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Effect of an indigenous AM and PGPR combination on chilli growth and productivity in lateritic soil

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ABSTRACT
Infertile lateritic soil is particularly deficient in phosphorus (P) and Nitrogen (N). Arbuscular Mycorrhiza (AM) has a key role to uptake bound P from the soil and provide to the plants growing under P-poor conditions and improve water and nutrient uptake. *Azotobacter* fixes free nitrogen and phosphate solubilizing bacteria (PSB) release bound phosphate, are the important groups of plant growth-promoting rhizobacteria (PGPR), sometimes they may act as mycorrhiza helper and applied with AM as biofertilizer. This pot experiment was conducted to determine the primary impact of singly and combined application of native bio-inoculants, the AM, *Acaulospora*, and the PGPR, *Azotobacter* and *Pseudomonas* sp. (PSB) on growth and yield of chilli (*Capsicum frutescens* L.), growing in acid lateritic soil. Inoculated treatments were compared for growth and productivity of chilli in terms of height, leaf number, leaf area, root collar diameter, number of flowers and number of fruits, final fresh and dry yield. The productivity of chilli showed a maximum in combined treatment of *Acaulospora*, *Azotobacter*, and PSB. Also the AM spore count and root colonization found maximum in that treatment. Hence the application of indigenous AM inoculation along with native PGPR, *Azotobacter* and PSB may present better productivity in low fertile lateritic soil.

Keywords: *Acaulospora*, *Azotobacter,* infertile soil, mycorrhiza, PSB

Efecto de una combinación indígena de AM y PGPR sobre el crecimiento y la productividad de los chiles en suelos lateríticos

RESUMEN
El suelo laterítico infértil es particularmente deficiente en fósforo (P) y nitrógeno (N). Las micorrizas arbusculares (AM) tiene un papel clave para absorber el P unido del suelo y proporcionar a las plantas que crecen en condiciones de P pobre y mejorar la absorción de agua y nutrientes. *Azotobacter* que fija el nitrógeno libre y las bacterias solubilizadoras de fosfato (PSB), son grupos importantes de rizobacterias que promueven el crecimiento de las plantas (PGPR). A veces pueden actuar de conjunto con micorrizas y aplicarse con AM como biofertilizante. Este experimento en maceta se realizó para determinar el impacto primario de la aplicación individual y combinada de bio-inoculantes nativos, AM *Acaulospora* y PGPR *Azotobacter* y *Pseudomonas* sp. (PSB) sobre el crecimiento y el rendimiento del chile (*Capsicum frutescens* L.), que crece en suelo ácido laterítico. Los tratamientos inoculados se compararon para el crecimiento y la productividad del chile en términos de altura, número de hojas, área foliar, diámetro de raíz, número de flores, número de frutos, rendimiento final fresco y seco. La productividad de los

chiles mostró un máximo en el tratamiento combinado de *Acaulospora*, *Azotobacter* y PSB. También el recuento de esporas de AM y la colonización de raíces encontraron el máximo en ese tratamiento. De ahí la aplicación de la inoculación de AM indígena junto con PGPR nativo, *Azotobacter* y PSB pueden presentar una mejor productividad en suelos lateríticos de baja fertilidad.

Palabras clave: *Acaulospora, Azotobacter*, suelo infértil, micorriza, PSB

INTRODUCTION

The fertility level of dry acid lateritic soil is very low. The soil is naturally deficient in phosphorus, nitrogen, and other essential nutrients like calcium, magnesium, etc. In this type of soil, nutrients remain unavailable to the plant, resulting in unfavourable conditions for plant growth (Koley, 2000). In conventional agricultural practices, uses of overdose agrochemicals day by day increasing of low fertility and toxicity in the soil in agricultural fields and pesticide-resistant pests throughout India. As a result of these real problems, adverse effects on health are being reflected (Mittal *et al*., 2013; Rahman and Debnath *et al*., 2015). Therefore, in modern agriculture strategy, the huge demand for producing organically toxic-free vegetables is increasing, which also cost-effective and environment-friendly (Madhusudhan, 2016; Pandey and Singh, 2012).

