Journal of Plant Nutrition

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/lpla20

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To cite this article: C. Sarathambal, R. Sivaranjani, V. Srinivasan, M. Alagupalamuthirsolai, K. P. Subila & B. Anamika (2023) Effect of arbuscular mycorrhizal inoculation on growth, mineral nutrient uptake, photosynthesis and antioxidant activities of black pepper cuttings, Journal of Plant Nutrition, 46:10, 2508-2524, DOI: 10.1080/01904167.2022.2160736

To link to this article: https://doi.org/10.1080/01904167.2022.2160736

JOURNAL OF PLANT NUTRITION
2023, VOL. 46, NO. 10, 2508–2524
https://doi.org/10.1080/01904167.2022.2160736

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Effect of arbuscular mycorrhizal inoculation on growth, mineral nutrient uptake, photosynthesis and antioxidant activities of black pepper cuttings

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi are one of most important soil microorganisms that can form mycorrhizal symbiosis with most of the terrestrial plants. In the present study, the effect of arbuscular mycorrhizal fungi inoculation on root colonization, growth, nutrition, photosynthetic gas exchange and antioxidant activities of black pepper cuttings were evaluated under polyhouse conditions. The single node cuttings of black pepper were grown in the presence and absence of AM combinations for 150 days under poly house. AM inoculated plants showed significantly higher mycorrhizal root colonization (95%) and spore numbers (312/50 g of sample). The effect of AM fungi was more prominent in improving root biomass than above ground biomass. Nutrient accumulations were higher in AM inoculated plants rather than uninoculated black pepper plants. Amount of acid phosphatase and dehydrogenase activity were significantly higher in AM inoculated soils. Net photosynthetic rate and stomatal conductance of AM colonized black pepper leaves were found to be significantly greater than uninoculated plants. The influence of AM was more prominent on poly phenol oxidase and β-glucanase activity in leaves than roots. In principal component analysis the scatter plot revealed variations of the effects of arbuscular mycorrhizal fungi on growth of black pepper cuttings. Based on these results, AM inoculation at the earlier stage of plant development could improve symbiosis, and increased plant growth in the nursery which may improve the performance after planting in the field.

ARTICLE HISTORY
Received 5 May 2021
Accepted 2 October 2022

KEYWORDS
Antioxidant enzymes; colonization; photosynthetic rate; nutrient uptake; soil enzymes

1. Introduction

Black pepper (*Piper nigrum* L.) is one of the most widely used spices in the world. It is a flowering vine belongs to the Piperaceae family, cultivated for its pungent and fragrant berries which is the main economic produce. Owing to higher demand in international markets, black pepper production and area is expanding both in traditional and nontraditional areas over a decade. However, the availability of quality planting material of high yielding varieties is one of the major constraints in cultivation of black pepper. The traditional black pepper propagation systems have certain restrictions due to high mortality rates, poor survival and poor rooting of transplanted cuttings. Therefore, there is a need to improve the traditional propagation system with an input management strategy to increase the production of quality planting material. In this regard, the

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addition of suitable bio-inoculants to the potting mixture will improve the performance of planting materials. Most horticultural nurseries now prefer the mycorrhizal inoculation in potting media to increase crop uniformity, reduce transplant mortality and increase productivity. Moreover, the use of arbuscular mycorrhizal application as bioinoculant has been recommended with the aim of increasing productivity and reducing use of chemical fertilizers. The typical AM forming plants include cereals, legumes, fruit trees, timber trees, horticultural crops, plantation crops and ornamental crops. Spice crops like black pepper, ginger, turmeric and cardamom also colonize endomycorrhizae fungi in its roots. Plants growing in natural soil are often associated with a wide range of microorganisms that influence their growth and health. It is estimated that approximately 95% of all vascular plants on earth are mycorrhizal. Plants and mycorrhiza have developed this mutually beneficial relationship as a mechanism to increase the survival rate. Arbuscular mycorrhizal (AM) fungi is a most abundant soil fungi belonging to *Glomeromycota*. AM fungi can increase the growth of several crop plants by improving uptake of nutrients and water use efficiency (Zhang et al. 2016) and also the symbiotic association eases crop vulnerability to biotic and abiotic stresses. In addition, AM have been reported to stabilize the structure of soil, which also boosts microbial growth. Apart from the nutritional benefit, AM fungi helps in increasing plant growth and yield by defending the plant against various abiotic and biotic stresses. Reports have revealed that colonization of the adventitious roots of the strawberry stem cuttings by AM fungi could improve their growth and vigor (Singh, Soni, and Kalra 2013). AM can also affect soil enzyme parameters by increasing soil microbial activities. They can directly exude up to 20–30% of soil microbial carbon and transfer more fresh plant carbon to soil microbes than root exudation (Kaiser et al. 2015).

As the result of improved plant nutrition, especially phosphorus (P) nutrition in mycorrhizal plants, photosynthetic rates are often higher in mycorrhizal than in non mycorrhizal plants (Miller et al. 2002; Auge, Toler, and Saxton 2016). Apart from nutritional benefits, physiological and gene expression measurements exhibited that the complete pathway of carbon movement from shoots to roots was also improved in AM inoculated tomato plants (Boldt et al. 2011).

Production of reactive oxygen species (ROS) is the common phenomenon when plants are exposed to various biotic and abiotic stresses. ROS are extremely toxic to biological cells causing oxidative damage to DNA and proteins. The symbiotic association with AM confers the host the resistance to conquer oxidative stress. Initial stages of AM fungi colonization trigger intracellular ROS burst in host plants; however, this effect is transient and is overcome by enhanced activities of antioxidant enzymes such as catalase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, superoxide dismutase and β-1,3-glucanase. These antioxidant and hydrolytic enzymes are helpful to alleviate the deleterious effects of various stress to plants (Garg and Chandel 2015). However, the influence and the underlying mechanism of AM fungi to improve the growth and vigor of black pepper cuttings remains unclear.

With this background, the purposes of the present study were planned as follows: (a) to assess the effects of AM fungi on the growth and physiological measurements of black pepper cuttings. (b) to evaluate the influence of AM fungi on the nutrient uptake and soil enzyme properties of black pepper cuttings and (c) to study the influence of AM fungi on antioxidant enzymes from leaves and roots of black pepper cuttings under polyhouse conditions.

