluted with heavy metals (Hildebrandt *et al.*, 2007). AMF are also involved in diverse functions such as soil health improvement, plant health improvement, and protection of plants against pathogen (Begum *et al.*, 2019). It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonisation of land by plants and in the evolution of the vascular plants (Brundrett, 2002). AMF are one of the few plant-fungus associations with a fossil record and may even have facilitated the origin of land flora (Simon *et al.*, 1993). The symbiosis is thought to have afforded green plants the opportunity to invade dry land ca 450 Myr ago and the vast majority of extant terrestrial plants retain this association. Phylogenetic analyses studies confirmed an estimate for the origin of VAM-like fungi of 353–462 Myr ago, which is consistent with the hypothesis that VAM were instrumental in the colonization of land by ancient plants (Simon *et al.*, 1993). Arbuscular mycorrhizal (AM) fungi perform various ecological functions in exchange for host photosynthetic carbon that almost always contribute to the fitness of hosts from an individual to community level (Hodge, 2003). Inoculation with Arbuscular Mycorrhizae Fungi (AMFs) has been identified as an eco-friendly approach to improve soil fertility (Dal Cortivo *et al.*, 2018). AMF is the most widespread soil microorganism that form a symbiotic relationship with more than 80% plants (Prasad *et al.*, 2017). This can be found in various ecosystems worldwide (Verbruggen *et al.*, 2012).

Screening of such wild herbaceous plants that promote AMF technology, will help us to judiciously grow only those plants which are beneficial for maintaining the diversity in the vicinity. Also it will help us to understand the role played by these fungi in the survival of wild plants. This will further provide information regarding the diversity of useful microbial symbiotic associations in rhizosphere of different herbaceous plants.

The present study is carried out to observe presence of AM fungi in some common wild herbaceous plants growing in the campus of Deshbandhu College and to identify various structures produced by them. The intensity of root colonization of these plants are also assessed.

The plants selected for this study are *Cannabis sativa*, *Vernonia cinerea*, *Ricinus communis*, *Amaranthus virides*, *Parthenium hysterophorus*, *Majus japonicus*, *Acalypha indica*, *Aerva lantana*, *Solanum nigrum* and *Euphorbia hirta*.

MATERIALS AND METHODS

The plant materials for the present study were collected from the soil in and around botanical garden of Deshbandhu College. The root clearing and staining methods to study AM colonization (modified procedure of Philips & Hayman, 1970) involves maceration of roots followed by staining and destaining to see arbuscules, vesicles and fungal hyphae. The roots are first macerated with an alkali (10% KOH) in order to make them soft. To neutralize the alkali, the roots are rinsed with 1% HCl to enhance the binding of stain. The stain, Trypan Blue binds to the carbohydrates present in the root and give them blue colour. Lactoglycerol is used to remove excess stain.

Root Clearing Procedure

Fine roots of the collected plants, around a centimeter each, are cut and then washed thoroughly under running tap water. Then the roots are placed in test tube and 10% KOH solution was added. This solution was heated for about 5-10 minutes, depending upon the age and tenderness of the roots. When the stellar region of the roots becomes visible, then KOH solution was poured off and the roots were rinsed well using distilled water (at least three complete washes with distilled water till no brown color appears). Then the roots were kept in 1% HCl and soaked for about 3-4 minutes. HCl solution was poured off and the roots were

incubated in 0.05% Trypan Blue for 30 minutes. Finally, the roots were de-stained in lactoglycerol (mounting medium) until no blue color appears.

OBSERVATIONS

Table 1: Mycorrhizal colonization in selected wild herbaceous plants of Deshbandhu College

S.N.	Name of the weed	Family	Fungal Hyphae	AM Colonization	VM Colonization
1.	<i>Cannabis sativa</i>	Cannabinaceae	+	+	+
2.	<i>Vernonia cinerea</i>	Asteraceae	++	++	+
3.	<i>Ricinus communis</i>	Euphorbiaceae	+++	—	+
4.	<i>Parthenium hysterophorus</i>	Asteraceae	++	+++	+++
5.	<i>Amaranthus viridis</i>	Amaranthaceae	+	+++	+
6.	<i>Solanum nigrum</i>	Solanaceae	++	—	—
7.	<i>Aerva lanata</i>	Amaranthaceae	++	—	++
8.	<i>Acalypha indica</i>	Euphorbiaceae	+	+++	-
9.	<i>Euphorbia hirta</i>	Euphorbiaceae	++	—	—
10.	<i>Majus japonicas</i>	Majaceae	++	+++	+++

