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Isolation of Arbuscular Mycorrhizal Fungi and Evaluation its Effect on Plant Growth over Chemical Fertilizers for Better Human Health

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Abstract

Background: Plants grown under the environment of chemical fertilizer exert ill effects on human health. The role of beneficial microorganisms can prove to be the most effective alternative to chemical fertilizers for enhancing growth and biomass production of crop plant. Therefore the present study has been undertaken to get better understand the effects of Arbuscular mycorrhizal fungi over chemical fertilizers in enhancing the growth and biomass production of plants with aim for better human

health. **Materials and Methods:** Arbuscular Mycorrhizal (AM) spores were isolated from rhizosheric soil by wet sieving and decanting method. The species of fungal spore were identified depending on morphological characteristics. To see the effect on plant growth, co-culture of AM fungal spores was performed with *Zea mayes*. Co-culture was done in six treatment groups where treatments (1, 2, and 3) were considered as control and treatments (4, 5, and 6) were sample. **Results:** Vesicles and arbuscules like structure were observed within the root of *Zea mayes*. The shoot and root weight of Treatments (T_1 - T_6) was found 36.25±0.86g and 17.00±0.88g; 26.50±1.47g and 13.50±1.11g; 13.5±1.11g and 7.25±0.62g; 39.75±0.56 g and 20.5±0.57g; 33.75±0.41 g and 18.25±0.41g; 19.50±0.63 g and 10.5±0.42 g respectively. The mean weight of shoot and root of *Zea mayes* in treatment 4 (T_4) were significantly ($p<0.001$) higher than treatment 1 (T_1). In case of T_5 and T_6, shoot and root weight were also significantly higher than treatment 2 (T_2, $p<0.001$) and treatment 3 (T_3, $p<0.001$) respectively. **Conclusion:** When arbuscular mycorrhizal fungal spores are used with chemical fertilizers, it reduces the usage of chemical fertilizer and increase plant growth and biomass.

Keywords: Arbuscular mycorrhizal fungi, Biofertilizer, *Zea mayes,* root weight, shoot weight, Human health

Introduction

In order to increase crop production for fulfillment of food requirements of the day by day increasing population, the systems is relying exclusively on the use of chemical fertilizers. Whereas, it is proved that use of chemical fertilizers and other pesticides are causing tremendous harm to the environment by pollution and contamination in water and soil and common men are suffering by many means, specially their health has been affected very much because of the situation (Weisenburger, 1993). Therefore, the need to increase production of crops, to preserve soil fertility and to protect the environment from detrimental, agronomic techniques has brought about a revision of productive systems in agriculture. Recently, the employment of beneficial microorganisms has gained popularity

(Pearson and Read, 1973; Giovanetti and Gianinazzi-Pearson, 1994; Perotti et al. 1996). Arbuscular mycorrhizal (AM) fungi are most important one. Its mutualistic association with roots can improve a plant's nutritional state by facilitating the absorption of the main elements in the soil (N, P, and K). It also increases the volume of soil explored by the root system, improve the plant's resistance to some diseases and increase its production of dry matter (Barber, 1995; Smith and Read, 1997; Giovanetti and Sbrana, 1998). The effects of Arbuscular mycorrhizas seem to increase in nonoptimal nutritional conditions. In environments with scarce precipitation, the presence of these fungi can make the plants more resistant to water stress and strengthen their ability to use the nutrients naturally occurring in the soil (Staddon et al. 2002; Koide and Dickie, 2002). Although rhizosphere microbes are well known to modulate plant nutrients, people are only just beginning to directly correlate soil health to human health (Morrissey et al., 2004; He and Nara, 2007). This is a very complex issue that spans agricultural practices, soil microbiology, food culture, and global food security. Human have been concerned with increasing the nutritional value of food issue since essential nutrients were first described (Kaluski et al., 2003). However, various current approaches (fertilize, diversified diet, fortify and biofortification) to boosting crop nutrient levels rely heavily on industrialized food production systems. While this is an effective way of producing nutritious food for countries that can pay for it, there remain economic and social barriers for many countries. A substitute to traditional ways of boosting nutrient may exist in soil. Interactions between beneficial microorganisms and plants have been well studied. These include root dwelling microbes as well as other endophytic and epiphytic microbesfound in roots and shoots (Rengel and Marschner, 2005). These symbiosis associations are well known to influence the nutrient status of plants based on their ability to access minerals, most importantly in nutritionally stressed environments (Jumpponen, 2001; Jeffrieset al., 2003; Johnson et al., 2010).Therefore the present study was undertaken to get better understand the effects of Arbuscular mycorrhizal fungi over chemical fertilizers in enhancing the growth and biomass production of plants.

