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# Growth Studies in Mangosteen (*Garcinia mangostana L.*). II. Activation of seedling growth in Mangosteen using Arbuscular Mycorrhizal Fungi and *Azospirillum*

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**Abstract: An experiment was undertaken in the central orchard at the main campus of the Kerala Agricultural University to address the slow growth in mangosteen, a highly potential crop of the humid tropics. *Glomus mosseae, Glomus fasciculatum* and *Azospirillum* individually and in combinations, as well as a control, formed the treatments. The treatments were adequately replicated in a completely randomized design. The best treatments for activating seedling growth were the combinations of *Glomus fasciculatum* (5 g) + *Azospirillum* (10 g) + single super phosphate (10 g) followed by *Glomus fasciculatum* (5 g) + *Azospirillum* (20 g) + single super phosphate(10 g) per plant. A rhythmic pattern was observed with the treatments giving the best seedling growth also yielding higher values of nitrogen, phosphorus, potassium, crude protein, chlorophyll a, b, total chlorophyll, total phenol total carbohydrates and abscisic acid content; treatments with intermediate growth recording also gave intermediate values except in the case of sodium. Control plants gave values that fell beween those of intermediate and the least growth. The highest spore count was observed in plants inoculated with *Glomus fasciculatum* (20 g) + single super phosphate (10 g) followed by *Glomus fasciculatum* (20 g) + *Azospirillum* (20 g) + single super phosphate (10 g). With regard to root infection, plants inoculated with *Glomus fasciculatum* (5 g) + *Azospirillum* (20 g) + single super phosphate (10 g) per plant and *Glomus fasciculatum* (20 g) + *Azospirillum* (10 g) + single super phosphate (10 g) revealed the maximum percentage of infection. The *Azospirillum* population was highest in the plants inoculated with *Glomus fasciculatum* (5 g) + *Azospirillum* (10 g) + followed by *Glomus mosseae* (20 g) + *Azospirillum* (20 g) + single super phosphate (10 g). The standard procedure for identification and quantification of abscisic acid was modified, as clear banding patterns were not obtained. Using the modified procedure, the characteristic-banding pattern corresponding to standard abscisic acid was obtained and confirmed when standards of abscisic acid were also simultaneously used with samples. Banding patterns and quantification of samples of the treatment with *Arbuscuar mycorrhizal fungi* and *Azospirillum*-inoculated plants were also successfully obtained and are presented. Growth measurements at the end of the first year revealed that all characters recorded were far superior to the established selection indices for the purpose.**

## 1. Introduction

Mangosteen (*Garcinia mangostana L.*), the "Queen of fruits" is a successful introduction into Kerala and flourishes well under the warm humid tropics (Yusuf and Kurien, 2012). It has high export potential but is limited by its long gestation period (Wiebel *et al.,* 1992, 1995). However, this long period of 10-15 years (Lim, 1984; Richards, 1990; Wiebel *et al.,* 1995) can be reduced by resorting to vegetative propagations; the problem of slow growth only gets magnified and the consequent low canopy volume leads to lower yield. Hence, mangosteen related work that will lead to activation of growth should get top priority in research.

The knowledge of symbiotic associations of mycorrhizal fungi with roots of vascular plants is a century old (Mohandas, 1993). Root infections by arbuscular mycorrhizal (AM) fungi have been reported in many perennial fruits such as grapes citrus, and apple. Root inoculated perennials with AM fungi effectively enhances the growth of plants such as plant height, number of leaves and leaf area. Inoculation of AM fungi in mangosteen resulted in significant changes in length-related characteristics (Masri *et al.,*

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1998). Other similar reports exist on increased growth in various crop plants including root growth enhancement as reported and reviewed by Gerdemann (1968) and Cherian (2001).

The role of AM fungi in increasing the mobilization and uptake of P and thereby the productivity of many crops is well documented and reviewed by Gerdemann (1968), Mosse, (1973), Meenakumari (1987), Nelsen (1987) and Bhandari *et al.* (1990). There are numerous reports of AM fungi increasing the N concentration in plant shoots and aiding in stimulating nodulation (Carling *et al.*, 1978). AM fungi directly enhances the uptake of micronutrients, viz. Zn, Cu and Fe (Gildon and Tinker, 1983; Kucey and Tanzen, 1987). Zinc deficiency can also be corrected by inoculating plants with an endomycorrhizal fungus (Gilmore, 1971). It was observed that AM fungi association resulted in a higher uptake of micronutrients in various plants, which was brought about by selective uptake and better utilization of N, Cu, Zn and S in various crops (Bhandari *et al.*, 1990)

With regard to *Azospirillum,* there is only scanty information available on its influence in perennial crops (Rao, 1982). Studies have been made in the rhizosphere and rhizoplane of cocoa and pepper (Govindan and Nair, 1984; Govindan and Chandy, 1985). However, most of the studies with *Azospirillum* are on field crops (Rao *et al.*, 1979). Rao and Dass (1989) found that soil inoculation with pure cell suspension of *Azospirillum brasilense* or *Azotobacter chrocaccum* resulted in growth enhancement of ber and pomegranate. Enhanced root elongation, root hair development and branching in a number of crops have been reported following *Azospirillum* inoculation (Kapulnik *et al.,* 1983). High crude protein content was noticed in inoculated plants (Patel *et al.*, 1993).

However, there are also reports on the dual inoculation of AM fungi and *Azospirillum* and its growth response in plants. Combined inoculation of *Azospirillum* and AM fungi significantly increased shoot biomass in mulberry (Nagarajan *et al*., 1989); plant height, shoot and root weight in pepper (Bopaiah and Khader, 1989); and shoot growth, and thereby biomass production, in *Tectonia grandis* (Sugavanam *et al*., 1998). Greater root colonization resulting in higher N and P and micro nutrients like Fe, Cu, Zn and Mn have been reported in coffee (Kumari and Balasubramanian, 1993). Sonowane and Konde (1997) revealed that co-inoculation of AM fungi and *Azospirillum* or *Azotobacter* resulted in the highest leaf area in studied grape vines and comparison between the two revealed that *Azospirillum* was superior when used in conjunction with a mixed culture of AM fungi (Sugavanam *et al*., 1998).

The probable reason for this increased dry weight was attributed to a higher photosynthetic rate (Estrada-Luna, 2000), or perhaps the production of growth promoting compounds namely auxin, gibberellins and cytokinins or vitamins (Miller, 1971; Crafts and Miller, 1974; Slankis, 1975).

