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Evaluation of mycorrhizae transplantation from *Panicum maximum* as biofertilizer for paddy cultivation in Sri Lanka

CHATHURIKA WANNINAYAKE[1]*, MEKHALA CHANDRASEKARA[1], SANATH HETTIARACHI[1], WASANTHA MALAVIARACHCHI[2], LORENZO VERGANI[3], SARA BORIN[3]

[1] *Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka*

[2] *Field Crop Research and Development Institute, Mahailluppallama, Sri Lanka*

[3] *Department of Food, Environmental and Nutritional Sciences, University of Milan, Milano, Italy*

**Correspondence details: umayanganincr@gmail.com*

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Abstract: Indiscriminate chemical fertilization in paddy fields is causing detrimental global impacts, therefore exploration of bio-based sustainable alternatives to compensate requirement of chemical fertilizers is necessary. This study was designed to evaluate use of Arbuscular Mycorrhizal Fungi (AMF) obtained from roots of a spontaneous weed grass (*Panicum maximum*), which demonstrated an average AMF root colonization of 75-85%, as self-produced biofertilizer for rice cultivation. Field trial was carried out in paddy field at Monaragala, Sri Lanka. Two traditional rice (*Oriza sativa* L.) varieties (Suwadel and Kuruluthuda) were selected, and Randomized Complete Block Design was employed with three replicates in eight different treatment plots per block. Different chemical fertilizer regimes (100%, 50% and 25% of recommended fertilizer dose) and no amendments as controls were applied. AMF inocula were applied alone or supplemented to fertilized treatments (100% +AMF, 50% +AMF, 25% +AMF, AMF alone). Treatment plan was implemented separately for two varieties. Rice yield and percentage of AMF root colonization were quantified at harvest. Results revealed that grain dry weight for Suwadel was significantly higher ($p<0.05$) in treatments supplying AMF in addition with chemical fertilizer at optimal dose (100%) or 50% reduced, compared to other treatments. Highest number of seeds was observed in plants treated with 100% fertilizer, but result was not significantly different ($p>0.05$) from 50% +AMF and 100% + AMF treatments. Treatments with AMF inocula alone showed highest percentages of AMF colonization in Suwadel. Inverse connection was observed between fertilizer level and AMF colonization. Differently from Suwadel, no AMF colonization was observed in Kuruluthuda variety and no significant differences were observed in harvest for treatments with and without AMF application. According to results, usage of AMF inocula as on-field prepared biofertilizer combined with 50% of chemical fertilizer is effective in improving sustainable agriculture but efficacy of application strictly depends on crop variety.

Keywords: Arbuscular mycorrhizal fungi, sustainable agriculture, rice, microbiome transplantation

