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Growth and Biochemical Changes of Vegetable Seedlings Induced by *Arbuscular mycorrhizal* Fungus

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ABSTRACT: The efforts were made to study the effects of *Arbuscular Mycorrhizal* fungi (AM) on the morphological and biochemical changes of four different vegetable seedlings grown under nursery conditions. The symbiotic association between AM fungi and plant roots provides a significant contribution to plant nutrition and growth. Hence, an attempt has been made to examine the effect of AM fungi on the four vegetable crops such as Tomato (*Lycopersicum esculentum* L.), Brinjal (*Solanum melongena* L.), Chilli (*Capsicum annuum* L.) and Bhendhi (*Abelmoschus esculentus* Moench.). The maximum increase in four plant's morphological parameters like root length, shoot length, fresh weight, dry weight, number of leaves, total leaf area and biochemical parameters like chlorophylls, proteins and nutrient content of nitrogen, phosphorus and potassium were observed in AM fungi treated seedlings when compared to non-mycorrhizal seedlings (controlled). The sugar and starch contents showed decrease in mycorrhizal seedlings than non-mycorrhizal one.

Key words: *Glumus fasciculatum*, Vegetables, Morphological parameters, Biochemical content

Introduction

Arbuscular mycorrhizal (AM) fungi have symbiotic relationship with roots of plants in terrestrial ecosystems and associating with about 80% of plant families world wide. AM fungi recognized as an important, widespread component of most terrestrial ecosystems and benefiting plant's well establishment by enhancing plant nutrient acquisition, improving soil quality and increasing resistance to environmental stresses (Smith and Read, 1997). They are also described to improve the absorption of several plant nutrients like N, P, K Mg, Cu, Ca and Fe by the roots of plants (Liu *et al.,* 2002). The mycorrhizal infection enhances plant growth by increasing nutrient uptake through increasing the absorbing area of root and by mobilizing sparingly available nutrient sources; or by excretion of chelating compounds or ecto enzymes (Marscher and Dell, 1994). The AM fungi can increase plant uptake of nutrients and consequently increase root and shoot biomass and improve plant growth and yield (Ryan and Angus, 2003). The inoculation of AM fungi improves the physico-chemical and biochemical properties of amended soil (Caravaca *et al.,* 2004). Phosphorus (P) solubilizing microorganisms increase the mycorrhizal colonization by production of specific metabolites like vitamins, amino acids and hormones (Barea *et al.,* 1997).
The AM mycelium in soil results in increase the efficiency of nutrient absorption particularly for slowly diffusing minerals, especially phosphorus by (Smith *et al.,* 2000). In addition to phosphorus AM mycelium also enhances the uptake of nitrogen in the form of NO_3 (Frey and Schuepp, 1993; Morte *et al.,* 2000) and also increases the potassium content in plants (Maksoud *et al.,* 1994). The present work aims at studying the effects of AM fungi on morphological and biochemical changes of the *Lycopersicum esculentum* L., *Solanum melongena* L., *Capsium annuum* L. and *Abelmoschus esculentus* Moench.

Materials and Methods

The experiment was conducted in the Botanical Garden of Botany Department, Annamalai University, Annamalainagar. The sand and loam soil (1:1) were air dried by passed them through 2 mm sieve and mixed thoroughly for getting homogenicity and sterilized by autoclaving at 120°C for 20 min to kill soil microbes. The seeds of *Lycopersicum esculentum* L., *Solanum melongena* L., *Capsicum annuum* L. and *Abelmoschus esculentus* Moench., were obtained from the Department of Horticulture of Tamil Nadu State Government, Dharmapuri. The seeds' sizes and weight were homogenous. They were surface sterilized with (0.1% $HgCl_2$ + 0.2 HCl for 4 min.) and finally washed with tap water.

