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EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON *Camellia sinensis* (L.) O. KUNTZE UNDER GREENHOUSE CONDITIONS

Deepa Sharma* and Highland Kayang

Microbial Ecology Laboratory, Centre for Advanced Studies in Botany, North-Eastern Hill University, Shillong-793022, Meghalaya, India

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Arbuscular mycorrhizal fungi

Camellia sinensis

Consortium

Inocula

plant growth parameters

ABSTRACT

The effects of arbuscular mycorrhizal fungi (AMF) on growth of tea both singly and in consortia were carried out in greenhouse environment for a period of 1 year. The effectiveness of native AMF species, viz., *Acaulospora scrobiculata* (*A.s.*), *Glomus macrocarpum* (*G.m.*), *Rhizophagus intraradices* (*R.i.*), on growth performance of *C. sinensis* plants were compared. The inoculating AMF noticeably increased the plant growth parameters such as number of leaves, leaf area, plant height, shoot length, root length, root and shoot weight (fresh and dry) and root: shoot (fresh and dry weight) ratio. The effect of individual and combined inocula of two as well as all the three different species of AMF on the growth parameters varied. In most of cases, consortium of all the three AMF species (*A.s.* + *G.m.* + *R.i.)* performed consistently better than the other inocula containing single as well as two AMF species. AMF colonization ranged from 30.69% to 81.26%. The study revealed the potential of AMF inoculation on growth of *C. sinensis*, regardless of single or mixed AMF consortium; thereby providing a promising resource for sustainable tea cultivation.

* Corresponding author

E-mail: bablideepa89@gmail.com (Deepa Sharma)

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1 Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze; Family: Theaceae] is a monoculture perennial evergreen shrub growing in a wide range of different soil types. Tea is of considerable interest being a widely consumed non-alcoholic beverage and a source of many pharmaceutically important compounds and possesses health benefits (Mondal et al., 2004). In India, the principal tea growing regions are located in the North-East and South-region. Assam is the major producer contributing about 53% of the total Indian production (www.nabard.org/.../plant_tea.asp). Tea cultivation has become a blooming business, playing a major role in the economy of this region.

In tea plantations, agrochemicals are extensively used for managing insects, pests, diseases and weeds. Considerable gain in leaf harvest has made the chemicals so popular that almost all of the tea estates adopt chemical inputs for production of tea. The dose of chemical fertilizers (N,P,K) depends on average yield of the previous pruning cycle. Insecticides such as Deltamethrin, Bifenthrin, Endosulfan and many more, fungicides such as Hexaconazole, Propiconazole and a few more are sprayed soon after plucking. These agrochemicals can be toxic to the non target organisms present in the system. However, exploitation of soil microorganisms offers an attractive alternative to the use of chemicals (Hazarika et al., 2003). The use of organic fertilizer and microbial inoculants or biofertilizers represents a sustainable alternative to high input of chemical fertilizers used in the conventional production systems (Tanwar et al., 2013).

Presence of biofertilizers in the soil enhances the availability of important nutrients to the plants and also protects the plants from soil pathogens. Biofertilizers reduce the need for costly plant fertilizer and do not pollute the environment. Bio-agents such as *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* are already being used in many tea plantations. Arbuscular mycorrhizal fungi (AMF) are considered as important components of agro ecosystems because of their potentiality to increase plant growth performance, improve soil-plant-water relations, enhance plant nutrient acquisition, resistance to disease and environmental stresses, improve soil quality, and productivity of the plants (Songachan, 2012). However, differences in AMF colonization rates relate to changes in the community composition of AMF and other variables such as the inoculum density, growth of roots, genetic compatibility between host plants and AMF, edaphic aspects and microbial activity (da Silva et al., 2017).

Due to the beneficial effects of mycorrhizal fungi on plant growth, inoculation of seedlings with mycorrhizal inoculants is becoming popular in agricultural as well as land reclamation industries (Wijesinghe, 2012). Therefore, the main objective of this study was to develop a strategy for promoting tea cultivation by introducing biofertilizer using AMF inoculation for substituting the chemical fertilizers, either partially or completely.

