Effect of *in vitro* Produced Arbuscular Mycorrhizal Fungi Inoculum on Anaerobic Direct Seeded and Transplanted Paddy

M. D. Iffah Haifaa[1] & Christopher Moses[2]

[1] Valent Biosciences, Innovation Way, Libertyville, Illinois, USA

[2] Sumitomo Chemical Enviro-Agro Asia Pacific Sdn Bhd, Senawang Industrial Park, Negeri Sembilan, Malaysia

Correspondence: Christopher Moses, Sumitomo Chemical Enviro-Agro Asia Pacific Sdn Bhd, Senawang Industrial Park, 70400, Seremban, Negeri Sembilan, Malaysia Tel: 60-126-080-393. E-mail: mcph.chris@gmail.com

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Abstract

It is widely accepted that the symbiotic arbuscular mycorrhizal fungi (AMF) play a key role in sustainable production systems in rice cultivation and they readily form a symbiotic relationship with these fungi. Four species consortium of AMF, *Funneliformis mosseae*, *Rhizophagus intraradices*, *Clariodeoglomus etunicatum* and *Glomus aggregatum*, produced through *in vitro* production system and formulated with organic biostimulants viz. humic acids and sea weed extract were tested in this experiment, both for direct seeded and transplanted paddy, under anaerobic cultivation system. AMF inoculated fields produced significantly higher yield than non-inoculated field. AMF inoculants formulated with a blend of humic acids and seaweed extract produced significantly higher grain yield than the inoculants formulated with humic acids alone, in both the cultivation systems. Effect of AMF inoculation was highly pronounced in transplanted paddy than direct seeded paddy, resulted in highest grain yield, highest grain weight, better grain filling rate and highest tiller production. It can be concluded that AMF inoculants can be potentially used for rice cultivation under anaerobic water management system, both for direct seeded and transplanted paddy and the nature of biostimulants used in the formulation also play a key role in the efficacy of AMF inoculants. Our findings contribute to the growing global consensus that mycorrhizal inoculants could play a role in sustainable rice production systems of the future, when used appropriately.

Keywords: *Funneliformis mosseae*, *Rhizophagus intraradices*, *Clariodeoglomus etunicatum*, *Glomus aggregatum*, biostimulants, grain weight, grain yield

1. Introduction

United Nations estimates predict that by the year 2050, the global population will increase from approximately 7.5 billion, to more than 9.7 billion, which will exert pressure on modern agriculture to become increasingly more efficient and to produce more food from the present cultivable area in an eco-friendly and sustainable way. Among the stable food crops, rice (*Oryza sativa* L.) is the most important food crop in the world, directly feeding more people than any other crop. It is estimated to fulfill the needs of over 3.5 billion people by providing over 20% of their dietary calories (Muthayya et al., 2014). It is also uniquely placed because it can grow in wet, anaerobic environments that other crops cannot grow or survive. More than 75% of global rice production is from lowland rice, cultivated under submerged conditions, and the remainder is from upland rice grown under non-submerged conditions (Maclean et al., 2013). Asia accounts for about 90% of rice production in the world. Hence, rice plays a pivotal role in food security, socio-economic values and governments' strategic interventions for several developing countries (Omar et al., 2019; Shah et al., 2019). Like any other country in South East Asia, it is a staple food for Malaysia and a defining feature of the country's culture. Malaysians consume the grain daily either as cooked rice or indirectly in some form of rice flour. On an average, Malaysians consumed 80 kg of rice per person per year, which is about 26% of the total caloric intake per day. Compared to the global rice production, Malaysia's total rice production area is only about 0.7 million ha, contributing a mere 0.4% of the total global output, which is the lowest in South East Asia (Firdaus et al., 2020). As per the latest available figure, over 2.7m MT of rice was consumed in Malaysia, wherein 67-70% is produced locally, and the remaining 33-30% is imported primarily from Thailand, Vietnam and Pakistan. Historically, Malaysia has always had production-driven agricultural targets. Indeed, over 30 years, the total production has increased, allowing the self-sufficiency level (SSL) to hover between 60-70% (Omar et al., 2019). In a developing country like Malaysia, agriculture plays a vital role in the nation's determination to attain the status of self-sufficiency by 2020 (Hashim et al., 2017). The Malaysian growth strategy plan, also known as Vision 2020 was developed aiming at developing the country in all socio-economic aspects, such as national unity, social cohesion, economics, social justice, political stability, a system of government, quality of life, social and spiritual values as well a national pride and confidence (Alam et al., 2016; Lee & Baharuddin, 2018). In its development to attain the vision, agriculture remains an essential sector of Malaysia's economy, contributing 12% to the national GDP and providing employment for 16% of the national population and uplifting life in rural communities through extensive extension activities (Mannan et al., 2017). Because of this, there is an urgent need to boost paddy production and productivity on national level. The expectation is to provide at least 75% of the countries needs ourselves in years to come and to reduce our dependency on imports and revisit the 70:30 policies during 2020. While the cultivable paddy area is expected to remain same, to meet the target, the additional grain yield should come from higher grain yield and productivity.