Mycorrhiza is the mutualistic root–fungus relationship between non-pathogenic soil fungi and the plant roots (Frank, 1885; van der Heijden *et al*., 2015). Arbuscular mycorrhiza (AM), belonging to the phylum Glomeromycota (Redecker *et al*., 2000; Kapoor *et al*., 2008), are the most common symbiotic association of mycorrhiza found in 90% of land plants (Schüßler *et al*., 2001; Brundrett, 2002; Smith and Read, 2008). AM colonizes in the cortical tissue of roots during active plant growth (Kirk *et al*., 2001) and procures nutrient form beyond the nutrients depletion zone of roots (Li *et al*., 1991). Organic acids and phosphatases produced by AM help to release of available phosphorous from unavailable complexes (Bagyaraj, 1984; Entry *et al*., 2002; Fomina *et al*., 2005). They also increase nitrogen and carbon content in soil (Almas *et al*., 2004). The fine extra-radical hyphae of arbuscular mycorrhizal fungi (AMF) involve in the nutrient process and uptake phosphorous (Bowen, 1973; Bucher, 2007), provide nitrogen (Madder *et al*., 2000; Chalot *et al*., 2006; Nuccio *et al*., 2013) by absorbing ammonium, nitrate and amino acids (Hodge *et al*., 2001), and deliver other plant micronutrients like copper, zinc, etc. (Smith and Read, 1997), and in exchange, withdraw organic sugar from plant roots (Kottke, 2002).

AM hyphae can absorb water from lower water potential than the plant roots (Bethlenfalvay *et al*., 1988; Püschel *et al.*, 2020) and form a mycelial mat which promotes retaining soil moisture. AM symbiosis is effective to overcome low pH in acidic soil which restrict plant growth (Clark, 1997; Muthukumar *et al*., 2014). The increasing water and nutrient uptake ability of AMF in low fertile and dry condition (Smith and Read, 1997; Aúge, 2001) provide improved plant growth in less-nutrient soil, such as acid lateritic soil (Weber *et al*., 1992; Brundrett, 2009). They also protect plants from pathogenic fungi and nematode disease (Bagyaraj, 1984). Nowadays, arbuscular mycorrhizal inoculation to the crops has become an appreciable alternative that increases the growth and yield (Mishra and Verma, 1982; Johansen *et al*., 1993; Ghosh and Verma, 2006; Samanta and Verma, 2006; Sengupta *et al*., 2006; Robinson *et al.*, 2016; Chukwuka *et al.*, 2017).

Acaulospora is the AM fungal genus belonging to the family Acaulosporaceae, has been distributed in more than 30 species (Simanungkalit, 2006). *Acaulospora* sp. are a common dominant group of arbuscular mycorrhiza at the lateritic ecosystem (Ghosh and Verma, 2011). A special feature, having the sporiferous saccule with the saccule subtending hypha present in *Acaulospora* (INVAM, 2018).

The plant-growth-promoting rhizobacteria (PGPR), present in plant mycorrhizosphere, promote the growth of the plant (Andrade, 2004; Giri *et al*., 2005). *Azotobacter* and phosphate solubilizing bacteria are the important plant growth promoters (PGP) and act as mycorrhiza helper, having an important role in the growth of hyphae from germinating

AM spores, colonization of plant roots by AM fungi and growth of external AM hyphae and dehydrogenase activity of the AM fungus (Burla *et al*., 1996). *Azotobacter* is free-living soil bacteria, fix free nitrogen directly from the environment. Inoculation of *Azotobacter* sp. to the plant has showed improved beneficial effects in seed germination, shoot, root biomass, and yield (Kumar *et al*., 2000; Kumar *et al*., 2009; Malik *et al*., 2009; Reddy *et al*., 2003). Phosphate solubilizing bacteria (PSB) can release bound phosphate to the available P form (Bhattacharyya and Jain, 2000; Ponmurugan and Gopi, 2006). Many research works related to plant growth promotion by the application of phosphate solubilizers have been reported earlier (Khiari and Parent, 2002; Saxena and Sharma, 2003; Yazdani *et al*., 2009).

