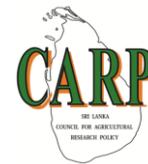


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## Research Paper

# Spore Abundance and Morphological Root Modifications of Arbuscular mycorrhizal Fungi-infected Black Pepper (*Piper nigrum* L) Plants in Reddish Brown Latesolic Soil of Matale in Sri Lanka

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**Abstract:** Arbuscular Mycorrhizae (AM) is a symbiotic association between fungi and roots of higher plants, which enhances plant growth and production. Black pepper (*Piper nigrum* L: Family Piperaceae), the second most valuable spice crop in Sri Lanka, is also identified as a host crop of mycorrhizae. However, existing indigenous AM species, their

spore abundance and root colonization is not yet well studied or documented. Therefore, the present study was conducted to collect data on AM spore density and morphological features of modified roots due to infection of existing AM species in black pepper fields. Rhizosphere soils of randomly selected, 20 well-grown black pepper plants in RBL soils of Matale in Sri Lanka were evaluated for pH, moisture and major nutrients while AM spore abundance and AM colonization in stained roots were examined microscopically. The soil pH (4.7-5.9), total N (0.18-0.23 %), and organic C (1.57-1.75%) levels in study sites were preferable for biological activities while variability was observed in available P (4.4-29 ppm), exchangeable K (188-788 ppm) and volumetric moisture (9.8-24%). The AM spore (size >50  $\mu$ m) density in examined soils were high and ranged from 1600-9000 spores  $\text{kg}^{-1}$  soil. Both live (slight yellowish to dark brown in colour) and dead (black) spores were present and globular or spherical in shape without any special hyphal attachments. About 95% of examined roots were infected with AM with modifications such as arbuscules or vesicles or both. Networks of non-septate, extra-radical hyphae and branched intra-radical hyphae could be seen. Arbuscules observed were in different maturity and highly branched. Both inter or intracellular vesicles found were oval or ellipsoid in shape. Thus, most of the mature black pepper plants in RBL soils of Matale were found infected with indigenous vesicular arbuscular mycorrhizae (VAM) and morphological features of their spores, hyphae, arbuscules and vesicles were similar to fungi of order Glomerales, which consisted with *Glomus* spp

**Keywords:** Black pepper, Root modifications, Spore abundance, Vesicular Arbuscular Mycorrhizae



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## Introduction

Mycorrhizae or fungi-root is a mutually beneficial symbiotic association between fungi in the Phylum Glomeromycota and roots of higher plants. The plant provides sugars to fungi and the fungi provide

nutrients such as P, Zn, N, Cu, etc., while protecting plants from drought stress, toxic effects of heavy metals and infections of some soil borne pathogens and nematodes, resulting in an increased plant

growth. In addition, this association enhances the formation of stable soil aggregates that improves the stability of soil structure restricting soil erosion (Singh *et al.*, 2010). The majority of economically important plants, about 95% of the world's present species of vascular plants or more than 80% of land families except Brassicaceae, Chenopodiaceae, Cyperaceae, Juncaceae, Urticaceae and Caryophyllaceae are hosts of arbuscular mycorrhizae (AM; Newman and Reddell, 1987).

Black pepper (*Piper nigrum* L: Family Piperaceae), the second most valuable spice crop in Sri Lanka, is a perennial vine and grows well on reddish brown latosolic soils. Black pepper is also identified as a host crop of mycorrhizae. Re-inoculation of nursery plants with AM isolates collected from different black pepper growing fields has been studied for good quality planting material production of black

## Materials and Methods

Rhizosphere soil samples from randomly selected 20 black pepper plants from three different fields located in Matale (IM3A), Sri Lanka were collected. The dominant soil type in the area was reddish brown latosol: Matale series (Mapa *et al.*, 1999). The soil samples were collected at a depth of 0-20 cm, and evaluated for soil pH (1:2.5 soil: water extract; Black, 1965) and the volumetric moisture content (%) using a Time Domain Refractrometer.