Introduction

Rice is one of the most consumed staple foods in the world and it is the main staple food of Sri Lanka. The health of agricultural land and water resources are vital factors for rice production, but contamination of those resources by indiscriminate application of chemical fertilizers is currently a huge environmental concern (Naher et al., 2018). Also, it creates other obstacles to cultivation, such as increasing the cost of production. The study done by Hardy et al. (2016) in India has demonstrated that System of Rice Intensification (SRI) production systems which emphasise the usage of organic fertilizers and manure, offered substantial environmental and economic benefits compared to conventional farming system. Moreover, that study concluded that higher yields result in smaller area could be observed in SRI system compared to intensive control. Furthermore, multi-purpose character of organic agriculture could enhance its cost-effectiveness due to its potentially lower transaction costs compared to more targeted agri-environmental measures. Therefore, the establishment of sustainable approaches for rice cultivation to reduce the harmful effects of chemical fertilizers while improving the food safety for consumers is currently gaining attention of agricultural scientists (Kuila & Ghosh, 2022). The application of biofertilizers to rice cultivation is a sustainable strategy with the potential to reduce chemical fertilizer dose while improving crop productivity and profitability (Naher et al., 2015; Panneerselvam et al., 2017). According to the literature, usage of biofertilizer with 30% reduced chemical nitrogen fertilizers, increased the rice grain yield by 69% and straw yield by 35% (Naher et al., 2015) compared to the usual practices of the farmers. Zhang et al., (2015) also provided evidence that AMF inoculation could be a promising tool for enhancing rice grain yield, especially under low nutrient conditions. Moreover, Campo et al. (2020) observed a positive response to mycorrhizae among different rice cultivars in terms of growth promotion and blast resistance, while emphasizing the notion that the effectiveness of AMF activities should be evaluated case-by-case in order to optimize their exploitation of in rice cultivation. Even though many biofertilizers are available in the market, the usage of Arbuscular Mycorrhizal Fungi (AMF) as biofertilizers for rice cultivation and their efficacy for a particular variety of rice under different environmental conditions should be further investigated. Also, the potential of AMF interaction with monocots, especially rice, and its effect to the harvest yield remains largely unexplored. Moreover, the main focus of the early work of researchers on AMF had been focused on plants growing on dry land, as the flooded and anaerobic soils are supposedly not conducive for AMF development. Preparation of inoculum is a vital point in usage of AMF as biofertilizers, which demands the selection of a promising host plant. Highly mycorrhized plant species that are naturally and readily available with a wide geographic distribution, could represent a good resource for collecting AMF inoculum. This would help to reduce the high costs in farming practices, as farmers will be able to make a fresh AMF inoculum by themselves, avoiding the purchase of inocula produced elsewhere and stored for a considerable period with possible loss of activity and with unreasonable price tags. In Sri Lanka, *Panicum maximum* has been recognized currently as invasive plant and is spreading under all climatic conditions throughout the country. It has become a huge burden on farmers and many other sectors in rural communities as they need to employ excess measures to control its presence in the farmlands, home gardens, fallow lands, roadsides etc. (Sandeepani et al., 2018). This species was introduced to Sri Lanka in the 1820's for forage and has become naturalized in most ecological zones, ecosystems, and habitats. It is now a spontaneous weed plant that overruns road and railway sides, natural forests, crop plantations, natural grasslands, and scrubland at low and mid elevations (Gajaweera et al., 2011). It has been proven that this grass can form extensive mycorrhizal association (Shruti et al., 2017), putatively responsible of *P. maimum* diffusion and high adaptability to different environments (Řezáčová et al., 2018). The rational of this work is to transform a burden in a resource by transplanting the *P. maimum* AMF to rice crops in a self-prepared biofertilizer. The root system of *P. maimum* together with the adhering soil has indeed a great potential as a natural AMF inoculum for crop cultivation since the source of inoculum is readily available in every climatic area of the country. Then usage of root system (already rich in mycorrhizae) and associated soil can be the best strategy to bypass time consuming trap culture or trap crop methods used to obtain AMF inocula. Colonization efficiency of AMF and the associated microbiome of this grass can be a key point behind its presence over a wide range of climatic conditions including the degraded lands. The aim of this research work is the usage of already available and well adapted *P. maimum* grass's root and adhering soil for the preparation of inocula to be used as a "do-it-yourself, zero-mile" biofertilizer. Exploration of the effectiveness of microbiome transplantation done by Jiang et al. (2022) reported it as a promising tool to effectively modulate protective microbiomes and promote plant health. Transplanting microbiome from a weed grass successfully adapted to the local soil and environmental conditions, to crop cultivation would in fact provide a high chance to mitigate and compensate the loss of microbial biodiversity through crop development and plant variety selection programs in modern agriculture. Furthermore, it is already proven that transplanting beneficial plant-associated microbiomes is a promising tool to gain desirable qualities for crop development and disease management (Mosqueda et al., 2023). The report issued by Food and Agricultural Organization in 2022 clearly mentioned that alteration of plant genotypes throughout the process of plant domestication and development has led to potential "missing microbes" in the root rhizosphere and soil microbiomes (Kendzior et al., 2022). Gutierrez and Grillo (2022) and Juan et al. (2016) also reported about the plant domestication process and its detrimental effect to change bio-diversity depletion of plants. Therefore, the aforesaid strategy of using on-field prepared AMF inocula by farmers themselves may provide high impact advantages not only in terms of cost reduction and easy access but would also improve the agricultural soil microbiota. However, the potential of spontaneous grass as a source of AMF has not been evaluated for its effectiveness and efficacy on improving growth and yield of different crop types and in different climatic conditions. Therefore, this study was performed to evaluate the effect of the AMF inoculum from *Panicum maximum* combined with decreasing chemical fertilizer regimes (as percentages of recommended fertilizer applications by the Department of Agriculture of Sri Lanka. Sirisena, D.N. 2013) on the harvest yield of two selected rice varieties, and therefore to assess its potential to represent a low cost, easily prepared and freely available biofertilizer for farmers.

