

MYCORRHIZAL FUNGI AS BIOFERTILIZER FOR FRUIT TREE PRODUCTION IN THAILAND

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Abstract

Arbuscular mycorrhiza (AM) species — Glomus mosseae and Glomus manihotis — were selected as biofertilizer for fruit tree production in Thailand. Field trials were established at three locations in Chantaburi, Petchaburi and Phrae provinces with three types of fruit tree — durian, cashew and longan. A randomized complete block design was employed with the following treatments: AM, full recommended rate and half rate of mineral fertilizer and the control. The results indicated enhanced growth of the three fruit types.

Introduction

Large amounts of mineral fertilizer have been used for enhancing fruit yields. Mycorrhizal fungi are an alternative biofertilizer to enhance fruit production instead of relying on mineral fertilizer. Moreover the fungi help to maintain and preserve soil and water resources for future generations. By far the most important and widely distributed type of mycorrhiza is arbuscular mycorrhiza (AM) fungi.

The rhizosphere benefits from AM fungi which form a mycelium around and in the roots. Internal and external fungal hyphae make contact with to ten entry points per cm of the root surface (Ocampo *et al.* 1980). The external mycelium considerably increases the contact of the root with the medium in which it grows. Without mycorrhiza, 1 cm of root can explore about 1-2 cm³ of soil using root hairs. Assuming radial growth of AM hyphae around the root, AM mycelium can increase this area from 5 to 200 times. A rhizospheric soil volume of 200 cm³ may be an exception, but 12-15 cm³ per infected root is common. The AM fungal mycelium appears to be more resistant than the root itself to abiotic stresses such as drought, toxic elements, and soil acidity (Sylvia & Williams 1992). A plant with mycorrhiza remains in close contact with the soil for a longer period of time than a plant without mycorrhiza. The lifespan of the external mycelium appears to decline rapidly three to four weeks after the first infection of the plant by the fungus (Schubert *et al.* 1987). The principal function of mycorrhiza is to increase the soil volume explored for nutrient uptake and to enhance the efficiency of nutrient absorption. It has been shown that mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non-mycorrhizal plants (Mosse *et al.* 1981; Smith & Dowd 1981). In peach, vesicular-arbuscular mycorrhizal (VAM) fungi increased growth 25-75% compared to the control plants (McGraw & Schenck 1980). Mycorrhizal plants also accumulate P, K, Ca, Cu and Mn in the leaf in higher concentrations than non-mycorrhizal plants (Ross & Harper 1970; Nopamombodi *et al.* 1987). However, little is known about the benefit of mycorrhiza in fruit trees in Thailand.

This study was conducted to establish technology for utilizing mycorrhizal fungi to increase the growth and yield of fruit trees in Thailand.

Keywords: Arbuscular mycorrhiza fungi, durian, longan, cashew, Thailand

Materials and methods

Inoculum preparation

Glomus mosseae and *Glomus manihotis* were selected as AM species. An inoculum of each species was prepared by growing a plant in steamed clay + sand at the ratio 1:1 in 12-inch clay pots. Soil was steamed two times on alternate days, each time for three hours at 85°C. *Sorghum vulgare* was used as the host plant — grown first in a paper cup for one week then transferred for growth in a clay pot. VAM spores were applied at the bottom of the transplant hole. Each pot was fertilized with half strength of Hoagland's nutrient solution, and watered on alternate days. At about 80% flowering stage of the host plant (about three months after germination), root and soil samples were collected to examine the percent root colonization and number of spores produced. After harvesting, the infected root and soil were ready to use as inoculum for further studies.

Experimental design and management

To study the effect of AM fungi on enhancing plant growth, yield and root colonization, AM spores produced in the soil grown with durian (*Durio zibethius* J. Murr.), cashew (*Anacardium occidentale* L.) and longan (*Dimocarpus longan*, Lour) were used. Field trials were established at three locations in Thailand: Chantaburi, eastern Thailand (for durian); Petchaburi, southern Thailand (for cashew); and Phrae, northern Thailand (for longan). The Chantaburi soil was a sandy clay loam with a pH of 4.8, organic matter at 2.4%, P (Bray II) at 25 ppm and K at 53 ppm. The soil at Petchaburi was a loamy sand with a pH of 7.5, organic matter at 1.11 %, P (Bray II) at 5.0 ppm and K at 30 ppm. The Phrae soil was a loamy sand with a pH of 6.2, organic matter at 1%, P (Bray II) at 33 ppm and K at 37 ppm.