A random selection of an area with dense herbaceous vegetation in Deshbandhu college fields was done. Screening of rhizosphere of wild herbaceous plants was carried out to analyze their symbiotic association with beneficial microbes. These beneficial microbes can be used as biofertilizers and can further help to encourage the growth of wild plants that are having such beneficial associations. Ten wild herbaceous plant species from mainly six angiosperm families namely Asteraceae, Solanaceae, Amaranthaceae, Cannabinaceae, Mazaceae and Euphorbiaceae were assessed for colonization of AM and VM fungi from the selected area namely *Cannabis*, *Vernonia*, *Ricinus*, *Amaranthus*, *Parthenium*, *Majus*, *Acalypha*, *Aerva*, *Solanum nigrum* and *Euphorbia hirta* were screened. Rhizosphere of all these plants was observed to have endomycorrhizal association with variable intensity of colonization. Both vesicles and arbuscules of endomycorrhiza could be observed in all the plants studied. However, significant difference was observed in the rate of AM and VM infection. (Table 1)

Observations on these plants show profusely formed arbuscules and vesicle in *Majus* (Fig.1 A and B). Well formed Y shaped hyphae are also prominent.

In *Parthenium* balloon shaped vesicles stain well and profusely formed arbuscules can be seen in root cortical cells (Fig.1 C and D).

Thick fungal hyphae and some arbuscules are observed in root cortical cells of *Vernonia* (Fig. 2A). Thick branched hyphae with vesicles are observed in *Aerva* (Fig. 2B and C). Root cortical cells of *Acalypha* show profusely formed arbuscules (Fig 2 D and E). In *Solanum nigrum* and *Euphorbia hirta* only thick branched hyphae are visible in root cortical cells. (Fig 3A and B). *Amaranthus* roots also show colonization with AM fungi. Well formed arbuscules are observed (Fig 3C).

In *Ricinus* thick branched, septate hyphae with vesicles are observed (Fig. 4A and B). Root colonization with arbuscules are observed in *Cannabis* (Fig. 4C).

Remarkable differences could be seen as both AM and VM colonization was maximum in *Majus*, *Parthenium*. In *Solanum nigrum* and *Euphorbia hirta* only hyphae are visible., *Aerva* and *Ricinus* show well-formed vesicles, whereas in other plants only AM colonization and dichotomous branching of fungal hyphae could be seen.

RESULTS AND DISCUSSIONS

AMF are soil-borne fungi that can significantly improve plant nutrient uptake and resistance to several abiotic stress factors (Sun *et al.*, 2018). The most widely recognised role of AMF in the plant-fungus

relationship is that of improving the uptake of nutrients by the host plant, **especially of Phosphorus (P)**. Indeed, P nutrition is generally regarded as the main controlling factor in the plant fungal relationship ((Thompson, 1987); (Graham, 2000)).

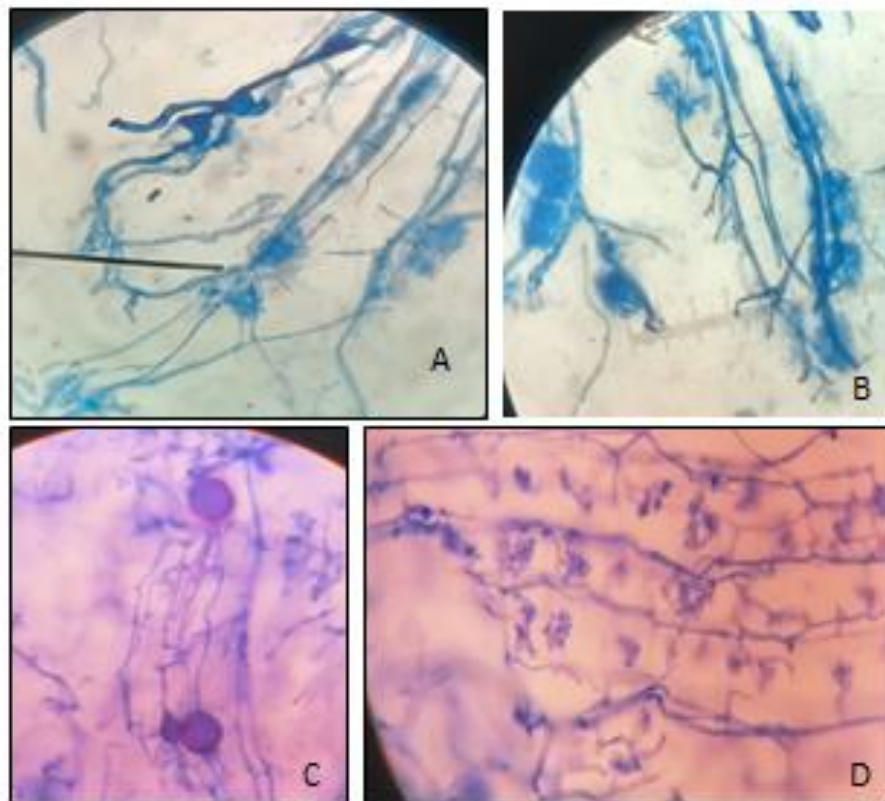


Fig. 1 Arbuscules and vesicles in roots of *Majus*(A and B); *Parthenium* (C and D)