Materials and Methods

Experimental Sites

The field experiment was carried out at selected farmer's paddy field in Monaragala (06.87°, 81.34° E, 151 m amsl) Sri Lanka, which is located in Low country, Intermediate zone, according to the agro-ecological division of the country (Figure 1). The annual average rainfall of this region is 1900-2500 mm and soil is constituted by reddish brown earth and immature brown loams.

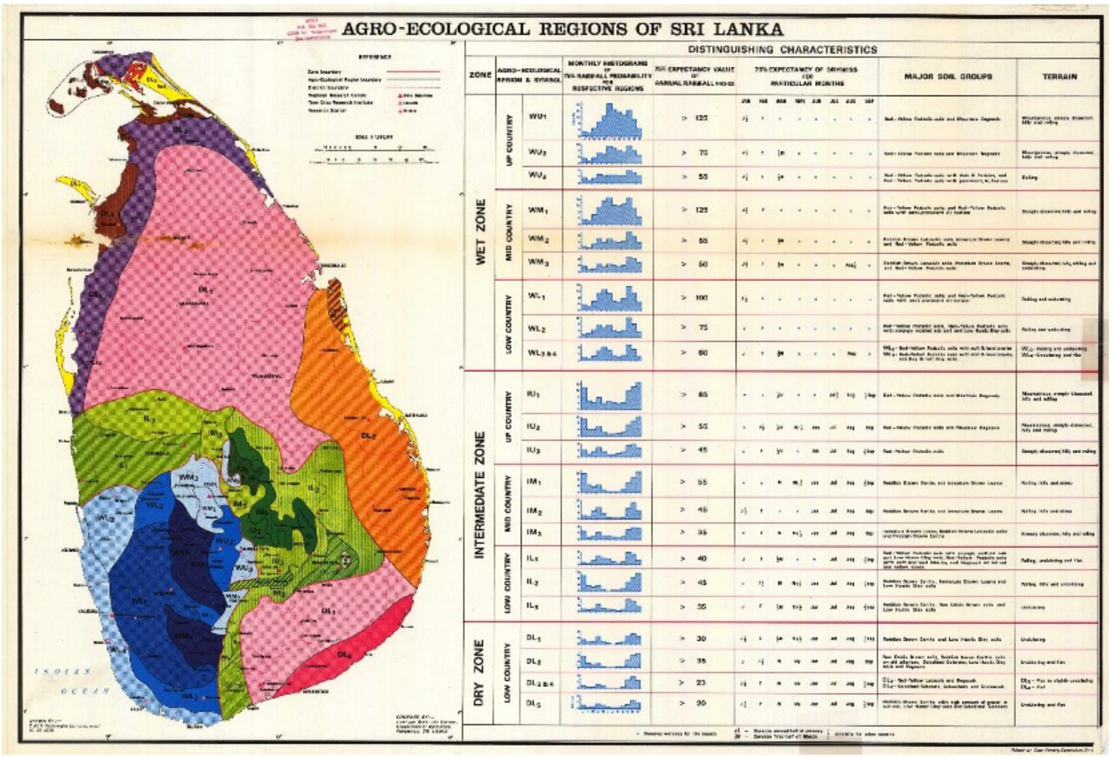

Figure 1 – Agro-ecological regions in Sri Lanka (map is online available in https://www.arcgis.com/apps/instant/minimalist/index.html?appid=d244641b5f3e4206a7c98f5cda1892e7)

Preparation of AMF inocula

Panicum maximum growing naturally close to field area was selected as host plant to prepare AMF inocula. Selection of *P. maximum* grass for this purpose was based on the results that obtained from preliminary screening (Random samples of roots were subjected to McGonigle staining procedure using tryphan blue as dye (McGonigle et al., 1990) to verify the colonization percentage of AMF in *P. maximum* plants. Well established and well grown *P. maximum* grasses were uprooted, and the root ball removed with adhering soil. Then, whole roots were cut into small pieces of about 1 cm in length and thoroughly mixed with the associated soil of root ball. The resulting mixture was used as AMF inoculum (average colonization percentage was in between 75%- 80% in selected root pieces from inoculum) by mixing 1 Kg of root pieces and associated soil with the field soil of the selected 3 m × 3 m plots of the trial.

