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Influence of indigenous arbuscular mycorrhizal fungus and bacterial bioinoculants on growth and yield of *Capsicum chinense* cultivated in non-sterilized soil

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Abstract

Despite the global importance of *Capsicum* species, there is limited information on the indigenous endomycorrhizal fungal association in this crop. Therefore, the diversity and colonization patterns of arbuscular mycorrhizal fungi (AMF) in roots of Naga King chilli (*Capsicum chinense*) were assessed during pre-flowering, flowering and fruit ripening growth stages under a sub-tropical shifting cultivation system of North Eastern India. All the roots examined had AMF colonization and the presence of *Paris*-type arbuscular mycorrhizal morphology is reported for the first time in *C. chinense*. A total of 11 AMF spore morphotypes were isolated from both field and trap culture soils. Maximum AMF spore density and root colonization were recorded during the pre-flowering and flowering stages, respectively. The influence of *Funneliformis geosporum*, individually or in combination with *Pseudomonas fluorescens* and *Azotobacter chroococcum*, on growth and yield of *C. chinense*, was evaluated in a pot experiment using sterilized and non-sterilized soils. The application of AMF and *P. fluorescens* to sterilized soil significantly increased the growth, flower and fruit production, and nutrient content of *C. chinense*. The highest growth rates and yields of *C. chinense* in non-sterilized soils were achieved when AMF was combined with both *P. fluorescens* and *A. chroococcum*. The results of the current study indicate the value of applying microorganisms to improve plant growth and performance in chillies. One of the mechanisms for this could be the facilitated assimilation of nutrients promoted by AMF and bacterial bioinoculants.

Introduction

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are a key functional component of soil biota in agroecosystems and form symbiotic associations with the majority of economically important crop plants (Smith and Smith, 2012) including vegetables (Baum *et al.*, 2015). Arbuscular mycorrhiza (AM) have been reported to improve plant growth and health by increasing the uptake of mineral nutrients and water and providing tolerance to biotic and abiotic stresses (Brundrett, 2009; Verbruggen *et al.*, 2012). A plant's demand for mineral nutrients, particularly phosphorus (P), is determined by its genes (Krishna *et al.*, 1985) and the fertility status of the soil. Some soil factors such as available nutrients, pH and organic carbon (OC) regulate the proliferation of AMF in the rhizosphere and secretion of the enzyme phosphatase by the fungal hyphae that help in hydrolysing phosphate from insoluble organic P compounds (Oehl *et al.*, 2010). Many AMF species are widespread in different terrestrial ecosystems (Öpik *et al.*, 2006), whereas others appear to be restricted to specific types of land use, vegetation or climates (Oehl *et al.*, 2010). Field crops depend obligately on AMF symbiosis and inoculation with these fungi has been found to be highly beneficial to crop yields in a range of soils (Rouphael *et al.*, 2015). Therefore, knowledge about the diversity of indigenous AMF prevalent in a specific region and associated with cultivated crops is an essential step for any application. The colonization patterns of AMF within the host roots tend to vary with the plant and fungal species involved (Dickson *et al.*, 2007). Furthermore, the ability of AMF to colonize roots and to provide nutrients to the host system may vary among different morphological types i.e. *Arum*-, *Paris*- or Intermediate (Dickson, 2004). Though the association of AMF has been reported in the majority of cultivated crops, their root colonization patterns are yet to be determined (Muthukumar and Tamilselvi, 2010).

Recent reviews have reported the significance of AMF inoculation on growth and yield improvements of vegetable crops through alteration in plant growth physiology and morphology (Baum *et al.*, 2015; Rouphael *et al.*, 2015). However, the characterized effect of AMF on plant growth and development in horticultural and agricultural crops is not always consistent, because of the complexity of interactions involved between the host plant genotypes and AMF, types of inoculation methods and differing environmental conditions (Baum *et al.*,

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2015). Application of microbial inoculants such as AMF and rhizobacterial communities, also termed biofertilizers and biostimulants, has attracted universal attention due to the negative impacts of excessive use of chemical fertilizers and pesticides, and to an increased awareness about sustainable organic agriculture (Gosling *et al.*, 2006; Srivastava *et al.*, 2016) and the beneficial association between rhizosphere microbiota and plants (Alori *et al.*, 2017). Among these, phosphate solubilizing bacteria (PSB), plant growth promoting rhizobacteria (PGPR) and AMF consortia have received notable interest due to their synergistic beneficial effects as growth promoters to improve plant health in agriculture and horticulture (Antoun, 2012). In this context, Pseudomonads and the nitrogen-fixing *Azotobacter* are popular bioinoculants used widely to promote the growth of a wide variety of crop species (Soleimanzadeh and Ghooshchi, 2013; Dey *et al.*, 2017; David *et al.*, 2018). These bacteria promote plant growth through making plant nutrients available, synthesis of phytohormones, solubilization of phosphate, production of siderophores, antibiotics and lytic enzymes that degrade the fungal cell wall, and degradation of the pathogenic toxins (David *et al.*, 2018). They may also act as mycorrhizal helper bacteria in promoting mycorrhizal formation and function. Previous studies have shown that co-inoculation of Pseudomonads and *Azotobacter* along with AMF can improve plant growth and yield in several plant species (Sabannavar and Lakshman, 2008, 2011; Pérez-de-Luque *et al.*, 2017).

Chilli (*Capsicum* spp., family Solanaceae) is among one of the most important horticultural and commercial crops in the Asian continent and its diverse cultivars are grown for vegetables, spices, condiments, etc., which are used mainly for colouring and pungency properties (Pereira *et al.*, 2016). India is the largest producer and consumer of chilli, contributing about 25% to the total global production, and remains in the prime position in terms of international trade by exporting 39% from its total production, earning about US$30 million per annum (Thilagar and Bagyaraj, 2015; SBI, 2017). Global production of chilli and hot peppers has increased gradually during 2000–2010 from 20.8 to 27.6 million tonnes (FAO, 2010). The agroclimatic conditions of North Eastern (NE) India are suitable for cultivation of profitable quantities of various spices and the area is also perceived as a hot spot for various indigenous chilli species (Mathur *et al.*, 2000). Naga King chilli/Umorok (*Capsicum chinense* Jacq. cv.) has been cultivated widely in north-east India by traditional shifting cultivation since time prehistoric, with an extensive amount of genetic variability between the landraces (Hazarika and Neog, 2014). It has received attention from the global scientific community due to its extremely high pungency [1 001 304 Scoville Heat Units (SHU)] and unique aroma (Meghvansi *et al.*, 2010) and has immense scope in domestic and international markets due to its remarkably high capsaicin content.

Recent studies in India have reported the positive role of AMF in the growth and yield of *C. annum* (Tanwar *et al.*, 2013; Thilagar and Bagyaraj, 2015) and *C. frutescens* (Dai *et al.*, 2011). Nevertheless, information on AMF diversity, root colonization level, the type of AM morphology produced therein and the impacts of AMF and/or bioinoculants on growth performances of *C. chinense*, in India, has not been explored to date. To our knowledge, only one previous study from Tabasco, Mexico has examined the response of *C. chinense* to inoculation with AMF and rhizobacteria (Constantino *et al.*, 2008). In view of the potential importance of AMF inoculation technology for sustainable agriculture, a detailed survey of indigenous AMF diversity in subtropical agroecosystems of the Indo-Burma biodiversity hotspot region would provide a basis for their multifunctional field applications in future. Therefore, an attempt has been made in the current investigation (1) to determine the spore density and species diversity of AMF in the *C. chinense* rhizosphere, (2) to record AM morphology as well as the root colonization patterns of AM during different stages of plant growth and (3) to evaluate the influence of the most abundant AM fungus on growth and yield response of Naga King chilli individually and along with rhizobacterial inoculants in sterilized and non-sterilized soils under greenhouse conditions of north-east India.

Materials and methods

Endomycorrhizal association in field cultivated C. chinense *plants*

Study site and crop growth conditions

The current work was conducted at a shifting cultivated agricultural field located on the gentle slope of a hillock along the Taphou Naga hill range of Senapati District (25°16′N, 94°01′E, 1117–1142 m asl) at a distance of 62 km north of Imphal, the capital city of Manipur, India. Geographically, about 90% of the total area of Manipur is covered by hill ranges that are offshoots of the eastern Himalayas, with ~1813 km^2 of the central valley area (Sehgal *et al.*, 1993). The sub-tropical humid climate of the area is characterized by three distinct seasons: warm, moist summer (April to June), wet, rainy months (July to September) and cool, dry winter (November to February). March and October comprise the transitional periods between winter/summer and rainy/winter seasons, respectively. The annual temperature of the study site ranged from 3.4 to 34.4 °C. The mean monthly averages of relative humidity (RH) varied from 76 to 92%, whereas the total annual rainfall was 1454 mm. According to United States Department of Agriculture classification (Soil Survey Staff, 1999), the soil is Ultisol developed from shale and sandstone on gently sloping narrow valleys to steep hill slopes and is heterogeneous in nature (Sehgal *et al.*, 1993). The soil of the study site was sandy loam in texture and slightly acidic in nature.