Chilli (*Capsicum frutescens* L.) is the part of the Solanaceae family, is a very essential ingredient in our daily vegetables. It is rich in volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, proteins, fibers, and minerals and acts as a natural bactericidal, having medicinal uses to treat muscle pain, cough, asthma, sore throat, etc. (Bosland and Votava, 2000). As it is an economically important crop, the average yield of chilli in India is lower as compared to other developed countries (APEDA, 2019; FAOSTAT, 2017). Cropping in unused barren land containing infertile soil such as lateritic soil may become a suitable solution.

The present research was conducted to determine the primary impact of singly and combined application of native bio-inoculants, the AM, *Acaulospora*, and the PGPR, *Azotobacter* and *Pseudomonas* sp. (PSB) on growth and yield of chilli (*Capsicum frutescens* L.), growing in acid lateritic soil.

MATERIALS AND METHODS

Plant material

Certified seed of chilli (*Capsicum frutescens* L.) were collected from District Agricultural Head office, Paschim Medinipur, West Bengal, India. Healthy seeds were selected manually and surface disinfected by immersion in an aqueous solution of 0.1% (w/v) mercuric chloride ($HgCl_2$) for 3-4 min followed by washing three times with autoclaved deionized water under aseptic conditions in a laminar flow chamber.

Experimental conditions

The experiment was conducted with chilli (*C. frutescens*) planted in sterilized lateritic soil (Sylvia, 1994). It was done in 22.30° N Latitude and 87.20° E Longitude in Midnapore subdivision of West Bengal in the pre-winter season. The primary soil characteristics were tested according to Jackson (1973), having pH 5.61, electrical conductivity (EC) of 0.18 m mohs/cm^2, moisture content of 2.7%, organic carbon (OC) of 0.63 g kg^{-1}, total nitrogen (N) of 0.04% and phosphate (P) of 0.03%.

Isolation of the AM propagules

Separation and isolation of indigenous AM propagules from the native ecosystem was done by following the wet sieving and decanting technique (Gerdermann and Nicolson, 1963). Spores of single species were separated morphologically using a LABOMED CSM2 microscope, and surface disinfected by 200 µg ml^{-1} streptomycin and 2% Chloramine-T solution (w/v) (Mosse, 1973).

The pure culture of each inoculum was cultured in sterilized sand and soil mixture. The soil: sand (1:1, v/v) mixture was sterilized by oven drying at 85 °C for 8 h with a gap of 48 h and again dried for 8 h (Sylvia, 1994). Pure cultures were done in funnels and mass cultures in surface-disinfected earthen pots, grown in an automated growth chamber with sorghum (*Sorghum vulgare* L.) plants. The mass cultures were maintained up to 90 days in the growth chamber at 26±2 °C under a photoperiod of 16 h light and 8 h dark, an irradiance provided by 110 W fluorescent lamps (Philips, India).

The successful mass culture of the indigenous AM inocula was identified morphologically by following Schenck and Perez (1990) and the INVAM web photo guide (INVAM, 2018) and confirmed as *Acaulospora* sp.

Bacterial cultures

Azotobacter was isolated from lateritic rhizospheric soil in Ashbey mannitol agar media for free-living nitrogen-fixing bacteria (Pelczar *et al*., 1957; Subba, 1977). Pikovskaya media for native phosphate

solubilizing bacteria (Pikovskaya, 1948; Sundara and Sinha, 1963; Subba, 1977) were used. *Azotobacter* sp. and *Pseudomonas* sp. (PSB) were confirmed according to Bergey manual (Holt and Krieg, 1984; Krieg *et al*., 1994).