2 Materials and methods

2.1 Isolation and identification of AMF

Soil samples were collected from the rhizosphere soil of *C. sinensis* growing in Ghagra of Udalguri district (105.16 m above MSL, 26.7452° N, 92.0962° E), Assam, India. Dominant AMF species were isolated by wet sieving and decanting method of Gerdemann & Nicolson (1963). The isolated spores were identified based on morphological descriptions published by International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu), AMF phylogeny (www.amf-phylogeny.com) and Oehl & Sieverding (2004). *Acaulospora scrobiculata* Trappe, *Glomus macrocarpum* Tul. & Tul. and *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schubler were found to be the dominating AMF species.

2.2 Propagation and preparation of AMF inocula

To obtain monospecific cultures of these native AMF species from the rhizosphere of *C. sinensis*, each fungi was used to inoculate *Oryza sativa* L. and *Zea mays* L. in sterilized sand - soil substrate in 1:1 ratio and were propagated following the method of INVAM (http://invam.caf.wvu.edu). After five months, the roots of the host plants were chopped and the air-dried root/soil mixture was stored in air tight gallon zip-loc plastic bags at 4°C, which was then used as inoculum for each individual target AMF species. Seven different inoculants (either singly or in combinations) were prepared from three different indigenous AMF species, viz., *A. scrobiculata* (*A.s.*), *G. macrocarpum* (*G.m.), R. intraradices* (*R.i.*), *A. scrobiculata* + *G. macrocarpum* (*A.s.* + *G.m.*), *A. scrobiculata* + *R. intraradices* (*A.s.* + *R.i.*), *G. macrocarpum* + *R. intraradices* (*G.m.* + *R.i.*), *A. scrobiculata* + *G. macrocarpum* + *R. intraradices* (*A.s.* + *G.m.* + *R.i.*).

Single nodal stem cuttings were obtained from the healthy mother bushes of *C. sinensis* (L.) O. Kuntze at Ghagra of Udalguri district (105.16 m above MSL, 26.7452° N, 92.0962° E), Assam, India. Surface sterilization of these stem cuttings were performed by treating them with Bavistin (0.1%; w/v) for 2 minutes. Individual cuttings were then transplanted in sterilized plastic bags containing sterilized sand-soil substrate (1:1 v/v) in the month of July, 2015. Inoculation with corresponding AMF was ensured by placing 10 g each of seven different inoculants in the soil just below the cuttings. Cuttings without inoculants, i.e., non-mycorrhizal (control) were also maintained. Each treatment as well as the control were maintained in six replicates. The pots were placed in green house and were watered whenever required. They were kept away from any kind of fertilizer treatment. Since *C. sinensis* is a slow growing woody plant (Singh et al., 2010), several growth parameters as well as AMF colonization percentage were assessed after one year following inoculation.

2.3 Analysis

Plant development of the inoculated plants as well as the control were evaluated by measuring number of leaves, leaf area, root length, shoot length, plant height, root and shoot weight (fresh and dry) as well as their ratio.

Numbers of leaves were recorded by visual observation at the end of the experiment, leaf area was measured with a leaf area meter, root length, shoot length and plant height were measured with the help of a measuring scale. After removing the plants from the polybags, the plant shoot was cut into approximate sizes of 10 cm and fresh weight was taken. Similarly fresh weight of roots was taken for each treatment. Shoots and roots were oven dried at 60° C for estimation of the dry weights.