Generally, *C. chinense* prefers deep, loose sandy loam or clay loam soil that is rich in organic matter with a pH ranging from 5.0 to 6.5 (Bhagowati and Changkija, 2009). The crop is cultivated as a sporadic intercrop in slash and burn agriculture or *Jhum* (shifting cultivation) fields in areas with an annual rainfall range of 1200–4050 mm and a temperature range of 6–36 °C. In the hilly region, planting of Naga King chilli is carried out during February/March and harvesting is from May/June onwards, whereas in plain areas this crop is planted in August/September and harvested in November/December (Meghvansi *et al.*, 2010). The negative effects of *Jhum* cultivation, such as loss of soil fertility and biodiversity, warrant efforts to reduce the reliance on such cultivation practices.

Sample collection

Fine roots and rhizosphere soil samples belonging to Naga King chilli were collected between February and June 2015 during three different plant growth stages: (1) pre-flowering, at 40–45 days after sowing (D1), (2) flowering, 85–90 days after sowing (D2) and (3) fruit ripening, 130–135 days after sowing (D3). The roots were excavated by digging a soil layer (0–15 cm) around

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five randomly selected chilli plants at each growth stage. Roots were washed gently and fixed in formalin : acetic acid : alcohol (FAA) solution (formalin : glacial acetic acid : 70% ethyl alcohol, 5 : 5 : 90 ml v : v : v) until processing. The soil surrounding and shaken from roots was placed in individual polythene bags, labelled and taken to the laboratory. After air drying in shade, one part of the soil samples belonging to each growth stage was used for extraction of AMF spores and another part was bulked together and used for assessment of soil properties and the establishment of trap cultures.

Analysis of soil properties

Soil physicochemical characteristics were determined using three sub-samples collected at each growth stage of chilli. Soil texture was analysed by the Bouyoucos hydrometer method (Allen *et al*., 1974), whereas soil pH and electrical conductivity (EC) were determined at room temperature in an aqueous solution of soil : water (1 : 1, v : v) using digital meters (ELICO, India). The OC was assessed by the Walkley and Black rapid titration method (Walkley and Black, 1934). Soil total nitrogen (N) and available P were determined according to Jackson (1971) and exchangeable potassium (K) was analysed after extraction with ammonium acetate (Jackson, 1971).

Extraction and identification of AMF spores

AMF spores were extracted by wet sieving and decanting methods (Gerdemann and Nicolson, 1963): In total, 100 g of each soil sample was dispersed in 1 litre of water and the suspension was decanted through a series of 710 to 37 µm sieves. Residues in the sieves were washed into beakers, whereas the filtrates were dispersed in water and filtered through filter papers with a grid drawn on it. Each filter paper was then spread on glass plates and observed under a light microscope at 40× magnification. All the intact and healthy AMF spores (non-collapsed spores with cytoplasmic contents and free from parasitic attack) were counted and the sporocarps and spore clusters were considered as one unit. Isolated AMF spores were transferred onto glass slides using a wet needle and mounted in polyvinyl alcohol-lacto glycerol with or without Melzer's reagent (Schenck and Perez, 1990). Identification of AMF spores was carried out based on the original description on the Schüssler's web site (http://www.amf-phylogeny.com/amphylo_species.html). Spore morphology was also compared with the cultural database of the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM; https://invam.wvu.edu/cultures). The AMF infective propagules in the soil were assessed by the most probable number (MPN) technique as proposed by Porter (1979).

Assessment of AMF colonization

Fixed roots were washed with distilled water to remove the FAA solution, cut into 1-cm long segments, processed for clearing in 2.5% potassium hydroxide (KOH) at 90 °C for 1–2 h, depending on the degree of lignification of the roots, in a water bath (Koske and Gemma, 1989) acidified with 5 N hydrochloric acid (HCl) solution for at least 15–20 min and stained with 0.05% of trypan blue-lacto glycerol overnight at room temperature. The stained root pieces were mounted on glass slides and examined for AMF structures with a compound microscope (Olympus BX 51, Japan). Percentage of root length colonization was estimated according to the magnified intersection method (McGonigle *et al*., 1990). Arbuscular mycorrhizal morphology was characterized based on the inter- or intra-cellular nature of fungal structures within the roots (Dickson, 2004).

Establishment of trap cultures

Five trap cultures were established using 2-litre earthenware pots filled with rhizospheric soil samples mixed with sterilized sand (1 : 1 v/v). The trap cultures were placed in the greenhouse and seeded with maize (*Zea mays* L.) as a host plant. The pots were watered on alternate days. Developed spores of AMF were isolated 120 days after culture initiation and identified as described above.

Bioinoculant potential on growth and yield performance of *C. chinense*

Experimental site and soil preparation

The study was carried out in the greenhouse of the Life Sciences Department, Manipur University, Imphal, India (24°45′N, 93° 55′E, 795 m asl). The mean minimum and maximum temperatures of the experimental site during the study period, i.e. February to June 2016, ranged from 9.6 to 27.5 °C, while the RH varied from 64 to 90.3%. The climate is monsoonal with a dry period between February and March. The soil used in the current experiment was sandy loam in texture, collected during the first week of February 2016 from a natural chilli field situated in Senapati District of Manipur, India. The soil was sieved to remove pebbles and root fragments. One part of the soil was autoclaved (sterilized, SS) three times at 121 °C for 1 h with a 24 h gap between each sterilization process, while the remaining portion was used as collected (non-sterilized, NS). Soil properties were assessed using standard procedures as outlined above, which revealed: 99.7 kg/ha total N, 80.5 kg/ha available P, 163.4 kg/ha exchangeable K and 2.26% of OC. The pH and EC of the test soil was 5.8 and 0.26 dS/m, respectively.

Isolation, pure culture and inoculum preparation of indigenous AMF

Dominant AMF were isolated from the rhizosphere soil of field-grown Naga King chilli by the wet sieving and decanting technique, as described by Gerdemann and Nicolson (1963). Field-collected soil had an indigenous AMF population of 18 propagules/g soil as assessed by MPN (Porter, 1979) and the indigenous AM flora consisted of *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Glomus ambisporum* G.S. Sm. & N.C. Schenck, *Glomus macrocarpum* Tul. & C. Tul., *Glomus* sp. and *Racocetra* sp., of which *F. geosporum* was the most dominant AMF.

The starter inoculum of *F. geosporum* was developed from a single spore by the funnel technique (Menge and Timmer, 1982) and multiplied for 30 days using maize as the host plant. The pure culture of *F. geosporum* was further mass-multiplied in earthenware pots of 22 cm diameter containing 3 kg of sterilized soil : sand (1 : 1 v/v) mixture as the substrate and *C. chinense* as the host plant. After 120 days of growth, roots of the host plant were assessed for AM colonization levels and their spore density in inoculum soil as described above. The percentage of root colonization of *C. chinense* by AMF ranged from 80 to 90% and the AM fungus spore population consisted of 115 ± 4 spores per

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10 g fresh soil, which included 65 infective propagules/g of soil as assessed by the MPN technique of Porter (1979). For mycorrhizal treatment, 100 g of fresh inoculum soil along with AMF spores and chopped colonized roots of chilli were prepared for each test pot.

Bioinoculant inoculum preparation

Two pure culture strains of rhizobacteria were used, i.e. *Pseudomonas fluorescens* (PSB) (LSDCC-21) and *Azotobacter chroococcum* (PGPR) (LSDCC-34) for this experiment. The bacterial inocula were procured from the Laboratory of Microbiology and Plant Pathology, Department of Life Sciences, Manipur University, Imphal, India. *P. fluorescens* and *A. chroococcum* were cultured in liquid King's broth and Mannitol agar medium, respectively and incubated at 28 ± 1.3 °C with orbital agitation (120 rpm) for 48 h. The medium was centrifuged (8000 ***g*** units/10 min) and the pellet resuspended in 50 ml sterile distilled water; 1 ml of the solution, adjusted to 1×10^8 colony forming units (CFU), was maintained for each rhizobacterial culture.