Seedling inoculation

Surface disinfected seeds of chilli were germinated in aseptic conditions and seeded with a 10 cm gap from each other in a seedling tray within a growing chamber under the environment at temperature 25-30 °C, humidity 65-70% and 15-16 h of lighting. Sterilized soil and sand mixture in 2:1 (v/v) ratio was used as growing substrate.

After germination, the healthy chilli seedlings were transferred to polythene bags (18 X 24 cm) filled with 3 kg sterilized soil (one seedling per pot) to grow for 90 days in the greenhouse. AM inoculation was done by soil-based inoculum obtained from mass culture (20 spores /g, 25 g /pot), applied under 4 cm from the soil surface. 10 ml of the bacterial cultures contained about 10^8 CFU ml^{-1} liquid medium were applied per pot.

The experiment was designed in a randomized block with eight treatments and three replicates. The treatments of this experiment consisted with T_0 = No inoculation (Control), T_1 = *Azotobacter* (AZO), T_2 = Phosphate solubilizing bacteria (PSB), T_3 = *Acaulospora* sp. (Ac), T_4 = *Azotobacter* + PSB (AZO+PSB), T_5 = *Acaulospora* sp. + *Azotobacter* (Ac+AZO), T_6 = *Acaulospora* sp. + PSB (Ac+PSB), T_7 = *Acaulospora* sp. + *Azotobacter* + PSB (Ac+AZO+PSB).

Plant parameters were measured at 30, 60 and 90 days after plantation (dap) in three replicates per treatment in terms of shoot height (cm), leaf number per plant, leaf area (cm^2), the total number of flower and fruit appeared per plant, and total fresh weight (g) and dry mass (g) of fruit yield per plant. The fresh yield was calculated by summing the total fresh weight of fruits obtained. The dry mass was measured by drying the fruits in a hot air oven at 65 °C for 48 hours until a constant weight was obtained.

Rhizospheric soil samples were tested for the AM spore population by wet sieving and decanting technique (Gerdermann and Nicolson, 1963). Plant root samples were treated with 10% KOH and stained with tryphan blue (Phillips and Hayman, 1970) to study the colonization percentage by the equation 1:

$$\text{Root colonization \%} = \left(\frac{\text{Number of root pieces colonized}}{\text{Total number of root pieces observed}}\right) X\ 100$$

Statistical analysis

Statistical analysis of data was done by comparison of means using the least significant difference (LSD) at $p < 0.05$ probability, after the preforming analysis of variance (ANOVA) using IBM SPSS 20 and MS Excel 2013. Pearson correlation coefficient between variables was analyzed at $p < 0.05$ and $p < 0.01$ level of significance.

RESULTS AND DISCUSSION

The inoculation of chilli seedlings with an indigenous AM and PGPR combination increased the growth since the early stage, with prominent effect of AM.

At 30 dap, the treatment T_7 having *Acaulospora* sp. + *Azotobacter* + PSB (Ac+AZO+PSB) showed height, leaf number, leaf area and root collar diameter slightly superior. Nevertheless, after 60 days of culture, the plants in treatment Ac+AZO+PSB (T7) were 23.66% higher than the control, followed by Ac+AZO (21.3%), Ac+PSB (15.5%) and Ac (10.6%). The same pattern was observed in the total leaf number and root collar diameter. Leaf area was in Ac+AZO+PSB 14.3%, in Ac+AZO 11.1% and in Ac 10.0% higher than the control.

The first appearance of flowering have few days of difference among the treatments. It was observed on 26th day after plantation in AZO+PSB and Ac+AZO+PSB, at 28th day in Ac+AZO and at 29th day in AZO, Ac, and Ac+PSB. On the other hands, first fruiting was observed at 33rd dap in AZO+PSB followed by Ac+AZO+PSB and Ac+AZO at the 36th day; and Ac and Ac+PSB at 41st day.