There are several potential approaches for enhancing the yield and productivity in paddy, of which the major intervention tools are developing high yielding hybrid rice varieties through plant breeding, plant nutrition management, improved agronomic practices and also judicious use of beneficial biostimulants based on microbial sources (arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, *Trichoderma* etc.) as well as non-microbial sources such as humic acids, chitosan, amino acids, protein hydrolysates, phosphites, seaweed extract etc (Raphael & Colla, 2020). All these tools form key components in sustainable agricultural practices.

Arbuscular mycorrhizal fungi (AMF) share a long history of coevolution with plants in various ecosystems, resulting in their adaptation to different agriculture system and forestry (Gosling et al., 2006). They form very important components of soil microbial communities and ecosystem. AMF fungi form symbiotic and mutualistic associations with roots of most terrestrial plants, including most agricultural crops, with few exceptions. These symbiotic associations have shown the potential of AM fungi to increase crop productivity in several crops, besides imparting several functional benefits, thereby playing a key role in the existence and sustainability of agroecosystems (Gianinazzi et al., 2010). In rice, the manifestation of AMF colonization in roots under different production regimes at diverse geographical locations, demonstrated variable and beneficial effects on the community compositions of AMF (Choi et al., 2018, Chen et al., 2017). The most important function of these symbiotic associations involves transfer of plant nutrients such as phosphorus (P), nitrogen (N) and other micronutrients to the plants by the fungi and transfer of organic carbon (C) in the form of sugars and lipids to the fungi by the plants (Smith & Read, 2008; Jiang et al., 2017; Luginbuehl et al., 2017). It is extensively studied and well documented that AMF-mediated improvement in mineral uptake, especially phosphorous, nitrogen, zinc, potassium, iron etc has led to increased growth and development of plants, and also confer resistance to several abiotic and biotic stress (Liu et al., 2007; Smith & Read, 2008; Gianinazzi et al., 2010).

In rice, the effects of mycorrhizal symbiosis are less studied, probably because rice is mostly cultivated in wetland areas, and plants in such anaerobic ecosystem have traditionally been considered as non-mycorrhizal. Ilag et al. (1987) reported that rice plants are capable of forming mycorrhizal association rapidly under dryland/upland condition, but the infection is rare under submerged condition, due to the presence of anoxic condition. Working on different cultivation system, Lumini et al. (2011) reported that AMF colonization in rice roots occurred only under dry conditions and not in the conventional paddy wet fields under anaerobic condition. However, there are studies which have sufficiently proved that mycorrhizal fungal species can effectively colonize rice grown in wetland and submerged ecosystem as well and have shown significant growth benefits and yield increase (Secilia & Bagyaraj, 1994; Paszkowski et al., 2002; Gutjahr et al., 2009). Studies have shown that these fungi can live in close association with paddy roots in different agro-ecological conditions and may have effects ranging from beneficial to detrimental, depending on the partners involved and other biotic and abiotic conditions in the rhizosphere (Bernaola et al., 2018).

Among the non-microbial biostimulants, humic acids and sea weed extracts are well known and extensively studied for different agricultural crops, including paddy (Olk et al., 2018; Sharp, 2018; Rouphael & Colla, 2020). Biostimulants are known as a natural organic compound, and when applied in small amounts, they can support plant growth, increase the efficiency of nutrient absorption, tolerance to stress, and improve crop quality. They are known to positively influence several metabolic processes such as respiration, photosynthesis, ion uptake, increase crop quality and productivity. Seaweed extract contains macro elements, micro elements, vitamins, fatty acids, amino acids, and phytohormones such as auxin, cytokinin, gibberellins and abscisic acid (du Jardin, 2015).