Experimental design and treatments for field trial

Traditional rice varieties, namely Suwadel and Kuruluthuda were used for this field trial at Monaragala. Each variety was established in 24 plots consisting of three replicates for eight treatments. Accordingly, 48 plots (8 × 3 replicates × 2 varieties) were prepared and irrigation system was established properly in order to avoid the contamination by water outflow of one plot from another. The size of a plot was 3 m × 3 m. Plots (8) in each block were completely randomized in eight treatments. Each plot was properly labeled with suitable codes depending on the rice variety and treatments.

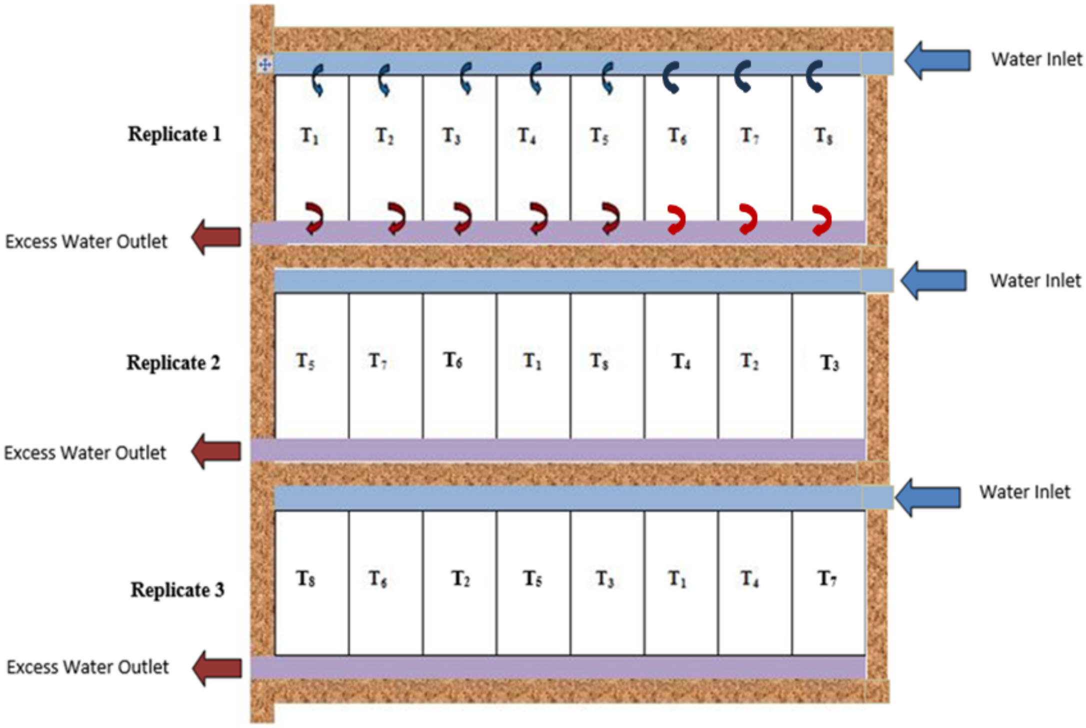

Figure 2 - Field layout for cultivation of Rice: presenting the way of plot establishment and water managing system: curved blue color arrows indicate the way that water is entering to the field via the irrigation line and the red color curved arrows indicate the way that excess water is passing through separate canal without mixing with other treatments. The same scheme was applied to replicate 2 and 3.