2. Materials and methods

2.1. Study site and plant material

The experiment was conducted at the poly house of the Division of Crop protection (11.29395°N, 75.82038°E), ICAR-Indian Institute of Spices Research, Kozhikode, India. Healthy single node black pepper (*cv. IISR Sreekara)* cuttings were used in the study with three leaves stage and even

thickness. Unsterilized potting mixture was used in this experiment to evaluate the AM inoculation effects under natural conditions. It consisted soil: sand: farm yard manure (1:1:1) and the soil used in the study was a red lateritic clay loam and collected from farm of the Indian Institute of Spices Research, Kozhikode. The initial nutrient composition of potting mixture was analyzed as per the standard procedures and the results were pH 6.23, organic carbon 2.1%, nitrogen 245 kg ha^{-1}, phosphorus 351 kg ha^{-1}, potassium 375 kg ha^{-1}, calcium 981 mg kg^{-1}, magnesium 375 mg kg^{-1}, iron 32 mg kg^{-1}, manganese 21.6 mg kg^{-1}, zinc 1.9 mg kg^{-1} and copper 1.2 mg kg^{-1}.

2.2. AM inoculum

The AM fungi (*Rhizophagus irregularis*) inoculum were maintained at repository of beneficial microorganisms at ICAR-IISR, Kozhikode was used in the study. The inoculum was prepared with vermiculite as the carrier, contained 100 propagules of per gram of the inoculum in the form of spores, hyphae, and mycorrhizal roots. The 2 g of inoculums were mixed with potting mixture as per the treatments. The black pepper rooted cuttings were planted in the AM inoculated and AM uninoculated mixtures and observed for monthly interval up to 5 months (0, 30, 60, 90, 120 and 150 days after inoculation) for improvement in growth measurements and biomass production.

2.3. Plant analysis

During early stage of plant development, AM symbiosis, can improve the growth and development of plant. Since, to study the initial responses of AM inoculation on black pepper, a batch of inoculated and uninoculated plants was used for analysis in monthly interval up to 5 months. The whole plants were separated and analyzed for biometric parameters like shoot length, root length, number of leaves and biomass (Newman 1966). Per cent of AM colonization was estimated by microscopically examination at 10X magnification, after clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to the technique described in Phillips and Hayman (1970). The mycorrhizal colonization was assessed by using following formula:

$$\text{Per cent of mycorrhizal colonization} = \frac{\text{Number of root sections colonized}}{\text{Total number of root sections observed}} \text{ x } 100$$

Extraction of AM spores were done by wet sieving and decanting process with following steps. In beaker 50 g of soil sample was taken and mixed in 500 mL water. After 1 h the contents of the beaker were decanted through the sieves which were arranged in a descending order from 600 μm to 37 μm size. The sieving was collected in a Petri plate and count the spore/sporocarps under stereo zoom microscope.

"The degree of plant growth change associated with arbuscular mycorrhizal (AM) colonization is expressed as mycorrhizal dependency (MD)" and was measured based in the method of Plenchette, Fortin, and Furlan (1983)

$$\}\text{MD } (\%) = \frac{\text{dry weight of mycorrhiza inoculated plant} - \text{dry weight of mycorrhiza un inoculated plant}}{\text{dry weight of mycorrhiza inoculated plant}} \text{ x } 100\}$$

For the nutrient uptake studies, plants were oven-dried at 60 °C and powdered using mixer grinder. The nitrogen (N) uptake was assessed using the Kjeldahl method (Nelson and Sommers 1973). For the estimation of the P, one-gram powdered plant sample were digested using a mixture of nitric acid (HNO_3) and hydrochloric acid (HCl 60%) with a ratio of 9:4 (v: v) and assessed using spectrophotometer at 660 nm (Jackson 1973). Exchangeable K, Ca and Mg were estimated using an atomic absorption spectrophotometer (Varian AA 240FS) (Thomas 1982). Fe,

Mn, Zn and Cu were analyzed by diethylene triamine penta acetic acid (DTPA) extraction method (Lindsay and Norvell 1978) and estimated by using atomic absorption spectrophotometer (Varian, AA 240FS).

2.4. Soil enzyme activities

Acid and alkaline phosphatase (E.C.3.1.3.2) activity was determined by incubating soils at 37 °C for 1 h in modified universal buffer (pH 6.5 or pH 11) with *p*-nitrophenyl phosphate as the substrate (Tabatabai and Bremner 1969). *β*-glucosidase (E.C. 3.2.1.21) activity was determined as above, but by using p-nitrophenyl *β*-d-glucopyranoside as the substrate (Eivazi and Tabatabai 1988). The amount of *p*-nitrophenol released was estimated spectrophotometrically.

Dehydrogenase [DHA, Enzyme Commission (EC) number 1.1.1.] activity was estimated by the addition of 3% aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) to 18 g soil followed by incubation at 37 °C for 24 h. The triphenyl formazan (TPF) was then extracted with methanol and color intensity was determined at 485 nm (Casida, Klein, and Thomas 1964). Urease (E.C.3.5.1.5) was analyzed by incubating soil with an aqueous urea solution (for 2 h at 37 °C), extracting NH_4 with $AgSO_4$-KCl mixture (2.5 M), and NH_4 determined by distillation (Mulvaney, et al. 1996).

2.5. Gas exchange measurements

During the early stage of AM fungi symbiosis, photosynthetic machineries were triggered and since the observations on physiological parameters were made from AM colonized and uncolonized plants at weekly interval in index leaf (4th leaf from the top, i.e., 3rd or 4th leaves are physiologically more active leaf in black pepper). Photosynthetic gas exchange parameters were recorded using a portable photosynthetic system (LCpro-SD Advanced Photosynthesis Measurement System, England). The parameters analyzed were net photosynthetic rate (Pn, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) under control saturating photosynthetic photon flux (900 µmol $m^{-2}s^{-1}$). During the experiment period, data were recorded between 9:30 and 11:30 am (i.e., During diurnal changes in photosynthesis black pepper showed higher photosynthesis between 9.30 to 11.30 am). The observations were taken when both Pn and gs were stable. Same leaf was used for chlorophyll (a) fluorescence measurements right after gas exchange measurements. The maximum PS II quantum yield was expressed in terms of chlorophyll fluorescence (F_v/F_m) using a chlorophyll fluorometer (Os-30p) in 10–15 min' dark adapted leaves between 9:30 and 11:30 am local time according to Strasser, Srivastava, and Govindjee (1995).