Depending on the source and method of extraction, Humic substances are known to contain humic acid, fulvic acid, humin, micro & macro nutrients and certain bioactive plant hormones. Humic substances are recognized as an indispensable part of physico-chemical soil properties, mainly for their role in improving the cation exchange capacity. They stimulate root growth and thus increase soil nutrient availability to the roots due to an increase in the area contact between soil and roots. In paddy, application of humic substances, enhanced the yield, by increasing early phase of growth, increasing the number of ears and increasing the number of grains per head (Hasegawa & Yazaki, 1988). Palanivell et al. (2015) reported that apart from improving growth of rice plants, acid soils can be ameliorated by the application of crude humic substances, besides increasing grain yield in rice.

Seaweed extracts are another extensively studied biostimulant on agricultural crops. Layek et al. (2018) reported that foliar spray of seaweed sap at a concentration of 5% and above, significantly increased plant height, dry matter accumulation, chlorophyll index, crop growth rate, yield attributes and yield of rice. Similarly, Pramanick et al. (2014) reported that the seaweed extracts are effective in increasing the growth parameters, yield attributes and yield of rice. Sriyuni et al. (2020) showed that the application of seaweed *Sargassum cristaefolium* applied together with combination of amino acid have the potential to promote growth and yield of upland rice. The authors also reported that application of *S. cristaefolium* added with amino acids increase growth higher than these biostimulants used alone. Beneficial effects of the most well studied seaweed *Ascophyllum nodosum* and pros and cons of different extraction methods are extensively reviewed by Shukla et al. (2019). Interestingly, the combination of humic acids and seaweed extract biostimulants improved early growth of few agricultural crops, as compared to seaweed extract and humic acids alone, showing synergistic effect and comprehensively documented by Cola et al. (2017).

In recent times, public and private research institutions and industries have been actively involved in investing and developing formulation of biostimulant blends using microbial and non-microbial sources. In a review on biostimulants, Rouphael and Colla (2018) opines that the approach of developing such biostimulant blend is mostly empirical rather than with any scientific evidence. Aim of our study was to find out suitability and benefits such blended commercial mycorrhizal formulations containing non-microbial biostimulants, in field condition, on wetland rice.

2. Materials and Methods

This study was conducted during the period September to December 2020, in paddy variety MR 297, which is a popular variety released by Malaysian Agricultural Research & Development Institute. The experimental site is located in Sungai Besar, Selangor situated in the Barat Laut Paddy Project and considered as one of the most important rice production areas in Malaysia. The area was selected due to it is plain flat coastal under Integrated Agricultural Development Authority (IADA) and considered as Rice Granary of the state of Kuala Selangor. The soil is considered as coastal soil, with mean soil pH 5.5, while the mean concentration of organic carbon is in excess of 2%. The mean concentration of total nitrogen is 0.25%, available P > 40 mg kg^{-1} and exchangeable K > 0.1 cmol kg^{-1}. It is located at 3°42′59.0976″N and 101°1′8.382″E latitude.

In this study, we investigated two different formulations of AMF containing different biostimulants as carrier and carried out under two cultivation system-direct seeded and transplanted paddy.

The AMF formulations chosen for the studies were produced and developed by Mycorrhizal Applications Inc, USA. These formulations have the following composition;

- Formula-1 (F-1): *In vitro* produced AMF consortium containing four species, namely, *Funneliformis mosseae, Rhizophagus intraradices, Clariodeoglomus etunicatum* and *Glomus aggregatum,* having a total of 450 propagules per gram of product. The propagules have been formulated using Humic acids as a carrier. The formulation is sprayable and suitable for drench or soil application.
- Formula-2 (F-2): *In vitro* produced AMF consortium containing *F. mosseae, R. intraradices, C. etunicatum* and *G. aggregatum,* containing a total of 900 propagules per gram of product. The propagules have been formulated with a blend of Humic acids and sea weed extract in equal proportion. The formulation is considered ideal for spraying, drench or soil application.

In this experiment, we tried to address two questions;

(1) Whether choice of biostimulant carriers in AMF formulation will have any role in host plant performance? Effects of two different biostimulant carriers (Humic acid alone and blend of Humic acid & seaweed extract) with AMF inoculants, while keeping the number of propagules inoculated per ha constant. Product F-1 was used at the rate of 250 gm per ha, which translates to 112,500 propagules per ha. While, F-2 was used at the rate of 125 gm per ha, which also translates to 112,500 propagules par ha.

(2) Whether planting method and timing of AMF application has any influence on the host plant performance? Effect of these two formulations on direct seeded and transplanted paddy was studied by inoculating at different stages of sowing/growth.