The samples were air dried and sieved, and the total N (%) was analysed by kjeldahl method (Bremmer and Mulvaaney, 1982), available P (ppm) by Olsen method (Olsen *et al.*, 1954), exchangeable K (ppm) by Ammonium acetate extraction method (Thomas, 1982) and organic C (%) by Potassium

## Results and Discussion

### Soil properties of study sites:

Soil pH of the studied rhizospheres ranged from 4.7 to 5.9 indicating that the study sites were slightly acidic. The mycorrhizae prefer slightly acidic PH (around 6.5) for their spore germination and growth (Widdowson, 2013). Volumetric

pepper (Mala *et al.*, 2010). However, mycorrhizal activity in fields with mature black pepper plants of Sri Lanka is not properly explored and documented up to date. Minimum anthropogenic disturbances in black pepper cultivations under Sri Lankan conditions and the low phosphate availability due to high phosphate immobilization may lead to higher indigenous mycorrhizal populations and diverse activities with a high species diversity. Presence of indigenous AM and their diversity in existing fields will affect the level of infection of introduced mycorrhizae, too. Therefore, this study was carried out to identify existing AM species and morphological modifications of colonized AM in black pepper roots in order to verify their involvement for a sustainable black pepper production on reddish brown latosolic soils in Matale in Sri Lanka.

dichromate method (Nelson and Sommers, 1982). The arbuscular mycorrhiza (AM) spore (size >50 µm) density in each location was assessed by the modified wet sieving and decanting technique with sucrose centrifugation using 270 (50 µm) and 40 (425 µm) mesh numbered sieves. The black pepper roots collected from selected plants were examined for mycorrhizal infection *via* trypan blue staining after clearing and acidification of roots (Brundrett *et al.*, 1996). Root colonization was visualized under the light microscope by the presence of modified root structures, namely, arbuscules and vesicles. Morphological characteristics of those structures including intra-radical and extra-radical hyphae were examined comparing morphological characteristics listed out for known species of AM.

moisture contents of soil collecting sites ranged from 9.8 % to 24 %. Total N and organic C were in the ranges of 0.18 % to 0.23 % and 1.57 % - 1.75 %, respectively, and was favourable for biological activities.

Available P ranged from 4.4 ppm to 29 ppm, but poor P availability was prominent in many locations. This may be due to high P immobilization in examined sites and the poor P levels may facilitate the AM infections. Different moisture and P contents may result in a variability in their colonization, too. Exchangeable potassium levels also varied among the plant rhizospheres, ranging from 188 ppm to 788 ppm, and this may be due to the application of different levels of organic and inorganic inputs to plants and different biological activities in the plant rhizosphere.

#### Extra-radical hyphae of AM fungi:

Less extra-radical hyphae, which were distributed in rhizosphere soil was observed microscopically as most of the non-attached mycelia has been removed during root clearing and staining steps. There were a few networks of hyphae identified in root samples examined under the light microscope (Figure 1A). The hyphae were relatively thin and highly branched, which are considered to be helpful in absorbing nutrients. As they were non-septate, the species of fungi may be members of order Glomerales.