Experimental design

The pot experiment consisted of a randomized complete block design (2 × 5 × 5) with two soil conditions, sterilized soil (SS) and non-sterilized (NS) field soil, and five inoculation treatments (C – control, T1 – *F. geosporum*, T2 – *F. geosporum* + PSB, T3 – *F. geosporum* + PGPR, T4 – *F. geosporum* + PSB + PGPR) each with five replicates. Ripened Naga King chilli fruits were harvested from the plants growing in the natural field, placed in a polythene bag and taken to the laboratory. The fruits were air dried under warm sunny conditions and room temperature for 1 week. The dried seeds were surface-sterilized with 2% sodium hypochlorite solution for 1 min, washed in several changes of sterilized distilled water, air dried and placed in plastic trays containing sterilized soil : sand mixture (1 : 1, v : v). After 20 days of growth, uniform chilli seedlings were transferred (1 seedling/treatment), individually, into pots (25 × 30 cm^2, height × diameter) filled with 4 kg of sterilized (SS) or non-sterilized (NS) soil. Before transplanting each seedling, 100 g of AMF inoculum was layered at a depth of about 5 cm. Rhizobacterial (PSB and PGPR) inoculations were made either individually or in combination as per the treatments by adding 3 ml cell suspension of each test inoculant (10^8 CFU/ml) adjacent to the chilli seedling root system at 5 min intervals. An equal quantity of heat sterilized microbial inocula was added to the control treatments. The pots with chilli seedlings were watered on alternate days to maintain the moisture at ∼60% of the water holding capacity of the soil.

Harvest and measurement

Naga King chilli plants were carefully uprooted 135 days after sowing and different growth parameters viz., shoot and root length (cm), number of leaves, shoot collar diameter (cm) and shoot and root dry weights (g), were determined. Similarly, days to flowering, flower number, fruit set (%), the number of fruits and fruit fresh weight (g) were also recorded. All tissue samples, i.e. shoots (leaves and stems) and roots were placed in separately labelled paper envelopes and oven-dried at 80 °C for 48 h to determine dry weights. The root/shoot (R/S) ratios were calculated from the dry weights of roots and shoots. Total root length was calculated according to Newman (1966) and fruit set (%) was calculated according to Wubs *et al.* (2009) as:

$$\frac{\text{No. set fruits}}{\text{No. flowers}} \times 100$$

Oven-dried shoots and roots were analysed for tissue N, P and K contents. The N content in shoot and root samples were determined by the micro-Kjeldahl digestion method, while total P was determined by the molybdenum blue method after triple acid (concentrated nitric acid [HNO_3], sulphuric acid [H_2SO_4] and 60% perchloric acid [$HClO_4$]) digestion (Jackson, 1971). Total K was estimated by flame photometry (Davis, 1962). Spore numbers of AMF in the test soil were estimated as described above. At sampling times, a weighed sub-sample of the fresh feeder roots was taken from each plant to assess the percentage root length AM colonization. Microbial inoculation effect (MIE) was calculated according to Bagyaraj (1992).

Statistical analyses

The AMF spore density, species richness, relative abundance (% RA) and isolation frequency (%IF) were calculated as indicated by Zhao and Zhao (2007). Analysis of variance was used to test the significance of variations between AMF variables in field-collected chilli roots and to compare growth and yield parameters evaluated in bioinoculant treatments in pot experiments (SPSS version 9, SPSS Inc., Chicago, Illinois). Pearson's correlations were carried out to assess the relationship between soil physico-chemical properties and mycorrhizal colonization and the relationship between plant growth variables, tissue nutrient contents and AMF colonization. To achieve the normalization prior to statistical analysis, the AMF spore numbers and the percentage values for root colonization by endomycorrhizal fungi were log and arcsine transformed, respectively.

Results

Soil properties

The soils of Naga King chilli during different growth periods had a pH range of 5.7–6.3, an EC of 0.26–0.30 dS/m and an organic C of 2.2–2.7% (Table 1). Total N, available P and exchangeable K of the soils varied from 95 to 113 kg/ha, 8.4 to 9.1 kg/ha and 195 to 211 kg/ha, respectively. Maximum concentrations of organic C, N, P, and K were recorded in the pre-flowering period. However, all the soil characters (except for EC) examined varied significantly ($P < 0.05$ to $P < 0.01$) among different growth periods.

The extent of AMF colonization

All the root fragments of Naga King chilli examined had an association of AMF (Table 2, Fig. 1). *Paris*-type AM morphology was observed in Naga King chilli with intracellular hyphal coils, arbusculate coils and vesicles. Significant ($P < 0.001$) differences were observed in the extent of AMF colonization and root length with AMF structures during different growth periods (Table 2). The percentage of root length with total AM colonization (% RLTC) ranged from 57 to 75%. Similarly, the percentage root length with hyphal coils (%RLHC) varied between 25 and 34%; arbusculate coils (%RLAC) ranged from 28 to 40%, and the vesicles (%RLV) varied between 3.9 and 4.8%. Overall, the percentage

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Table 1. Soil properties in the upper 15 cm soil profile of *Capsicum chinense* during different plant growth stages

	Soil factors‡					
Sampling phase†	pH	EC (dS/m)	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)
D1	6.3 ± 0.11^{b}	0.30 ± 0.021^{a}	2.7 ± 0.23^{b}	113 ± 4.5^{c}	9.1 ± 0.28^{b}	211 ± 7.5^{b}
D2	5.7 ± 0.23^{a}	0.26 ± 0.020^{a}	2.2 ± 0.15^{a}	95 ± 2.8^{a}	8.4 ± 0.15^{a}	195 ± 4.9^{a}
D3	6.2 ± 0.23^{b}	0.27 ± 0.028^{a}	2.4 ± 0.19^{ab}	104 ± 4.7^{b}	8.7 ± 0.30^{ab}	198 ± 6.5^{a}

†D1, D2 and D3 indicate pre-flowering (at 40–45 days after sowing), flowering (at 85–90 days after sowing) and fruit ripening (at 130–135 days after sowing), respectively.
‡pH, EC, OC, N, P and K indicate percentage hydrogen ion concentration, EC, OC, total nitrogen, available phosphorus and exchangeable potassium, respectively.
Means ± standard error in a column followed by different superscript letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 2. Extent of AMF colonization in roots of *C. chinense* during different plant growth stages

	AMF‡				
	Colonization				
Sampling phase†	%RLHC	%RLAC	%RLV	%RLTC	SPN
D1	25 ± 1.5^{a}	28 ± 1.7^{a}	3.9 ± 0.59^{a}	57 ± 2.0^{a}	455 ± 26.7^{b}
D2	31 ± 1.6^{b}	40 ± 2.7^{c}	4.2 ± 0.66^{a}	75 ± 3.7^{b}	232 ± 16.0^{a}
D3	34 ± 2.6^{c}	33 ± 1.6^{b}	4.8 ± 0.88^{a}	71 ± 2.9^{b}	325 ± 18.3^{a}

†D1, D2 and D3 indicate pre-flowering (at 40–45 days after sowing), flowering (at 85–90 days after sowing) and fruit ripening (at 130–135 days after sowing), respectively.
‡%RLHC, %RLAC, %RLV, %RLTC and SPN indicate percentage root length with AMF hyphae, hyphal coils, arbuscules/arbusculate coils, total colonization and spore number (per 100 g soil), respectively.
Means ± standard error in a column followed by different superscript letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

root length colonization with hyphal coils and vesicles were highest during the fruit-ripening stage, while that of arbusculate coils and total AM colonization were maximum during the flowering period. Significant ($P < 0.001$) variations existed among the different growth stages of Naga King chilli for %RLHC, %RLAC and % RLTC. However, there was no significant variation in %RLV among three different growth periods.

AMF spore density

The highest spore population of AMF was recorded in root-zone soils of Naga King chilli during the pre-flowering stage, while that of the flowering stage revealed the lowest spore counts (Table 2). Moreover, AMF spore density varied significantly ($P < 0.01$) among the different growth periods and was significantly ($P < 0.05$) and negatively correlated with the AMF root colonizing variables (Table 3).

The relationship between endomycorrhizal fungal and soil variables

Pearson's correlation analysis revealed the existence of significant ($P < 0.05$) positive correlations between SN and soil pH, organic C and P (Table 3). The %RLAC and %RLTC were significantly ($P < 0.05$) and negatively correlated with soil pH and total N and available P. A similar correlation also existed for %RLHC and soil exchangeable K, and that of %RLV as well as %RLTC with K and P concentrations. Likewise, AMF spore numbers were significantly ($P < 0.05$) and negatively correlated with %RLAC and % RLTC, while %RLAC and %RLHC were significantly ($P < 0.05$) and positively correlated with %RLTC.