To evaluate influence of AMF inoculum on rice productivity, two field experiments were conducted simultaneously, for direct seeded and transplanted, in the same location, each had a total area of 2000 square meter. Each of the experiment had 3 treatments (F-1 inoculum, F-2 inoculum and untreated control) and each treatment had 6 replications, and each replicated plot size being 40 sq. meter (5 × 8 meter). Buffer rows were kept between the plots on all the four sides in order to prevent cross movement of nutrients or water or any overlapping of treatment effect. Fertilizer programme and other plant protection measures were uniform for all the treatments.

Seed rate for direct seeding was as per the recommendation and farmer practice (150 kg/ha). For transplanting, 15 days old seedlings were used. The seedlings were planted at a density of 3 seedlings per hill and 24 hills per square metre, with a spacing of 25 × 16.5 cm. Our earlier studies with these formulations clearly revealed that the best rate for application for achieving highest grain yield in paddy was 112,500 propagules per ha (Iffah Deris & Christopher, 2021) and hence we opted to use the same application rate for this study. For direct seeded paddy, AMF inoculation was done 7 days after sowing, by drenching together with pre-emergent herbicide (pretilachlor). For transplanted paddy AMF inoculation was given 2 days after transplanting, by drenching together with the same pre-emergent, which is a recommended chemical by the Department of Agriculture in Malaysia.

Grain weight and yield were calculated adjusting to 14% grain moisture content. Grain weight assessment and milling analysis were carried at Laboratory of Food and Security, Universiti Putra Malaysia, Kuala Lumpur. Analysis of variance were conducted to determine the effect of treatments on the response variables and treatment means were compared by Tukey test, using Minitab Version 18 test.

3. Results

3.1 Total Tillers Numbers

Effect of AMF inoculants on the tiller production in transplanted paddy is presented in Table 1a. AMF treated plants produced significantly higher number of total tillers and productive tillers. Among the different formulations tested, plants treated with F-2 formulation produced more tillers than with F-1 formulation. We have not made any tiller count observation for the direct seeded paddy, due to expected variation in the seedling density. Results of the present study indicate that AMF inoculum in the presence of humic acids and kelp is far more effective in terms of tiller production, than AMF inoculum with humic acid alone. Perhaps, seaweeds present in AM Fungi F-2 formulation would have provided an abundant source of natural growth substances and that could possibly have stimulated better plant growth directly and indirectly through enhanced mycorrhization, resulting in higher production of tillers and panicles.

Table 1a. Effect of AMF formulations on the tiller production and grain qualities in the transplanted paddy

Method	Treatment	Total no of tillers	No. of productive tillers	1000 GW (g)	Grain yield (MT/ha)	% yield increase
	UTC	21.8a	15.8a	26.0a	7.6a	0
Transplanted paddy	F-1	23.5b	17.5b	26.2a	8.4b	10.1
	F-2	29.8c	23.8c	27.0a	9.9c	29.8

Note. Means in column followed by different letter(s) are significantly different at $p < 0.05$.

Table 1b. Effect of AMF formulations on the tiller production and grain qualities in direct seeded paddy

Method	Treatment	Total no of tillers	No. of productive tillers	1000 GW (g)	Grain yield (MT/ha)	% yield increase
	UTC	0	0	26.2a	6.6a	0
Direct seeded paddy	F-1	0	0	26.4a	6.9a	4.2
	F-2	0	0	26.7a	7.8b	17.8

Note. Means in column followed by different letter(s) are significantly different at $p < 0.05$.

3.2 Grain Yields

Data on grain weight is presented in Table-1a and 1b. We could not record any significant differences in thousand grain weight (TGW), although there is numerical increase in TWG in treatments received AMF inoculation, as compared to the untreated control. Nonetheless, grains harvested from transplanted fields have higher grain weight than grains from direct seeded plots. Grain weight in F-2 treated plots are numerically higher and more consistent both in direct seeded and transplanted paddy. Grain weight is mostly dependent on the genetics of the crop and variety and it is not conditioned by the status of nutrients (Counce & Wells, 1990).