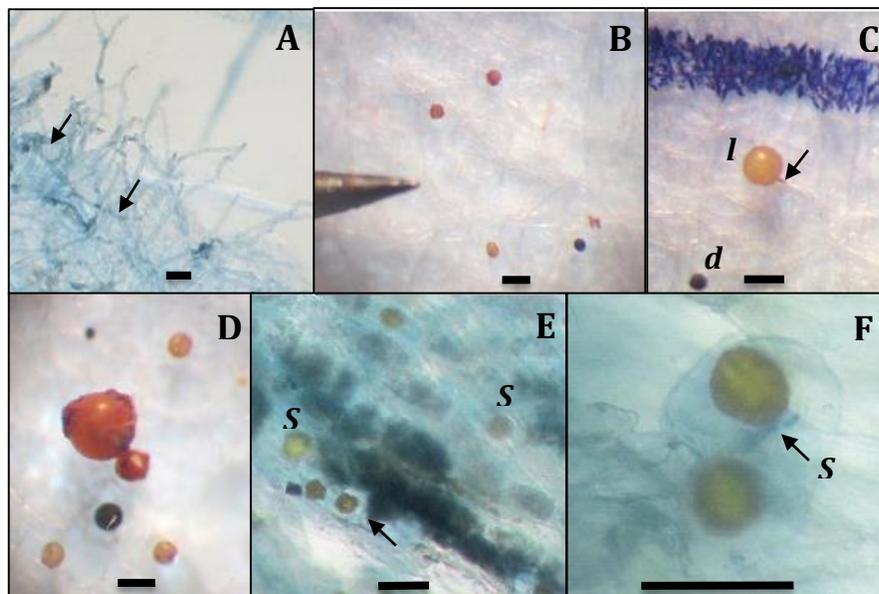


Figure 1: Section of a root of black pepper stained in trypan blue showing an A - Extra-radical hyphae, B - Free or single spores, C - Live (*l*) and dead (*d*) spores without special hyphal attachment, D - differently coloured and different sized spores, E - Intercellular spores (*S*), and F - spores without formation of any special hyphal attachments (horizontal bar size:100 µm)

#### AM spores and their abundance:

The AM spore (size > 50 < 425 µm) density in examined soils were ranged from 1600 to 9000 spores kg<sup>-1</sup> soil. Dhanapal (2013) reported that the spore abundance of AM in *Piper nigrum* ranged from 12.0 to 90.3 g<sup>-1</sup> soil in different locations in India, and found a positive significant correlation with the soil nitrogen, and negative correlation with rainfall and relative humidity. Mafaziya and Madawala (2015) showed that the number of AM spore morphotypes belonging to different genera in four different land use types of Sri Lanka such as restored Pinus stand,

Paraserianthes stand, degraded grassland, and natural forest patch and semi-natural landuse types in Upper Hanthana area ranged from 1,573 to 8,234 spores kg<sup>-1</sup> soil, with the highest recorded in the natural forest patch. Therefore, a comparatively higher number of AM spores or high spore abundance could be seen in black pepper fields at the Matale study sites.

Further, spores identified in this study were free and single spores (Figure 1B) and no sporocarps or fruiting bodies were observed. Both dead (and parasitoid) and live spores (Figure 1C) were

observed. Size of the live spores varied from 50 to 425  $\mu\text{m}$  and colour was transparent or slightly brownish to dark brown. Colour of the dead spores was dark black. Spore shape was globular or spherical (Figure 1D). There were some inter-cellular spores in the AM infected roots (Figure 1E). A characteristic feature described by Redecker (2006) of the mycorrhizae fungi family Acaulosporaceae (*Acaulospora* and *Entrophospora* spp), i.e. formation of spores on a hyphal attachment called “sporiferous saccules” or a distinguish feature of Gigasporaceae (*Scutellospora* and *Gigaspora* spp; presence of hyphal attachment called “bulbous suspensor”) was not observed in the identified spores (Figure 1F). Therefore, examined spores should belong to order Glomerales, which consists with *Glomus* spp.

Root colonization and intra-radical structures in roots:

The characteristics of root modifications were observed to detect AM colonization in roots. The intra-radical structures examined in black pepper roots were arbuscules, vesicles and the intra-

radical hyphae. Intra-radical hyphae observed were not much coiled but had H or Y branches (Figure 2A). They were darkly stained with vital stain, Trypan blue, confirming their phylogenetic origin as order Glomerales.

Visualization of arbuscules and vesicles in roots:

About 95 % of examined roots were infected with AM showing root modifications such as arbuscules or vesicles or both. The hyphae, which ramify within the cortex of roots forming intricately branched haustoria called arbuscules (Figure 2B), were prominent in most of the examined roots. Arbuscules were at different growth stages or at different maturity (Figure 2C) and they were highly branched structures (Figure 2D). In many roots, storage structures called vesicles were also observed. Vesicles were oval or ellipsoid in shape (Figure 2E). They were found between root cortex cells in branched intra-radicular hyphae. Both inter- or intracellular vesicles could be seen, but more intra-cellular vesicles were observed with a few inter-cellular vesicles. In some roots, there were many vesicles present as clusters in branched hyphal structures.