Materials and Methods

The experiment was done at Department of Biochemistry of Molecular Biology, University of Chittagong, Bangladesh during the period of September'2011-December'2011. Five kilogram rhizospheric soil was collected from maize field at Hathazari, Chittagong, Bangladesh.

Isolation of AM fungal spores

The rhizospheric soil was first mixed thoroughly breaking lumps, if any, between the thumb and fingers and 200 g soils from it was kept in 8 liter capacity bucket filled in three quarter with tap water. The materials in bucket were agitated vigorously by hand and left to settle down for about ten second. The suspension was then sieved by wet sieving and decanting method (Schenck and Perez, 1990). Two sieves of 250 μm and 100 μm (micromesh) were used sequentially in sieving. The solution with spores was evenly distributed in two equal sized test tubes balancing up the tubes to equal weights. The tubes were plugged properly and centrifuged for five minutes at 3000 rpm. The supernatant was poured, the tubes filled with 60% sucrose solution and stirred vigorously with a round-ended spatula to re-suspend the precipitate. The plugged test tubes were again centrifuged for 2 minutes at 1800 rpm, supernatant sucrose poured through a 100 micromesh (μm) sieve and washed rapidly with water to remove the sucrose from mycorrhizal spores. The materials were then transferred from sieve through washing to Petridis for observation.

Arbuscular Mychorrhizal (AM) inoculums preparation

Mychorrhizal spores were isolated from soil and about 500 spores were separated in a Petridis for each treatment.

Germination of seed

Seeds of *Zea mayes* (maize) were collected from the market. Almost equal in size, good quality seeds were then separated out. The maize seed was germinated in petridishes with wet filter paper. The Petridis was covered and was kept at room temperature. After three days about 70-80 % seeds were germinated.

Preparation of potting materials for co-culture

For pot experiment, different sizes sand particles (250μ to 1mm) were collected. Sand

mixture was then autoclaved for 60 minutes to make it microbes free. It was done three times for better result.

Co- culture of Arbuscular mycorrhizal fungi (AMF) with Zea mayes (Maize)

Experimental design for co-culture

Six different treatments were used for co-culture. In each treatment there were five replications. The treatments were

T_1 : 100 % (N+ P+ K)

T_2 : 75 % (N+ P+ K)

T_3 : 50 % (N+ P+ K)

T_4 : 100 % (N+ P+ K) + AMF

T_5 : 75 % (N+ P+ K)+ AMF

T_6 : 50 % (N+ P+ K)+ AMF

N= Nitrogen, P= Phosphorous, K= Potassium, AMF=Arbuscular mychorrhizal spores

Fertilizer recommendation

As Sand is nutrient deficient so it was taken as potting mix. Fertilizers were recommended following Fertilizer Recommendation Guide (2005) by Bangladesh Agricultural Research Council (Miah et al. 2005). 100% (N, P and K) = 89 mg N/kg sand (200kg/ha), 28.6 mg P/kg sand (64kg/ha) and 57 mg K/kg sand (128kg/ha). Only percentages of N, P & K were varied in different treatments. Micronutrients were recommended as 18 mg S /kg sand (40kg/ha), 5.35 mg Mg /kg sand (12kg/ha), 1.78 mg Zn/kg sand (4kg/ha), 0.89mg B /kg sand (2kg/ha) without considering any variation within the treatments (BARC, 2005). Urea (46%), TSP (20%) and Muriate of Potash (50%) were used as a source of N, P and K respectively (BARC, 2005).

Doses of fertilizers

100%, 75% & 50% (N, P and K) were divided into six parts. Initially one part was mixed with sand and after that the rest of the parts were given after 15 days up to 90 days.

Co- culture

Pots were filled with 3 kg sand. For treatment T_4, T_5 and T_6, 500 AM fungal

spores/pot were added below 3 cm from the surface. After that, maize seedling was sown in each pot. Small volume of water was also given to each pot. After 15 days of co-culture, chemical fertilizer was given to each pot in accordance to treatment plan. The plants were kept to grow for 90 days. After then the plants were pulled out to collect the roots and shoots.