Mycorrhizal spores: The spores of AM fungus *Glomus fasciculatum* were collected from the Department of Agricultural Microbiology, Annamalai University. For getting more spores of *G. fasciculatum* it was cultured along with the plant *Zea mays.* After culturing, the spores were extracted by using wet sieving and decanting method (Daniels and Skipper, 1982). The spore suspension was diluted with tap water and each ml has 50 spores and this was the optimal range for inoculation. 5 ml of spore suspension was mixed with soil and they were filled in the pots and the pots were arranged in randomized block design. For this study, inoculated with AM fungi and uninoculated (control) plants were raised. Fifteen seeds were sown in each pot at equal distance and regular cultural operations namely irrigation, weeding and plant protection measures were carried out. At the end of the growth period, plant samples were carefully up rooted and washed thoroughly with tap water. After washing, the roots and leaves were separated and the necessary analyses were carried out.
The shoot (cm) and root length (cm), the fresh and dry weight (mg/fr. wt.), the number of leaves per plant and total leaf area (cm^2) were determined. The pigment contents, chlorophyll 'a', 'b' and total chlorophyll were estimated spectrophotometrically (Metzner et al., 1965). The biochemical content of protein was estimated by using the method of (Lowry et al., 1995). The starch was determined following the method of (Mc Cready et al., 1950). The sugars were estimated following the method of Dubois et al. (1956). The Total Kjeldahl Nitrogen (TKN) was determined after digesting the sample with concentrated H_2SO_4 (1:20, w/v) followed by distillation method (Bremner and Mulvaney, 1982). Total phosphorus (TP) and total potassium (TK) contents of plants were analysed by wet digest method (tri acid (HNO_3 –H_2SO_4 –$HClO_4$) mixture was used for digestion) (Jackson, 1973). In this process, the total phosphorus (TP) was estimated by colorimetric method the and total potassium (TK) was determined by flame photometer method (Bansal and Kappoor, 2000).

Results and Discussion

AM fungi are ecologically important symbiont of most terrestrial plant's root system and they are critical component of terrestrial ecosystems and have important effects on nutrient acquisition by most of land plants. It is frequently suggested that AM may improve P nutrition, enhance N uptake, improve disease resistance in their host plants or adaptation to various environmental stresses. The agriculturally important symbiotic microorganisms play a remarkable role in nutrients (nitrogen, phosphorus, potassium and microelements) acquisition for plants (Rabie et al., 2005).
The results indicate that the inoculation of AM fungi results in significant increase in germination percentage, shoot and root length, fresh and dry weight, number of leaves per plant and total leaf area, when compared to uninoculated plants (Figs. 1-5). The increase in plant growth may be attributed to increased uptake of

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phosphorus by AM fungi, which levels to the increase of shoot and root lengths (Vejsadova et al., 1992; Linderman and Davis 2004).

Fig. 1. Impact of AM fungi on germination percentage of vegetable seedlings studied (on 7th day)

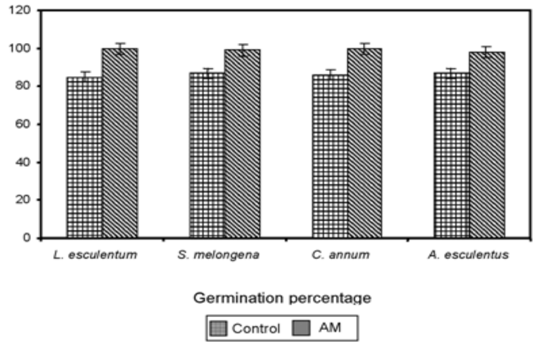


Fig. 2. Impact of AM fungi on root length of four different vegetable seedlings (on 30th day)

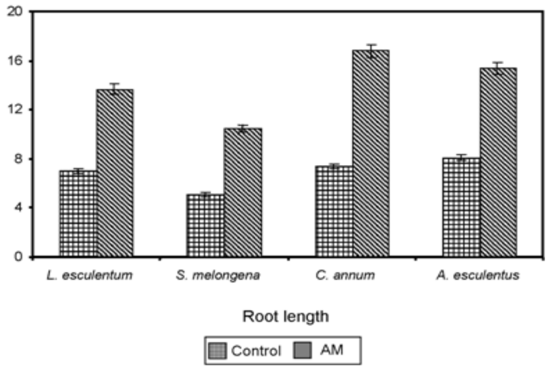


Fig. 3. Impact of AM fungi on shoot length of four different vegetable seedlings (on 30th day)

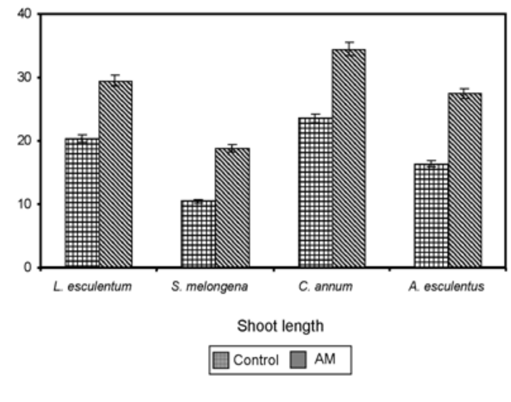


Fig. 4. Impact of AM fungi on number of leaves of four different vegetable seedlings (on 30th day)