Masri *et al.* (1998) observed that arbuscular mycorrhiza enhanced the growth and reduced the nursery period of mangosteen (*Garcinia mangostana* L.) seedlings. In mangosteen (*Garcinia mangostana* L.), alteration of root system characteristics and nutrient uptake in response to AM fungal inoculation have been studied. Arbuscular mycorrhizal inoculation induced significant changes in root characteristics and this was accompanied by a tremendous increase in nutrient uptake. Uptake of P was increased by 67-88% in inoculated seedlings (Masri and Azizah, 1998).

The present study was undertaken with the prime objective of ascertaining as to whether the growth rate in mangosteen can be increased through symbiotic association of AM fungi and Azospirillum. However, its presence and beneficial effects in cultivated crops of Kerala have been reported by various workers (Potty, 1978; Sivaprasad *et al*., 1982, 1984; Girija and Nair, 1985; Nair and Girija, 1986).

## 2. Materials and Methods

The experimental site experiences a warm humid tropical monsoon climate. It is situated at 12°32'N latitude and 74°20'E longitude at an altitude of 22.5 m above mean sea level. The study was carried out at the Kerala Agricultural University Central Orchard, Thrissur. The area receives an average rainfall of 2150 mm distributed over a year's period. The mean maximum temperature ranged 28-36°C and the mean minimum temperature 12.8-20.6°C. The relative humidity was 90-98% with a mean of 94%.

The soil type is typical sandy clay loam with a pH of 5.4, EC of 1.25 $dsm^{-1}$ and belongs to the order Ultisols with 8 $p^H$ ranging from 5.5-5.8. The soil is low in available N and $P_2 05$ and high in $K_2O$.

Fruits were collected from plants belonging to the same age group (25-50 years) from the Pariyaram area of the Thrissur District in Kerala and seeds were extracted. The seeds were sown in black polythene bags (45x30 cm) filled with potting mixture comprised of farmyard manure, sand and cow dung in the ratio 2:2:1. The weight of the potting media was 5 kg, which was uniformly maintained. Seedlings were subjected to a secondary selection for uniformity in growth. Three months after germination of the seeds, the treatments were inoculated in the potting media and a uniform dose of 10 g of single super phosphate was added to the potting media of each polybag. The treatments were as follows:

1. *Glomus mosseae* (G.m.) - 5 g
2. *Glomus mosseae* - 10 g
3. *Glomus mosseae* - 20 g
4. *Glomus fasciculatum* (G.f.) - 5 g
5. *Glomus fasciculatum* - 10 g
6. *Glomus fasciculatum* - 20 g
7. Azospirillum (Az.) - 5 g
8. Azospirillum - 10 g
9. Azospirillum - 20 g
10. Azospirillum - 5 g + *Glomus mosseae* - 5 g
11. Azospirillum - 10 g + *Glomus mosseae* - 5 g

12. Azospirillum - 20 g + *Glomus mosseae* - 5 g
13. Azospirillum - 5 g + *Glomus mosseae* - 10 g
14. Azospirillum - 10 g + *Glomus mosseae* - 10 g
15. Azospirillum - 20 g + *Glomus mosseae* - 10 g
16. Azospirillum - 5 g + Glomus *mosseae* - 20 g
17. Azospirillum - 10 g + *Glomus mosseae* - 20 g
18. Azospirillum - 20 g + *Glomus mosseae* - 20 g
19. Azospirillum - 5 g + *Glomus fasciculatum* - 5 g
20. Azospirillum - 10 g + *Glomus fasciculatum* - 5 g
21. Azospirillum - 20 g + *Glomus fasciculatum* - 5 g
22. Azospirillum - 5 g + *Glomus fasciculatum* - 10 g
23. Azospirillum - 10 g + *Glomus fasciculatum* - 10 g
24. Azospirillum - 20 g + *Glomus fasciculatum* - 10 g
25. Azospirillum - 5 g + *Glomus fasciculatum* - 20 g
26. Azospirillum - 10 g + *Glomus fasciculatum* - 20 g
27. Azospirillum - 20 g + *Glomus fasciculatum* - 20 g
28. Single Super Phosphate (SSP) - 10 g alone
29. Control

The morphological observations recorded were the plant height, girth at collar, total number of leaves, number of new flushes per year, number of leaves/flush and total leaf area and survival rate of the seedlings at one-year stage after germination stage. Leaf area was calculated by standardizing a common factor (0.6727) and then multiplying the length breadth with this factor (i.e. L×B×Factor). The leaf area was expressed as $cm^2$. The factor was pre-standardised taking 100 leaves and measuring the length and breadth. The leaf area of the corresponding leaf was measured using a leaf area meter to work out the factor value. Thus, the factor value (0.6727) was derived using the formula

Factor = (Leaf area/Length x breadth)

Using the factor value, the leaf area of a leaf/whole plant was calculated.

Survival rate was calculated by counting the established plants and expressing it as a percentage of the total number of seedlings observed after germination. Shoot and root fresh weight and dry weight, root to shoot dry weight ratio, length of longest root, number of primary, secondary and tertiary roots, and total number of roots were also recorded.

To estimate fresh weight of shoot and root, the seedlings were uprooted one year after germination. The plants were immediately cut and separated into shoots and roots. The fresh weights were recorded separately and the average expressed in grams. To obtain the dry weights, the samples collected to determine the fresh weights were dried in an oven maintained at 60°C till the weight of the samples remained constant. Dry weights were recorded separately and the average expressed in grams.

Dry weight ratio of root to shoot was calculated as follows:

Dry weight ratio = (Root dry weight/Shoot dry weight)

Length of the longest root (tap root) was measured from the collar region to the growing tip using a scale and expressed in centimeters.

For biochemical studies, leaf samples from seedlings were collected one year after germination; leaf samples from mother plants were also collected. The third leaf from the tip was collected and oven dried at 60°C, ground and used to estimate the content of N, P, K and Na. The following methods were applied, as described by Jackson (1973): for total nitrogen the microkjeldhal method was used and the average expressed as percentage; phosphorus content was determined using di-acid extract method; potassium content was determined with di-acid extract, then read in an EEL flame photometer, at 548 nm, and the average expressed in percentage; nitrogen content was estimated by microkjeldhal method; the value of nitrogen content was multiplied by the factor 6.25 to obtain the crude protein content and the average expressed in percentage; sodium content was determined with di-acid extract, then read in an EEL flame photometer at 598 nm and the average expressed in percentage.

The chlorophyll content (total chlorophyll, chlorophyll a and chlorophyll b) was estimated in leaf samples using Arnon's Acetone method (Sadasivam and Manickam, 1996) and the average expressed in milligrams.