2.6. Antioxidant activities

In general, antioxidant enzymes will be stimulated, during the early stages of AM fungi symbiosis. Since the Leaves and root samples were collected at weekly interval from control and treated plants and used for enzymatic analysis. Plant parts were cleaned of soil and dirt and were homogenized in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 12,000 g for 10 minutes. The supernatants were used to determine the peroxidise activity. The guaiacol peroxidase (POD) activity was measured as oxidation of guaiacol in the presence of hydrogen peroxide at 470 nm according to the method by Putter (1974). The activity was expressed as the amount of enzyme required to increase the absorbance min^{-1} mg $protein^{-1}$.

In polyphenol oxidase (PPO) assay, samples were extracted using Tris HCl pH 7.2, 0.4 M sorbitol and 10 mM NaCl and centrifuged and the resultant supernatant was used for the PPO activity. The reaction mixture contained 2.7 mL of substrate solution consists of 20 mL of 0.01 M phosphate buffer (pH 6.5) and 5 mL of 0.5 M catechol. The response was started by the addition

of 300 µl of the enzyme extract. The increase in the absorbance was measured at 420 nm and specific activity was calculated as the amount of enzyme required to increase the absorbance to 0.01 min^{-1} mg $protein^{-1}$ (Halpin and Lee 1987).

The homogenate prepared in potassium acetate buffer (pH 5) was used for measuring β-1,3-glucanase activity. The reaction mixture consisted of 100 µl sample, 900 µl buffer (0.05 M potassium acetate, pH 5), and 1 mL of 2% laminarin as substrate. The reaction mixture was incubated at 40 °C for 1 h. After incubation, the amount of glucose releases was measured (Gupta, Ravi, and Sharma 2013). The β-1,3-glucanase activity was calculated as the function of glucose release using the standard curve of glucose.

2.7. Statistical analysis

All the treatment combinations were replicated three times. Data was subjected to complete randomized design with 2 factors (Factor 1 is AM inoculation with 2 levels and Factor 2 is duration of inoculation with 6 levels as mentioned the section 2.2) with $p < 0.05\%$ level of significance using analysis of variance (ANOVA) with SAS software (SAS Institute Inc 2011). The Principal Component Analysis (PCA) was done with IBM SPSS 20 with the correlation technique and Varimax with Kaiser Normalization as the rotation technique (IBM Corp 2011). PCA was used to evaluate the twenty-two variables such as mycorrhizal root colonization, growth, uptake of nutrient, photosynthetic rate and gas exchange of black pepper cuttings, dry mass production over different day's interval.

3. Results

3.1. Arbuscular mycorrhizal colonization and growth of black pepper

The percentage of root colonization was significantly higher in AM inoculated roots (95%) compared with non AM inoculated roots (19%). (Figure 1A). Significant number of spore load (164.2 spores 50 g^{-1} substrate) were also observed in AM inoculated treatment (Figure 1B). Mycorrhizal dependency (%) used to express the degree of growth transformation related with arbuscular mycorrhizal colonization and it was recorded between 20 and 24% of inoculated black pepper (Figure 1C).

In the present study, black pepper cuttings inoculated with AM had significantly higher root length and dry biomass when compared to uninoculated plants (Table 1). The effect of AM on biomass was more prominent in root biomass than aerial biomass. The dry biomass was significantly higher in inoculated cuttings compared to non-inoculated control at 0, 30, 60 and 90, 120 and 150 days after AM inoculation, which might probably increase uptake of nutrients. The mean shoot length of inoculated black pepper (124.6 cm) was significantly higher than the control (117.4 cm). However, shoot length and number of leaves were not significantly improved by AM treatment.

3.2. AMF on the nutrient uptake and soil enzymes

Nutrient uptake was positively correlated by the mycorrhizal treatments in black pepper plants. On 120 days after inoculation of AM, nitrogen uptake was observed to be significantly high in AM inoculated plants (34.72 g dry wt $^{-1}$) when compared to uninoculated (25.2 g dry wt of the $plant^{-1}$) (Figure 2A).

As expected P uptake was significantly increased by AM inoculated plants from 90 days after inoculation onwards (3.19 g dry wt^{-1}) (Figure 2B). Apart from N and P, AM fungi able to increase the potassium (30.4 g dry wt^{-1}), calcium (31.0 g dry wt^{-1}), magnesium (10 g dry wt $^{-1}$),

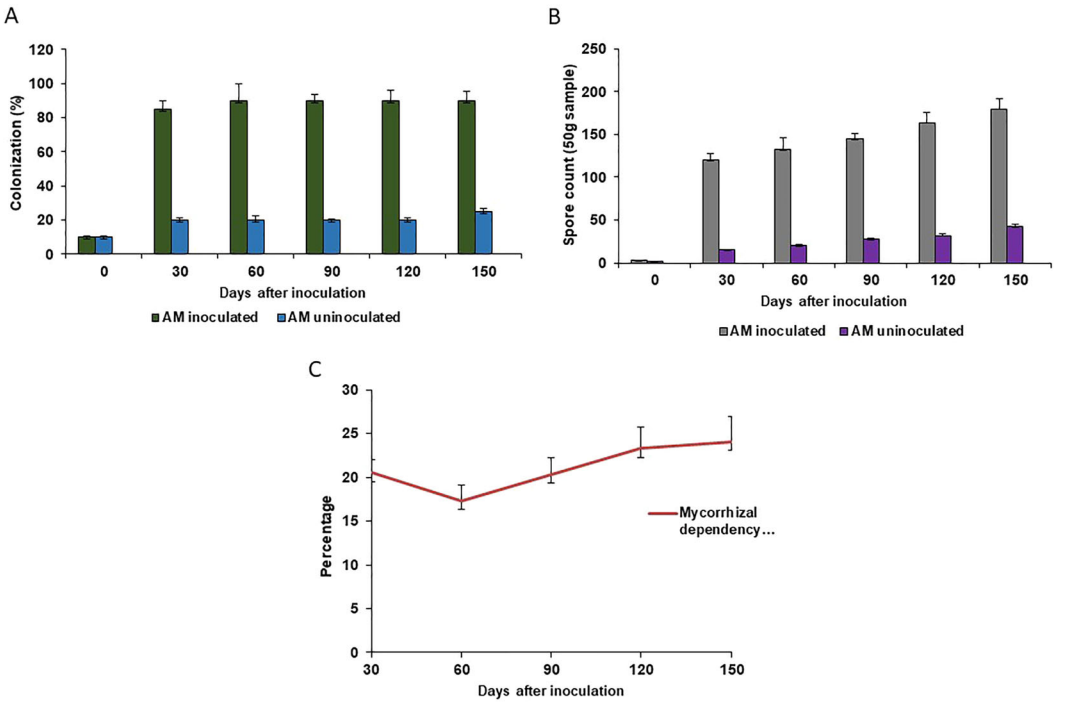


Figure 1. Effect of AM inoculation on (A) colonization (B) spore count and (C) mycorrhizal dependency (%) of black pepper cuttings. Values are means ± 1 SE ($n = 3$).