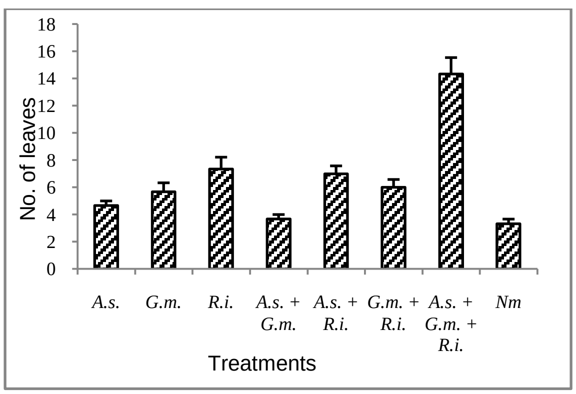
To evaluate the mycorrhizal colonization, roots were cut into approximately 1 cm segments and then cleared in 10% (w/v) KOH by heating at 90° C for 1 to 2 hours, depending on the degree of hardness and lignifications of the roots. Alkaline hydrogen peroxide (0.5% NH4OH and 0.5% H2O2 v/v in distilled water) was used for post-clearing bleaching. Distilled water was used to rinse the roots, treatment with 1% HCl was performed and staining was carried out with 0.05% w/v Trypan blue (Phillips & Hayman, 1970). The percentage of root length colonized by AMF and DSE was estimated according to the magnified intersection method (McGonigle et al., 1990). Estimation of phosphorus content of soil, roots and shoots were carried out by following the molybdenum blue method of Allen et al. (1974).

Means and standard errors were calculated for plant growth parameters and also for mycorrhizal colonization in AMF inoculated plants. Variation between plant growth parameters in AMF inoculated and control plants was analyzed using one way analysis of variance (ANOVA).

3 Results

AMF inoculated plants showed higher growth as compared to that of non-mycorrhizal (control) plants, in terms of all the growth parameters such as number of leaves, leaf area, plant height, shoot length, root length, root and shoot weight (fresh and dry) and root: shoot (fresh and dry weight) ratio (Figure 1).

The effect of individual and combined inocula of three different species of AMF on all the growth parameters varied. Combined inoculum of all the three AMF species (*A.s.* + *G.m.* + *R.i.*) performed consistently better than the other inocula containing single as well as two AMF species, except for leaf area, which was found to be highest in *A.s.* + *R.i.* inoculated plants. The phosphorus (P) content in the shoots ranged from 0.10% to 0.21%. It was lowest in non-mycorrhizal plants and highest in the plant inoculated with combination of *A.s.* + *G.m.* + *R.i.* Further, root P content ranged from 0.03% to 0.18%, whereas soil P content ranged from 0.03% to 0.09%. Both root and soil P content were lowest in control plants and highest in *A.s.* + *R.i.* inoculated plants.



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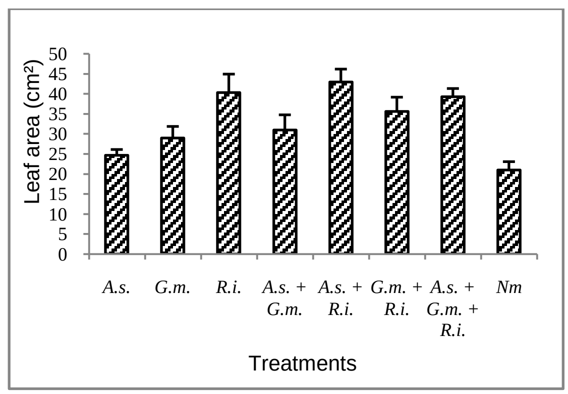

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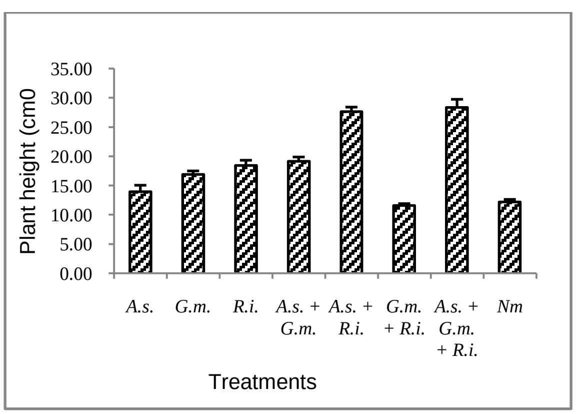