AMF species distribution

A total of 11 AMF spore morphotypes corresponding to five genera, i.e. *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis* and *Racocetra*, were extracted from the natural field soil of Naga King chilli (Table 4, Fig. 2). Six of these AMF spore morphotypes belonging to four genera were also isolated from trap cultures of *C. chinense* (Table 4). *Glomus* was the dominant genus that was represented by six different species, out of which, *G. multicaule* was recovered from the soil samples collected during all three growth periods, while *G. microaggregatum* and *G. macrocarpum* were extracted from the soils belonging to pre-flowering and fruit ripening periods of crop growth. Spore morphotypes of genera such as *Sclerocystis* were represented by two species, whereas a single species each of *Funneliformis*, *Rhizophagus* and *Racocetra* were isolated from Naga King chilli rhizosphere. Of these, *F. geosporum* and *G. multicaule* were recovered from all the three growth periods with highest percentages of RA and IF (Table 4). *Rhizophagus fasciculatus* and *Racocetra* sp. were exclusively found only during the pre-flowering stage, while *Sclerocystis rubiformis* and *S. taiwanensis* were specifically isolated during the flowering and fruit ripening stages.

Effect of bioinoculants on growth performance of Naga King chilli

The current findings revealed significant ($P < 0.05$ to $P < 0.001$) enhancement in different evaluated growth parameters and fruit yield compared with the control chilli plants. At final harvest (130–135 days), microbial inoculated Naga King chilli seedlings had greater shoot and root lengths, collar diameter, biomass and leaf number, compared with uninoculated controls under SS and

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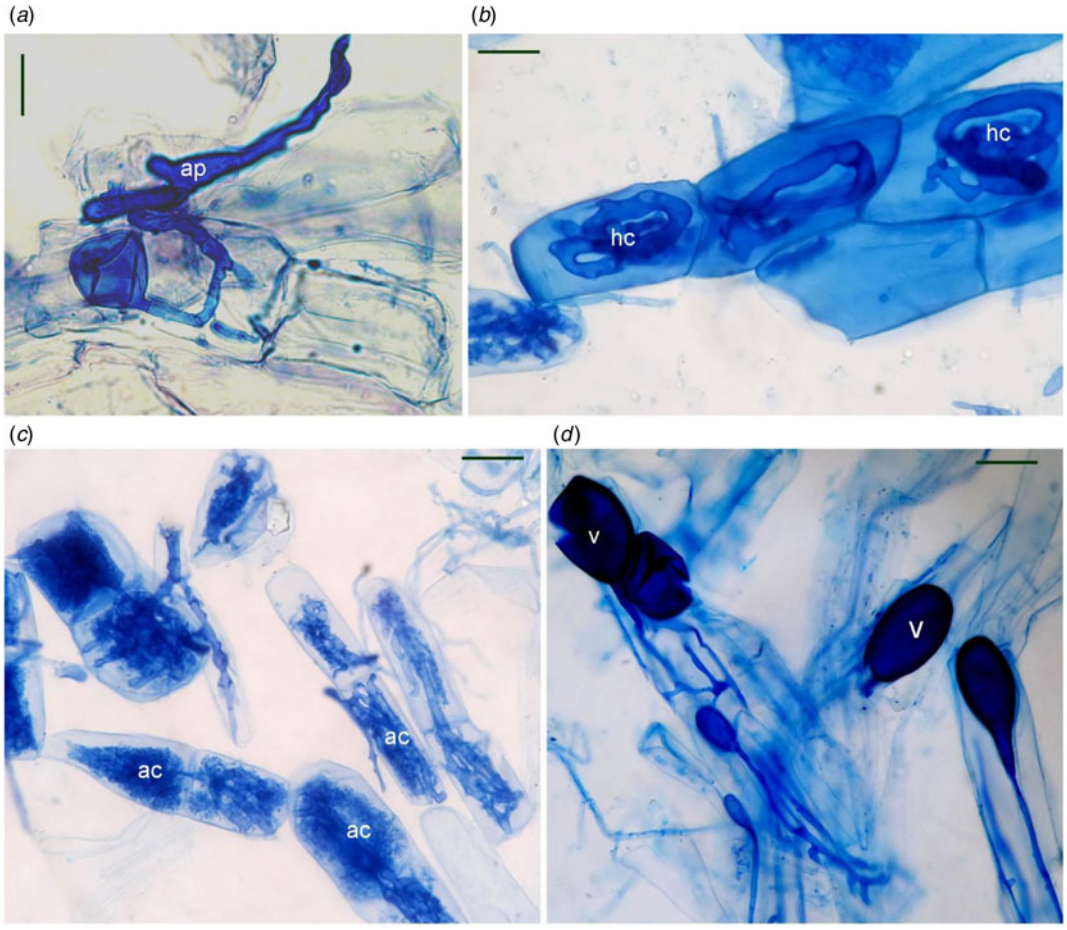

Fig. 1. (Colour online) AMF structures in *Capsicum chinense* roots. Appressorium (a); hyphal coils (b); arbusculate coils (c); vesicles (d). ap, appressorium; ac, arbusculate coil; hc, hyphal coil; v, vesicle. Scale bars: 20 µm.

Table 3. Pearson's correlation coefficient values for root colonizing structures of AMF and soil variables ($n = 45$)

		AMF[a]				
Variables		SPN	%RLHC	%RLAC	%RLV	%RLTC
Soil	pH	0.325*	−0.035	−0.483*	0.106	−0.321*
	EC	0.279	−0.154	−0.274	−0.228	−0.305
	%OC	0.555*	−0.116	−0.322*	0.069	−0.267
	N	0.254	−0.260	−0.478*	−0.220	−0.497*
	P	0.345*	−0.235	−0.337*	−0.059	−0.365*
	K	0.106	−0.452	−0.224	−0.366*	−0.462*
AMF	SPN		−0.275	−0.352*	0.032*	−0.382*
	%RLHC			0.273	0.314*	0.795**
	%RLAC				−0.192	0.783**
	%RLV					0.211

[a]SPN, %RLHC, %RLAC, %RLV and %RLTC indicate AMF spore numbers, percentage root length with AMF hyphal coils, arbusculate coils, vesicles and total colonization, respectively.
* and **: Significant at $P < 0.05$ and $P < 0.01$, respectively.

NS soil conditions (Table 5). Maximum shoot length of chilli plants was recorded when all three microbes i.e. AM + PSB + PGPR were combined under the SS soil conditions, whereas other growth parameters viz., leaf number, root length, shoot collar diameter and shoot and root dry biomass were higher under NS soil conditions. The most effective treatment in SS soil was AM + PSB, which revealed significantly ($P < 0.001$) higher root length, shoot collar diameter, shoot and root dry weights compared

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Table 4. Occurrence, %RA and %IF of AMF during different growth stages of *C. chinense*

	RA (%)					
	Sampling phase[b]				Isolated from soil of[a]	
AMF species	D1	D2	D3	IF (%)	Natural chilli field	Trap culture
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	24.03	31.96	22.22	88.88	+	+
Glomus microaggregatum Koske, Gemma & P.D. Olexia	12.37	–	18.13	66.67	+	–
Glomus ambisporum G.S. Sm. & N.C. Schenck	15.90	29.90	–	66.67	+	+
Glomus macrocarpum Tul. & C. Tul.	7.77	–	15.20	66.67	+	+
Glomus multicaule Gerd. & B.K. Bakshi	9.89	12.89	19.88	66.67	+	–
Glomus sp. 1	5.30	5.67	–	22.22	+	–
Glomus sp. 2	–	16.49	14.62	44.44	+	–
Rhizophagus fasciculatus (Thaxt.) C. Walker & A. Schüßler	11.66	–	–	33.33	+	+
Sclerocystis rubiformis Gerd. & Trappe	3.89	3.09	–	44.44	+	–
Sclerocystis taiwanensis C.G. Wu & Z.C. Chen	5.65	–	9.94	55.55	+	+
Racocetra sp.	3.53	–	–	22.22	+	–
	100	100	100		11	05
Species richness	10	6	6			

[a]+ and – indicate presence and absence of a particular AMF species.
[b]D1, D2 and D3 and indicate pre-flowering (at 40–45 days after sowing), flowering (at 85–90 days after sowing) and fruit ripening (at 130–135 days after sowing), respectively.

with uninoculated controls. The interaction between soil × treatments was not significant in the case of root length and root dry weight, whereas Naga King chilli seedlings inoculated with AMF individually or together with PSB and PGPR had lower R/S ratios under NS soil conditions compared with controls.