The field experiment was arranged in a randomized complete block design with four replications and five treatments as follows. Treatment 1: inoculated with *Glomus mosseae* + *G. manihotis* (M); Treatment 2: applied with fertilizer at 93.75-93.75-93.75 kg/ha of N-P₂O₅-K₂O at the rate of 250 g/plant (F); Treatment 3: applied with fertilizer at 93.75-93.75-93.75 kg/ha of N-P₂O₅-K₂O at the rate of 250 g/plant and inoculated with M (M+F); Treatment 4: applied with fertilizer at 50-50-50 kg/ha at the rate of 250 g/plant and inoculated with M (M+F/2); Treatment 5: Control (Ck). Two hundred spores of AM from the pot culture were applied to each plant. The plot size for durian and longan was 24 x 32 m, plant spacing was 8 x 8 m. The plot size for cashew was 24 x 24 m, plant spacing was 6 x 6 m. All treatments were applied with organic fertilizer at 50 kg/plant and rock phosphate (5% P₂O₅) at 1.5 kg/plant.

Extraction of AM spores from soils

Soil and root plant samples were collected at the same time. Soils were sampled at the depth of 15 cm by digging beside the root plant. Soil was collected from the field at each of the three sites. Five composite samples were collected to represent each plant. All of the samples were stored individually in polyethylene bags at 2-5°C until the spores were extracted and counted.

Counting spore propagules in soils

The AM spores were extracted from the soil samples by wet sieving and decanting as described by Gerdemann (1963). Soil samples of approximately 250 gm were suspended in one litre of water which was gently stirred. Heavier particles were allowed to settle for a few seconds and the liquid was decanted through a sieve (250 microns) to remove the large particles of organic matter and allow the desired spores to pass through. The suspension was passed again through sieves (100 microns and 63 microns). The spores and small amounts of debris remaining on each sieve were transferred from the sieve to a centrifuge tube containing water (Smith & Skipper 1979); they were centrifuged for three minutes at 2,000 rpm. The upper solution was poured out and the debris at the bottom was supplemented with 40% sucrose

and centrifuged for one minute at 2,000 rpm. The upper solution was separated for examination under a stereoscopic microscope.

The spores which were collected under the stereoscopic microscope were stored in Ringer's solution for further study. Spores from each sample were counted based upon 100 grams of soil. The propagule numbers for every sample were recorded.

Examination of vesicles and arbuscules in the root tissue

After the soil was washed from the plant roots, the feeder roots were removed from each plant. They were cleared and stained to determine the percent of root colonization using the Phillips & Hayman method (1970). The roots were cleared in 10% KOH, boiled at 90°C for about 15 minutes then stained in lactoglycerol trypan blue. Roots were cut into 1-cm lengths (100 pieces) randomly and placed on slides for counting. A stereoscopic microscope (x100) was used to estimate the percentage of root colonization by counting the arbuscules or surface mycelia or clusters of echinulate vesicles.

Results and discussion

The study was a long-term experiment. The results showed the effect of AM fungi on plant growth, root colonization and number of AM spores at 30 months of planting. Table 1 shows the effect of AM fungi on the growth of durian, cashew and longan. Statistical differences in the height of durian and longan were recorded. The highest heights were found in durian and longan applied with fertilizer. No statistical difference was found in cashew; the optimum height for cashew resulted from application with AM fungi and fertilizer (M+F), being 11.21% higher than the control.

Statistical difference in the stem diameter of longan was recorded. The largest stem diameter was found in longan applied with AM fungi and fertilizer (M+F). No statistical differences were found in durian and cashew. The largest stem diameter for durian resulted from fertilizer application, being 30.21% larger than the control. The largest stem diameter in cashew resulted from AM fungi and fertilizer (M+F) application, being 19.18% larger than the control.

No statistical differences in bush diameter were found among the three plants. The widest bush diameter for durian resulted from application with fertilizer, being 24.55% wider than the control. The widest bush diameter for cashew resulted from application with AM fungi and fertilizer (M+F), being 20.09% wider than the control. The widest bush diameter for longan resulted from application with AM fungi (M), being 24.27% wider than the control.

Figures 1 and 2 show that the non-inoculated plants were colonized by indigenous species. From the number of inoculated species, the spores showed superimposition of effective AM isolates over the indigenous AM population, which effectively stimulated growth.