The total sugars were estimated using standard procedure (AOAC, 1980), the total carbohydrates using Anthrone method (Dubois *et al*., 1951), and total phenol content using Folin-Ciocalteau method (Sadasivam and Manickam, 1996), all expressed in milligrams. The procedure adopted for quantification of abscisic acid was a modification of the standard method of Little *et al.* (1972). The modification became imperative as bands were not obtained. The procedure was standardized and bands were obtained corresponding to the standard abscisic acid. Further quantification was carried out using a U-V spectrophotometer and standards of known concentration from which a standard graph was obtained.

*Microbial observations*

Estimation of the spore population in the rhizosphere was carried out (Gerdemann and Nicolson, 1963) and expressed as number of spores/100/g soil.

Percent infection by AM fungi was calculated as described by Philips and Hayman (1970). The infection percentage was worked using the standard formula:

Percent infection= (Number of infected root segments/ Total number of root segments observed) x 100.

The *Azospirillum* population in the rhizosphere was estimated using serial dilution technique. The population was then calculated using an MPN table or chart (Cochran, 1950).

The study of all morphological and biochemical characters using a combination of arbuscular mycorrhizal fungi (AMF) + *Azospirillum* + single super phosphate was carried out as a completely randomized block design using Analysis of Variance techniques. Another set of plants receiving identical treatments were maintained for destructive analyses for taking root, shoot characters and biochemical analysis. The significance was tested by F test and the treatments were compared by Duncan's multiple range test (Snedecor and Cochran, 1983).

## 3. Results

*Morphological characters of seedlings*

All mean data on morphological characters of seedlings are presented in Table 1. At the twelve-months stage, maximum height was observed in the plants inoculated with G.f. (5 g )+ Az. (10 g )+ SSP (10 g), which was significantly superior to all other treatments except the next best combination of inoculation, G.f. (5 g) + Az. (20 g) + SSP (10 g). The greatest significant increment in height was recorded in plants inoculated with the same treatment as at the six-months stage, followed by plants treated with SSP (10 g) alone. These treatments were statistically superior in terms of rate of height increment compared to other treatments. Maximum girths at twelve months were recorded in the same treatment combinations as above, with both treatments statistically significant. Also at the twelve-months stage, the greatest increment was observed in the treatment combination G.f. (5 g) + Az. (20 g) + SSP (10 g), which was significantly superior to G.m. (10 g) + Az. (20 g) + SSP (10 g). These two treatments were significantly superior to all other treatments including the control.

With regard to the total number of leaves at the twelve-months stage, maximum leaf count was recorded with

Table 1 - Morphological characters of twelve-month-old mangosteen (*Garcinia mangostana* L.) seedlings inoculated with arbuscular mycorrhizal fungi (AMF) and *Azospirillum*

| Treatments (g) | Twelve month stage (Nine months after inoculation) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Height (cm) | Increment in height (cm) | Girth (cm) | Increment in girth (cm) | Total number of leaves | Increment in total number of leaves | New flushes/ year (No) | Leaves/ flush (No) | Total leaf area ($cm^2$) | Survival rate (%) |
| G.m. 5 | 10.13 c | 2.43 bcd | 1.31 d | 0.09 d | 12.67 abcde | 2.00 abcd | 1.57 ab | 2.00 | 82.61 bc | 100.00 a |
| G.m. 10 | 7.73 c | 1.33 d | 1.62 abcd | 0.11 cd | 8.00 e | 1.33 abcd | 1.12 b | 2.00 | 18.93 c | 100.00 a |
| G.m. 20 | 11.23 bc | 1.33 d | 1.77 abcd | 0.25 abcd | 12.33 abcde | 3.33 ab | 1.67 ab | 2.00 | 31.66 c | 100.00 a |
| G.f. 5 | 8.93 c | 1.27 d | 1.38 cd | 0.08 d | 11.00 abcde | 1.67 abcd | 1.54 ab | 2.00 | 42.43 bc | 100.00 a |
| G.f. 10 | 11.57 bc | 1.67 d | 1.68 abcd | 0.21 abcd | 14.33 abcd | 1.67 abcd | 2.00 a | 2.00 | 107.00 abc | 100.00 a |
| G.f. 20 | 8.43 c | 1.60 d | 1.52 abcd | 0.23 abcd | 8.67 de | 0.67 cd | 1.22 ab | 2.00 | 40.29 c | 100.00 a |
| Az. 5 | 10.50 c | 2.75 bcd | 1.65 abcd | 0.24 abcd | 11.00 abcde | 1.33 abcd | 1.60 ab | 2.00 | 111.10 abc | 100.00 a |
| Az. 10 | 10.47 c | 2.07 d | 2.13 abcd | 0.50 abcd | 9.67 bcde | 0.33 d | 1.42 ab | 2.00 | 84.49 bc | 100.00 a |
| Az. 20 | 10.33 c | 2.30 bcd | 1.60 abcd | 0.18 bcd | 10.33 abcde | 1.67 abcd | 1.55 ab | 2.00 | 47.77 bc | 100.00 a |
| Az. 5 + G.m. 5 | 8.70 c | 3.63 bcd | 1.51 abcd | 0.18 bcd | 9.00 cde | 1.00 bcd | 1.21 ab | 2.00 | 20.00 c | 100.00 a |
| Az. 10 + G.m. 5 | 11.23 bc | 3.10 bcd | 1.76 abcd | 0.30 abcd | 13.00 abcde | 2.00 abcd | 1.80 ab | 2.00 | 89.48 abc | 100.00 a |
| Az. 20 + G.m. 5 | 8.83 c | 2.70 bcd | 1.54 abcd | 0.20 abcd | 11.33 abcde | 2.00 abcd | 1.68 ab | 2.00 | 63.95 bc | 100.00 a |
| Az. 5 + G.m. 10 | 11.73 abc | 4.00 abcd | 1.80 abcd | 0.31 abcd | 14.33 abcd | 1.67 abcd | 1.93 ab | 2.00 | 119.80 abc | 100.00 a |
| Az. 10 + G.m. 10 | 9.33 c | 2.00 d | 1.64 abcd | 0.23 abcd | 12.00 abcde | 2.00 abcd | 1.75 ab | 2.00 | 76.21 bc | 100.00 a |
| Az. 20 + G.m. 10 | 14.10 abc | 3.90 abcd | 2.18 abc | 0.58 ab | 16.33 a | 3.00 abc | 2.00 a | 2.00 | 181.90 abc | 100.00 a |
| Az. 5 + G.m. 20 | 13.27 abc | 4.00 abcd | 1.91 abcd | 0.38 abcd | 14.67 abcd | 2.00 abcd | 1.64 ab | 2.00 | 205.20 abc | 100.00 a |
| Az. 10 + G.m. 20 | 13.07 abc | 3.57 bcd | 1.91 abcd | 0.37 abcd | 14.67 abcd | 2.00 abcd | 1.68 ab | 2.00 | 168.50 abc | 100.00 a |
| Az. 20 + G.m. 20 | 11.00 bc | 3.00 bcd | 1.43 bcd | 0.14 cd | 12.00 abcde | 1.67 abcd | 1.34 ab | 2.00 | 70.01 bc | 100.00 a |
| Az. 5 + G.f. 5 | 11.70 abc | 2.97 bcd | 1.82 abcd | 0.41 abcd | 12.33 abcde | 3.00 abc | 1.43 ab | 2.00 | 133.40 abc | 100.00 a |
| Az. 10 + G.f. 5 | 18.30 a | 6.47 abc | 2.32 a | 0.52 abcd | 15.00 abc | 3.00 abc | 2.00 a | 2.00 | 287.50 a | 100.00 a |
| Az. 20 + G.f. 5 | 17.77 ab | 7.77 a | 2.28 ab | 0.63 a | 15.33 ab | 2.33 abcd | 2.00 a | 2.00 | 211.60 abc | 100.00 a |
| Az. 5 + G.f. 10 | 11.43 bc | 3.00 bcd | 1.81 abcd | 0.32 abcd | 14.00 abcde | 2.00 abcd | 1.99 a | 2.00 | 106.90 abc | 100.00 a |
| Az. 10 + G.f. 10 | 11.80 abc | 2.57 bcd | 1.67 abcd | 0.25 abcd | 13.00 abcde | 2.33 abcd | 1.83 ab | 2.00 | 246.60 ab | 100.00 a |
| Az. 20 + G.f. 10 | 9.47 c | 2.23 cd | 1.64 abcd | 0.13 cd | 11.33 abcde | 1.33 abcd | 1.75 ab | 2.00 | 78.29 bc | 100.00 a |
| Az. 5 + G.f. 20 | 9.47 c | 1.10 d | 1.59 abcd | 0.23 abcd | 11.00 abcde | 3.00 abc | 1.54 ab | 2.00 | 143.70 abc | 66.67 b |
| Az. 10 + G.f. 20 | 13.17 abc | 4.00 abcd | 2.23 abc | 0.53abc | 14.33abcd | 3.67 a | 2.00 a | 2.00 | 223.30 abc | 100.00 a |
| Az. 20 + G.f. 20 | 10.23 c | 1.90 d | 1.44 bcd | 0.27abcd | 11.67abcde | 1.33 abcd | 2.00 a | 2.00 | 54.21 bc | 100.00 a |
| SSP10 alone | 14.50 abc | 6.53 ab | 2.08 abcd | 0.52abcd | 14.00abcde | 3.33 ab | 1.68 ab | 2.00 | 123.90 abc | 100.00 a |
| Control | 12.30 abc | 2.63 bcd | 1.83 abcd | 0.32abcd | 12.33abcde | 2.33 abcd | 1.12 b | 2.00 | 169.60 abc | 100.00 a |
| Mean values | 11.41 | 3.03 | 1.76 | 0.30 | 12.40 | 2.03 | 1.66 | 2.00 | 115.19 | 97.70 |
| CD. ($p < 0.05$) | 5.63 | 3.51 | 0.69 | 0.35 | 5.03 | 1.96 | 0.69 | NS | 166.20 | 24.78 |