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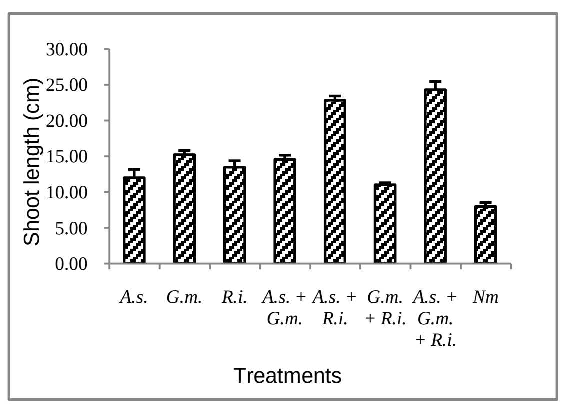

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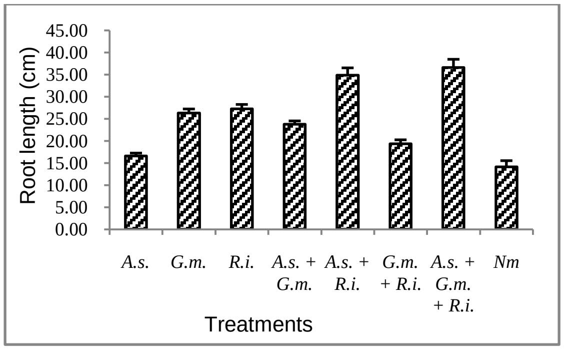

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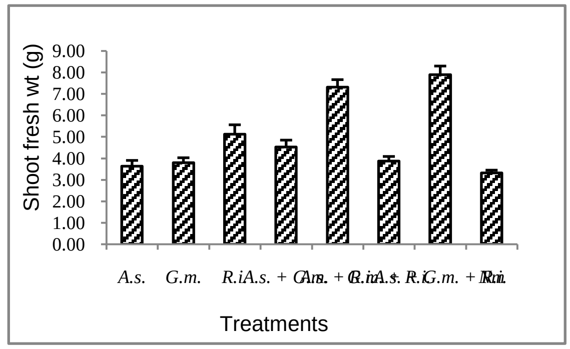

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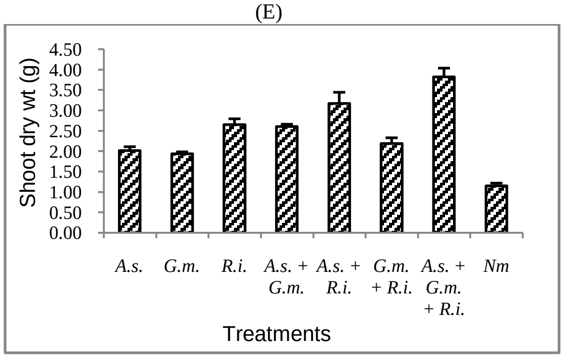

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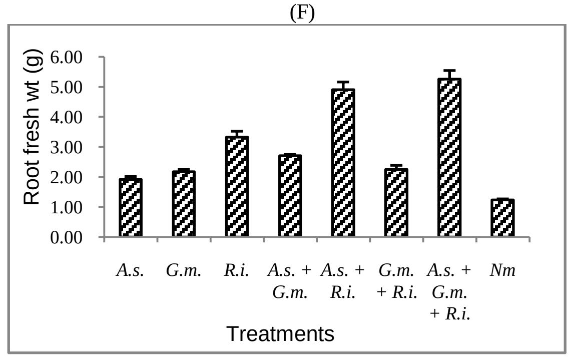

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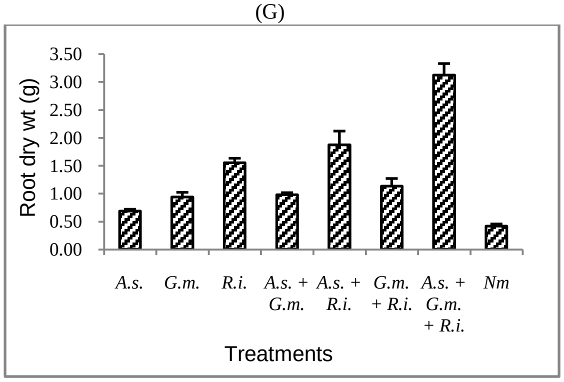