Flowering and fruit yield

The current results showed that Naga King chilli flowering and fruit yield parameters under SS and NS soil conditions were highly influenced by different microbial inoculations (Table 6). Early flowering was recorded in the inoculated ones compared with that in the controls in which flowering was delayed. The flowers were first seen in AM + PSB + PGPR-treated chilli plants under NS soil, followed by AM + PSB under SS soil conditions compared with control seedlings, in which flowers appeared at 85 days. Similarly, the number of flowers and fruits along with fresh weight (g) of fruit yield varied significantly ($P < 0.001$) among the two soil conditions, treatments and soil × treatments. Maximum numbers of flowers, fruits and the fruit yield were recorded in all three microbial consortia, i.e. AM + PSB + PGPR-treated Naga King chilli plants under NS soil conditions. However, AM + PSB had more influence on fruit yield parameters under SS soil conditions. In contrast, there was no significant difference in fruit set (%) between soil × treatments ($P > 0.05$).

Tissue nutrient concentrations of Naga King chilli plants

Significant differences in N, P and K concentrations of shoots and roots were found between soil ($P < 0.05$ to $P < 0.01$) and treatments ($P < 0.01$ to $P < 0.001$) (Table 7). Seedlings inoculated with AM + PSB + PGPR had a significantly ($P < 0.01$) higher accumulation of nutrients in their shoots and roots compared with other treatments under NS soil conditions. However, AM + PSB inoculated chilli seedlings had maximum N, P and K concentrations in SS soil compared with the control. Root P and K concentrations revealed significant ($P < 0.05$ to $P < 0.01$) variation between soil conditions, treatment and soil × treatments (Table 7).

AMF colonization and spore density in treated Naga King chilli plants

The percentage of root length colonization in *C. chinense* plants increased significantly ($P < 0.001$) with inoculation of AMF when compared with the control treatments (Table 8). The % RLTC in Naga King chilli varied between 42 and 77% in different treatments. Among all, the highest %RLTC by AMF was observed in the plants inoculated with AM + PSB + PGPR under NS soil conditions. PSB or PGPR along with AMF and the triple microbial combinations increased mycorrhizal colonization significantly ($P < 0.001$) over AM alone and uninoculated controls. However, AMF spore density was higher in AM alone in SS soil and AM + PSB + PGPR for NS soil conditions, as compared with other treatments (Table 8).

The relationship between Naga King chilli growth parameters and AMF structures

Pearson's correlation coefficient analysis revealed a significant ($P < 0.05$ to $P < 0.001$) positive correlation between chilli plant growth variables, tissue nutrient concentrations and the AMF structures (Table 9). In case of tissue P concentrations, it had a significant ($P < 0.05$) negative correlation with a plant R/S ratio. Similarly, the days of flowering (NDF) were significantly ($P < 0.05$, $P < 0.001$) and negatively correlated with AMF structures

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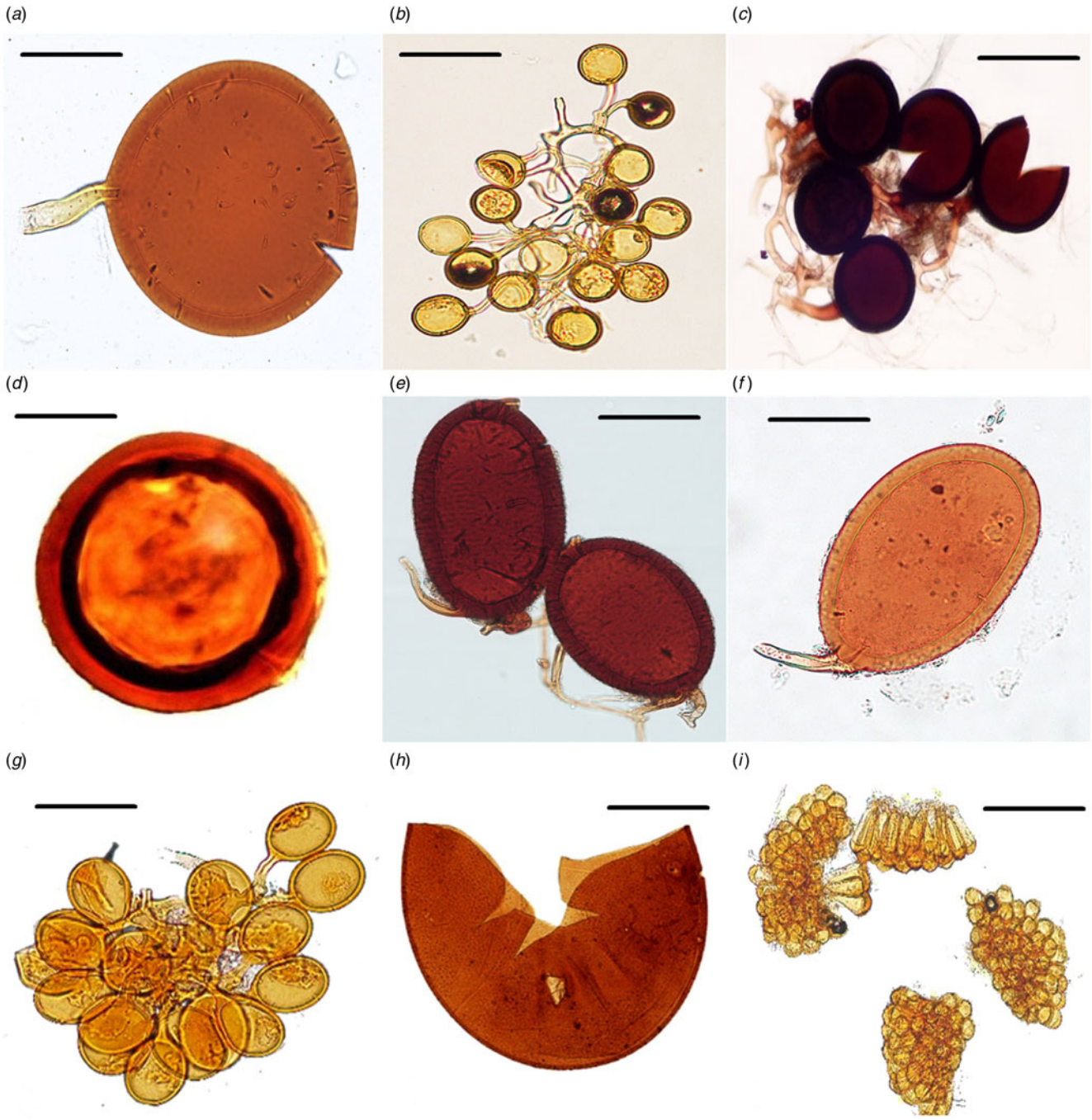

Fig. 2. (Colour online) AMF spores isolated from rhizosphere of *C. chinense*. (a) *Funneliformis geosporum*, (b) *Glomus microaggregatum*, (c) *Glomus ambisporum*, (d) *Glomus macrocarpum*, (e) *Glomus multicaule*, (f) *Rhizophagus fasciculatus*, (g) *Sclerocystis rubiformis*, (h) *Racocetra* sp., (i) *Sclerocystis taiwanensis*. Scale bars: 20 µm (b, c, e, g, h, i) and 40 µm (a, d, f).

and the tissue nutrients. Significant ($P < 0.05$ to $P < 0.001$) and positive correlations also existed among the different yield variables of Naga King chilli and AM structures together with tissue nutrient concentrations.

Microbial inoculation effect

MIEs of Naga King chilli under different treatments and soils did not vary significantly with each other. The higher MIE was observed in the chilli seedlings colonized by AM + PSB (62%) under SS soil conditions (Fig. 3).