After 90 dap, in treatment with the combination of AM and PGPR (Ac+AZO+PSB, T7) all the parameters reached the maximum, without significantly differences with Ac+AZO (T5) in plant height, leaf number and root collar diameter. As well as T7 treatment was similar to T6 (Ac+PBS) in root collar diameter (Table 1). The shoot height in Ac+AZO+PSB was 31.64% superior to control and the leaf number 37.8%. The total fruit number was found maximum in Ac+AZO+PSB, 127.5% better than control and significantly higher ($p<0.05$) all the rest. A similar result was observed in the total fresh and dry mass of fruits yield per plant (Figure 1).

Table 1. Effect AM and PGPR on chilli (*Capsicum frutescens* L.) plants parameters after 90 days after plantation.

Treatments	Height (cm)	Leaf Number	Leaf Area (cm^2)	Root Collar Diameter (cm)	Flower Number	Fruit Number
T_0 Control	17.38 c	28.3 b	2.58 b	0.98 c	5 b	3.66 d
T_1 (AZO)	19.5 b	30.6 b	2.68 b	1.13 b	7 b	5 c
T_2 (PSB)	18.74 c	29 b	2.63 b	1.07 c	6 b	5 c
T_3 (Ac)	20.52 b	31.3 b	2.79 b	1.29 a	7 b	6.33 b
T_4 (AZO+PSB)	20.12 b	31 b	2.77 b	1.2 b	6.6 b	5 c
T_5 (Ac+AZO)	21.18 a	36.6 a	2.95 b	1.35 a	8 a	7 b
T_6 (Ac+PSB)	20.76 b	34.6 b	2.82 b	1.3 a	7.6 a	6.33 b
T_7 (Ac+AZO+PSB)	22.88 a	39 a	3.05 a	1.38 a	8.6 a	8.33 a

Data with different letters in the same column indicate significant differences at $p<0.05$ according to LSD test. AZO-Azotobacter *sp., Ac*- Acaulospora *sp., PBS*-Pseudomonas *sp.*