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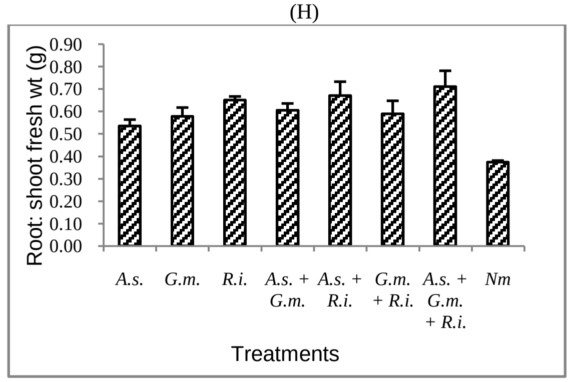

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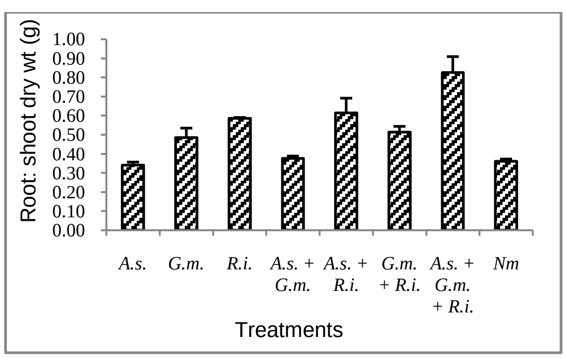

(K)

Figure 1 Effects of AMF inoculation on number of leaves (a), leaf area (b), plant height (c), shoot length (d), root length (e), shoot fresh wt (f), shoot dry wt (g), root fresh wt (h), root dry wt (i), root: shoot fresh wt (j) and root: shoot dry wt (k) in *Camellia sinensis.* (Note: *A.s.* = *Acaulospora scrobiculata*, *G.m.* = *Glomus macrocarpum*, *R.i.* = *Rhizophagus intraradices* and Nm = non-mycorrhizal).

The plants inoculated with AMF showed mycorrhizal colonization which was characterized by the presence of hyphae, arbuscules and vesicles, while in uninoculated plants no mycorrhizal colonization was detected. Highest AMF colonization was observed in plants inoculated with *A.s.* + *G.m.* + *R.i.* (81.26%), which was followed by *A.s.* + *R.i.* inoculated plants (74.17%), *A.s.* + *G.m.* inoculated plants (62.75%), *G.m.* + *R.i.* inoculated plants (58.53%), *R.i.* inoculated plants (52.46%), *G.m.* inoculated plants (32.82%) and *A.s.* inoculated plants (30.69%) (Figure 2).

There was a significant variation ($p < 0.05$) in number of leaves among different treatments of mycorrhizal and non-mycorrhizal plants, however, no variation was observed between *A.s.* + *G.m.* inoculated plants and non-mycorrhizal ones. Significant variation was observed in leaf area of control plants and *R.i.*, *A.s.* + *R.i.*, *G.m.* + *R.i.* and *A.s.* + *G.m.* + *R.i.* inoculated plants. Plant height varied significantly among different AMF inoculated plants and non-mycorrhizal plants, except in case of *A.s.* and *G.m.* + *R.i* inoculated plants. No significant variation was seen in root length between plants inoculated with *A.s.* and control plants. Shoot fresh weight of control plants and *R.i.*, *A.s.* + *G.m.*, *A.s.* + *R.i.* and *A.s.* + *G.m.* + *R.i.* inoculated plants showed variation. The root: shoot dry weight ratio has a variation between control plants and *G.m.*, *R.i.*, *G.m.* + *R.i.* and *A.s.* + *G.m.* + *R.i.* inoculated plants. Parameters such as shoot length, shoot dry weight, root fresh weight, root dry weight and root: shoot fresh weight ratio showed variations among different AMF treated plants and non-mycorrhizal plants.