Chemical fertilization was started before seed introduction to the plots based on the treatment plan. The recommended rates of chemical fertilizers for paddy soils (Government Agriculture Department) are 12 kg/hectare for urea, 112 kg/ hectare for triple superphosphate (TSP), 50 kg/hectare for muriate of potash (MOP) as basal and 86 kg/hectare, 136 kg/ hectare, 74 kg/hectare for urea after 2, 5 and 7 weeks from sowing, respectively. Paddy seeds were soaked in water for 24 hours and were allowed to germinate for 48 hours after draining the water before broadcasted in the prepared field. 7.4 kg of seeds were soaked in water and sown in each plot. The treatments were T1 – no treatment (no fertilizer addition), T2 – 25% of the recommended chemical fertilizer, T3 – 50% recommended chemical fertilizer, T4 – 100% recommended chemical fertilizer, T5 – 25% recommended chemical fertilizer + AMF, T6 – 50% recommended chemical fertilizer + AMF, T7 – 100% recommended chemical fertilizer + AMF and T8 - AMF inoculum only.

Measurements and Data Collection

Harvest data were collected by randomly picking ten plants for each of the three replicated plot, obtaining three average yield values for each treatment that were than used for statistical analysis. Five root samples per plot were collected and stored in tubes containing Formaldehyde Acetic Acid for AMF verification. Determination of AMF colonization percentage was done following the modified grid transects method (McGonigle et al., 1990) using Tryphan blue as dye.

Statistical Analysis

All statistical analyses were performed using SAS 9.4. software package. One way ANOVA was used to analyze the results. Duncan's Multiple Range Test method and 95.0% confidence was used to test for differences among treatment means at a significance level of $p < 0.05$.

Results

Preliminarily analyses demonstrated that *P. maximum* root apparatus was densely colonized by AMF, with an average colonization percentage of 75% - 80% in prepared inoculum with weed root pieces and surrounding soil. At harvest time, AMF root colonization and grain yield were analyzed and compared with plants cultivated in control plots which were not amended by AMF and/or chemical fertilizer. The measurement of the grain yield of the Suwadel variety showed that 25% and 50% of chemically fertilized plants produced higher grain yield values than non-treated control plants (Figure 3). However, no statistically significant difference was observed among non-treated control, reduced chemical fertilizer percentages (25%, 50%), and the recommended fertilizer dose (100%). AMF inoculation did not significantly increase rice yield in plants that did not receive chemical fertilizer or received only 25% of the recommended dose. AMF combined with 50% and 100% chemical fertilizer showed the highest grain yield, without a significant difference in between these two treatments ($p > 0.05$). The calculated yield for the negative control treatment was 3.6 t/ha (Table No.1), while 50% fertilizer with AMF inocula and 100% fertilizer with AMF inocula improved the harvest by 233% and 242% respectively. Moreover, a significant increment was observed comparing these two treatments and 100% fertilization alone ($p < 0.05$), while the increase was not significant compared to 50% chemical fertilization (Figure 3).

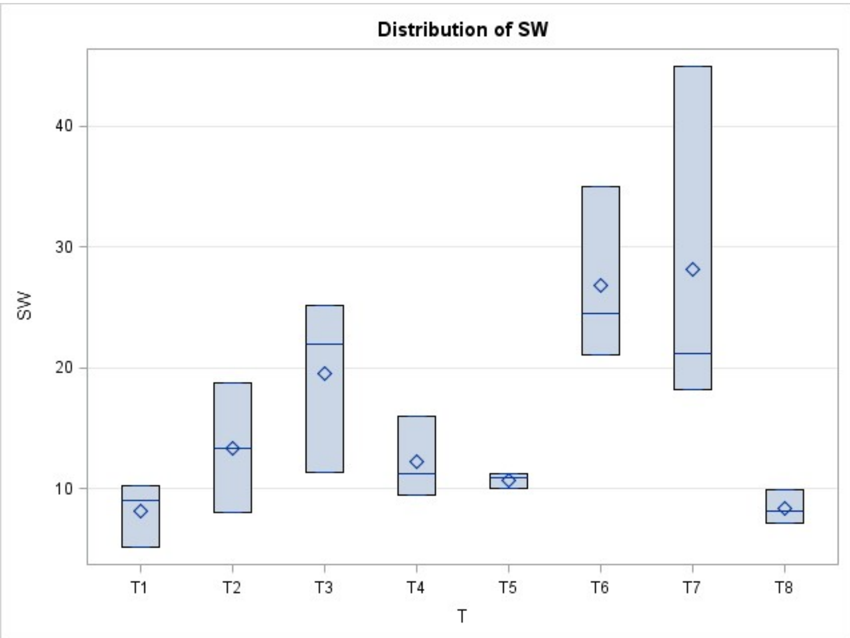

Figure 3 - Distribution of Mean values for yield of randomly collected ten plants per treatment (Suwadel variety): SW denote the Seed Weight

