



ASSESSMENT OF ROOT COLONIZATION BY VAM FUNGI IN VEGETABLE PLANTS IN CENTRAL INDIA

Poonam Verma¹, Sagar Barle², Mridul Shakya¹ and Sardul Singh Sandhu^{1*}

¹BioDesign innovation Centre, Ekatm Bhawan, Rani Durgavati University Jabalpur (MP), 482001

²Bio²Fungal Biotechnology and Invertebrate Pathology Laboratory, Department of Biological Sciences, Rani Durgavati University, Jabalpur – 482001, M.P.

*Corresponding author's email: ssandhu@rediffmail.com

ABSTRACT

In the present study, nine vegetables of four family namely as Amaranthaceae, Cucurbitaceae, Poaceae and Solanaceae were observed for maximum colonization of Arbuscular mycorrhizal (AM) fungal. The highest rate of colonization was observed in *Solanum melongena* belong to Solanaceae followed by *Solanum lycopersicum* (Solanaceae), *Allium cepa* (Amaryllidaceae) and minimum was observed in case of *Cucumis sativus* (Cucurbitaceae). The seedlings of the test plants were treated with AM fungi and the vesicle formation were observed in case of all seedlings, but arbuscule was detected only in onion and brinjal. The maximum average of colonization percentage was also recorded during the study and the highest frequency was showed in Solanaceae family about 93.47% and minimum was found in Cucurbitaceae family about 66.7%. This study first time observed the maximum AM colonization with vesicle in *S. oleracea* (Amaranthaceae).

KEYWORDS: Biofertilizer, Myco-rhizosphere, Growth promoter, population.

INTRODUCTION

Arbuscular mycorrhizal (AM fungi) associations are the most frequent symbioses found in nature because of their broad association with plants and cosmopolitan distribution (Harley and Smith, 1983; Verma, 2010). These are beneficial soil inhabiting fungi that establish symbiotic association within the roots of plants. AM fungi create relationship within the extracellular spaces of root cortical tissues and increase the uptake of water, phosphorus, nitrogen and micronutrient in the host plant (Brundrett, 1991). AM fungi benefits host plant not only by improving nutrient uptake but also by increasing production of growth hormones, etc. Several plants colonized by AM fungi showed increased advantages include drought tolerance, activation of plant defense mechanism, increased growth, reduced pathogen pressure and general benefits to plant health (Brundrett, 1991; Verma and Jamaluddin, 1994; Mukerji *et al.*, 1996). It's also provides a useful measure of relative soil quality and health (Klingeman *et al.*, 2002).

The association of mycorrhiza in environment is ubiquitous like aquatic plants (Seerangan and Thangavelu, 2014), freshwater (Miller, 2000), wetlands (Bauer *et al.*, 2003), agriculture land (Hedlund and Gormsen, 2002), forest land (Devi *et al.*, 2017), degraded land (Verma and Verma, 2017), garden soil (Johnson *et al.*, 1992), forest Nursery (Verma and Verma, 2016; Verma *et al.*, 2016; Verma *et al.*, 2017). These were indicates that the biology and ecology of this association deserves further research, especially with respect to the role of fungi in plant nutrition and tolerance of habitat conditions. Now a day's application of AM fungi during raising seedlings is helpful for growth of different seedling in nurseries condition (Verma and Verma, 2016; Verma *et al.*, 2016; Verma *et*

al., 2017). In the present investigation, cucumber, Wheat, Onion, Spinach, Eggplant, Garlic, Chilli, Red chaoli and Tomato were selected. These were widely cultivated and used in Indian traditional medicine since ancient times. All have medicinal properties like low in calories, antidiabetic, lipid lowering, antioxidant activity, several bioactive compounds, proteins, carbohydrate, source of multiple nutrients and dietary fiber and amount of nutrients, including iron; calcium; magnesium; amino acids; and vitamins A, C and E (Howard *et al.*, 2000; Mattina *et al.*, 2003; Khan *et al.*, 2010; Cicitelli *et al.*, 2010; Bois *et al.*, 2005; Huang *et al.*, 2011; Wilde *et al.*, 2009; Okigbo *et al.*, 2009; Berruti *et al.*, 2015; Gutjahr and Paszkowski, 2013; Kumar *et al.*, 2013; Mauseth, 2014).

MATERIALS AND METHODS

Survey and sample collection

Soil sample were collected from campus of Rani Durgavati University, Jabalpur according to Parkinson, (1979). A soil auger used which as washed thoroughly before starting of sampling procedure. The sampling was done in 10-20cm depth in soil horizon and collected in polyethylene bags tied with rubber bands and brought to the laboratory (Clayton *et al.*, 2009). Sample were homogenized and stored at 4°C for further use. Soil sample was sterilized with 30% formaldehyde solution.