Numbers followed by the same letter do not differ significantly at 5% level.
G.m.= Glomus mosseae, G.f.= Glomus fasciculatum, Az.= Azospirillum.

G.m. (10 g) + Az. (20 g) + SSP (10 g), which was significantly higher than G.f. (5 g) + Az. (20 g) + SSP (10 g). These two treatments were significantly superior to all other treatments including the control. The maximum increment at this stage was observed in the treatment G.f. (20 g) + Az. (10 g) + SSP (10 g).

The greatest number of new flushes/year was recorded in treatment plants inoculated with G.f. (10 g) + SSP (10 g), G.m. (10 g) + Az. (20 g) + SSP (10 g), G.f. (5 g) + Az. (10 g) + SSP (10 g), G.f. (5 g) + Az. (20 g) + SSP (10 g), G.f. (20 g) + Az. (10 g) + SSP (10 g) and G.f. (20 g) + Az. (20 g) + SSP (10 g) which produced the same number of flushes, followed by G.f. (10 g) + Az. (5 g) + SSP (10 g) that were statistically at par with each other. All the above treatments were significantly superior to the other treatments. There were no variations observed among the treatments with regard to the number of leaves/flush and all the treatments gave an average of two leaves. The maximum leaf area was recorded in the plants inoculated with G.f. (5 g) + Az. (10 g) + SSP (10 g), followed by G.f. (10 g) + Az. (10 g) + SSP (10 g). These two treatments were at par with each other and were relatively superior to all other treatments, including the control.

A 100% survival rate was observed in all treatments except G.f. (20 g) + Az. (5 g) + SSP (10 g), which had only a 66.67% survival rate.

*Biomass and root characters*

All mean data related to the characters of shoots and roots of one-year-old mangosteen seedlings are presented in Table 2. The maximum fresh weight of shoot was recorded

Table 2 - Morphological characters of twelve-month stage mangosteen (*Garcinia mangostana* L.) seedlings inoculated with arbuscular mycorrhizal fungi (AMF) and *Azospirillum*