Table 1 - Representing harvest data after conversion of weight in kilograms per hectare (Suwadel variety). Means with same letters are not significantly different (p > 0.05). T1 – Negative control, T2 – 25% fertilizer, T3 – 50% fertilizer, T4 – 100% fertilizer, T5 – 25% fertilizer + AMF, T6 – 50% fertilizer + AMF, T7 – 100% fertilizer + AMF, T8 – AMF only

Treatments	Total harvest (kg/ha)
T1	3600[b]
T2	6000[b]
T3	8700[ab]
T4	5400[b]
T5	4500[b]
T6	12000[a]
T7	12300[a]
T8	3800[b]

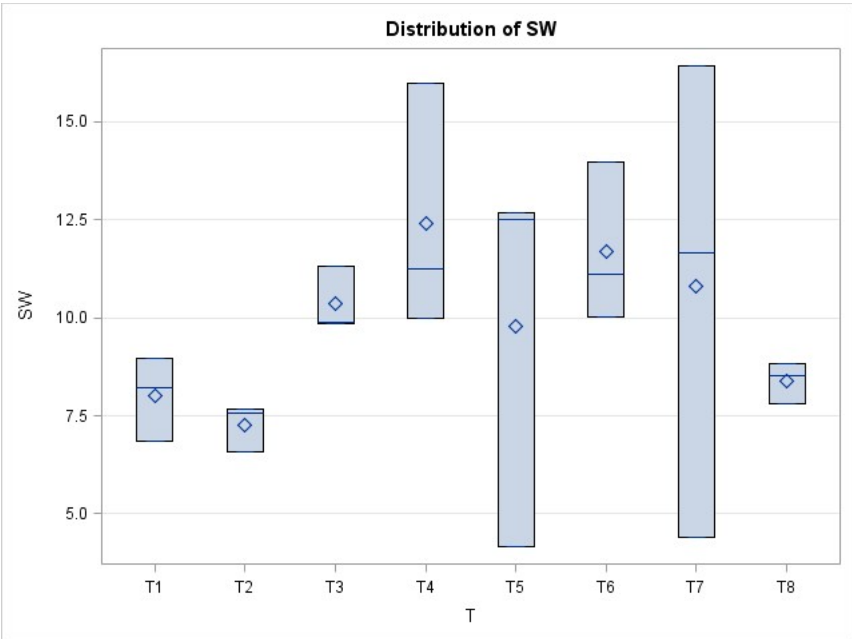

Figure 4 - Distribution of Mean values for yield of randomly collected ten plants per each treatment (Kuruluthuda variety): SW denote the Seed Weight

Table 2 - Representing harvest data after conversion of weight in kilograms per hectare (Kuruluthuda variety). Means with same letters are not significantly different (p > 0.05). T1 – Negative control, T2 – 25% fertilizer, T3 – 50% fertilizer, T4 – 100% fertilizer, T5 – 25% fertilizer + AMF, T6 – 50% fertilizer + AMF, T7 – 100% fertilizer + AMF, T8 – AMF only

Treatments	Total harvest (kg/ha)
T1	3600[a]
T2	3200[a]
T3	4600[a]
T4	5500[a]
T5	4300[a]
T6	5200[a]
T7	4800[a]
T8	3700[a]

AMF colonization on variety Suwadel (Table 3) was observed on all the inoculated treatments except 100% fertilizer + AMF. The highest average root colonization by AMF was observed in AMF inocula alone applied treatment (T8) as a value of 82.2% (Table 3). However, this highest colonization rate by AMF alone added treatment for Suwadel variety did not correspond to a yield increase when compared to the results of harvest data (Figure 3). The treatment of AMF added with 25% of fertilizers showed 14.4% of root colonization rate but also in this case there was no increment in the harvest data.