Values on grain yield increase is presented in Table-1a and 1b. Plots treated with AM fungi F-2 formulation have recorded the highest grain yield, both in direct seeded and transplanted paddy. The treatment response in transplanted paddy is more pronounced than direct seeded. Interestingly, highest grain yield of 9900 kg/ha was recorded in plots treated with F-2 formulation, followed by 8400 kg/ha in plots received inoculation with F-1 formulation, in transplanted paddy, which is 2300 kg/ha and 800 kg/ha more than their respective controls (Figures 1 and 2). In terms of percentage grain yield increase, highest yield increase of 29.8% was recorded in transplanted paddy followed by 17.8% increase in direct seeded paddy, where F-2 formulation was inoculated. These results clearly indicate that there is a greater synergy between AM Fungi, humic acids and seaweed extract, present in F-2 formulation, which is found to be superior to F-1 formulation, containing only AM fungi and humic acids.

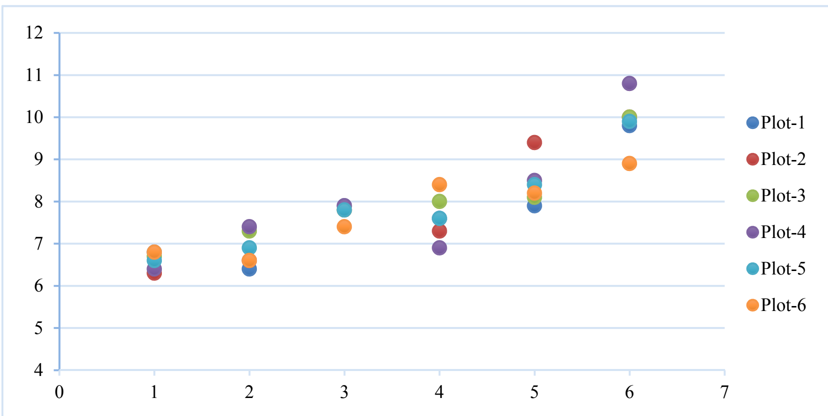
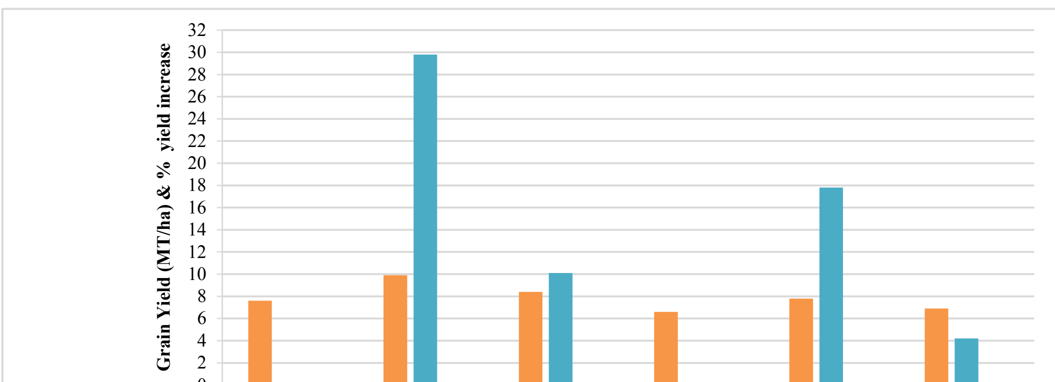


Figure 1. Effect of planting method and AMF formulations on grain yield (MT/ha)

Note. Vertical bar 1-3 direct seeded = 1: UTC; 2: F-1; 3: F-2; & 4-6 transplanted = 4: UTC; 5: F-1; 6: F-2.



	UTC	F-2	F-1	UTC	F-2	F-1
		Transplanted			Direct seeded	
Yield (MT/ha)	7.6	9.9	8.4	6.6	7.8	6.9
% Yield increase	0	29.8	10.1	0	17.8	4.2
Standard Error Mean	0.217	0.253	0.215	0.08	0.075	0.168

Figure 2. Mean grain yield (MT/ha) and yield increase (%) as influenced by planting method and AMF formulation

3.3 Grain Filling

Data on grain filling is presented in Table-2a and 2b. While, significant increase in percentage of grain filling was recorded in all AMF treated plots, the increase was more pronounced in transplanted paddy (both F-1 and F-2 formulations) than in direct seeded paddy. Increase in percentage grain filling was over 9% and consistent in case of transplanted paddy, while it was less than 3% in case of direct seeded paddy. Lowest percentage of grain filling was observed in the control plot of transplanted paddy. Among the yield components, the most contributing factors for yield increase are the number of productive tillers and percentage filled grains. AMF treated plants, especially the transplanted paddy has shown consistent increase in these two parameters, over the direct seeded paddy.