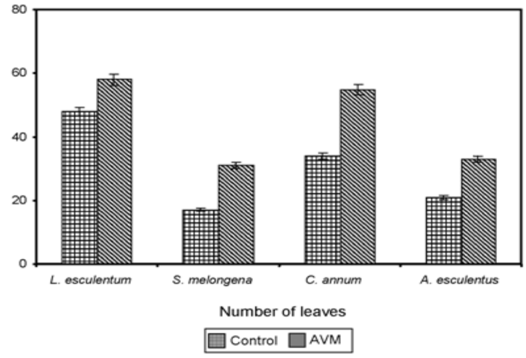
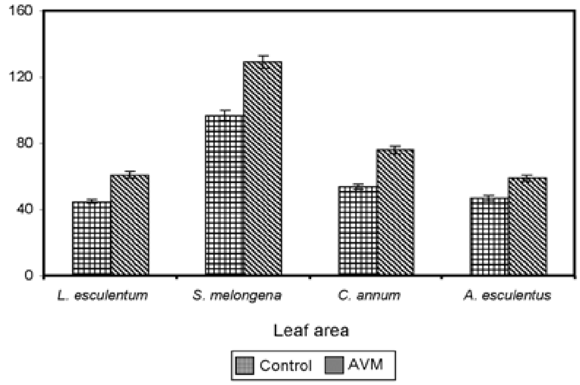


Fig. 5. Impact of AM fungi on leaf area of four different vegetable seedlings (on 30th day)



The mycorrhizal infection is known to enhance the plant growth by increasing nutrients uptake (Marschner and Dell, 1994). The increasing of height registered with inoculated plants could be as a result of enhanced inorganic nutrients absorption (Cooper, 1984). The greater rates of photosynthesis which obviously could have given rise to an increase in plant growth (Allen et al., 1984). The chlorophyll 'a', 'b', and total chlorophyll contents increased in mycorrhizal inoculated plants when compared to uninoculated plants (Figs. 6-8). The AM symbiosis increased leaf gas exchange and photosynthetic rate (Ruiz-Lozano et al., 1996), AM inoculated seedlings show a greater increase in the rate of photosynthesis than uninoculated plants (Wright *et al.,* 1998) and it suggests that AM colonization increase photosynthetic activity.

Fig. 6. Impact of AM fungi on chlorophyll 'a' content of four different vegetable seedlings (on 30th day)

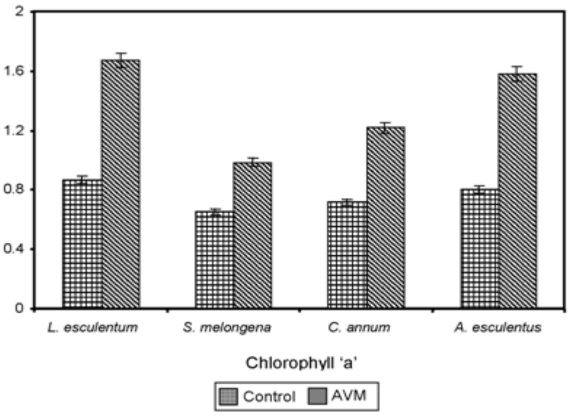


Fig. 7. Impact of AM fungi on chlorophyll 'b' content of four different vegetable seedlings (on 30th day)