4 Discussion and conclusions

Growth parameters of tea cuttings were recorded after a year of inoculation, since tea being a woody plant exhibits slow growth (Singh et al., 2010). The inoculated AMF plant had a significant positive effect on all growth parameters regardless of single or mixed-species inoculation (Singh et al., 2008; Cho et al., 2009; Sharma et al., 2009; Gogoi & Singh, 2011 and Ortas & Ustuner, 2014). The effect of individual and combined inoculates of three different species of AMF on the parameters such as plant height, number of leaves, root length, shoot length, root and shoot weight (fresh and dry) and their ratio varied.

The combined inoculum of three AMF species, i.e., *A.s.* + *G.m.* + *R.i.* consistently outperformed the other inocula containing single as well as two AMF species, which may be ascribed that more AMF species means more functions fulfilled and more opportunities for beneficial relationships to develop (Sharma et al., 2009) and as compared to a single mycosymbiont, AMF consortium delivers unwavering benefits to the host plants (Singh et al., 2010; Ortas & Ustuner, 2014). Following inoculation with AMF, plant growth is enhanced, as inoculation amplifies the macronutrient as well as micronutrient contents in roots (van der Heijden et al., 2006).

All inoculated plants showed mycorrhizal colonization whilst no AMF were detected in the non-inoculated plants (Orlowska et al., 2012; Majewska et al., 2017). The extent of root colonization in plants treated with different AMF inoculants varied. Consortia of two or more AMF species had higher colonization levels as compared to single AMF species. Among different treatments, AMF colonization was highest in *A.s.* + *G.m.* + *R.i.* inoculated plants. Gai et al. (2006) postulated that the ability of AMF to colonize a specific host determines the magnitude of colonization and functioning of the AMF symbiosis. Increment of colonization rate in plant roots leads to exploration of a higher volume of soil by the mycorrhizal root system, and thus, the ease in taking up of mineral nutrients from the soil by the more extensive root system (Cho et al., 2009). Results of present investigation shows that AMF inoculated plants have a high P concentration as compared to the control, which is in line with the results obtained by Ortas & Ustuner (2014). High P content is probably due to more efficient uptake of available P from the soil and possibly to mineralization of organic phosphorus, due to a higher phosphatase production by AMF plants (Songachan, 2012).

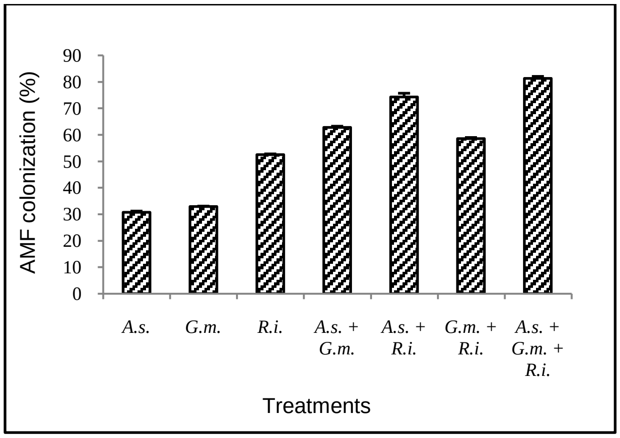

Figure 2 AMF colonization in different treatments of *Camellia sinensis*. (Note: *A.s.* = *Acaulospora scrobiculata*, *G.m.* = *Glomus macrocarpum* and *R.i.* = *Rhizophagus intraradices*).

The inoculation of tea cuttings with AMF enhances the growth of tea seedlings (Singh et al., 2010). Besides, the AMF species isolated from the rhizosphere of local *C. sinensis* plantations have an added advantage of being locally adapted to the soil used in the experiment. Similar observations were reported by Sharma et al. (2009) that the home fungi associations have higher mycorrhizal effectiveness in plants. They also further suggested that commercial single species inocula are generally ineffective as compared to native or locally adapted field inocula.

The AMF species had a significant positive impact on the seedling growth of tea. Thus, it can be concluded that soil amendment with AMF have the potential to possibly reduce the application of phosphorus fertilizer, and therefore AMF can be used as an efficient biofertilizer to improve productivity of the plant, thereby offering a convenient alternative to the conventional method of tea cultivation. However, further work needs to be performed to determine the effect of AMF inoculation under field conditions.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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