Collection of seeds and surface sterilization

To conduct pot experiment seeds of Cucumber, Wheat, Onion, Spinach, Brinjal, Garlic, Chili, Tomato and Red chaoli were collected from seed stored shop, Jabalpur, healthy seeds were sorted and surface sterilization was done by using 1% sodium hypochlorite (NaOCl) solution for 10 minutes and after that sample was washed three

times with distilled water and then subjected to hot water treatment for break the dormancy of seeds (Kaushish *et*

al., 2011). The plants used in the investigation are shown in Table no. 1.

TABLE 1: List of vegetable plants

S. No.	Common Name	Botanical Name	Family
1.	Cucumber (Khira)	<i>Cucumis sativus</i> L.	Cucurbitaceae
2.	Wheat (Gahu)	<i>Triticum aestivum</i> L.	Poaceae
3.	Onion (Payaj)	<i>Allium cepa</i> L.	Amaryllidaceae
4.	Spinach (Palak)	<i>Spinacia oleracea</i> L.	Amaranthaceae
5.	Brinjal (Began)	<i>Solanum melongena</i> L.	Solanaceae
6.	Garlic (lahsun)	<i>Allium sativum</i> L.	Amaryllidaceae
7.	Chili (Mirch)	<i>Capsicum annuum</i> L.	Solanaceae
8.	Tomato (Tamatar)	<i>Solanum lycopersicum</i> L.	Solanaceae
9.	Red cholai (Lal bhaji)	<i>Alternanthera</i> sp. Forssk.	Amaranthaceae

Nursery experiment

AM fungal inoculums were applied on selected vegetable species to study whether these microbes worked as biofertilizer and play a positive role in establishment of seedling in nurseries or not.

Experimental design

For this purpose seeds were sown in Biological Science Department, RDVV, Jabalpur. Soils were mix properly and sterilized with formaldehyde and filled in polyethene bags. Seeds were sown in 22 May 2017. The experimental seedlings received the following treatments: (1) Control, (2) AM fungi.

Assessment of AM Fungal Root Colonization (RC)

Development of mycorrhizal fungi in term of RC was detected by Phillips and Hayman (1970) root staining

method. Fine root were washed in tap water to detach the soil and external mycelium. Root were cut into 1cm segments and simmered at about 90°C for 15-30 minute in 10% KOH solution on a water bath. Then the root were rinsed 3-4 time in tap water and were acidification by immersing in 1% HCl for 5min. Acid was poured and stain (0.5% trypan blue in lactophenol) was added. Roots were boiled for 5 minutes in stain. Stain is poured off and lactic acid and glycerol (1:1) was added and was kept overnight to de-stain the root tissues. Then washed with distilled water and squashed roots were examined to observed hyphae, vesicles and arbuscular under microscope. Hundred root bits were examined and the present of RC was calculated by following the formula mentioned below:

$$\% \text{ root Colonization} = \frac{\text{Number of root bits infected with AM} \times 100}{\text{Total number of root bits examined}}$$

RESULTS & DISCUSSION

A total nine vegetables of four family namely as Amaranthaceae, Cucurbitaceae, Poaceae and Solanaceae were observed for maximum colonization of Arbuscular mycorrhizal (AM) fungal. The highest rate of colonization

was observed in *Solanum melongena* belong to Solanaceae followed by *Solanum lycopersicum* (Solanaceae), *Allium cepa* (Amaryllidaceae) and minimum was observed in case of *Cucumis sativus* (Cucurbitaceae).

TABLE 2: Root colonization (RC) in different plants

S. No.	Name of plants	Percentage of Root colonization							
		VAM inoculated			Control				
		RC %	V	A	H	RC %	V	A	H
1	Cucumber	66.7		x	x	20		x	x
2	Wheat	80		x		6.7		x	x
3	Onion	93.3			x	13.3		x	x
4	Spinach	86.7		x	x	26.6		x	x
5	Brinjal	100			x	6.7		x	x
6	Garlic	80		x		26.6			x
7	Chili	86.7		x		33.3			x
8	Tomato	93.7		x		0	-	-	-
9	Red cholai	80		x		13.3		x	x
Mean		85.23±9.9	-	-	-	16.28±11.1	-	-	-
SE		3.301	-	-	-	3.7	-	-	-

RC= root colonization percentage by AM fungi; A=arbuscule, V=vesicle, H=hyphae

The seedlings of the test plants were treated with AM fungi and the vesicle formation were observed in case of all seedlings, but arbuscule was detected only in onion and brinjal (Table 2; fig 2). The maximum average of colonization percentage was also recorded during the

study and the highest frequency was showed in Solanaceae family about 93.47% and minimum was found in Cucurbitaceae family about 66.7% (Fig. 1). This study first time observed the maximum AM colonization with vesicle in *S. oleracea* (Amaranthaceae).