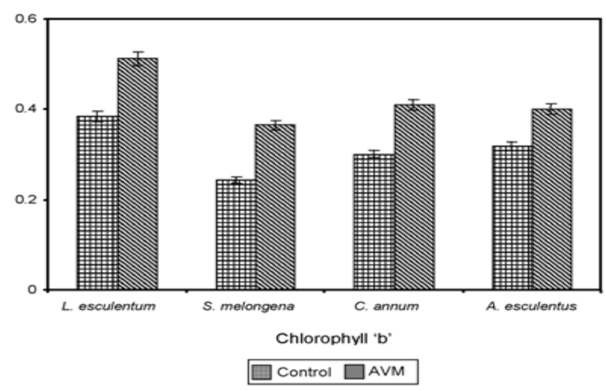
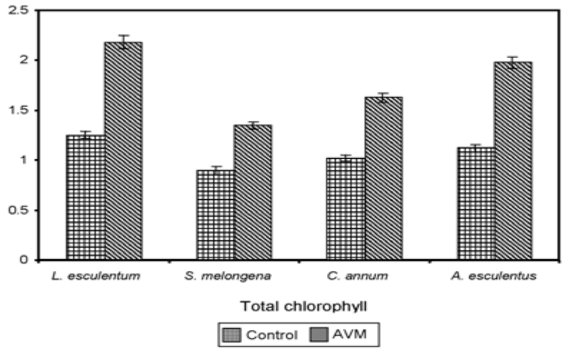


Fig. 8. Impact of AM fungi on total chlorophyll content of four different vegetable seedlings (on 30th day)



Increased levels of protein in the inoculated plants could be attributed to either the presence of fungal proteins or post infectional stimulation of protein synthesis in the host plant (Krishna and Bagyaraj, 1983). The mycorrhizal and non-mycorrhizal plants are known to differ in their biochemical constitution particularly in the amino acid and protein fractions (Nermic and Meredith, 1981). The sugar and starch contents in the leaves of the selected vegetable plants of this study showed a decrease in mycorrhizal seedlings than non-mycorrhizal seedlings (Figs. 10-11). The decrease in sugars, starch may be due to the translocation of carbohydrates produced by the host to the fungal partner (Johnson et al., 1997). The decrease in sugars and starch in the leaves may be due to the fact that the AM fungi utilize of net photosynthate in exchange for the nutrients to the host to lead a symbiotic life (Allen, 1991).

Tyrosin and Phenylalanine are important precursors to the production of Rosmarinic and Caffci Acids (RA and CA) (Peterson and Simmonds, 2003). Therefore, possible higher N assimilation in AM plants (Toussaint et al., 2004) might have contributed to the production of these amino acids, subsequently to higher production of the phenylalanine ammonia-lyase a one of the main enzymes involved in the production of CA and RA. Another possible mechanisms could reside in the potential of AM *i.e.,* inducing changes in phytohormone levels in the host plant, such as cytokinins for gibberllin (Allen et al., 1982).

Fig. 9. Impact of AM fungi on protein content of four different vegetable seedlings (on 30th day)

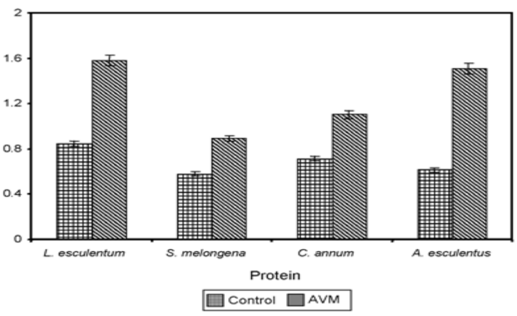


Fig. 10. Impact of AM fungi on sugar content of four different vegetable seedlings (on 30th day)

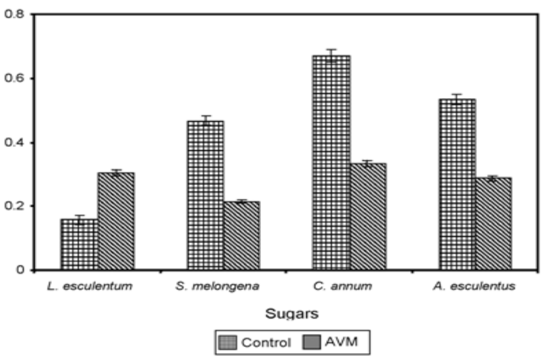


Fig. 11. Impact of AM fungi on starch content of four different vegetable seedlings (on 30th day)

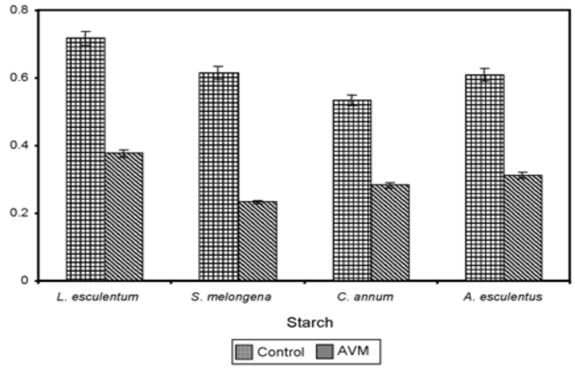


Fig. 12. Impact of AM fungi on nitrogen content of four different vegetable seedlings (on 30th day)