| Treatments (g) | Shoot fresh weight (g) | Root fresh weight (g) | Shoot dry weight (g) | Root dry weight (g) | Root to shoot dry weight ratio | Root length (cm) | Primary roots (No) | Secondary roots (No) | Tertiary roots (No) | Total roots (No) |
|---|---|---|---|---|---|---|---|---|---|---|
| G.m. 5 | 6.00 ij | 1.00 lmn | 3.13 kl | 0.65 ij | 0.21 b | 10.2 k | 12.0 kl | 16.0 g | 9.0 no | 37.0 l |
| G.m. 10 | 3.05 lmn | 0.89 mno | 0.97 r | 0.35 mno | 0.36 b | 23.2 efgh | 21.0 fghijk | 36.0 d | 15.0 jklm | 72.0 ghij |
| G.m. 20 | 4.79 jkl | 1.08 lm | 0.22 t | 0.51 jklm | 2.62 a | 22.8 efgh | 31.0 abcde | 43.0 cd | 19.0 ijk | 93.0 efgh |
| G.f. 5 | 3.09 lmn | 0.70 op | 1.20 q | 0.31 nop | 0.26 b | 20.7 hi | 14.0 jkl | 31.0 def | 12.0 lmno | 57.0 ijkl |
| G.f. 10 | 10.54 f | 2.05 g | 4.74 h | 0.86 g | 0.18 b | 23.2 efgh | 22.0 efghij | 38.0 cd | 10.0 mno | 70.0 ghijk |
| G.f. 20 | 7.54 hi | 1.56 i | 3.27 k | 0.68 hi | 0.21 b | 24.6 cdefgh | 26.0 defghi | 39.0 cd | 26.0 gh | 91.0 efgh |
| Az. 5 | 1.53 n | 0.42 qr | 0.67 s | 0.21 opq | 0.30 b | 17.6 ij | 19.0 hijkl | 28.0 defg | 11.0 lmno | 58.0 ijkl |
| Az. 10 | 3.94 klm | 1.06 lm | 1.81 op | 0.50 jklm | 0.28 b | 21.2 ghi | 20.0 ghijk | 32.0 def | 15.0 jklm | 67.0 hijkl |
| Az. 20 | 3.36 lm | 0.58 pq | 1.19 q | 0.17 pq | 0.14 b | 20.3 hi | 15.0 jkl | 19.0 efg | 8.0o | 42.0 jkl |
| Az. 5 + G.m. 5 | 5.57 jk | 1.13 kl | 1.94 o | 0.38 lmn | 0.20 b | 18.3 ij | 20.0 ghijk | 32.0 def | 14.0 klmn | 66.0 hijkl |
| Az. 10 + G.m. 5 | 8.32 gh | 1.83 h | 3.77 j | 0.90 g | 0.24 b | 21.5 ghi | 23.0 defghij | 38.0 cd | 24.0 hi | 85.0 fghi |
| Az. 20 + G.m. 5 | 6.42 ij | 1.37 ij | 2.55 m | 0.55 ijk | 0.22 b | 14.6 j | 19.0 hijkl | 52.0 bc | 30.0 g | 101.0 defg |
| Az. 5 + G.m. 10 | 11.04 ef | 2.51 f | 4.79 h | 1.10 f | 0.23 b | 20.3 hi | 29.0 bcdefg | 86.0 a | 46.0 de | 161.0 b |
| Az. 10 + G.m. 10 | 5.56 jk | 1.49 ij | 2.21 n | 0.57 ijk | 0.26 b | 26.8 bcde | 36.0abc | 94.0 a | 58.0 ab | 188.0 a |
| Az. 20 + G.m. 10 | 12.52 de | 2.40 f | 5.90 f | 1.16 f | 0.20 b | 27.4 bcd | 30.0bcdef | 59.0 b | 38.0 f | 127.0 cd |
| Az. 5 + G.m. 20 | 2.49 mn | 0.26 r | 0.92 r | 0.11 q | 0.11 b | 16.2 j | 10.0 l | 18.0 fg | 12.0 lmno | 40.0 kl |
| Az. 10 + G.m. 20 | 4.08 klm | 0.83 no | 1.68 p | 0.36 lmno | 0.21 b | 21.7 fghi | 22.0 efghij | 33.0 def | 14.0 klmn | 69.0 hijk |
| Az. 20 + G.m. 20 | 8.47 gh | 1.95 gh | 3.69 j | 0.81 gh | 0.22 b | 28.4 bc | 16.0 jkl | 44.0 cd | 16.0 jkl | 76.0 ghi |
| Az. 5 + G.f. 5 | 7.72 hi | 1.30 jk | 3.00 l | 0.52 ijkl | 0.17 b | 22.5 fgh | 20.0ghijk | 34.0 de | 20.0 ij | 74.0 ghi |
| Az. 10 + G.f. 5 | 19.72 b | 6.31 a | 10.55 b | 3.03 a | 0.29 b | 36.4 a | 40.0 a | 58.0 b | 60.0 a | 158.0 b |
| Az. 20 + G.f. 5 | 18.60 b | 3.32 de | 6.20 e | 1.42 e | 0.23 b | 23.2 efgh | 18.0 ijkl | 28.0 defg | 30.0 g | 76.0 ghi |
| Az. 5 + G.f. 10 | 11.36 ef | 2.49 f | 5.39 g | 1.36 e | 0.25 b | 25.8 cdef | 28.0 cdefgh | 40.0 cd | 47.0 de | 115.0 cde |
| Az. 10 + G.f. 10 | 19.65 b | 2.49 f | 8.76 c | 2.15 c | 0.25 b | 18.2 ij | 15.0 jkl | 33.0 def | 42.0ef | 90.0 efgh |
| Az. 20 + G.f. 10 | 9.80 fg | 3.97 c | 4.49 i | 1.82 d | 0.41 b | 23.2 efgh | 16.0 jkl | 30.0 defg | 18.0 jk | 64.0 hijkl |
| Az. 5 + G.f. 20 | 16.95 c | 4.01 c | 6.92 d | 1.67 d | 0.24 b | 26.8 bcde | 32.0 abcd | 43.0 cd | 50.0 cd | 125.0 cd |
| Az. 10 + G.f. 20 | 25.53 a | 3.39 d | 11.09 a | 2.56 b | 0.23 b | 30.2 b | 30.0 bcdef | 42.0 cd | 53.0 bc | 125.0 cd |
| Az. 20 + G.f. 20 | 13.91 d | 5.23 b | 5.43 g | 1.35 e | 0.25 b | 23.8 defgh | 29.0 bcdefg | 38.0 cd | 43.0 ef | 110.0 def |
| SSP10 alone | 16.27 c | 3.15 e | 6.89 d | 1.79 d | 0.26 b | 30.4 b | 38.0 ab | 44.0cd | 58.0 ab | 140.0 bc |
| Control | 10.54 f | 3.91 c | 3.65 j | 0.45 klmn | 0.12 b | 25.4 cdefg | 28.0 cdefgh | 32.0 def | 18.0 jk | 78.0 ghi |
| Mean values | 9.60 | 2.16 | 4.04 | 0.98 | 0.31 | 22.93 | 23.41 | 40.0 | 28.138 | 91.552 |
| C.D. ($p< 0.05$) | 1.63 | 0.19 | 0.19 | 0.15 | 0.33 | 3.60 | 8.17 | 13.08 | 4.903 | 26.15 |

Numbers followed by the same letter do not differ significantly at 5% level.
G.m.= *Glomus mosseae,* G.f.= *Glomus fasciculatum,* Az= *Azospirillum.*

in the plants inoculated with G.f. (20 g) + Az. (10 g) + SSP (10 g), which was significantly superior to all other treatments. This was followed by G.f. (5 g) + Az. (10 g) + SSP (10 g), G.f. (10 g) + Az. (10 g) + SSP (10 g) and G.f. (5 g) + Az. (20 g) + SSP (10 g). These three treatments were at par with each other and significantly superior to all the other treatments including the control. The highest fresh weight of the root was recorded in the treatment G.f. (5 g) + Az. (10 g) + SSP (10 g) followed by G.f. (20 g) + Az. (20 g) + SSP (10 g). The differences between the treatment means were significant and both these treatments were also significantly superior to all other treatments including the control.