Maximum RC was observed in brinjal followed by Onion, tomato and Spinach, Chili. Brinjal show 100% colonization with arbuscule and vesicle. But in control treatment 6.7% colonization was observed. Motha *et al.*,

(2015ab) observed the percentage of RC varied from location to location. Percentage RC was varied maximum (90%) and minimum (54%). 30.12% to 81.93% RC variation observed in Marathwada district (Sawant, 2013).

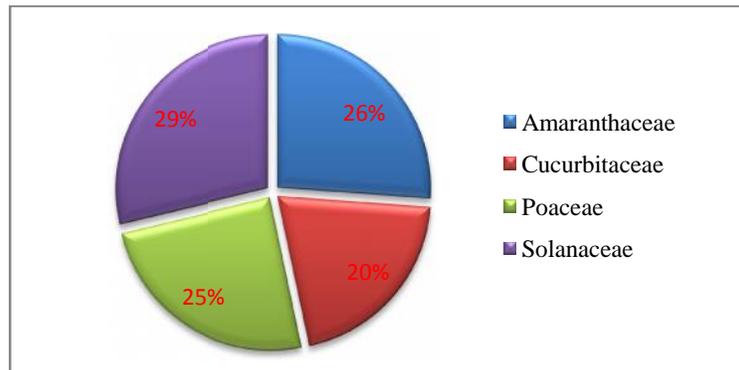


FIGURE 1: Root percent colonization in family

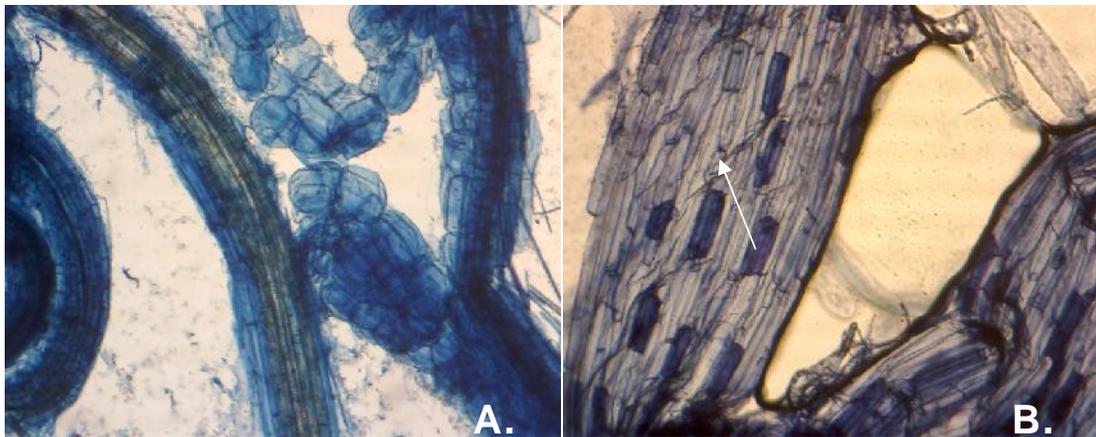


FIGURE 2: Structure of AM fungi in root: (1) vesicle; (2) arbuscule

Colonization by AM fungi in onion was observed 15% to 50% by Afek *et al.* (1990). Abdullahi and Sheriff, (2013) recorded 11.2% RC in uninoculated plants. Galvan *et al.* (2009) observed average 60% for arbuscular colonization (AC) and 84% for hyphal colonization. The presence of vesicles was much lower, namely, 7% on average. In present result vesicles, arbuscule was present with 93.3% and hypha was absent.

Tomato has 93.7% RC with vesicle and hyphae. Bhuiyan, (2013) observed 50 to 60% colonization. Similarly Copetta *et al.* (2011) observed no colonization in control and 15.3±2.1% in treated plants, with 13.4±1.5% arbuscule abundance.

Gashua *et al.* (2015) observed varied level of colonization ranging between 35 to 65%. Gurumurthy *et al.* (2014) recorded AM fungi colonized the chili plants to varying degree. Maximum observed in *Glomus macrocarpum* with 92% and 39.67% in Un-inoculated Control. Raza and Chaudhry (2017) observed colonization in *C. annuum* at different varieties and at different concentration of CuSO_4 . Muthukumar and Sathya (2017) observed 35.83% RC in *C. annuum*. But in present reading 86.7% colonization recorded with vesicle and hyphae. In control plants 33.33% colonization observed with arbuscule and vesicle.