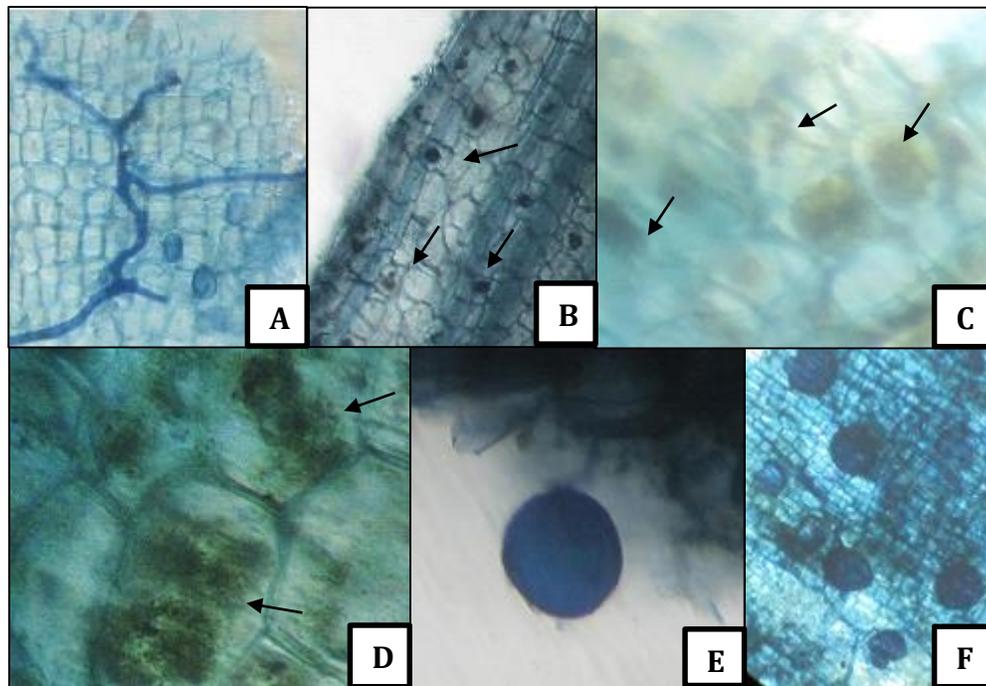


Figure 2: Section of a root of black pepper stained in trypan blue showing an A - Darkly stained intra-radical hyphae (100X), B - Arbuscules in root cortex cells (100X), C - Arbuscules at different maturity (400X), D - Highly branched nature of arbuscules (400X), E - Oval shaped vesicles (400X) and F - Vesicles as clusters in branched hyphal structures (100X)

In studied root samples, rate of colonization was different however, about 95 % of roots observed under the microscope were infected with arbuscular mycorrhizae. Thus, the presence of vesicles or arbuscules were common in studied roots. About 30 % of the samples contained both vesicles and arbuscules. The density of arbuscules or vesicles varied in root tissues of study sites but the relationship between rate of colonization in root tissues and existing environmental conditions are

## Conclusion

Most of the mature black pepper plants in RBL soils of Matale in Sri Lanka were found to be infected with indigenous species of vesicular arbuscular mycorrhizae (VAM) forming high spore abundance in soil and root modifications such as arbuscules and vesicles in infected black pepper roots.

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not discussed in this paper. However, a higher rate of root colonization of AM or vesicular arbuscular mycorrhizae (VAM) in roots of black pepper plants grown in reddish brown latosolic (RBL) soils of Matale indicated the successful implication of this symbiotic association in existing black pepper cultivations. Further studies are needed to verify their functions in various soil and environmental conditions in order to use sustainable production of black pepper.

Morphological features of spores, hyphae, arbuscules and vesicles of infected AM were similar to fungi of order Glomerales, which consisted *Glomus* spp.