Maximum dry weight of shoot and root were recorded in plants inoculated with G.f. (20 g) + Az. (10 g) + SSP (10 g) and G.f. (5 g) + Az. (10 g) + SSP (10 g), followed respectively by G.f. (5 g) + Az. (10 g) + SSP (10 g) and G.f. (20 g) + Az. (10 g) + SSP (10 g). In both cases (i.e. dry weight of shoot and root), the means of the two treatments which gave maximum dry weight not only significantly differed between them but was also superior to all other treatments. A critical analysis revealed that only the relative positions of the best and second-best treatments in the case of root and shoot dry weight inter changed.

Maximum dry weight ratio of roots and shoots were recorded in plants inoculated with G.m. (20 g) + SSP (10 g), which was significantly superior to all other treatments, which were at par with each other including the control.

The maximum root length was observed in plants inoculated with G.f. (5 g) + Az. (10g) + SSP (10 g), which was significantly superior to all other treatments. This was followed by plants treated with SSP (10 g) alone and G.f. (20 g) + Az. (10 g) + SSP (10 g), which were statistically at par. These treatments produced better root length, giving significantly superior results compared to all other remaining treatments including the control.

Maximum and significantly higher primary root count were recorded in the plants inoculated with G.f. (5 g) + Az. (20 g) + SSP (10 g) followed by SSP (10 g) alone. The greatest number of secondary roots was observed in the plants inoculated with G.m. (10 g) + Az. (10 g) + SSP (10 g), which was at par with G.m. (10 g) + Az. (5 g) + SSP (10 g), the next best treatment and significantly superior to all other treatments. This was followed by G.f. (5 g) + Az. (10 g) + SSP (10 g) and G.m. (5 g) + Az. (20 g) + SSP (10 g). The means of the latter two treatments were at par with each other. The highest tertiary root count was observed in the treatment G.f. (5 g ) + Az. (10 g) + SSP (10 g), which was statistically at par with the treatment combination G.m. (10 g) + Az. (10 g) + SSP (10 g) and SSP (10 g) alone. The latter two treatments were at par with G.f. (20 g) + Az. (10 g) + SSP (10 g). The above treatments were significantly higher than all other treatments including control.

The total number of roots was greatest in plants inoculated with G.m. (10 g) + Az. (10 g) + SSP (10 g) , which was superior to all other treatments. This was followed by G.m. (10 g) + Az. (5 g) + SSP (10 g), which was at par with G.f. (5 g) + Az. (10 g) + SSP (10 g) and SSP (10 g) alone. The means of these treatments were significantly superior to the means of all other treatments including the control.

*Biochemical characters of seedling leaves*

For the purpose of analyzing the biochemical characters, the treatments were categorized as those showing the best growth, intermediary growth and the least growth (Fig. 1). Typical treatments for each group were selected and are presented in Table 3: best growth, G.f. (5 g) + Az. (10 g) +

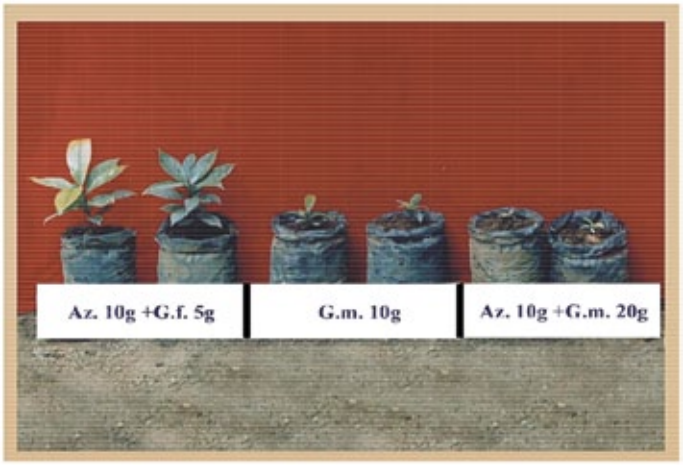


Fig. 1 - Seedling of treatments, showing the best, the intermediate and the least growth response to arbuscular mycorrhizal fungi (AM fungi) and *azospirillum* in Mangosteen (*Garcinia mangostana* L.).

Table 3 - Biochemical characters of the leaf samples in one-year-old mangosteen (*Garcinia mangostana* L.) seedlings treated with arbuscular mycorrhizal fungi and *Azospirillum*

| AMF and *Azospirillum* inoculated plants | N (%) | P (%) | K (%) | Crude protein (%) | Na (%) | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Chlorophyll a/b ratio | Total chlorophyll (mg/g) | Total phenols (mg/g) | Total carbohydrates (mg/g) | Abscisic acid (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Best (Az. 10 + G.f. 5) | 2.35 | 0.19 a | 0.34 | 14.67 a | 0.34 | 0.95 a | 0.42 a | 2.30 | 1.37 a | 0.64 | 7.85 a | 0.21 a |
| Intermediate (G.m. 10 g) | 2.08 | 0.05 b | 0.30 | 12.99 b | 0.34 | 0.66 b | 0.29 a | 2.38 | 0.95 ab | 0.46 | 6.59 ab | 0.16 ab |
| Least (Az. 10 + G.m. 20) | 1.79 | 0.04 b | 0.28 | 11.22 c | 0.34 | 0.17 c | 0.11 b | 1.88 | 0.27 b | 0.47 | 4.85 b | 0.08 b |
| Control | 1.94 | 0.04 b | 0.29 | 12.10 bc | 0.34 | 0.41 c | 0.20 ab | 2.05 | 0.61 ab | 0.46 | 5.72 ab | 0.14 ab |
| Mean values | 2.04 | 0.08 | 0.30 | 12.75 | 0.34 | 0.55 | 0.25 | 2.15 | 0.80 | 0.51 | 6.25 | 0.15 |
| C.D. ($p<0.05$) | NS | 0.09 | NS | 1.46 | NS | 0.25 | 0.17 | NS | 0.80 | NS | 2.13 | 0.096 |

Numbers followed by the same letter do not differ significantly at 5%.
G.m.= *Glomus mosseae,* G.f.= *Glomus fasciculatum,* Az= *Azospirillum.*

SSP (10 g); intermediate growth, G.m. (10 g) + SSP (10 g); least growth, G.m. (20 g) + Az. (10 g) + SSP (10 g).

The nitrogen content was highest in the maximum growth treatment G.f. (5 g) + Az. (10 g) + SSP (10 g). A distinct trend was noticed, with maximum values found in treatments with the best growth, the intermediary values being recorded in treatments showing intermediate growth, and the lowest values in treatments showing the least growth. However, there were no significant differences observed between the treatment means.