Spinacia oleracea had no signs of mycorrhizal colonization in the roots (Toprak *et al.*, 2017; Sinigani and Yeganeh, 2017). Thus, the mycorrhizal fungi relationship of these plants needs further exploration. *S. oleracea* was not good photosymbiont for mycorrhiza symbiosis (Veiga *et al.*, 2013). But in current study *S. oleracea* show 86.7% colonization with vesicle only and it's only reported in this study.

T. aestivum show 73% RC percentage in hydroponic condition (Hawkins and George, 1997). Sharma *et al.* (2011) and Sadhana *et al.* (2016) observed varied level of colonization rang 12.05% and 92 ±1.24% respectively. Biswas *et al.* (2000) suggested that certain strains of VAM fungi can promote wheat growth and yield through mechanisms that improve single leaf net photosynthetic rate rather than biological N_2 fixation. In a field experiment in Iran, yield improvements of more than 20% have been obtained for wheat as a result of mycorrhiza inoculation (Biswas *et al.*, 2000). It is well known that wheat roots secrete carbonaceous exudates, which could help in proliferation of VAM (Biswas *et al.*, 2000). However, intense VAM infected roots even at moderate nutrient deficiency are important during early plant growth when roots are too small to provide a high demand for

minerals for shoot growth. In present investigation wheat show 80% colonization with arbuscule and hyphae.

In present investigation garlic show 80% colonization with arbuscule and hyphae, similarly percentage of RC in garlic roots was analyzed by Shinde *et al.* (2015), It was recorded minimum 63±1.83 and maximum 91±1.83% at Niphad Tehsil of Nashik District. Borde *et al.* (2009) observed after 60 days of AM inoculation it was 28.33%, after 90 days of AM inoculation it was 45%, after 120 days of AM inoculation 61.66% and after harvest 75%. AM fungal inoculation increased the level of AMF root colonization of garlic. This increase in colonization is very important for the growth and nutrient uptake by the plant and the higher number of spores which may compete with native AM spores (Borde *et al.* 2009). Moustafa *et al.* 2010 observed RC were distinctly higher in cultivated crops than wild plants. Regarding RC rate, *Allium sativum* scored 93% colonization among cultivated crops, but in wild plant no colonization observed. Similarly in present investigation 26.6% colonization was observed.

Debnath *et al.* (2015) observed 16.93 ±3.39% vesical; 40.81 ±6.52% hypha and 38.95 ±6.45% dark septet in *Alternanthera dentata*. But in this study 80% colonization was observed with vesical and hyphae. 13.3% colonization was observed in un-inoculated plants. Chandra and Kehri (2006) conducted a study at the meerut college campus, in Uttar Pradesh to determine the occurrence of mycorrhizal associations in 62 species of wild (including weeds) and cultivated plants. Of the 62 species evaluated, 54 showed mycorrhizal associations. The vesicular arbuscular mycorrhizal associations in species of *Alternanthera* spp. constitute the first reports from Meerut.

In present investigation *Cucumis sativus* show 66.7% RC, But in another experiment Bruce *et al.* (1994) and Kubota *et al.* (2004) recorded 81% association with root.

Smith and Read (1997) reported that AM fungi grow in the cortical root tissues and also grow out from the roots into the surrounding soil, forming an external hyphae network which increases uptake of mineral nutrients and consequently promotes plant growth. AM fungi have a wider host range and exert several positive effects on colonized host plants when grown in pot cultures (Chandra and Kehri, 2006). AM fungi has been reported to be involved in the improvement of growth and biomass production in a range of hosts such as monocots and dicots, shrubs and trees, medicinal plants (Giovannetti and Mosse, 1980) and several economically important crops (Varma and Verma, 2016).

This implies the ubiquitous nature of the fungi, occurring naturally in most agricultural soils (Cabello, 1999; Caimey *et al.*, 1999; FrancoRamirez *et al.*, 2007) and, the poor root colonization by the native AM Fungi compared to the applied inoculums as observed in this study could be attributed to, reduction in population of the native AMF due to different soil management practices in the research field (Gryndler *et al.*, 2005a, b; Valentine *et al.*, 2001).

CONCLUSION

AM fungi show positive plant-microbes interactions. The contribution of AM fungi in plants establishment as well as nutrition is now well understood. Whether or not we can use the mycorrhizae in our interest will depend on

how best we understand and value the equity relations of nature.

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