Mycorrhizal colonization is very common in majority of angiosperms. The present study reveals the natural occurrence of AM and VM mycorrhiza in all the 10 wild herbaceous plants selected from our college flora. Although variations could be observed in the type of mycorrhizae and intensity of colonization of rhizosphere of all the selected genera. Hundred percent colonization indicates effective symbiosis with the weeds enriching the soil with various nutrients. Mycelium with various types of hyphae, vesicles and arbuscules are observed in these plants.

In *Majus* (low-growing perennial plants generally found in damp habitats) Y shaped hyphae with vesicles are observed in the cortical cells. *Ricinus* and *Aerva* root cortex also show Y shaped thick branched hyphal structures. Jabbar and Thomas (2020) have reported the occurrence of similar Y-shaped hyphae, and hyphae with clamp connections in *Aerva lantana*. Thick branched fungal hyphae are abundant in *Solanum* and *Euphorbia* also but no prominent arbuscules or vesicles were observed.

In *Parthenium* balloon shaped vesicles stain well and profusely formed arbuscules can be seen in root cortical cells (Bhale, 2018) have reported presence of hyphae, arbuscules and vesicles in roots of *Parthenium hysterophorus*(L.) growing on degraded land both in Rabi and kharif season. Weedy plants have the potential for the association of beneficial mycorrhizae for 'P' uptake. When mycorrhizal residing weeds are present in

degraded land, such lands becomes fertile for the further cultivation of crop plants. Some weeds are helpful for reestablishing crop plants. Thus, weeds plant will benefit directly from the AM symbiosis through