In Kuruluthuda rice variety cultivated in parallel plots we observed an increase in the weight of seeds in 50% and 100% fertilized treatments, both with and without AMF, compared to the non-treated control (Table 2). However, on the contrary of Suwadel, no significant differences in the average grain yield among the different treatments given by chemical fertilizer or by AMF were detected ($p > 0.05$) (Table 2). Moreover, there was no observable root colonization, regardless of the fertilization regime, demonstrating that this rice variety did not constitute a host for AMF.

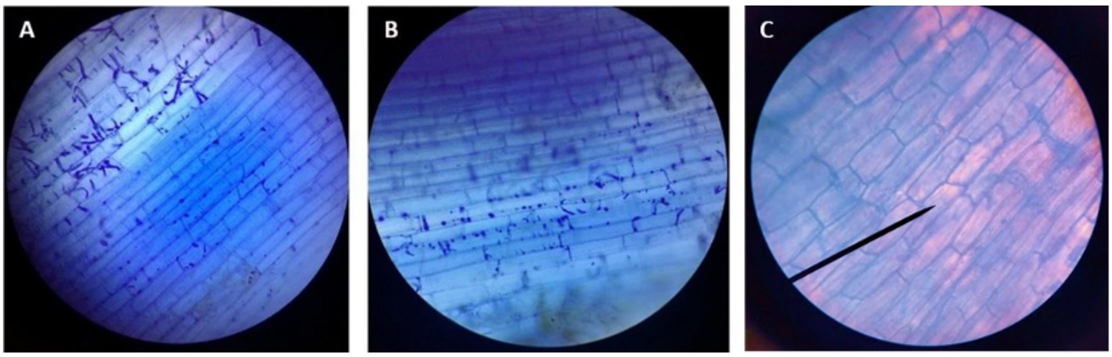

Figure 5 - Observation under microscope after staining the Suwadel roots (roots indicating the presence of AMF invasion in root cells by hyphae and small vesicles can be identified in (a and b) while a root without colonization of AMF in control root (c), scale bars represent 200μm in a and b, 150μm in c)

Table 3 - Average percentage of AMF colonization within Suwadel roots. ND (Not detected).

Treatments	Average percentage of AMF colonization (%)
T1 – Negative control	ND
T2 – 25% fertilizer	ND
T3 – 50% fertilizer	ND
T4 – 100% fertilizer	ND
T5 – 25% fertilizer + AMF	14.4
T6 – 50% fertilizer + AMF	38
T7 – 100% fertilizer + AMF	ND
T8 – AMF only	82.2