In the case of phosphorus, a pattern nearly similar to that of nitrogen was observed. However, the mean content in the treatment, which showed the best growth, was significantly superior to other treatments including the control.

The potassium content also revealed a similar trend: the treatment with maximum growth also had the highest potassium content. However there were no significant differences observed between the various treatment means including the control. The control treatment gave the values between intermediate growth and least value treatments.

The highest crude protein content was also observed in the treatment which showed best growth. It was significantly superior to other treatments and also to the control. The treatments which showed intermediate growth also showed higher values for this parameter and they were significantly higher than the treatment with least growth and control plants.

Treatments showing the best, intermediate and least growth, as well as the control, all showed the same level of sodium content.

Maximum chlorophyll a and b and total chlorophyll content were observed in the treatment that recorded the maximum growth. In the case of chlorophyll a, this was significantly higher than the means observed in plants of other categories and the control. The content of the plants with the least growth was at par with control. In the case of chlorophyll b content , the trend observed was similar to that of growth. The treatment with maximum growth had the highest chlorophyll b content, which was on par with the treatment showing intermediate growth. These two levels were significantly superior to the level observed in the plants with the least growth, but it was statistically at par with the control.

Contrary to what is commonly believed, the total phenol content was highest in the treatments with maximum growth (Fig. 2). However, there were no significant differences observed between treatment means and the control.

The greatest amount of total carbohydrate was found in the treatment that had maximum growth. This was at par with the treatment means that showed intermediate growth and also with the control.

The content of abscisic acid, which normally goes hand in hand with growth inhibition, was also contrary to normal lines of thought. The treatments which revealed more growth also had the highest abscisic acid content. The means of the group with the highest abscisic acid content were also at par with those of the treatment with intermediate growth and the control, but the treatment means of the first group was significantly superior to the category which showed the least growth (Fig. 1, 2, and 3).

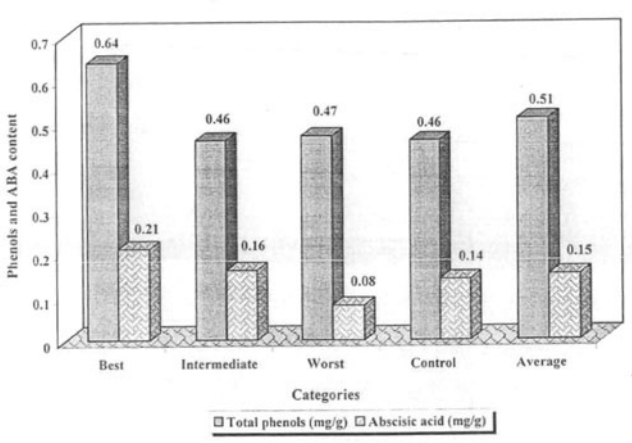


Fig. 2 - Total phenols and abscisic acid content of the AM fungi and Azospirillum treated plants showing best, intermediate and least growth in mangosteen (Garcinia mangostana L.).

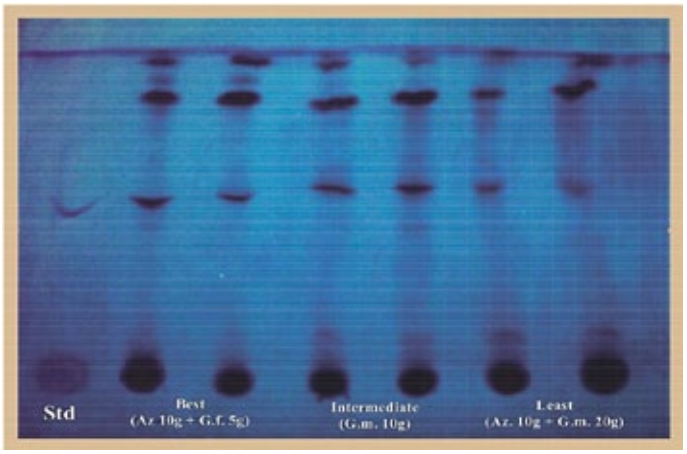


Fig. 3 - Banding pattern observed in TLC used to quantify abscisic acid content in various treatments using arbuscular mycorrhizal fungi (AM fungi) and *azospirillum* in Mangosteen (*Garcinia mangostana* L.)

*Microbial population and percent root infection*

The mean data related to microbial population and the percent root infection by AM fungi are presented in Table 4.

*Total spore population of arbuscular mycorrhizal fungi (AMF)*

The highest number of spores was recorded in the rhizosphere of plants inoculated with G.f. (20 g) + SSP (10 g), followed by G.f. (20 g) + Az. (20 g) + SSP (10 g). These two treatments were on par with all other treatments except the control, proving the superiority of the treatment in colonization.

*Percent root infection*

Root infection, which gave a clear picture of colonization, was also studied. The highest percentage of root infection was recorded in plants inoculated with G.f. (5 g) + Az. (10 g) + SSP (10 g) and G.f. (20 g) + Az. (10 g) + SSP (10 g), with both registering the highest values of mean infection percentage. These treatments were at par with all other treatments except G.m. (5 g) + SSP (10 g),

Table 4 - Microbial population in seedlings rhizosphere and infectivity in roots after one year of inoculation in mangosteen (*Garcinia mangostana* L.)