Weight of Shoots and Roots

Shoots and roots were cut with sharp blade. To avoid damage, collected roots with adhered sand were emerged in water in a white clean bowl to allow the sand particles to separate away. Water was changed several times for a complete wash and dried by soaking with filter paper. The weight of shoots and roots were then taken by digital balance.

Determination of Arbuscular mycorrhizal (AM) infection capability

Roots were collected from all the treatment after weighting roots of harvesting maize plants. AMF colonization was determined by using a modified root staining (Khade and Rodrigues, 2002) procedure (Koske and Gemma, 1989). Fine root samples of each species were washed under tap water and cut into small segments of approximately 1cm length, from which 100 root segments were randomly selected for staining. These root samples were then cleared in 2.5% KOH, acidified in 5 N HCl and stained in lactoglycerol with 0.05% Trypan blue. The stained roots were observed under a compound microscope for the presence or absence of mycelium, vesicle and arbuscule or combination of one, two and all of these structures.

Statistical analysis

The weight of shoots and roots were analyzed statistically by the software SPSS 11.5. Here the mean and standard error of mean (SEM) were calculated. The means of shoot and root weight in different treatment group were compared statistically by independent sample t-test.

Results

Infection capability of Arbuscular Mycorrhizal fungus

During co-culture we observed the formation of vesicles in root which is an important characteristic of *glomus sp* of Mycorrhizal fungus (Fig 1).

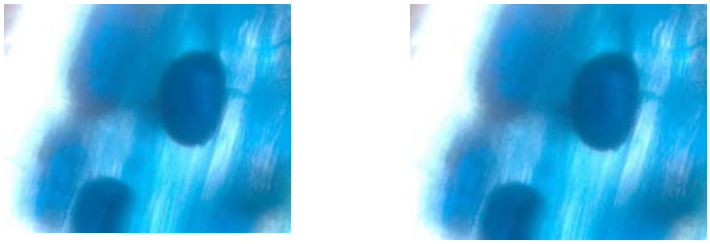

Fig 1: Vesicles formed in *Zea mays* root

Shoot and root weight (Mean±SEM) of Zea mays in different treatment

In Treatment 1 (T_1) shoot and root weight was found 36.25±0.86 g and 17.00±0.88 g respectively. In Treatment 2 (T_2) shoot and root weight was found 26.50±1.47g and 13.50±1.11g respectively. In Treatment 3 (T_3) shoot and root weight was found 13.5±1.11 g and 7.25±0.62 g respectively. In Treatment 4 (T_4) shoot and root weight was found 39.75±0.56 g and 20.5±0.57 g respectively. In Treatment 5 (T_5) shoot and root weight was found 33.75±0.41 g and 18.25±0.41 g respectively. In Treatment 6 (T_6) shoot and root weight was found 19.50±0.63 g and 10.5±0.42 g respectively (Table 1).

Mean comparison of different treatments taking treatment 1, 2 and 3 as control

The mean shoot weight of *Zea mays* in treatment (T_4) was significantly higher than those in controls (T_1, T_2 & T_3; $p=0.004$, $p<0.001$ & $p<0.001$ respectively) and treatments (T_5, & T_6; $p<0.001$ in both). In treatment (T_5) this was significantly higher than those in controls (T_2 & T_3; $p<0.001$ in both) and treatment (T_6; $p<0.001$) but significantly lower than those in control (T_1, $p=0.02$) and treatment (T_4, $p<0.001$). In treatment (T_6) this was also significantly higher than those in control (T_3; $p<0.001$) but significantly lower than those in controls (T_1&T_2, $p<0.001$ & $p=0.001$ respectively) and treatments (T_4, &T_5; $p<0.001$ in both) (Table 1, 2). The mean root weight of *Zea mays* in treatment (T_4) was significantly higher than those in controls

Table 1: Root and shoot weight of *Zea mays* in case of different treatments

Treatment	Shoot weight(gm) (Mean±SEM)	Root weight(gm) (Mean±SEM)
T_1 : 100% feritilizer (Urea+ TSP+Murat of Pothash)	36.25±0.86	17.00±0.88
T_2 : 75% feritilizer (Urea+ TSP+Murat of Pothash)	26.50±1.47	13.50±1.11
T_3 : 50% feritilizer (Urea+ TSP+Murat of Pothash)	13.5±1.11	7.25±0.62
T_4 : 100% feritilizer (Urea+ TSP+Murat of Pothash) + Micorrhizal spores	39.75±0.56	20.5±0.57
T_5 : 75% feritilizer (Urea+ TSP+Murat of Pothash) + Micorrhizal spores	33.75±0.41	18.25±0.41
T_6 : 50% feritilizer (Urea+ TSP+Murat of Pothash) + Micorrhizal spores	19.50±0.63	10.5±0.42