Table 2a. Effect of AMF formulations on grain filling pattern in transplanted paddy

Method	Treatments	Total grains-No (100 g)	Filled grains-No (100 g)	% Filled grains
Transplanted	UTC	4265a	3379b	79.2a
	F-1	4269a	3764a	88.5b
	F-2	4176a	3695ab	88.5b

Note. Means in column followed by different letter(s) are significantly different at $p < 0.05$.

Table 2b. Effect of AMF formulations on grain filling pattern in direct seeded paddy

Method	Treatments	Total grains-No (100 g)	Filled grains-No (100 g)	% Filled grains
Direct seeded	UTC	4246a	3605a	84.9a
	F-1	4221a	3701a	87.7b
	F-2	4287a	3651a	85.3ab

Note. Means in column followed by different letter(s) are significantly different at $p < 0.05$.

3.4 Milling Yield

Milling yield is an important quality indicator in the value chain, especially from the commercial viewpoint. Milling yield includes milled rice yield as well as head rice yield. Milling yield is the estimate of the quantity of total milled rice obtained from a unit of rough rice (paddy) which is produced by removing the hulls, germ, and most of the bran. Milled rice includes intact and broken kernels and generally expressed as percentage. Head rice is the intact or whole kernels and includes milled kernels having equal to or more than three-fourth of whole grain. After adjusting the moisture content to 14%, paddy was subjected to milling and grading and the data are presented in Table-3a and 3b. Milling recovery and percentage of head rice was not influenced greatly, due to any treatments or method of planting. The milling recovery was in the range of 62.4 and 66.8%, whereas the head rice percentage was 93 and 96.9% and the values are not statistically significant. Milling rice recovery and head rice recovery by and large is determined by the fertilizer and nutrient availability, stage of harvesting and importantly, the cultivars.

Table 3a. Effect of AMF formulations on milling recovery and head rice% in transplanted paddy

Method	Treatments	Milling recovery (%)	Head rice (%)
Transplanted	UTC	65.7a	96.5a
	F-1	64.4a	94.3a
	F-2	66.0a	96.9a

Note. Means in column followed by different letter(s) are significantly different at $p < 0.05$.

Table 3b. Effect of AMF formulations on milling recovery and head rice% in direct seeded paddy

Method	Treatments	Milling recovery (%)	Head rice (%)
Direct seeded	UTC	62.4a	93.0a
	F-1	66.8a	94.6a
	F-2	62.5a	94.3a

Note. Means in column followed by different letter(s) are significantly different at $p< 0.05$.

4. Discussion

In this experiment, inoculation of AMF formulations was done together with pre-emergent herbicide. The manufacturer of the product claim that AMF and the formulations are compatible with certain herbicides. Our earlier studies confirm that these AMF formulations are safe to apply together with pretilachlor, a pre-emergent herbicide (Iffah Deris & Christopher, 2021). It is reported that most herbicides do not harm AMF spores or hyphae and there are reports that some have even shown compatibility and synergy between AMF and herbicide application (Ahmed et al., 2019). Pannerselvam et al. (2016b) reported that application of Bispyribac sodium herbicide significantly enhances the mycorrhizal colonization in rice fields.

Effect of humic acid and sea weed extract on the plant growth and number of tillers have been reported and well documented (Sivakumar et al., 2007; Palanivell et al., 2015). Kumar et al. (2014) reported that humic acid treatment significantly increased tiller production and other plant growth parameters in transplanted paddy. Similarly, Suntari et al. (2015) reported significant increase in tiller production in rice due to humic acid treatment. Layek et al. (2018) reported that foliar spray of seaweed extract increased the number of panicles per hill and number of effective grains per panicle, when compared to the untreated control. Similar results of increased yield attributes such as enhanced tiller production and panicles etc were reported for rice by other researchers as well (Patel et al., 2015; Arun et al., 2020). Foliar application of humic acid on leaves effected in increase in plant height, more tillering, ratio of effective branch, index of leaf area, volume of dry matter and number of rice flower/unit of land, number of filled grains or good seeds/rice flowers, ratio of filled grains and 1000 grains weight (Tan et al., 2004). The authors reported that spraying humic acid on leaves of rice of C70 variety had effected in increased productivity and post-harvest quality of rice. Nadjilom et al. (2020) reported significant increase in tiller production due to AMF treatment in two African paddy verities viz., Tox-728-1 and Madjitolngar. Working on resource allocation and grain yield as altered by AMF inoculation, Zhang et al. (2015) found that AMF inoculation markedly increased biomass allocation to shoots.