Discussion

The current study provides the first comprehensive report on AMF species diversity in rhizosphere soil, as well as the morphology and colonization patterns of AMF in the roots of *C. chinense* during three different stages of crop growth in the sub-tropical habitat of NE India. Total spore density of AMF (232–455 spores per 100 g air-dried soils) recorded in the current study was much higher than those reported in *C. annuum* rhizosphere (16–24 spores per 100 g dried soil) by Muthukumar and Tamilselvi (2010). In contrast, Boonlue *et al.* (2012) reported

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Table 5. Growth response of *C. chinense* plants inoculated with native arbuscular mycorrhizal fungus, phosphate solubilizing and growth promoting rhizobacteria under pot conditions

Treatment	Shoot length (cm/plant)	Leaf number (per plant)	Shoot collar diam. (cm/plant)	Root length (cm/plant)	Dry weight (g/plant)		R/S ratio
					Shoot	Root	
Sterilized soil (SS)							
Control	36 ± 4.3^{a}	27 ± 2.6^{a}	1.4 ± 0.12^{a}	22 ± 1.9^{a}	2.3 ± 0.19^{a}	1.7 ± 0.15^{a}	0.74 ± 0.089^{bc}
AM	51 ± 3.7^{bc}	51 ± 4.2^{c}	2.1 ± 0.19^{bcd}	30 ± 2.9^{bcd}	4.6 ± 0.43^{bc}	3.5 ± 0.29^{bc}	0.78 ± 0.019^{c}
AM + PSB	66 ± 3.5^{ef}	69 ± 3.1^{efg}	2.6 ± 0.24^{def}	38 ± 3.6^{de}	6.1 ± 0.73^{cde}	4.8 ± 0.61^{cd}	0.79 ± 0.041^{c}
AM + PGPR	58 ± 2.5^{cd}	62 ± 4.2^{de}	1.9 ± 0.14^{bc}	27 ± 2.9^{abc}	4.9 ± 0.53^{bc}	3.6 ± 0.45^{bc}	0.74 ± 0.040^{bc}
AM + PSB + PGPR	76 ± 4.2^{h}	74 ± 3.6^{fg}	2.4 ± 0.26^{cdef}	32 ± 3.5^{bcde}	5.3 ± 0.34^{cd}	3.9 ± 0.30^{bc}	0.74 ± 0.031^{bc}
Non-sterilized soil (NS)							
Control	45 ± 5.5^{ab}	41 ± 4.2^{b}	1.8 ± 0.22^{ab}	26 ± 3.0^{ab}	3.3 ± 0.57^{ab}	2.5 ± 0.33^{ab}	0.78 ± 0.049^{c}
AM	63 ± 4.1^{ef}	58 ± 3.3^{cd}	2.2 ± 0.18^{bcde}	35 ± 3.9^{cde}	5.6 ± 0.69^{cd}	3.9 ± 0.59^{bc}	0.70 ± 0.035^{abc}
AM + PSB	64 ± 4.7^{ef}	66 ± 5.3^{def}	2.4 ± 0.23^{cdef}	37 ± 4.3^{de}	6.7 ± 0.75^{de}	4.4 ± 0.66^{cd}	0.65 ± 0.028^{ab}
AM + PGPR	71 ± 3.6^{gh}	72 ± 5.2^{fg}	2.7 ± 0.27^{ef}	36 ± 4.6^{de}	7.3 ± 1.1^{ef}	4.6 ± 0.94^{cd}	0.62 ± 0.037^{a}
AM + PSB + PGPR	72 ± 5.9^{gh}	78 ± 3.7^{g}	2.9 ± 0.18^{f}	40 ± 3.4^{e}	9.0 ± 1.1^{f}	5.8 ± 0.96^{d}	0.66 ± 0.038^{ab}

AM, *Funneliformis geosporum*; PSB, *Pseudomonas fluorescens*; PGPR, *Azotobacter chroococcum*.
Means ± standard error in a column followed by different letter(s) are significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 6. Yield of *C. chinense* inoculated with native arbuscular mycorrhizal fungus, phosphate solubilizing and growth promoting rhizobacteria under pot conditions

Treatment	Number of days of flowering (per plant)	Number of flowers (per plant)	Fruit set (%)	Number of fruits (per plant)	Fruit fresh weight (g/plant)
Sterilized soil (SS)					
Control	85 ± 3.3^{g}	14 ± 2.9^{a}	46 ± 5.2^{a}	6.2 ± 0.86^{a}	9 ± 0.87^{a}
AM	58 ± 2.7^{de}	32 ± 4.2^{b}	73 ± 8.0^{b}	23 ± 3.0^{b}	38 ± 5.6^{b}
AM + PSB	47 ± 2.8^{bc}	41 ± 3.1^{cd}	83 ± 3.6^{bcd}	34 ± 3.6^{c}	56 ± 2.2^{d}
AM + PGPR	52 ± 3.3^{cd}	31 ± 4.0^{b}	74 ± 4.8^{bc}	24 ± 4.3^{b}	35 ± 3.8^{b}
AM + PSB + PGPR	61 ± 1.3^{e}	35 ± 2.9^{bc}	82 ± 4.7^{bcd}	29 ± 3.37^{bc}	43 ± 2.6^{bc}
Non-sterilized soil (NS)					
Control	68 ± 3.2^{f}	20 ± 2.2^{a}	54 ± 1.4^{a}	11 ± 1.1^{a}	15 ± 1.5^{a}
AM	52 ± 3.1^{bcd}	35 ± 3.3^{bc}	78 ± 4.1^{bc}	27 ± 3.3^{b}	37 ± 2.5^{b}
AM + PSB	47 ± 3.0^{bc}	42 ± 4.6^{cd}	85 ± 5.9^{cd}	36 ± 3.6^{cd}	49 ± 2.8^{cd}
AM + PGPR	45 ± 3.6^{b}	49 ± 2.2^{d}	86 ± 3.1^{cd}	42 ± 2.4^{d}	69 ± 4.8^{e}
AM + PSB + PGPR	38 ± 2.2^{a}	57 ± 3.5^{e}	92 ± 4.0^{d}	52 ± 2.3^{e}	76 ± 3.8^{f}

AM, *F. geosporum*; PSB, *P. fluorescens*; PGPR, *A. chroococcum*.
Means ± standard error in a column followed by different superscript letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

a range from 270 to 790 of AMF spores per 100 g dried soil of *C. frutescens* cultivated in organic farms of Thailand. The findings of the current study indicate that traditional shifting cultivation by slash and burn practice does not adversely affect AMF communities in the long run, as observed by Aguilar-Fernández *et al.* (2009). However, crop rotation and tillage generally influence the composition of AMF communities as well as the spore population and mycelial density in tropical and temperate agro-ecosystems (Priyadharsini *et al.*, 2012), indicating that agricultural practices are important factors that affect AMF diversity (Li *et al.*, 2007). Soil properties such as texture, pH and nutrient availability therein are also determining variables for the development of AMF. In the current study, soil samples collected during the pre-flowering stage of Naga King chilli revealed maximum AMF spore density followed by the fruit ripening stage. Rajeshkumar *et al.* (2013) reported that soil pH influences the status of AM spores in crop rhizosphere soil, and slightly acidic soils (pH 6.0–6.3) generally harbour a significantly greater number of AM propagules than soils with more acidic pH (5.3–5.7). Spore population of AMF was significantly and positively correlated

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Table 7. Nutrient concentrations in *C. chinense* tissues as influenced by inoculation of arbuscular mycorrhizal fungus, phosphate solubilizing and growth promoting rhizobacteria

	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
Treatment	Shoot	Root	Shoot	Root	Shoot	Root
Sterilized soil (SS)						
Control	1.3 ± 0.16^{a}	1.0 ± 0.12^{a}	0.17 ± 0.029^{a}	0.13 ± 0.014^{a}	2.3 ± 0.32^{a}	1.9 ± 0.26^{a}
AM	2.4 ± 0.16^{abc}	1.7 ± 0.19^{ab}	0.23 ± 0.017^{ab}	0.20 ± 0.015^{abcd}	4.2 ± 0.41^{cd}	2.4 ± 0.30^{abc}
AM + PSB	3.5 ± 0.33^{cd}	2.4 ± 0.25^{bc}	0.33 ± 0.036^{bcd}	0.24 ± 0.022^{cde}	4.8 ± 0.42^{cd}	3.2 ± 0.33^{bcde}
AM + PGPR	2.5 ± 0.25^{abc}	1.5 ± 0.13^{ab}	0.22 ± 0.030^{ab}	0.17 ± 0.018^{abc}	3.5 ± 0.22^{bc}	2.2 ± 0.24^{ab}
AM + PSB + PGPR	2.7 ± 0.24^{bcd}	1.8 ± 0.17^{ab}	0.26 ± 0.024^{abc}	0.21 ± 0.010^{bcd}	3.7 ± 0.37^{bc}	2.5 ± 0.30^{abcd}
Non-sterilized soil (NS)						
Control	2.0 ± 0.20^{ab}	1.3 ± 0.11^{a}	0.19 ± 0.025^{a}	0.16 ± 0.024^{ab}	2.7 ± 0.47^{ab}	1.9 ± 0.18^{a}
AM	3.1 ± 0.43^{bcd}	2.2 ± 0.28^{bc}	0.30 ± 0.031^{bcd}	0.23 ± 0.020^{bcde}	4.0 ± 0.44^{c}	2.7 ± 0.40^{abcd}
AM + PSB	2.9 ± 0.36^{bcd}	2.1 ± 0.33^{bc}	0.34 ± 0.052^{cd}	0.25 ± 0.030^{de}	4.8 ± 0.63^{cd}	3.2 ± 0.43^{cde}
AM + PGPR	3.4 ± 0.55^{cd}	2.4 ± 0.42^{bc}	0.38 ± 0.067^{d}	0.30 ± 0.059^{e}	4.8 ± 0.31^{cd}	3.4 ± 0.27^{de}
AM + PSB + PGPR	3.8 ± 0.61^{d}	2.7 ± 0.37^{c}	0.38 ± 0.079^{d}	0.29 ± 0.041^{e}	5.4 ± 0.34^{d}	3.9 ± 0.25^{e}