Table 2: Result of Independent t-test analysis of mean differences of shoot weight between groups

	T_2	T_3	T_4	T_5	T_6
T_1	0.000	0.000	**0.004**	**0.02**	0.000
T_2	-	0.000	0.000	**0.000**	0.001
T_3	-	-	0.000	0.000	**0.000**
T_4	-	-	-	0.000	0.000
T_5	-	-	-	-	0.000

Table 3: Result of Independent t-test analysis of mean differences of root weight between groups.

	T_2	T_3	T_4	T_5	T_6
T_1	0.028	0.000	**0.005**	**0.222**	0.000
T_2	-	0.000	0.000	**0.001**	0.025
T_3	-	-	0.000	0.000	**0.001**
T_4	-	-	-	0.006	0.000
T_5	-	-	-	-	0.000

P value<0.05 was considered as significant

T_1=100% (U+T+M); T_2=75% (U+T+M); T_3=50% (U+T+M); T_4=100% (U+T+M) + AMF; T_5=75% (U+T+M) + AMF; T_6=50% (U+T+M) + AMF;

U= Urea, T= TSP, M= Murat of Potash, AMF= Arbuscular Mycorrhiza Fungi

(T_1, T_2 & T_3; $p<0.005$, $p<0.001$ & $p<0.001$ respectively) and treatments (T_5, & T_6; $p=0.006$ & $p<0.001$ respectively). In treatment (T_5) this was significantly higher than those in controls (T_2 & T_3; $p=0.001$ & $p<0.001$ respectively) and treatment (T_6; $p<0.001$). The mean roots weight between treatment (T_5) and control (T_1) did not show any significant difference. In treatment (T_6) this value was also significantly higher than those in control (T_3; $p=0.001$) but significantly lower than those in controls (T_1&T_2, $p<0.001$ & $p=0.025$ respectively) (Table 1 & Table 3)

Discussion

Arbuscular Mycorrhizae (AM) is an integral part of most plants in nature (Atlas, 1982) and occurs on 83% of dicotyledonous and 79% of monocotyledonous plant investigated (Wilcox, 1996). All gymnosperms are reported as being mycorrhizal (Newman and Reddell, 1987). Infection of the root system of the plant by these fungi creates a symbiotic (beneficial) relationship between the plant and fungus. Upon root infection and colonization, AM fungi develop an external mycelium which is a bridge connecting the root with the surrounding soil (Toro et al. 1997). One of the most dramatic effects of infection by AM fungi on the host plant is the increase in phosphorus (P) uptake (Kothari et al. 1991) mainly due to the capacity of the AM fungi to absorb phosphate from soil and transfer it to the host roots (Asimi et al. 1980). In addition, AMF infection results in an increase in the uptake of copper (Lambert et al. 1979), zinc (Lambert et al. 1979), nickel (Killham and Firestone, 1983), chloride and sulphate (Buwalda et al. 1983).

AM fungi are also known to reduce problems with pathogens which attack the roots of plants (Gianinazzi-Pearson and Gianinazzi, 1983). The benefits are greatest in P-deficient soils and decrease as soil phosphate levels increase (Schubert and Hayman, 1986). It is well established that infection by AM fungi is significantly reduced at high soil phosphorus levels (Amijee et al. 1989; Koide and Li, 1990), the addition of phosphate fertilization results in a delay in infection as well as a decrease in the percentage of infection of roots by AM fungi (De Miranda et al. 1989). Therefore the role of Arbuscular mycorrhizal (AM) fungi in the growth and multiplication of crop

plant can prove to be the most effective alternative to fertilizers for enhancing growth and biomass production.