Table 1. Influence of AMF on the growth and colonization of black pepper cuttings.

Treatment (A)	DAI (B)	Shoot length (cm)	Root length(cm)	No of leaves	Dry biomass(g)
+AM	0	15	28	3	5.3
	30	50	30	8	7.8
	60	85	38	12	9.2
	90	155	41	15	10.3
	120	208	43	34	12.4
	150	235	32	39	14.3
	Mean	124.6	35.3	18.5	9.88
-AM	0	16	11	4	5.7
	30	58.3	23	8	7.2
	60	71.3	26.6	13	8.6
	90	150	27	16	9.2
	120	190	30	30	10.5
	150	219	34	34	10.6
	Mean	117.4	25.6	17.5	8.66
$^*p < 0.05$	Treatment (A)	NS	2.60	NS	0.75
	DAI (B)	17.42	4.50	2.68	1.31
	A*B	24.64	6.37	NS	NS

DAI-Days after inoculation, NS-Non significant.

zinc (0.47 mg dry wt^{-1}) and iron (6.3 mg dry wt^{-1}) uptake (Figure 2C–H) under inoculated treatments on 150 days after inoculation. In case of manganese (1.05 mg dry wt^{-1}) (Figure 2G) uptake was significantly increased on 120 days after inoculation of AM. In turn, copper uptake was increased significantly on 60 and 90 days after inoculation and the uptake was considerably reduced 120 days onwards (Figure 2I).

The highest acid phosphatase (Figure 3A) and dehydrogenase activity (Figure 3D) were observed at 120 days and 150 days after inoculation in AM inoculated soil samples. In turn alkaline phosphatases (Figure 3B), β-glucosidase (Figure 3C) and urease (Figure 3E) activities were not significantly enhanced in AM uninoculated soil samples.

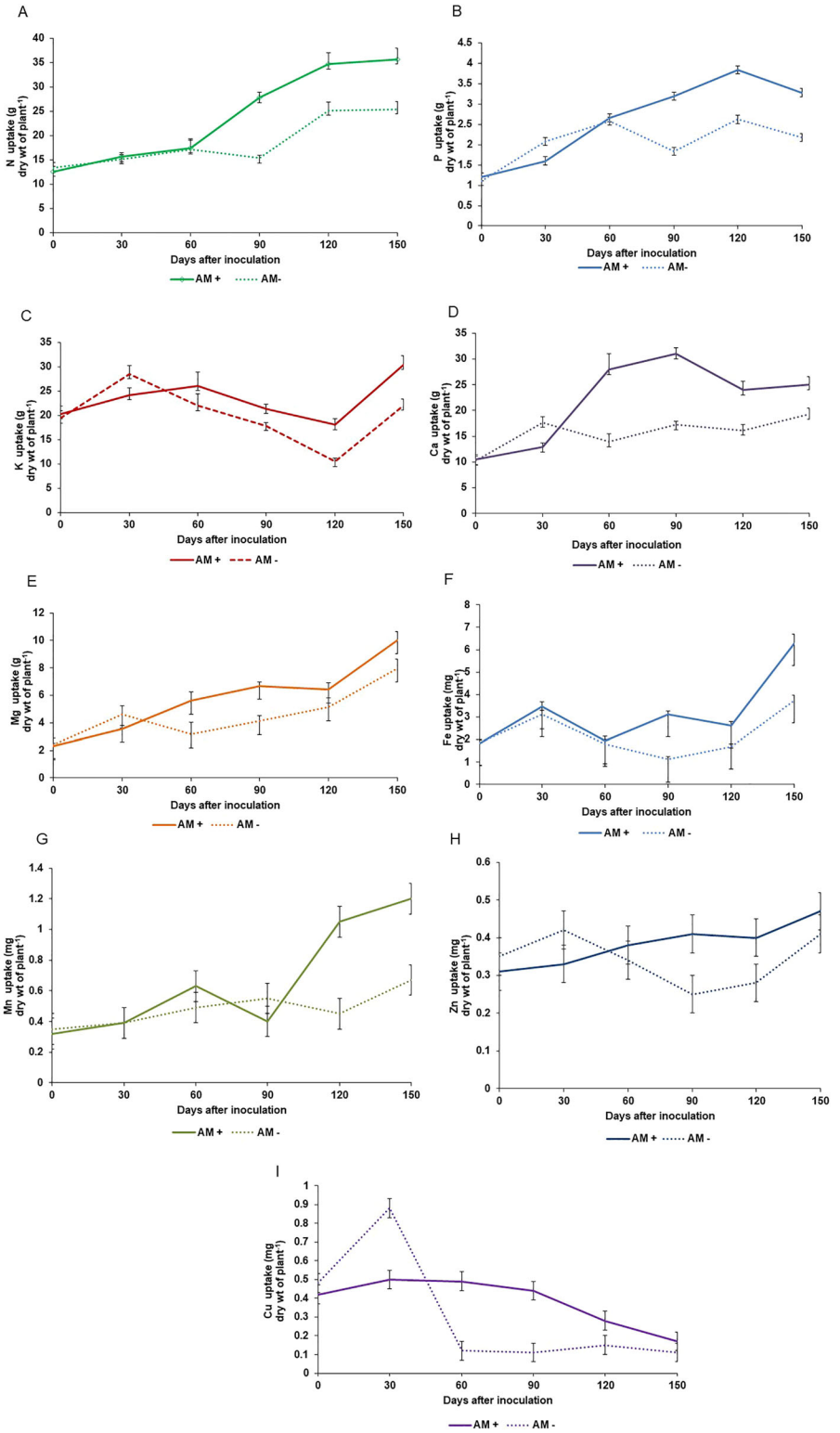


Figure 2. Effect of AM on uptake of nitrogen (A), phosphorus (B), potassium (C), calcium (D), magnesium (E), iron (F), manganese (G), zinc (H) and copper (I) in black pepper cuttings. Values are means ± 1 SE ($n = 3$).