Discussion

The reduction of chemical fertilizer application in agricultural fields is a major concern in sustainable agriculture. Nevertheless, in the present study, the AMF inoculum supplied alone did not demonstrate the potential to reduce the chemical fertilization in both the rice varieties, which showed similar harvest yields to non-inoculated plants. The current study on Suwadel variety shows that no significant difference was seen between 50% and 100% chemical fertilizer applications even in plants without AMF. This result suggests that in this soil 50% of the recommended fertilizer dose could be optimal for this rice variety, making the use of 100% dose not necessary. Further, there was a significant yield increment in treatments where AMF was combined with 50% and 100% chemical fertilizers dose, compared to 100% fertilizer application with no AMF amendment. This phenomenon has been described in the literature using same experimental design for maize cultivation, where plants needed both fertilization and AMF inoculation to achieve optimal growth and yield and the application of AMF could compensate for the reduction in chemical fertilizers up to 50% (Fall et al., 2023). Coherently, in the present work a higher rice yield was reported in the treatment of AMF inocula added with 50% fertilizers compared to that of the 50% fertilizers alone, even though the increment was non statistically significant. Similar results have been reported in the literature for pineapple cultivation and oil palm seedlings, where the AMF inoculation and the application of half of the fertilizer dose were related with the better harvest (Rini et al., 2021; Trejo et al., 2021). Notably, a similar pattern of harvest increment has been reported in a maize experiment done by Fall et al. (2023) providing evidence about the possibility of this application for cereal cultivation. Similarly, the positive effect regarding the reduction of chemical fertilizer application via inoculation of AMF for maize cultivation has been reported by Qian et al. (2024). Moreover, Mulyadi and Jiang (2023) reported that the combined application of AMF and N fertilizer increased the physiochemical properties, rice growth, rice productivity, and N uptake of rice grown in polybag. These observations suggest the occurrence of some synergistic effect between AMF and chemical fertilizers, and one possible reason is that AMF increase the availability of inorganic nutrients for plant uptake, improving plant nutrient absorption and thus fertilization efficiency. These results support the effectiveness of the combined application of AMF inoculum as a sustainable strategy to reduce the indiscriminate application of chemical fertilizers. According to the results of AMF colonization potential, despite being obligate aerobic, AMF could probably survive in association with rice roots under anaerobic conditions by obtaining O_2 from the atmosphere through the plant's aerenchymatous tissues (Watanarojanaporn et al., 2013). The highest colonization rate by AMF was observed in the Suwadel variety when crops were treated with *Panicum* root pieces without added fertilizer, but this was not combined with an increase in the yield. One possible clarification for these results is that, under nutrient scarcity, AMF must compete for limiting nutrients for their hyphae development against the host plants, reducing the resources that host plants can allocate to the grains and increase productivity (Chen et al., 2022). Wang et al., 2022 reported that the differences in growth and reproduction characteristics of cherry tomato related to different AMF inoculation treatments were regulated by the available nutrient levels. In the present study, an inverse relationship was observed between AMF colonization and the amount of chemical fertilizer application, indicating some challenge for AMF to colonize the roots with increased amounts of inorganic fertilizers. Similar decline of AMF colonization along with higher doses of inorganic fertilizer application has been previously reported for corn cultivation (Ishaq et al., 2021). However, even with no observed AMF colonization in AMF+100% treatment, Suwadel harvest significantly increased when compared to 100% chemical fertilizer alone treatment. A recent study suggests that even though the root infection by AMF does not occur directly on the crop of interest, their beneficial effect can be mediated via tripartite interaction with the assistance of other neighboring plants (such as weeds) which can be colonized by AMF (Wang et al., 2022). It is therefore crucial to investigate the potential of AMF colonization within a particular selection of crop before recommending their usage to reduce the application of chemical fertilizers. Otherwise, there is a chance to fail biofertilizer applications if the crop of interest is not responsive.

This study also suggested AMF host specificity, since only one of the two tested rice varieties (Suwadel) was colonized and showed an increase in productivity. The Kuruluthuda variety, on the contrary, did not show any AMF root colonization and significant effect on the plant harvest yield. This result was confirmed in other two experiments that were conducted for the evaluation of the potential AMF colonization in traditional and improved rice varieties; even in this case no colonization was observed in Kuruluthuda variety (unpublished data). This observation can be explained by the fact that AMF colonization is dependent on plant genotype (Yang et al. 2010) and it could therefore differ in between two variety of the same species.

Conclusion

Application of AMF inocula, transplanted to crops from the weed *P. maximum*, was effective in increasing the harvest yield of rice var. Suwadel even with a half dose of recommended chemical fertilization, showed a good potential to be applied as biofertilizer with the effect of increasing crop productivity. The method adopted to prepare the AMF inoculum is particularly easy and low-tech, and can be self-prepared directly on-site by farmers, thus representing a highly sustainable approach with environmental and economic benefits, especially for rural communities. The bio-inoculum effect was nevertheless variety-dependent, since it was demonstrated that AMF from *Panicum* grass colonized the roots of only one of the two rice varieties and, coherently, showed plant growth promotion only on the colonized one. The use of AMF proved to be promising as an efficient way to reduce the economic and environmental cost of production, by improving crop yield with a significant decrease in the use of inorganic fertilizer and therefore environmental pollution. However, the application of AMF inocula for some varieties of rice such as Kuruluthuda may not be much effective because of the lack of capacity to establish an association with the plant. The results of the present work point out the necessity of further field studies to better investigate the relationship among AMF colonization, effectiveness and chemical fertilizer application, and to evaluate the AMF-prone rice varieties before attempting to use AMF as fertilizer supplement.

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