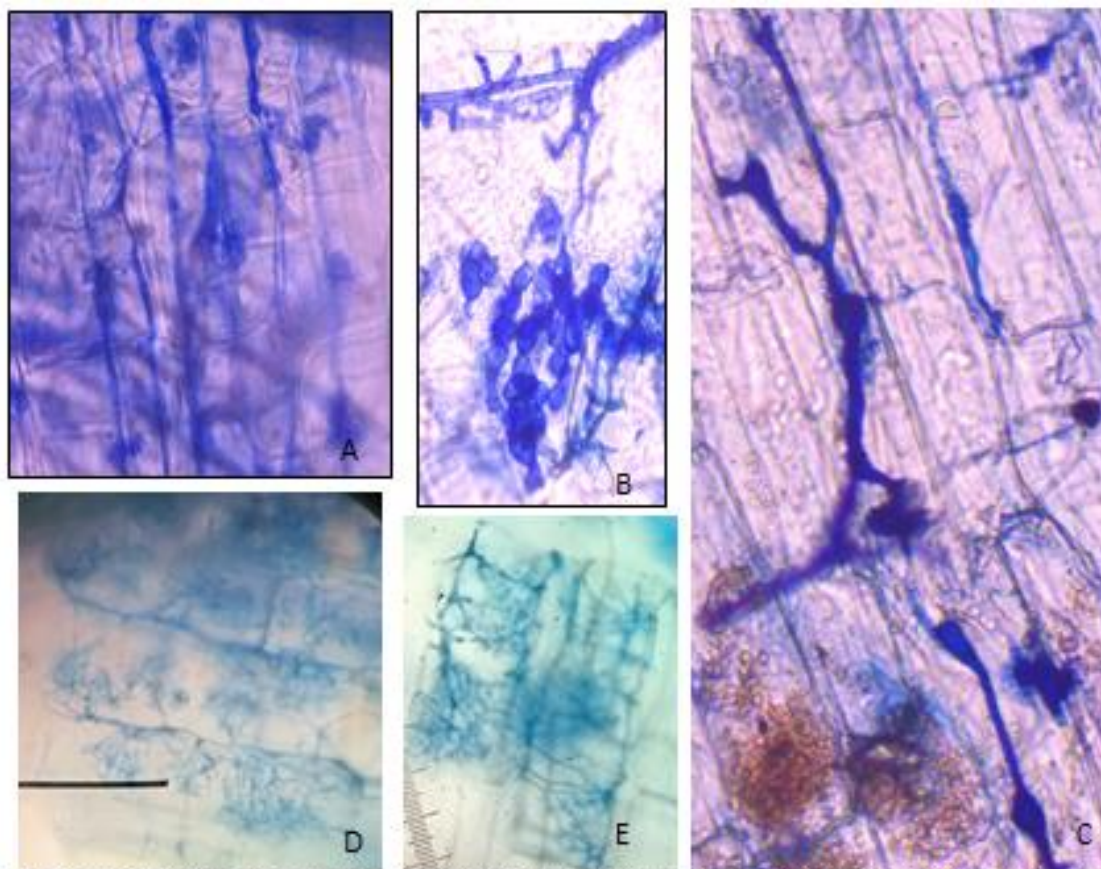


Fig. 2 Fungal hyphae, vesicles and arbuscules in roots of *Vernonia* (A); *Aerva* (B and C); *Acalypha* (D and E)

increased nutrient uptake and inevitably increased growth. According to (Bhale, 2018) Weeds are an important variable in crop productivity, economically, ecologically and also may serve to maintain diversity and agronomic beneficial species of AM fungi. It has been observed in the weedy plants such as *Dichanthium caricosum*, *Parthenium hysterophorus*, *Cynodon dactylon*, *Dinebera retroflexa* and *Chrochrous capsularis* in crop fields, it would be support for higher AM colonization and directly increased the biomass and yield (Bhale, 2018). These plants have a tendency for mycorrhizal association for the uptake of phosphorus. When such weeds are present in a land, the land becomes fertile for further cultivation of crop plants and other economically important plants. Also, these mycorrhizal associations may be responsible for the survival and fast growth of these weeds particularly *Parthenium*.

Arbuscules can be dense and compact in structure and appearance (See slide view above). Arbuscules are considered to be the major site of exchange between the fungus and host. This assumption is based on the large surface area of the arbuscular interface, but has not been confirmed (Smith, 1995). Well developed and profusely formed arbuscules observed in *Acalypha* and *Amaranthus* as observed in present study may be the reason for extensive growth of these plants in not so fertile soils. AMF can play a significant role in crop growth in soil with low phosphorus availability. However, it has become increasingly evident that AMF also

have an important role in the uptake of a range of other nutrients particularly zinc (Zn), but also including copper (Cu), iron (Fe), nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg) (Smith & Read, 2010) (Clark & Zeto, 2000). Vesicles are usually formed between root cortex cells and known to accumulate

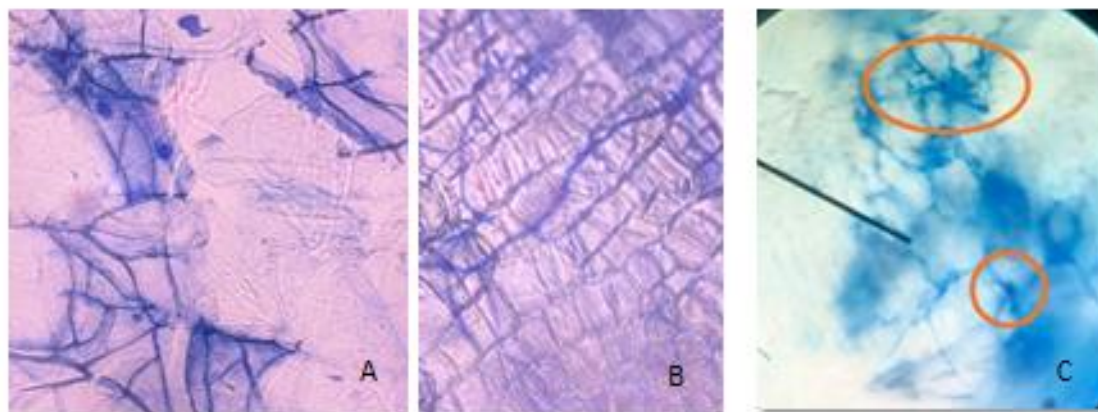


Fig. 3 Dichotomously branched hyphae and vesicles in roots of *Solanum* (A); *Euphorbia* (B) and *Amaranthus* (C)