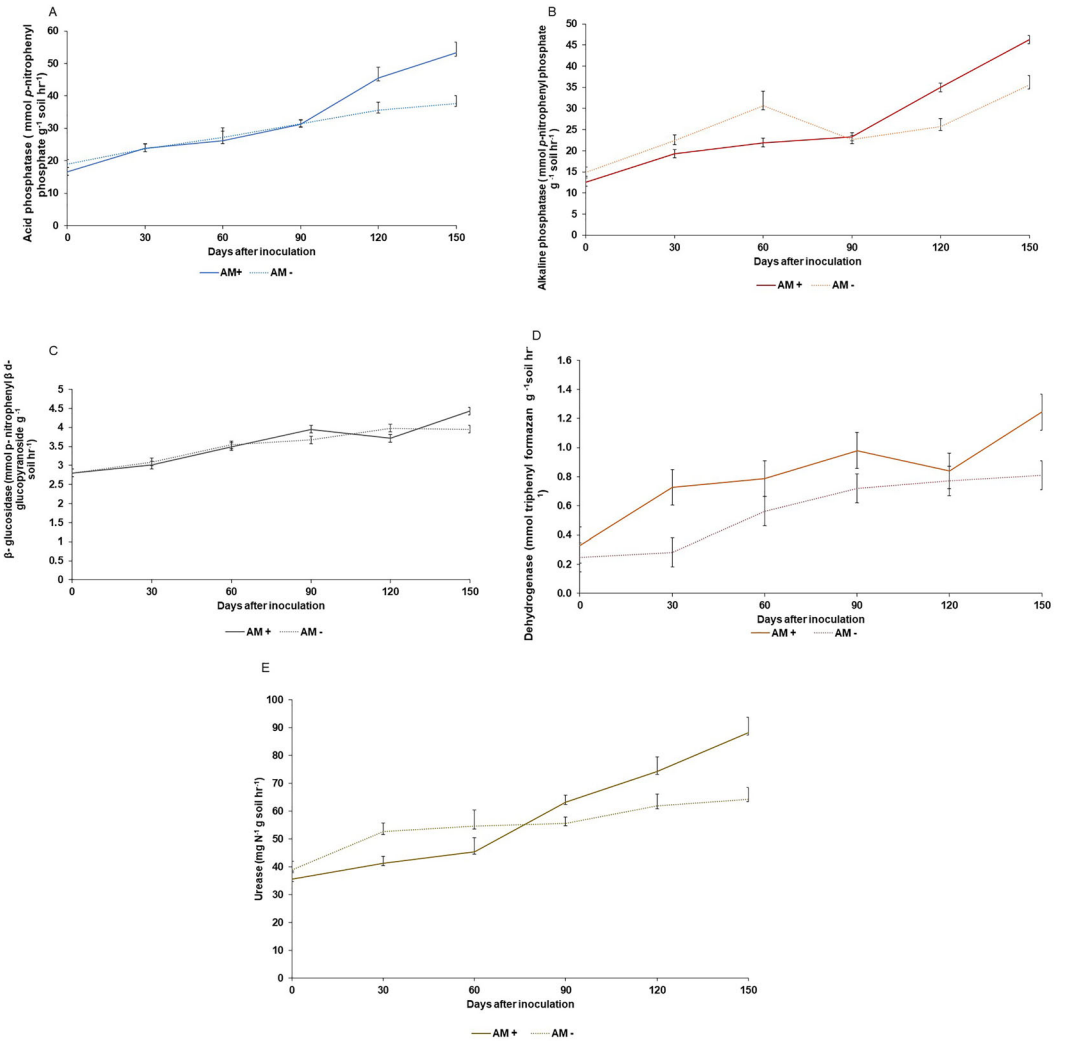


Figure 3. Effect of AM on activities of A) acid phosphatase, B) alkaline phosphatase, C) β-glucosidase D) dehydrogenase and E) urease in rhizosphere soils of black pepper. Values are means ± 1 SE ($n = 3$).

3.3. Physiological parameters

Net photosynthetic rate (Pn) and stomatal conductance (g_s) varied significantly in AM plants over the uninoculated plants at 4th week after treatment. Whereas they were not significant till 3rd week after inoculation of AM (Table 2). At 4th and 5th week Pn and gs value were higher (6.76 and 6.32; 0.136 and 0.163 respectively) than in AM uninoculated plants. Till 3rd week after AM inoculation difference in Pn and gs between inoculated and non-inoculated plants were not significantly different. The results showed that AM fungi increased the leaf gas exchanges indicating role of AM fungi by increasing the stomatal conductance in leaves.

3.4. AMF on antioxidant activity

AM inoculation has shown significant positive influence in the stimulation of POD activity during initial stages of colonization. In case of leaves, the AM inoculated samples showed significantly higher POD activities on second week after inoculation (Figure 4b). On the contrary, root

Table 2. Influence of AMF on gas exchange measurements.

Treatment	WAI	Pn (μmol CO_2 m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	Chlorophyll fluorescence (F_v/F_m)
+AM	0	4.092	0.048	0.692
	1	3.643	0.065	0.708
	2	3.957	0.075	0.726
	3	5.326	0.122	0.691
	4	4.847	0.093	0.675
	5	5.267	0.119	0.719
	Mean	4.52	0.087	0.701
-AM	0	3.784	0.047	0.691
	1	4.096	0.075	0.712
	2	3.442	0.067	0.718
	3	5.769	0.094	0.710
	4	6.764	0.137	0.667
	5	6.334	0.146	0.702
	Mean	5.03	0.094	0.700
$^*p < 0.05$	Treatment (A)	0.350	0.008	0.013
	WAI(B)	0.606	0.013	0.023
	A*B	0.857	0.019	NS

WAI – Weeks after inoculation, Pn – Photosynthetic rate, gs-stomatal conductance, NS-Non significant