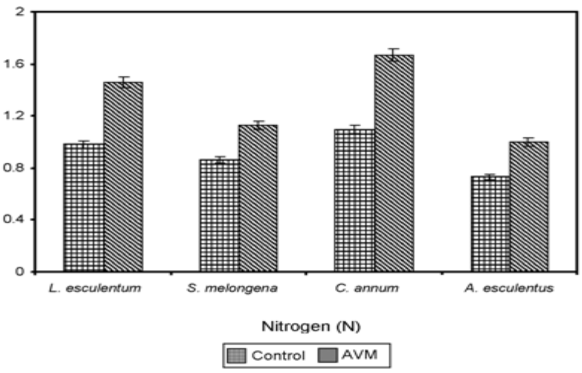


Fig. 13. Impact of AM fungi on phosphorus content of four different vegetable seedlings (on 30th day)

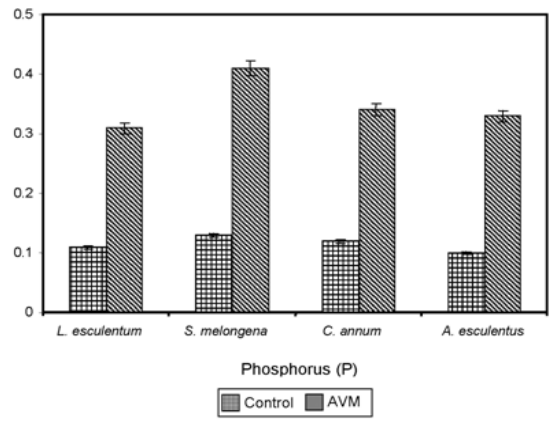
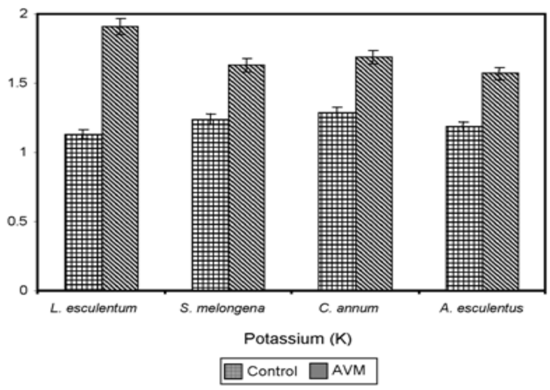


Fig. 14. Impact of AM fungi on potassium content of four different vegetable seedlings (on 30th day)



AM fungi are obligate biotrophs, the host substantial impact on the AM fungi through the regulation of 'C' supply (Schaeffer et al., 1997). The leaf protein content of all mycorrhizal inoculated seedlings showed a greater increase than the non-mycorrhizal seedlings (Fig. 9). It is suggested that AM mycorrhizal hypae may have a potential for supplying N to mycorrhizal plants through atmospheric N fixation (Rabie and Humiany, 2004) and increasing in the percentages of N, P in plant, organic acids added to soils increased the plant uptake of P from a water soluble P (Bolan et al., 1994). The phosphorus with in the soil is taken up via a phosphate transporter located in the extra-radical hyphae of this fungus (Harrison and Van Buuren, 1995). The phosphate is then condensed into polyphosphate (Poly-P) and translocated back towards the plant by cytoplasmic streaming into the intradical hyphae (Tinker, 1976). Finally the Poly-P may be hydrolyzed and released as phosphate to the plant across the fungal membrane probably at the arbuscule.

Conclusion

The present study showed an increase in the elements content of Nitrogen (N), Phosphorus (P) and Potassium (K) with of AM fungi inoculated plant than controlled one. AM inoculated plants recorded higher concentrations of N, P, K, nutrients content and this may be due to AM hyphae net work which is substantiated by the observed increase in nutrient content. AM inoculation significantly increased the potassium content in four vegetable seedlings. The mycorrhizal plants accumulate more potassium than the non-mycorrhizal plants. The beneficial effects of AM fungus attributed to the major role in phosphorus uptake and translocation through the phosphatases in the transport of phosphorus.

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