AM, *F. geosporum*; PSB, *P. fluorescens*; PGPR, *A. chroococcum*.
Means ± standard error in a column followed by different letter(s) are significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 8. AMF root colonization, spore numbers (SPN) and infective propagules of AMF in the rhizosphere of *C. chinense* as influenced by microbial inoculations under pot conditions

	AMF†					
	Colonization					
Treatment	%RLHC	%RLAC	%RLV	%RLTC	SPN (25 g soil)	No. of infective propagules (25 g soil)
Sterilized soil (SS)						
Control	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}
AM	25 ± 2.3^{bcde}	21 ± 2.3^{bc}	3 ± 0.80^{ab}	49 ± 3.8^{bc}	81 ± 5.4^{de}	25 ± 3.0^{b}
AM + PSB	34 ± 4.4^{fg}	28 ± 3.7^{de}	5 ± 1.6^{b}	67 ± 6.9^{e}	74 ± 4.6^{cd}	32 ± 3.2^{bcd}
AM + PGPR	24 ± 1.7^{bcd}	24 ± 2.1^{cd}	5 ± 0.88^{b}	52 ± 3.5^{cd}	71 ± 4.0^{cd}	25 ± 4.0^{b}
AM + PSB + PGPR	22 ± 1.4^{bc}	25 ± 2.1^{cde}	7 ± 2.5^{b}	54 ± 3.8^{cd}	65 ± 3.3^{c}	38 ± 3.0^{de}
Non-sterilized soil (NS)						
Control	21 ± 1.8^{b}	18 ± 2.6^{b}	3 ± 0.78^{ab}	42 ± 2.3^{b}	52 ± 6.8^{b}	29 ± 3.1^{bc}
AM	28 ± 1.3^{def}	26 ± 2.1^{cde}	5 ± 1.6^{b}	59 ± 1.8^{de}	104 ± 7.8^{f}	51 ± 6.1^{f}
AM + PSB	26 ± 2.6^{cde}	30 ± 2.9^{ef}	6 ± 1.8^{b}	63 ± 2.2^{e}	90 ± 4.5^{e}	34 ± 3.4^{cde}
AM + PGPR	30 ± 1.9^{ef}	29 ± 2.4^{ef}	5 ± 1.3^{b}	64 ± 3.4^{e}	87 ± 4.2^{e}	25 ± 2.3^{b}
AM + PSB + PGPR	37 ± 2.9^{g}	34 ± 2.5^{f}	5 ± 1.2^{b}	77 ± 5.3^{f}	118 ± 6.2^{g}	41 ± 2.5^{e}

†%RLHC, %RLAC, %RLV, %RLTC and SPN indicate percentage root length with hyphal coils, arbusculate coils, vesicles, total AMF colonization and spore number of AMF, respectively.
AM, *F. geosporum*; PSB, *P. fluorescens*; PGPR, *A. chroococcum*.
Means ± standard error in a column followed by different letter(s) are significantly different according to Duncan's multiple range test ($P < 0.05$).

with soil pH and %OC, which is in accordance with Singh *et al.* (2003) who reported a similar relationship between these variables from a natural forest of NE India. However, an array of environmental, host and fungal growth factors influence AMF diversity under natural field soils (Smith and Read, 2008).

Spores of 11 AMF species belonging to five genera associated with soils of *C. chinense* were isolated. A similar range of AM morphospecies (9–17 species) and dominance of *Glomus* species as that observed in the current study have recently been reported from rhizosphere of *C. annuum* and *C. frutescens* cultivated in different geographical areas with varied soil conditions of China (Chen *et al.*, 2012), Thailand (Boonlue *et al.*, 2012), India (Vyas and Vyas, 2012) and Vietnam (Vo *et al.*, 2015), whereas Carballar-Hernández *et al.* (2017) extracted a higher number of AMF morphotypes (33 species) from *C. annuum* rhizosphere in Puebla State, Mexico. Such variations in the occurrence of AMF

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Table 9. Pearson's correlation coefficients for plant growth, reproductive parameters, AMF structures and tissue nutrient concentrations ($n = 10$)

Variables		Plant growth parameters						Plant reproductive parameters				
		SL	NL	SD	RL	TDW	R/S	NDF	NF	FS	FN	FFW
AMF structures	SPN	0.732*	0.786**	0.810**	0.848**	0.873**	−0.474	−0.924***	0.852**	0.867**	0.832**	0.781**
	MPN	0.698*	0.670*	0.628*	0.694*	0.636*	−0.234	−0.685*	0.581*	0.679*	0.570*	0.483
	%RLHC	0.864**	0.922***	0.904***	0.899***	0.926***	−0.450	−0.964***	0.902***	0.939***	0.888**	0.857**
	%RLAC	0.770**	0.848**	0.881**	0.880**	0.894***	−0.308	−0.953***	0.871**	0.875**	0.849**	0.842**
	%RLV	0.895***	0.896***	0.766*	0.761*	0.748*	−0.320	−0.776***	0.707*	0.850**	0.705*	0.660*
	%RLTC	0.842**	0.905***	0.901***	0.898***	0.916***	−0.381	−0.964***	0.891***	0.922***	0.873**	0.852**
Nutrient (%)	TN	0.842**	0.879**	0.961***	0.971***	0.969***	−0.522	−0.939***	0.957***	0.928***	0.952***	0.939***
	TP	0.807**	0.830**	0.946***	0.946***	0.947***	−0.715*	−0.881***	0.958***	0.895***	0.955***	0.950***
	TK	0.779**	0.842**	0.952***	0.964***	0.974***	−0.592	−0.918***	0.978***	0.926***	0.974***	0.966***

SPN, spore number; MPN, most probable number; %RL HC, root length with hyphal coils; %RLAC, root length with arbusculate coil, %RLV, root length with vesicles; %RLTC, total root length colonization; TN, total plant nitrogen; TP, total plant phosphorus and TK, total plant potassium; SL, shoot length; NL, number of leaves; SD, shoot diameter; RL, root length; TDW, total dry weight; NDF, date of flowering; NF, number of flowers; FS, fruit set; FN, fruit number; FFW, fruit fresh weight.
*, **, ***: Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

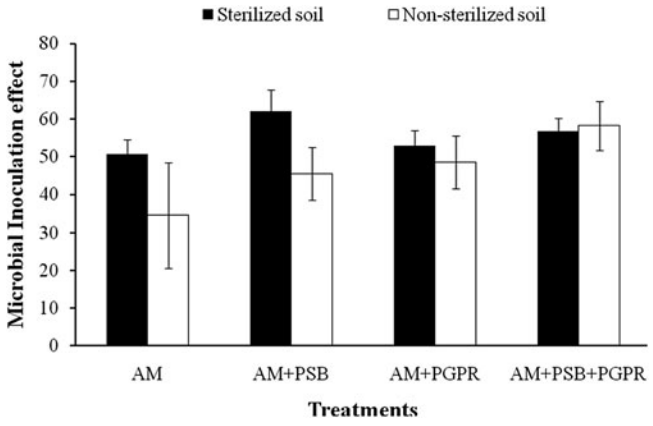

Fig. 3. MIE of *C. chinense* plants inoculated with native AMF, phosphate solubilizing and growth promoting rhizobacteria under pot conditions using sterilized and non-sterilized soils. Error bars indicate ± standard error.

species diversity in different habitats is in agreement with reports for a variety of agroecosystems, land use types and crop production systems (Oehl *et al.*, 2010; Verbruggen *et al.*, 2012). *Glomus* species possess the smallest spores among AMF taxa, which enables them to disperse easily and sporulate in large numbers within a short period. The high RA and IF of *F. geosporum* in the shifting cultivated field soil of NE India was interesting and agreed with the findings of Zhao and Zhao (2007), Oehl *et al.* (2010), Pandey *et al.* (2016) and Carballar-Hernández *et al.* (2017), suggesting that a large number of spore-producing AMF species can be considered as generalists showing ability to adopt to a wide range of habitats.