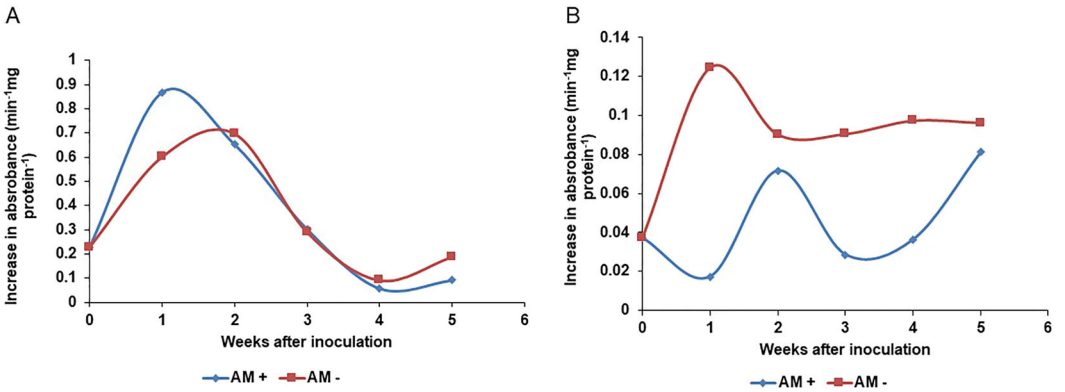


Figure 4. Activity of peroxidase (POD) in root (a) and leaves (b) of AM inoculated and uninoculated black pepper cuttings. Values are means ± 1 SE ($n = 3$).

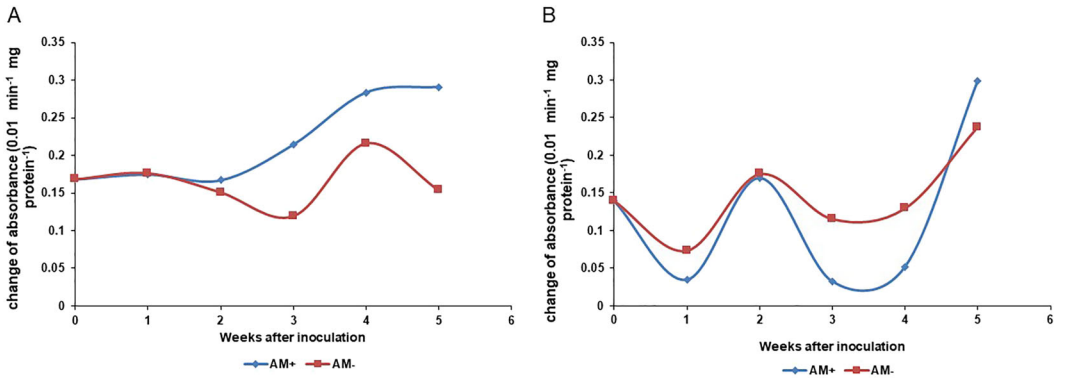


Figure 5. Activity of polyphenol oxidase (PPO) in root (a) and leaves (b) of AM inoculated and uninoculated black pepper cuttings. Values are means ± 1 SE ($n = 3$).

samples have not shown any significant variation between AM inoculated and un-inoculated samples for POD activity (Figure 4a). In our study, significant stimulation of PPO activity in AM colonized roots were noticed when compared to non-AM roots (Figure 5a). The maximum PPO

activity was found in 5th week after inoculation in pepper roots. The leaf sample did not show any AM induced influence in PPO activity (Figure 5b). In case of β-1,3-glucanase activity among the treatments, significant difference could not be observed in roots during early phase. But, moderately significant increase in the activity was observed in root samples at 5th week after inoculation (Figure 6a). However, in leaves pronounced increase in its activity was observed in 2nd, 3rd and 4th week after inoculation while comparing inoculated and uninoculated samples (Figure 6b).

To reveal similarities and differences between sample plots and the relationships between different variables, Principal Component Analysis (PCA) was performed on all data.

In a PCA model, the objects (samples) are represented by their scores and the variables are represented by their loadings. The scores and loadings can be presented in a graph where three components are plotted against each other. In a score plot, similar samples will be positioned close to each other and in loading plots, positively correlated variables will be positioned close to each other and negatively correlated will be opposite to each other. Samples that are high in a specific variable will be pulled toward the area of the score plot where the variable in the corresponding loading plot is located.

The scatter plot showed variations of the principal component analysis of the effects of arbuscular mycorrhizal fungi on growth, nutrition, and biomass of black pepper seedlings (Figure 7). The PC 1 explained 73% of the total variations and PC 2, PC 3 accounted for 13.7% and 8.31% respectively. Among the variables shoot length, number of leaves, N uptake, Mg uptake, Fe uptake, Mn uptake, Zn uptake as well as soil enzymes were positively ordinated with significant contribution to the PCs. In PC 2, the variable such as Cu uptake, Ca uptake and root length also significantly contributed. However, the variables like K uptake showed minor contribution to PC loadings.

4. Discussion

The considerable variation in root colonization of black pepper between control and AM inoculated treatments is an evidence of the ability of the inoculum used in this study to colonize with beneficial influence on host. Structural colonization of AM was recorded in black pepper roots from the inoculated samples (Figure 8). On the other hand, uninoculated samples had very few number of spore counts mainly due to the presence of autochthonous group of AM flora which does not contribute much on enhancement of growth and nutrient uptake. The degree of

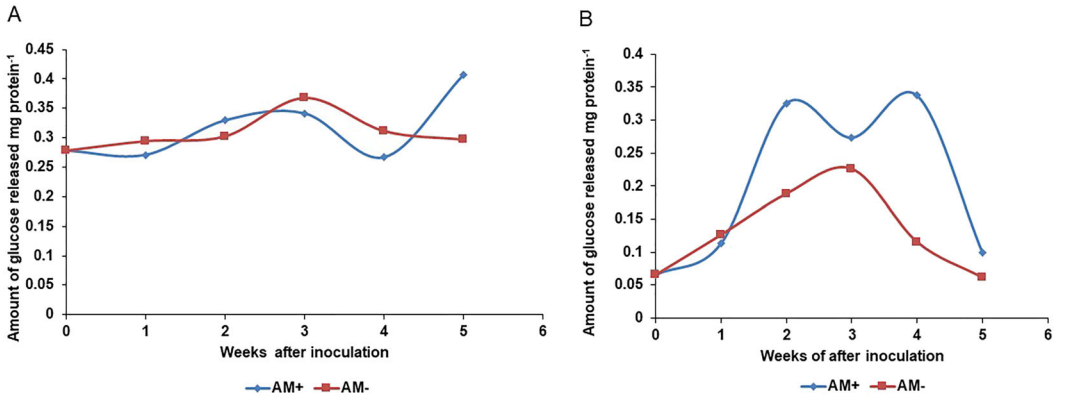
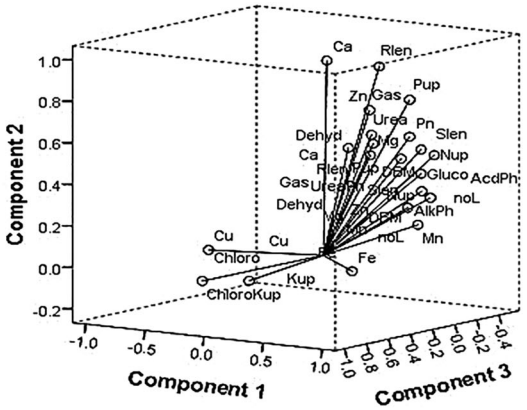