| Treatments | Total AMF Spores (No./100 g of soil) | Root Infection by AMF (%) | *Azospirillum* population (x 106 cfu/g of soil) |
|---|---|---|---|
| G.m. 5 | 198.00 ab | 40.00 cdef | 0.22 bc |
| G.m. 10 | 214.00 ab | 55.00 abcdef | 0.24 bc |
| G.m. 20 | 219.00 ab | 65.00 abcde | 0.26 abc |
| G.f. 5 | 206.00 ab | 40.00 cdef | 0.27 ab |
| G.f. 10 | 222.00 ab | 60.00 abcdef | 0.28 ab |
| G.f. 20 | 228.00 a | 70.00 abcd | 0.30 ab |
| Az. 5 | 136.00 bc | 35.00 def | 0.34 ab |
| Az. 10 | 142.00 abc | 40.00 cdef | 0.35 ab |
| Az. 20 | 148.00 abc | 30.00 ef | 0.37 ab |
| Az. 5 + G.m. 5 | 200.00 ab | 45.00 bcdef | 0.31 ab |
| Az. 10 + G.m. 5 | 213.00 ab | 55.00 abcdef | 0.36 ab |
| Az. 20 + G.m. 5 | 215.00 ab | 50.00 abcdef | 0.37 ab |
| Az. 5 + G.m. 10 | 204.00 ab | 50.00 abcdef | 0.33 ab |
| Az. 10 + G.m. 10 | 218.00 ab | 65.00 abcde | 0.37 ab |
| Az. 20 + G.m. 10 | 222.00 ab | 70.00 abcd | 0.34 ab |
| Az. 5 + G.m. 20 | 206.00 ab | 60.00 abcdef | 0.32 ab |
| Az. 10 + G.m. 20 | 220.00 ab | 75.00 abc | 0.38 ab |
| Az. 20 + G.m. 20 | 224.00 ab | 55.00 abcdef | 0.39 ab |
| Az. 5 + G.f. 5 | 204.00 ab | 80.00 ab | 0.32 ab |
| Az. 10 + G.f. 5 | 216.00 ab | 90.00 a | 0.36 ab |
| Az. 20 + G.f. 5 | 219.00 ab | 85.00 ab | 0.40 a |
| Az. 5 + G.f. 10 | 210.00 ab | 80.00 ab | 0.33 ab |
| Az. 10 + G.f. 10 | 220.00 ab | 85.00 ab | 0.37 ab |
| Az. 20 + G.f. 10 | 224.00 ab | 85.00 ab | 0.38 ab |
| Az. 5 + G.f. 20 | 213.00 ab | 85.00 ab | 0.37 ab |
| Az. 10 + G.f. 20 | 224.00 ab | 90.00 a | 0.35 ab |
| Az. 20 + G.f. 20 | 226.00 ab | 80.00 ab | 0.39 ab |
| SSP10 alone | 150.00 abc | 30.00 ef | 0.23 bc |
| Control | 90.00 c | 25.00 f | 0.12 c |
| Mean values | 201.10 | 61.21 | 0.32 |
| C.D. ($p< 0.05$) | 73.55 | 32.69 | 0.14 |

Numbers followed by the same letter not differ significantly at 5% level.
G.m= *Glomus mosseae,* G.f= *Glomus fasciculatum*.

G.f. (5 g) + SSP (10 g), all the *Azospirillum* + SSP alone treated plants, G.m. (5 g) + Az. (5 g) + SSP (10 g), SSP (10 g) and also the control.

*Azospirillum population in soil*

The maximum *Azospirillum* population was recorded in the rhizosphere of plants inoculated with G.f. (10 g) + Az. (20 g) + SSP (10 g), which was on par with all other treatments except the combination of G.m. (5 g) and (10 g) alone with SSP (10 g) absolute control of SSP (10g) alone and the control plants.

## 4. Discussion and Conclusions

The best treatments in case of microbial inoculation was a combination of G.f. (5 g) + Az. (10 g) + SSP (10 g) followed by G.f. (5 g) + Az. (20 g) + SSP (10 g).

Critical analysis of the data revealed that the percentage of AM fungi infection was the highest in treatments with maximum growth. The next best in order of growth also showed a high infection percentage. Though the total spore count was not the highest the treatments were on par with the treatments that yielded the highest spore count namely G.f. (20 g) treated plants. These two-treatment combinations also showed a high *Azospirillum* population. These three characters proved, beyond a doubt, that the combination was best among the treatments for maximum growth. This is a reflection of mycelial mat formation on absorbing roots, which in turn leads to higher P, N and K uptake as observed in the study. The effects were most pronounced with regard to P as the content registered a three-fold increase. This should have been due to two factors, namely the external apply of SSP (10 g) and a proven concept that mycorrhiza gives out an organic acid secretion which is capable of solubilising as well as mobilizing the acid soluble phos-

phate (i.e. semi-soluble phosphate) into soluble form. Some of the previous reports substantiate these findings (Bartlett and Lewis, 1973). Increases in selective uptake of major nutrients have also been reported by several workers (Hatch, 1937; Umesh *et al.,* 1988; Rizzardi, 1990). The capability of transferring it into the soluble form has also been reported (Bolan *et al*., 1984). Another possible reason is that they can enhance the storage capacity, and the continuous disintegration of arbuscules leads to the availability of more mineral nutrients to the host (Gerdemann, 1968).

The higher levels of P, almost three times, should have been the reason for quantitative improvement of the root characters. This can be observed in the best treatment which induced the highest fresh and dry weight of roots, as well as characters such as length of longest root and improved characters of number of roots. In mangosteen, Masri and Azizah (1998) reported alternation of root characters such as root density and branching density, which led to 67-88% higher uptake of P. Studies on alteration of rooting density are of paramount importance in mangosteen owing to the fact that the crop produces only magnolioid roots, an unique feature of mangosteen among other fruit crops. Due to lack of production of root hairs, absorption of nutrients and water is less and even survival at nature's mercy or benevolence. As such any treatment, which improves the qualitative aspects of rooting such as root length, root branching and root density will certainly influence all aspects of growth and productivity and hence should be the prime consideration in crop management.

The production of growth promoting substances by *Glomus mosseae* and *Glomus fasciculatum* is well documented (Miller, 1971; Crafts and Miller, 1974; Slankis, 1975). Greater nutrient uptake and growth promoting substances should have been the reason for the increased chlorophyll content and higher leaf area, which together accounted for higher carbon assimilation (Estrada-Luna *et al*., 2000) leading to higher carbohydrate content as observed in the study. Higher crude protein content also points to the level of protein synthesis due to AM fungi inoculation, which is a new area worth to be probed further.

The greater nutrient uptake and carbohydrate accumulation resulted in greater shoot and root biomass and dry weight content. Differences observed in growth increment during intervals of observation are basically due to infectivity and colonization of AM fungi on feeder roots. This is partly an efficiency factor of the fungi and secondly is influenced by soil ecology factors as well as the host. In mangosteen, Masri *et al.* (1998) observed increased growth due to AM fungi inoculation and thereby reduced nursery period for mangosteen.

As in the case of activation of growth using bioregulators, here also the content of inhibitors - namely phenols and ABA - were higher in the best treatment and a general decrease was observed in the treatment showing the least growth. This can also be argued only on the lines of correlative inhibition. The higher content did not inhibit the growth, as the balance of various growth regulators should have been more towards the plant growth promoters than growth inhibitors. More detailed investigation encompassing the whole endogenous levels of plant hormones at the critical stages of bud dormancy, bud activation, flushing and post flushing can only answer this vital question.

The present study convincingly proves the efficacy of the two treatments in activating growth of mangosteen. The mean values of growth characters obtained in the best treatments are far superior to the selection indices standardized for seedling growth (Yusuf and Kurien, 2012). This investigation is of great practical application and gains more importance as mangosteen is a crop which lacks fine root hairs (Richards, 1990) while the study revealed that colonization of AM fungi and *Azospirillum* takes place on the network of fine ramified roots enabling better uptake of nutrients and thereby growth.

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