These authors also infer that AMF inoculation did not increase TGW in wetland rice. Consistent with the above studies, our study also confirm that AMF inoculation increased the tillers and number of panicles but has no effect on TGW. However, in contrast, inoculating with *Glomus hoi* in rice pants grown in saline soils, Fernandez et al. (2011) reported that AMF treatment increased one thousand grain weight significantly. Sisaphaithong et al. (2017) also showed that rice seedlings when pre-colonized with AMF exhibited increase in one thousand grain weight.

Different application methods and benefits of AMF for sustainable rice production is extensively reviewed by Pannerselvam et al. (2016a). When AMF is applied at nursery stage, Solaiman and Hirata (1997) reported grain yield increase in rice up to 21%. Studies by Zhang et al. (2015) showed that AMF inoculation had a significant effect on all biomass production and allocation traits, with plant biomass, shoot biomass and root biomass. Sandepogu et al. (2019) ascribed that among different biostimulants combinations tested in lettuce and spinach, 0.25% of seaweed extract together with 0.2% of humic acids produced significantly higher fresh and dry biomass, compared to the other treatments and concentrations. While the synergistic effect of seaweed extracts and humic acids have been known and documented (Zhang et al., 2003), mutualistic effects between mycorrhizal fungi and biostimulants like seaweed extract and humic acid have also been well documented in review by Rouphael and Colla (2018) and appear to be an emerging area of research.

Previous studies have reported that AMF inoculation increased grain yield both in transplanted paddy as well as in direct seeded paddy. In a pot culture study, Solaiman and Hirata (1997) showed that application of AMF at the nursery stage (akin to transplanting stage) was found to increase grain yield by 14-21% in the wetland rice cultivar Nipponbare. In another pot culture study, Secilia and Bagyaraj (1994) recorded grain yield increase of 35-62% under flooded condition when treated with different AM fungi, such as *Glomus fasciculatum*, *G. mosseae* or *Acaulospora* sp. In a field study, Watanarojanaporn et al. (2013) reported that rice plants harvested from the Systemic Rice Intensification (SRI) managed plots showed significantly higher total biomass, root dry weight, and grain filling than those obtained from conventional flooded, direct seeded paddy. Rice seedlings of European cultivars such as Loto and Gines varieties, when pre-inoculated with AM fungi *R. irregularis*, transplanted into flooded fields, and grown until maturity, showed significant increase in grain yield in mycorrhiza inoculated plants compared with non-mycorrhizal plants, wherein the yield increase was attributed to increase in the number of panicles (Campo et al., 2020). Consistent with the earlier studies, our study reconfirms the above findings that AMF could be potentially used in anaerobic paddy cultivation, both for direct seeded and transplanted paddy.

Sisaphaithong et al. (2017) reported that ARC5955 rice variety, pre-colonized with AMF exhibited significant increase in grain yield, which was associated with the increase in the percentage of filled grains per panicle. Working on several ecotypes of rice, Diedhiou et al. (2016) reported that AMF application positively influence grain filling (spikelet fertility), but also ascribed that it can differ with the ecotype. Working with different farmer fields in Senegal River valley, Wopereis et al. (2002) conclude that the milling recovery and head rice quality is largely influenced by fertilizer status and timing of application. The milling yields and grain quality are mostly determined by variety and less to do with agronomic practices (IRRI, 2015). Our study does confirm that AMF inoculation enhances grain filling significantly, but does not indicate any correlation to the milling quality or percentage of head rice.

Roots of rice plants in agricultural fields exhibit higher affinity of colonization by native AM fungi than other crops in a different environment (Mader et al., 2000). However, when AM fungi is inoculated to the field at different stages of growth, the colonization is largely influenced by the flooding of the field. Dhillion (1992), and Secilia and Bagyaraj (1994) opined that the period from seeding until flooding highly favors the colonization of root systems by AM fungi. Seed priming with AM fungal spores for direct seeded paddy has been studied and reported to be very effective. Kavitha Mary et al. (2018) reported significant plant growth in AMF treated paddy seedlings. However, recent study by Bernaola and Stout (2020) reports that seed treatment with AM fungi not always favors grain yield increase but seeds treated with AM fungi did not reduce percent yield losses and help overcome herbivory injury. Interestingly, grain yields of rice cultivars in Europe, Loto and Gines varieties, inoculated with *R. irregularis* were 41.61% and 28.68% greater than non-inoculated plants, respectively. Also, the number of panicles per plant increased by 30.13% and 14.77% in mycorrhiza treated plants.