Figure 6. Activity of β gluconase in root (a) and leaves (b) of AM inoculated and uninoculated black pepper cuttings. Values are means ± 1 SE ($n = 3$).



Component vector labels are as follows- Slen: Shoot length, Rlen: Root length, noL: No of leaves, N up: N uptake, P up: P uptake, K up: K uptake,Ca: Ca uptake,Mg: Mg uptake,Fe: Fe uptake,Mn: Mn uptake, Zn: Zn uptake,Cu: Cu uptake, Acid Ph: Acid phosphatase, Alkaline Ph: Alkaline phosphatase, Gluco: Glucosidase, Urea: Urease, Dehy: Dehydrogenase, Pn: net photosynthetic rate, gs :stomatal conductance, Chlor: Chlorophyll fluorescence, DBM: Dry biomass

Figure 7. Principal component analysis on interactions of AMF on the growth characters, soil nutrition and biomass of black pepper.

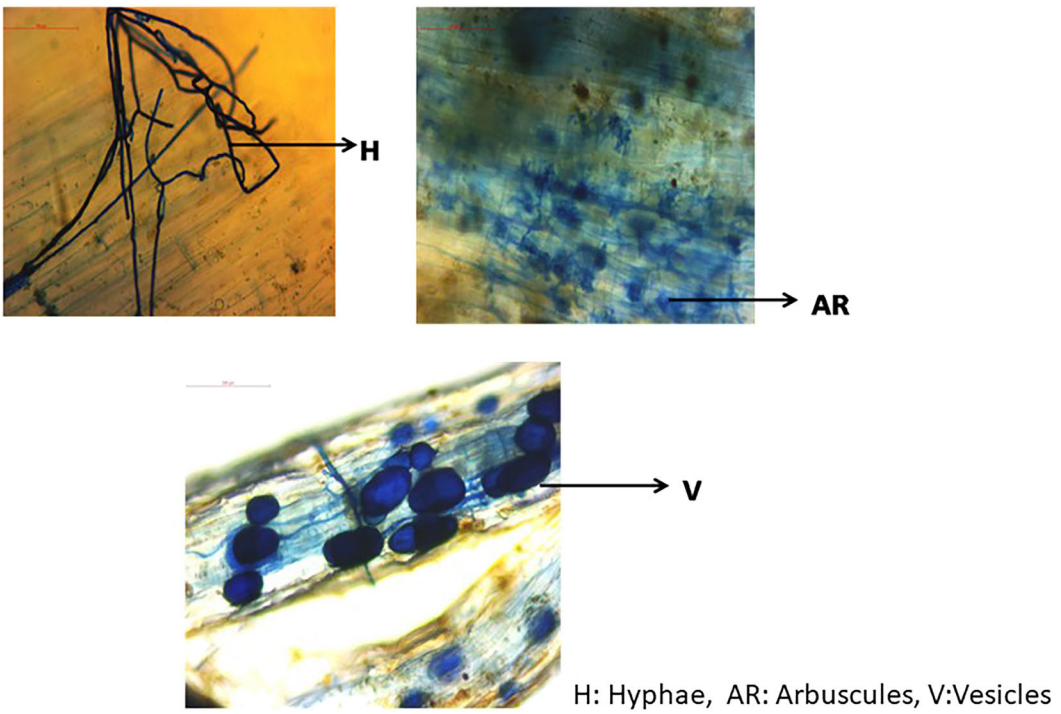


Figure 8. Microphotographs of structural colonization of arbuscular mycorrhizae in black pepper roots.

mycorrhizal dependence is an inherent trait of a given crop species and is very essential for it response to inoculation (Urcoviche, Castelli, and Gimenes 2014).

AM inoculation effect was assessed up to 5 months of growth of black pepper cuttings in nursery. The nutrient uptake may be the main cause of growth reaction found in AM inoculated plants. AM could enhance their growth and vigor of the stem cuttings through colonization of

their adventitious roots. Compared to control, the root length and dry biomass was greater by 37.8% and 13.9% respectively. However, Kandiannan et al. (2000) observed significant increase in shoot length, number leaves of black pepper cuttings over the uninoculated control. AM inoculation at initial stage of plant development can enhance symbiosis, which leads to improve in growth of plant in the nursery and improving performance after planting in the field (Singh, Soni, and Kalra 2013).

Enhanced nutrient uptake is due to inoculation of AM and the symbiotic relationship for nutrient exchanges is established between mycorrhizal fungi and plants, where plants benefit from fungi. AM symbiosis mainly regulating nutrient cycling and play a vital role in the change of sustainable agriculture (Wang et al. 2017). Nitrogen from soil taken up by the AM hyphae outside the roots is assimilated into amino acids, translocated to the intraradical mycelium as arginine, but transferred to the plant exclusive of carbon (Govindarajulu et al. 2005; Larimer, Clay, and Bever 2014). Mycorrhizal mutualism are vital for the growth of several plant species and are responsible for up to 80% of nitrogen (N) and phosphorus (P) uptake by plants (van der Heijden et al. 2015; Suri et al. 2011).