In our study, by using sand as potting materials, the concentration of phosphorous, nitrogen and potash was reduced. The chemical fertilizer was applied after 15 days of co-culture so that phosphorous level does not increase and AM fungal spore can infect the root system as increased phosphorous level inhibit mycorrhizal infection(Happer,1983). Upon visualization maize roots were found to be yellowish which was an indication of AMF infection as mycorrhizal infected roots turns into yellow (Karthikeyen et al. 1995). After staining with dye, arbuscles and vesicles like structures were observed within the maize roots (Fig 2). So these fungi may be vesicular arbuscular mycorrhizal fungi. These structures are responsible for the transfer of absorbed nutrients from the fungus to the roots and ultimately enhance plants growth and yield. Control maize plants didn't show any AMF infections in roots and spore productions was also not observed in control maize plants containing sand. Internal hyphae, arbuscules and vesicles were observed from roots of all the treatment except controls (Fig 2). Maize plant growth was found to be significantly increased in AM fungal spore treated maize seedlings than control. Mean shoot and root weight of 75% fertilizer and AM fungi (T_5) treated maize plant was found significantly higher than the maize plant treated 75% fertilizer alone (T_2) and almost similar to the maize plant treated 100% fertilizer alone (T_1). Seedlings treated with 50% fertilizer and AM fungi (T_6) also showed good results as compared to 50% fertilizer alone (T_3) (table 2, 3 &4). From this data it can be stated that use of AM fungi may reduce 25% chemical fertilizer usage. This may be because AM fungi help to increase nutrient uptake by increasing the surface area of the plant absorptive system (roots) that was evident from our experiment and exploring soil by extraradical hyphae beyond the root hair and P-depletion zone and thus facilitates the absorption of the main elements in the soil (N, P, and K), improves the plant's resistance to some diseases, and increasing its production of dry matter (Barber, 1995; Smith and Read, 1997; Giovanetti and Sbrana, 1998). Higher root biomass production in mycorrhizal plants compared to non mycorrhizal plants has been frequently

reported (George, 2000). Similar results were seen in our study. Results of the experiments confirm various reports on enhanced plant growth due to AM inoculation to medicinal plants (Nisha and Rajeshkumar, 2010) and forest tree species (Rajan et al. 2000).

It has been reported that even if AM fungi are considered to have a wide host range, there is some degree of ecological specificity between AM fungi and plants (Rosendahl et al. 1992). The efficiency of the fungus to increase plant growth in a phosphate-deficient soil depends on the ability to form extensive and well-distributed hyphae in soil to form extensive colonization in the root system and to absorb P from soil. Hence, the need for selecting efficient AM fungi that can be used for inoculating different mycotrophic plants has been stressed by different workers (Bagyaraj and Varma, 1995). That is why our study was performed to investigate whether AM fungal spore can infect Zea mays or not and its role over chemical fertilizer. In our experiment we found positive result for root colonization of AMF in Zea mays. An experiment in peach by McGraw and Schenck (1980) found that vesicular-arbuscular mycorrhizal (VAM) fungi increased growth 25-75% compared to the control plants. A significant improvement in the plant height, plant canopy, pruned material and fruit yield was evident in 5-year-old pomegranate plants in field conditions. In view of the above results, use of biofertilizer technology may be adopted for the establishment and development of other horticultural plant species in arid regions.

Studies conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India, on neem (Azadirachta indica) showed that combined inoculation of neem plants with AM fungi and phosphobacterium (Pseudomonas striata) enhanced the dry matter production, AM colonization, and plant nutrient uptake significantly as compared to individual inoculation and uninoculated controls(Karthikeyen et al. 1995). In our study AM fungi reduce the use of chemical fertilizer by 25% (Table 2). It was also observed that when chemical fertilizer was used in combination with mycorrhiza, the plant growth occurs highest. Many of the compounds produced by plants in response to AM fungal colonization function as antioxidants in the plant and also in our diet (Toussaint et al., 2007). There

is mounting evidence that these compounds are critical for optimal human health and disease prevention (Halliwell, 1996). Considering that these compounds have important pharmacological effects, it is surprising that very little is known about relationship between the AM symbiosis and their synthesis. The present study is a first step to explore the beneficial health effects of AM fungi.

Conclusion

From the above study, it can be concluded that Arbuscular mycorrhizal fungi (AMF) can effectively reduce the requirements of chemical fertilizers and increase the growth of plants. Further study like nutritional status of plants that grown with chemical fertilizer have to be compared with the nutritional status of plants grown with biofertilizer, for better understanding the role of biofertilizer on human health.

Conflict of interest: The authors declare that they have no conflict of interest.

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