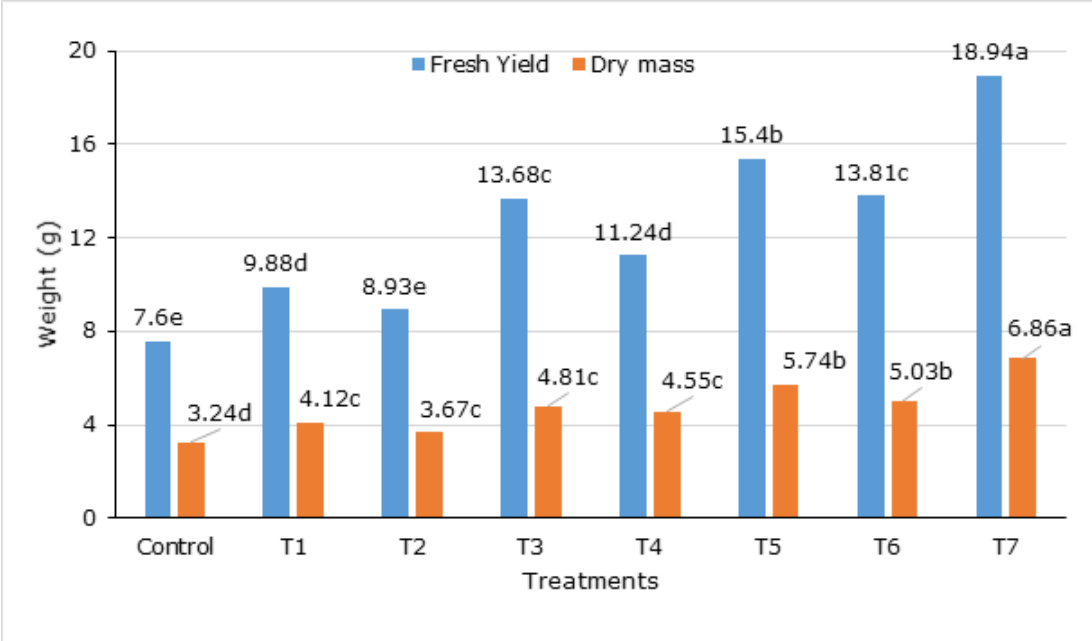

Figure 1. Fresh yield and dry mass of *Capsicum frutescens* L. plants inoculated with AM and PGPR. Treatments T_1 (AZO), T_2 (PSB), T_3 (Ac), T_4 (AZO+PSB), T_5 (Ac+AZO), T_6 (Ac+PSB), T_7 (Ac+AZO+PSB). AZO-*Azotobacter* sp., Ac- *Acaulospora* sp., PBS-*Pseudomonas* sp.

In single inoculation, the effect of AM was superior than *Azotobacter* and PSB in root collar diameter and fruit number. In dual inoculation, the combination of AM and *Azotobacter* was better than AM and PSB in height and leaf number. Plants inoculated with *Azotobacter* sp. have shown an increase in shoot and yield effects. It have been tested on different crops (Reddy *et al*., 2003; Singh and Rana, 2005; Kumar *et al*., 2009; Malik *et al*., 2009). The dual effect of inoculation of AMF and *Azotobacter* was found encouraging in earlier research work also (Paul *et al*., 2011; Dal *et al*., 2018; Shaimaa and Massoud, 2017). *Azotobacter* adds only nitrogen in soil and PSB release available phosphate, AM mainly translocate nutrients including micronutrients through the extensive mycelial network (Kayama and Yamanaka, 2014; Rouphael *et al.*, 2015) and also release phosphates by phosphatase (Zhang *et al.,* 2014).

Hence, AM alone is found almost sufficient for nutrition rather than *Azotobacter* or PSB in single inoculation. In dual treatments, also uptake ability of AM was in advance than the only supply of nutrients, and *Azotobacter* as a source of N and AM as P substitute worked best. Interaction between the AM, *Acaulospora* with *Azotobacter* and PSB, in this triple application may provide the development of plant growth and yield (Nadeem *et al*., 2014; Hashem *et al*., 2016). Enhanced supply of N and P in the rhizosphere by PGPR boosts up AM uptake in the triple inoculation resulting establishment of better mycorrhizal activity (Selvakumar *et al*., 2012; Vafadar *et al*., 2014; Raklami *et al*., 2019).

The mycorrhizal infection and spore population were found maximum in Ac+AZO+PSB (T7) treatment followed by MA combined with PGPR (Ac+AZO, Ac+PSB) (Figure 2). Mycorrhizal colonization was mostly mycelial and arbuscular.

Mycorrhizal colonization with class – III intensity produced in Ac+AZO+PSB and Ac+AZO. In these treatments, colonization percentage were observed higher than the inoculation formed in Ac alone. A strong significant positive correlation ($r=0.997$, $p<0.05$ and $p<0.01$) was observed between AM root colonization and spore density. They were also showed a significant positive correlation ($p<0.05$ and $p<0.01$) with all the measured plant parameters, including fresh yield and dry yield. The last one were also positively and significantly correlated ($p<0.05$ and $p<0.01$) with plant height.