Up-regulation of a plant K + transporter in arbuscular mycorrhizal roots was reported in *Lotus japonicus* (Guether et al. 2009; Berruti et al. 2015). In tomato AM Fungi *Rhizophagus irregularis* inoculation improved the uptake of various nutrients including K, Mg and Ca (Cimen et al. 2010). The role of mycorrhizal inoculation on uptake of Zn for several crop species has been demonstrated by several workers (Marschner 1998; Ortas and Akpinar 2006; Cavagnaro 2008). Since Indian soils are Zn deficient, mycorrhizal association is a very important tool for managing this problem. However, Kothari, Marschner, and Romheld (1991) stated the reduced uptake of Mn in AM inoculated plants. The availability of Mn and Fe in soil is subject to oxidation-reduction potential and pH of soil. The reduced forms of these elements are further available to plants. AM were reported to decrease the number of Mn-reducing bacteria thereby indirectly reducing oxidation-reduction potential and availability of Fe and Mn in the rhizosphere (Posta, Marschner, and Romheld 1994). This possibly may be attributed to absorption and mobilization of nutrients, which in turn raised nutrient uptake of respective nutrients.

Soil enzymes are known to play significant roles in improving soil health and its environment. AM fungi developing a hyphal network that reaches into the level finest soil pores producing different enzymes such as phosphatases, β-glucosidase, urease and dehydrogenase (Figure 4) which improve the availability of nutrients and hence their following uptake by the host crop (Miransari 2003). AM fungi mobilize micronutrients by affecting the root morphology and physiology of the host plant and also producing soil enzymes. Mycorrhizal fungi might have extracellular enzymes, in that way subsequent in release of phosphatases and dehydrogenase and could induce variations in the quantity and composition root exudates (Xiao et al. 2009). Nevertheless, pertinent studies report significantly variable results for the influence of AM on soil enzyme activities (Wu, Zou, and He 2011; Xie et al. 2014; Ye et al. 2015). Based on the trade balance model, the energy reachable to mycorrhizal fungi depends on the assistances that the host plant obtains from the symbiosis (Koide 2010).

With respect to photosynthetic rate our data agree with Ibrahim et al. (1990). The AM inoculated plants attributed to enhanced inorganic nutrient absorption (Cooper 1984) and greater rates of photosynthesis (Allen et al. 1981). Chlorophyll is vital for photosynthesis, which enables plants to get energy from light (Zai et al. 2012). During photosynthesis both the growth of the AM fungi and the plant photosynthetic rate improve due to the mutual Carbon –Phosphorus relationship between host plants and AM fungi (Kiers et al. 2011; Jiang et al. 2017). The efficiency of Photosystem II (F_v/F_m) did not differ significantly in AM inoculated plants throughout the observations under well-watered condition.

The improvement in leaf gas processes might be related to the increase in nutrient and water uptake caused by AM fungi (Zhu, Song, and Xu 2010). The increase of photosynthetic rate might

be related to the increase in phosphorus (P) and magnesium (Mg) uptakes caused by AM fungi (Zhu et al. 2014). A high magnesium (Mg) uptake indicated that AM fungi increased the synthesis of chlorophyll and leaf photosynthesis, which is consistent in the study. In addition, El-Mesbahi et al. (2012) reported that enhanced potassium absorption in mycorrhizal plants could be an instrumental for water transport by the hyphal network, because the supply of extra K always improved hydraulic conductivity of roots in mycorrhizal plants.

During the early stage of AM fungi symbiosis development, stimulation of antioxidant enzymes was noticed by several workers (Blilou, Ocampo, and Garcia-Garrido 2000; Hajiboland and Joudmand 2009). The stimulation of ROS-scavenging enzymes, such as POD, polyphenol oxidase, catalase and superoxide dismutase is the key mechanism to scavenge detrimental ROS during stress conditions (Evelin and Kapoor 2014). The activation of two main hydrolytic enzymes, i.e., β-1,3-glucanase and chitinase is associated with biotic stress tolerance which enhance fungal resistance in crop plants (Kirubakaran and Sakthivel 2007). The result of their meta-analysis proved that AM fungi colonization tended to increase production of antioxidant enzymes by about 16%. The polyphenol oxidase (PPO) catalyzes the oxidation of phenolic compounds present in the plants converting them into quinones which is toxic to plant pathogens. There by PPOs play an important role in developing plant immunity.

Peroxidase (POD) was maximum constantly connected with positive priming by AM than other antioxidant enzymes (Lokhandwala and Houseman 2019). Priming of plant responses to stress occurs when plants change their production of these antioxidant enzymes upon AM fungi colonization (Bora and Lokhandwala 2016; Younesi, Moradi, and Namdari 2013). Thus, antioxidant enzymes are important indicators of efficiency of mycorrhiza-induced priming (Pozo et al. 1999). Several studies have confirmed that AM symbiosis can also serve to defend the host plants against oxidative damage during abiotic and biotic stress conditions (Garg and Manchanda 2009; Wu et al. 2010; Borde, Dudhane, and Jite 2011; Estrada et al. 2013). Several reports were already mentioned the increase in PPO activity in AM inoculated plants (Tang, Chen, and Shang 2000; Nelson and Achar 2001; Panwar and Vyas 2002). There is also a view that alteration in the pattern of antioxidative enzymes such as PPO in mycorrhizal roots may indicate that oxidative components are produced during initial phase of colonization (Blilou, Ocampo, and Garcia-Garrido 2000).

The increase in β-glucanase could be due to their function as antioxidant activity for any oxygen molecules developed during initial process of colonization. Build-up of these enzymes due to their localized regulation of the protection mechanism. Earlier studies done by several authors also point out the increase in β-1,3-glucanse activity in response AM inoculation is the innate adaptive mechanism to fight against plant pathogens (Pozo et al. 1999; Dumas-Gaudot et al. 1996; Vierheilig et al. 1994)

Based on the results we found that the percentage of root colonized by AM fungi was positively related to performance of black pepper cuttings. AM inoculation had synergistic effects on the growth and nutrient uptake of black pepper cuttings. Mycorrhizae inoculated plant had increased leaf photosynthetic rate and stomatal conductance by facilitating the uptake of mineral nutrients, and also improve the activity of antioxidant enzymes. However, there were no significant differences of peroxidase activity in roots was noticed between AM inoculated and uninoculated plants. Whereas higher activity of polyphenol oxidase in roots and β-1-3, glucanase activity in leaves was observed in AM inoculated black pepper samples. Generally, it is concluded that inoculation of mycorrhizal fungi in black pepper cuttings in the nursery stage was the most effective and eco-friendly treatment to produce more quality planting materials in future and also the effects on yield and quality in field condition may be tested in future experiments.

Acknowledgments

Authors wish to thank Ms. PK Chandravally and O Shajina for their technical support.

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