Combining seaweed extracts and humic acids is reported to have synergistic effect and it is well documented in a review by Rouphael and Colla (2018). In other words, they work better in combination than used alone, magnifying their benefits to the plant. Virginia Tech, USA did a long term study on organic bio-stimulants, and they found that combining humic acids and seaweed extracts at a specific ratio, worked 50% better than either product alone (Zhang et al., 2003). They also reported that plants treated with the humic acids/seaweed combination produced 50% more SOD (superoxide dismutase). SOD is a powerful antioxidant enzyme and a plant protection and stress reducing agent. SOD is known to protect the cell membranes, mitochondrial membranes and chlorophyll membranes from harmful oxidative stress. The application of humic acids alone or the application of mycorrhizal inoculum (*R. intraradices*) resulted in a significant increase in dry weight of onion roots and leaf carotenoids by 43.9 and 12.1% (for humic acids) and by 29.6 and 57.1% (for mycorrhizal fungi), respectively, whereas the application of both humic acids and mycorrhizal fungi together, induced a synergistic effect with the highest accumulation of the two parameters, root weight and leaf carotenoid content by 106.7 and 123.6%, respectively (Bettoni et al., 2014). The apparent mode of action involved in the stimulation of crop performance was linked to enhanced nutrient availability driven by the synergistic action of humic acids and mycorrhizal fungi applied in combination. Similarly, in perennial ryegrass the combination of mycorrhizal fungi (*R. intraradices*) and humic acids spray when applied on the substrate as pre-sowing was more effective in enhancing root biomass and chlorophyll biosynthesis than either products applied alone (Nikbakht et al., 2014). Similar synergistic action between mycorrhiza, sea weed extract and humic acids also has been reported in tobacco (Moradi et al., 2019).

Chemical fertilization is the common practice in rice to address the demand for higher productivity. However, there are concerns with regard to the use of excessive chemical fertilizers like improper use, excessive use, imbalanced fertilization etc., which can lead to poor fertilizer use efficiency, run-off, and eutrophication and eventually increased cost of fertilizer inputs in the hands of farmer besides adversely affecting the environment and sustainability. This can be largely mitigated by the judicious use of beneficial microbes like AMF. The appropriate use of AMF and a suitable delivery mechanism together with compatible, yet synergistic biostimulants can contribute significantly in enhancing rice productivity and at the same time, reducing the environmental impact. *In vitro* produced AMF fungi has received lot of attention in recent times due to its advantages like production of contamination-free propagules, mass production, scaling up possibility which are the major constraints faced by substrate based (*in vivo*) production system. Another major advantage of *in vitro* produced propagules is the possibility of using in irrigation and spraying system, which is not possible with the propagules produced through substrate based production system. The *in vitro* produced AMF formulations tested in this experiment holds certain advantage when resort to application methods like drenching or spraying in paddy cultivation.

5. Conclusion

Rice yield and quality are the primary focus of this experiment—given this, it is vital to have a better understanding how effective AMF inoculants would be, when delivered with different biostimulant blends for different cultivation systems. Biostimulants (microbial and non-microbial) are increasingly becoming viable options for solving the problem of ineffective uptake of nutrients from fertilizers by plants and to use them as potential tools to enhance fertilizer use efficiency. Our study reports that AMF inoculation in paddy is beneficial both for direct seeded and transplanted paddy, albeit the best result was recorded in transplanted paddy which recorded highest grain weight increase. This study also proved that biostimulant carrier in the form of humic acids and sea weed extract is best suited for enhanced efficacy of AMF inoculation, which has an edge over the formulation, where humic acid alone was used. We suggest that *in vitro* produced AMF inoculation delivered through biostimulants like humic acids and sea weed extract could be a powerful tool for improving rice grain yield, especially for flooded anaerobic cultivation system both for direct seeded and transplanted paddy. However, while choosing humic acids and seaweed extract as a suitable carrier for enhancing efficacy of AMF inoculants, the source and method of extraction does matter in the efficacy of the product. Humic acids are obtained from different sources, like mines, peat sources, organic matter, vermicompost etc, wherein the composition and presence of bioactive compounds varies greatly based on the sources. Same way, source of seaweed extract, different extraction methods and the species used determine the presence and activity of bioactive compounds and finally the field efficacy (Goni et al., 2018). Besides, as stated earlier, ratio of humic acids and seaweed extract blend is another determining factor on the product efficacy and hence while developing AMF formulations it would be critical to consider these parameters.

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