In the current study, *C. chinense* had typical *Paris*-type of AM morphology. In contrast, Muthukumar and Tamilselvi (2010) reported an intermediate- type of morphology in *C. annuum*. However, the ability of the AMF to form different morphologies in species of the same plant genera could be due to the influence of plant growth rates and environmental factors such as light intensity, temperature and soil moisture (Yamato and Iwasaki, 2002). Dickson *et al.* (2007) also indicated that AM morphology is influenced strongly by the identity of the host plant and may vary with ecosystems. The roots of Naga King chilli were colonized heavily by AMF throughout the crop growth period, which reveals the ubiquity of mycorrhizal association in tropical and sub-tropical agroecosystems. Nevertheless, the incidence of percentage root colonization, i.e. 57–75% in the current study, was lower compared with that of 90% in other *Capsicum* spp. reported by Muthukumar and Tamilselvi (2010). Boonlue *et al.* (2012) reported the AMF root colonization ranging from 46 to 60% in *C. frutescens* growing in organic farms of Thailand. Such variation in AMF colonization usually occurs due to the influence of edaphic and climatic factors such as elevation, temperature, rainfall, soil nutrient level and host plant specificity in different ecosystems, which may be altered by a variety of potential mechanisms including biological characteristics of the rhizosphere under host species, mycorrhizal dependency and host plant-mediated changes in soil microenvironments, all of which are known to affect the mycorrhization patterns (Smith and Smith, 2008; Wang *et al.*, 2013). At higher altitudes, extreme environments such as cool dry winter and late rainy season with heavy rainfall followed by soil erosion can also affect AMF sporulation and subsequently reduce root colonization by AMF. Differences in AMF communities and their root colonization

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patterns have been observed among plants, ecosystems, locations and seasons, and it seems inherent, therefore, that the coexisting AMF have distinct functional capabilities (Öpik *et al.*, 2006; Oehl *et al.*, 2010). Bhattarai and Mishra (1984) reported that percentage AM colonization increases with the age of the plant, as found in the current study.

The current results showed that AM colonization had a negative correlation with soil pH, N, P and K which is in accordance with the finding of Li *et al.* (2007). AMF could enhance uptake of P and other mineral elements by the plant, especially in a nutrient-deficient environment. Increasing soil P is known to suppress AMF formation, which may either be due to the direct effect of P on the external hyphal growth or an indirect effect associated with the P status of the plant (Muthukumar and Udaiyan, 2002). The AMF spore population was significantly and negatively correlated with total AM root colonization. This indicates clearly that the spore density of AMF community does not reflect exactly their root colonizing ability because of the possible existence of some non-sporulating AMF species (Daniell *et al.*, 2001). Furthermore, AM spore density may also be affected by the uneven spatial distribution of fungal spores and the complex structure of the underground root component.

Naga King chilli seedlings inoculated with biofertilizers revealed better growth compared with uninoculated controls under both sterilized and non-sterilized soil conditions. Okon (2014) also recorded a similar result in okra [*Abelmoschus esculentus* (L.) Moench] plants inoculated with AM fungus in sterile and non-sterile soils. Higher biomass and fruit yield as observed in bio-inoculated Naga King chilli plants corroborates the findings of earlier studies related with *C. chinense* and red bell pepper (Constantino *et al.*, 2008; Tanwar and Aggarwal, 2014). In the current study, inoculation with the AMF species i.e. *F. geosporum* alone was also found to improve chilli growth and yield significantly compared with uninoculated ones. Similar results were also observed in other studies with *C. annuum* (Davies *et al.*, 2002) and *C. frutescens* (Elahi *et al.*, 2012), even when AM were applied along with *P. fluorescens* (PSB) and *A. chroococcum* (PGPR), thus exhibiting high biological specificity between AMF strain and the host plant species (Constantino *et al.*, 2008). Free-living soil bacteria such as *P. fluorescens* and *Azotobacter* sp. can increase microbial populations in the rhizosphere of mycorrhizal plants and are known to affect the growth of the AMF hyphae through their influence on spore germination (Barea and Jeffries, 1995). The current study also confirmed a similar interaction between AM fungus and *P. fluorescens* or *A. chroococcum*. *P. fluorescens* was more compatible with the AM fungus compared with the consortium involving *A. chroococcum* in colonizing host roots. The dual inoculation with AM and *P. fluorescens* showed stimulatory effects on chilli plant growth and biomass compared with uninoculated control, which may be due to the acquisition of more P from the soil and its accumulation resulting in increased plant root length and shoot and root dry biomass in sterile soil (SS) treatments. However, higher shoot length was recorded in the treatments with combined inoculation of AM + PSB + PGPR, which is in accordance with the findings of Okon (2014). Further, the low soil P might have increased the plant's responsiveness to AM colonization.

The current findings revealed that chilli plant height was higher in SS than in NS soil. Due to the mobilization of ammonium and nitrate-like soil nutrients, in particular, autoclaved soils reduced competition between soil-borne microorganisms and the plant roots to obtain more nutrients (Ortas, 2003). Whereas inoculation with all three bioinoculants under NS soil conditions showed a maximum increase in all studied plant growth parameters compared with the control. These results differ from those of Okon (2014) and Ortas (2015), who reported that plant growth response including shoot and root dry biomass decreased randomly in NS treatments compared with SS conditions. The presence of either PSB or PGPR along with mycorrhizal inoculum can synergistically improve plant growth (Gamalero *et al.*, 2004). Such improved plant growth and yield parameters could be possible due to the establishment of higher root colonization by native AMF species that increased the water and nutrient uptakes from soil and decreased the transplanting shock of chilli pepper (Castillo *et al.*, 2013).

In the current study, combined inoculation of PSB and PGPR along with AMF reduced the number of days for flowering and increased the number of flowers, fruit set and fruit yield of Naga King chilli. Indeed, fruits appeared in the uninoculated control also but were less in number and weight. Tanwar and Aggarwal (2014) also found a better result in plant reproductive parameters such as increased number of flowers and fruit yields in red bell pepper (F1 hybrid, Indam Mamatha) under pot experiments. AMF might have stimulated the population of bioinoculants such as *P. fluorescens* and *A. chroococcum* in the soil rhizosphere by directly providing energy-rich carbon compounds derived from the host assimilation and transported through fungal hyphae to the mycosphere, changes in suitable pH and also by secretion of some stimulatory substances (Johansson *et al.*, 2004). Plant nutritional status of AM inoculated plants was significantly different under various treatments and soil conditions. In SS, AM + *P. fluorescens* inoculated plants had better shoot and root P, N and K concentrations compared with uninoculated control. This could be due to the secretion of phosphatases by AMF and *P. fluorescens*, which is also considered a common mode of conversion of insoluble P to soluble forms, thus enhances the nutrient uptake efficiency of plant roots from the soil and directly influences plant growth and yield (Alori *et al.*, 2017). Further, solubilization of inorganic P sources by synthesis of organic acids is possible by *P. fluorescens* inoculation. The presence of either PSB or PGPR or both along with mycorrhizal inoculum can synergistically improve plant growth.

In conclusion, the current findings suggest that the studied *C. chinense* rhizosphere soil harbours a diverse AMF community relative to other tropical and sub-tropical habitats. *Glomus* was the dominant genus which was found during all three growth stages of Naga King chilli grown under shifting cultivated lands. Roots of Naga King chilli were found to be heavily colonized by AMF throughout the crop growth period which reveals the ubiquity of mycorrhizal association in this region of the agro-ecosystem. The studied chilli cultivar treated with different bioinoculants revealed enhanced growth and yields, which could be attributed to improvement in the physiological and reproductive status of plants, as well as the nutrient contents in shoots and roots under sterilized and non-sterilized soil conditions. Hence, the application of indigenous AMF along with efficient bioinoculants during seedling transplantation can be considered as potential bioagents that increase the production of chillies in NE India.

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