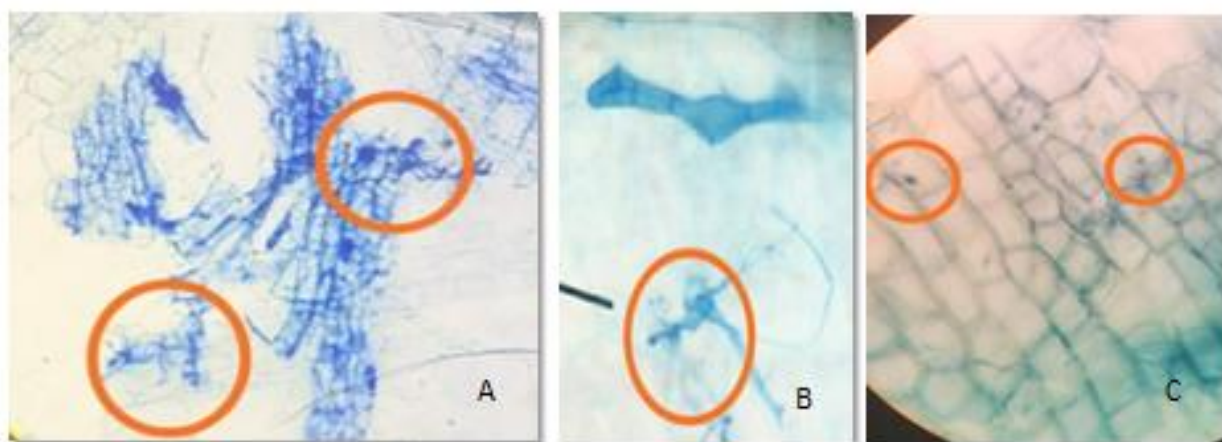


Fig. 4 Fungal hyphae, vesicles and arbuscules in roots of *Ricinus* (A and B) ; *Cannabis* (C)

storage products in many VAM associations. Vesicles are initiated soon after the first arbuscules, but continue to develop when the arbuscules senesce. Vesicles are hyphal swellings in the root cortex that contain lipids and cytoplasm. These may be inter- or intracellular. Vesicles can develop thick walls in older roots and may function as propagules ((B. Biermann & Linderman, 1983)(B. J. Biermann & Linderman, 1983). Some fungi produce vesicles which are similar in structure to the spores they produced in soil, but in other cases they are different. *Aerva* and *Ricinus* show such vesicles along with thick hyphae. Pawaar *et al.* (2012) have reported the presence of AM fungi in 11 of the 15 medicinal plants screened. In their study also, colonization was observed in the form of mycelium, arbuscules, vesicles and chlamydospores.

AMF may also be necessary for the long-term sustainability of Ecosystems, particularly due to their role as biofertilizers, bioregulators and bioprotectants in the maintenance of soil structure and plant diversity. The mycorrhizal associations therefore play an effective role in plant productivity. AMF-mediated growth

promotion is not only by improving water and mineral nutrient uptake from the adjoining soil but also by safeguarding the plants from fungal pathogens (Smith & Read, 2010, Jung *et al.*, 2012; Evelin *et al.*, 2012). They are of key importance for sustainable crop improvement (Gianinazzi & Schüepp, 1994). AMF benefits to the crop includes improved nutrition (Lambert *et al.*, 1979; Thompson, 1987; Graham, 2000), enhanced resistance to pests and disease (Schonbeck, 1979; Linderman, 1992; Borowicz, 2001, Calvet *et al.*, 2001) and improved water relations (Ruiz-Lozano *et al.*, 1995; Smith, 1995; Mohammad *et al.*, 2003; Evelin *et al.*, 2012).

Role of AM fungi in alleviation of salt stress has been emphasized by various workers in different plant species (Evelin *et al.*, 2009; Evelin *et al.*, 2019; Srivastava *et al.*, 1996). Several physiological, biochemical and mechanisms at molecular level are responsible for salt stress tolerance in plants induced by arbuscular mycorrhizae (Evelin *et al.*, 2019). It is quite possible that mycorrhizal association of these wild plants are responsible for their survival and growth even in harsh environmental conditions.

AMF might act as a potential tool in replacing inorganic fertilizers in near future. Different research studies conducted on AMF during the past two decades have highlighted their countless benefits on soil health and crop productivity. Therefore, it is widely believed that AMF could be considered as a replacement of inorganic fertilizers in the near future, because mycorrhizal application can effectively reduce the quantitative use of chemical fertilizer input especially of phosphorus (Srivastava *et al.*, 1996, Ortas, 2012). Continuous use of inorganic fertilizers, herbicides, and fungicides has caused various problems to soil, plants, and human health, through their damaging impact on the quality of food products, soil health, and air and water systems (Yang *et al.*, 2014). These fungi are a cheap and environment friendly source of biofertilizers that can help in increasing the plant productivity without harming the ecosystem.

ACKNOWLEDGEMENTS

This project work was undertaken at Deshbandhu College under the DBT star College scheme of Department of Biotechnology, Government of India.

Conflict of interests

The authors declare that there is no competing interest.

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