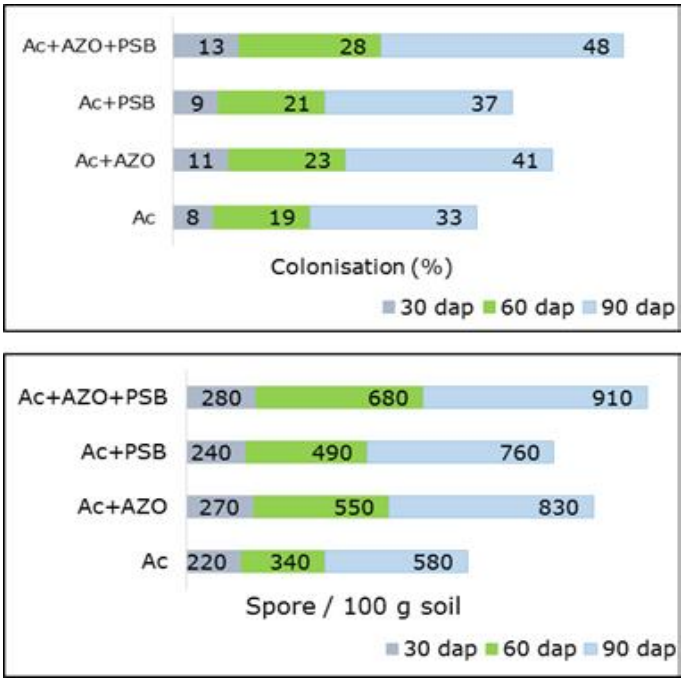

Figure 2. Mycorrhizal colonization and spore population in root and rhizosphre of chilli plant inoculates with AZO-*Azotobacter* sp., Ac- *Acaulospora* sp., PBS-*Pseudomonas* sp.

In all the AM related treatments, arbuscular colonization and spore formation occurred. Formation of arbuscles and high-intensity colonization in the plant roots indicates active symbiosis between *Acaulospora* and *C. frutescens* in those treatments. As the natural propagules in lateritic soil are low, the mycorrhizal application to the plant increased the growth and yield (Ghosh and Verma, 2006; Hempel *et al*., 2013). AM are more active in nutrient-poor dry soils, plants depended more on mycorrhizae for nutrients and moisture in this condition (Brundrett, 2009; Begum *et al*., 2019). The mycorrhization facilitates an effective hyphal network that spreads throughout the rhizosphere and improves the nutrient absorbing ability of the host plant (Avio *et al*., 2006; Püschel *et al*., 2020). Active mycorrhizal colonization in the plants alters some physiological processes and increases water and nutrient uptake which enhances crop growth and yield (Oyetunji *et al*., 2003; Mardukhi *et al*., 2011; Douds *et al*., 2005), which also influenced the chilli plant (Gaur *et al*., 1998; Vyas and Vyas, 2014).

The test plant, *C. frutescens* establish showed AM dependency on the dominant indigenous AM genus, *Acaulospora,* in this lateritic zone (Sieverding, 1991; Ghosh *et al*., 2008), which shows active adaptation and efficacy in native soil (Oliveira *et al*., 2005; Samanta and Verma, 2006; Akib *et al.*, 2018). The triple application of AM, *Azotobacter*, and PSB were found to influence the growth of the crops too (Shwetha and Lakshman, 2013; Lallawmkima *et al*., 2018), though with other AM species, not indigenous or in lateritic soil.

Enhancement in productivity of chilli through the mycorrhization established in earlier works (Selvakumar and Thamizhiniyan, 2011; Bhuvaneswari *et al.*, 2014). The mycorrhizal inoculation can enhance the yield of chilies significantly and reduced the application of chemical fertilizers by up to 50 percent (Bagyaraj and Sreeramulu, 1982). Application of AM with such mycorrhiza helping rhizobacteria develops a synergistic microbial interaction (Artursson *et al*., 2006) and maybe a substitute for chemical fertilizer particularly in this dry acidic soil.

CONCLUSIONS

Application of indigenous AM, *Acaulospora* sp. along with beneficial and native plant growth-promoting rhizobacteria, *Azotobacter* and PSB facilitate *C. frutescens* L. better growth and enhanced the yield grown in infertile dry and acid lateritic soil. Such AM and microbial consortium could be benefit other crops also in this soil type and organic agriculture and horticultural practice particularly in other poor nutrient soil and in surely reduced cost in comparison to chemical fertilizers.

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Conflict of interest

This work was done all together and no potential conflict of interest present among the authors.

Author contributions

Conceptualization SG, Data curation DK and BM, Formal analysis DK, Funding acquisition SG, Investigation SG, Methodology DK, BM and SM, Project administration SG, Resources SG and GB, Supervision SG, Software DK and SM, Validation SG, Visualization GB, Writing—original draft DK, Writing—review & editing SG.

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