Conclusion

The results of this experiment showed that AM fungi seem to increase plant growth, but the plants took more than 30 months to yield. Since AM fungi increased the yield of pineapple by 73.57% compared to the control (Thamsurakul *et al.* 2000), increasing fruit tree yield may be possible. This experiment will be continued and effective AM fungi will be used as biofertilizer for fruit tree production in Thailand.

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Table 1. Average height, stem diameter and bush diameter of durian, cashew tree and longan (30 months)

Treatment	Height (cm)			Stem diameter (cm)			Bush diameter (cm)		
	Durian	Cashew tree	Longan	Durian	Cashew tree	Longan	Durian	Cashew tree	Longan
M	200.5 abc	265.1	229.0 b	19.5	33.7	22.9 ab	136.0	341.2	290.9
F	243.7 a	238.5	236.0 a	23.5	31.2	24.9 a	173.5	312.1	284.5
M + F	172.0 bc	280.9	221.0 b	17.6	39.1	25.9 a	142.6	392.3	251.5
M + F/2	225.0 ab	229.7	217.4 b	21.3	30.8	23.5 ab	148.1	306.9	209.5
CK	159.5 c	249.4	222.0 b	16.4	31.6	18.6 b	130.9	313.5	220.3
F-test	*	ns	0	ns	ns	*	ns	ns	Ns
CV (%)	15.1	10.1	23.4	17.2	9.2	16.5	20.8	11.4	19.7

In a column, means followed by a different letter are significantly different at the 5% level by Duncan's multiple range test.

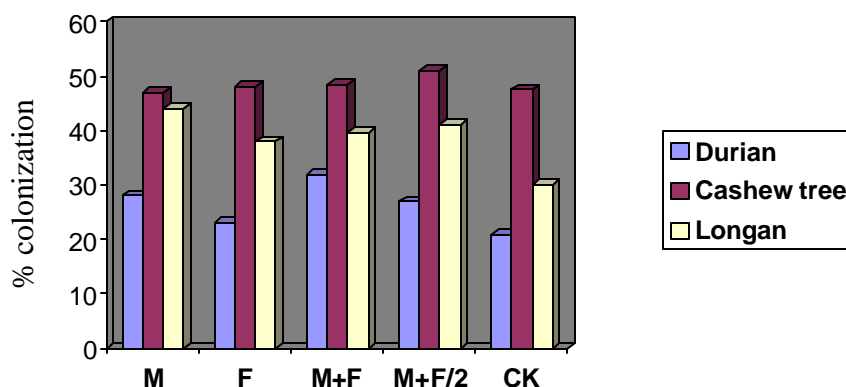


Figure 1. Percentage of colonization in durian, cashew tree and longan roots at 30 months after planting

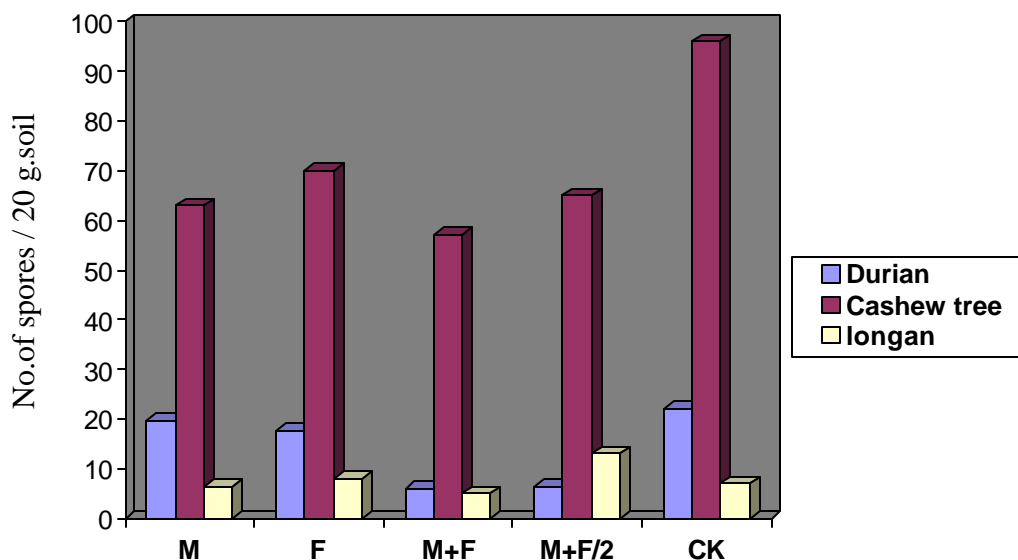


Figure 2. Number of spores in durian, cashew tree and longan soil at 30 months after planting