

Article

Mycorrhizal Inoculation and Chemical Fertilizer Interactions in Pineapple under Field Conditions

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Abstract: Excessive inorganic fertilizers applied to pineapple crops in Mexico cause the progressive degradation and pollution of soils in the short- and long-term, and they also increase production costs. An alternative to reduce excessive fertilization is its partial substitution by nutrition and growth enhancing arbuscular mycorrhizal fungi (AMF). The goal of this research was to compare the effect of AMF inoculation combined with different fertilizer doses and full chemical fertilization on pineapple yield variables in a commercial plantation. We used a randomized block design with six treatments: a non-inoculated control with 100% chemical fertilization, and five treatments with AMF inoculation and fertilization doses of 0%, 25%, 50%, 75%, and 100% chemical fertilization. There were four replicates of each treatment containing 30 plants in each experimental unit (plot). We measured the dry weight of the D-leaf 9 months after planting, and the root mycorrhizal colonization percentage, yield, and fruit quality after 18 months. Mycorrhizal inoculation equated to 100% chemical fertilization already when combined with 25% fertilization and surpassed it when combined with 50% fertilization in most of the yield variables measured. The fruit mass and organoleptic variables were significantly higher in mycorrhizal plants with 50% fertilization than in the non-inoculated control and the treatments inoculated with AMF and combined with 0%, 25%, 75%, and 100% of a dose of chemical fertilizer. Inoculation with mycorrhizal fungi in the field could reduce chemical fertilizer application by 50%, with no yield loss and with improved fruit quality.

Keywords: D-leaf; quality fruits; yield; mycorrhizal



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1. Introduction

Mexico is the 10th largest pineapple (*Ananas comosus*) producer in the world. Seventy percent of the national production is concentrated in the southeast [1]. Large quantities of fertilizers are used (3.5–5 ton/ha/cycle) to achieve the yield required by the market, not including other agrochemical products. This situation not only affects the economy of the producers but also the environment [1] as continuous application of chemical fertilizers contributes to the emission of greenhouse gases and the pollution of soil and water [2].

On the other hand, uninterrupted monoculture for more than 30 years has increased the population of plant pathogens in the soil, especially nematodes [3], that reduce the flow of water and nutrients to the plant [4]. To deal with them, farmers increase pesticide input, which affects microorganisms that contribute to soil fertility and plant nutrition [5]. Within this group, we can find arbuscular mycorrhizal fungi (AMF) that form a symbiotic association with 80% angiosperms and contribute to plant nutrition and development (Smith and Read, 2008). They play a fundamental role as they translocate phosphorus and other nutrients to the plant [6] and increase its tolerance to nematodes [7].

The response of pineapple to mycorrhizal fungi inoculation has been widely demonstrated by different authors [8–10], but generally in in vitro conditions during acclimatization. Jaizme-Vega and Azcón [11], found that the inoculation with *Funneliformis mosseae* or

G. fasciculatum (now *Rhizophagus fasciculatus*) increased plant growth up to 322% and 294%, respectively. On the other hand, the response of pineapple to mycorrhizal inoculation can be higher when phosphorus levels in the soil are not high [12,13]. In the Cayenne, Champaka, and MD2 varieties, Trejo et al., [14] showed that P fertilization up to 80 mg kg⁻¹ not only inhibited AM colonization but reduced plant growth. Shoot and D-leaf biomass reductions suggest that current management with excessive P fertilizer is highly cost-inefficient and possibly leads to yield losses and environmental degradation.

P is an important nutrient in pineapple production; its deficiency diminishes the size of the fruit and the postharvest quality [15]. Mexican pineapple producers apply high phosphorus doses that have led to soil P accumulation up to 200% higher than in mulch soils [1]. AM fungi have a relevant function in phosphorus translocation, plant health, and fruit quality [16]. Inoculation with AMF, drastically reduced in soil by management practices, may help in reducing the use of synthetic fertilizers and the economic costs for pineapple producers. The goal of this work was to evaluate the effect of AMF mycorrhizal inoculation under field conditions combined with growing fertilizer doses on the yield and fruit quality of pineapple in field conditions.

2. Materials and Methods

2.1. Plant Material and Mycorrhizal Inoculum

Pineapple suckers of the variety “Regional Smooth Cayenne” were obtained from commercial plantations from the Loma Bonita region in Oaxaca, Mexico. The AM inoculum used was a consortium denominated Rizofermic-UV containing *Acaulospora morrowiae*, *A. spinosa*, *A. scrobiculata*, *Cetrasporaspora pellucida*, *Claroideoglossum etunicatum*, *Funneliformis mosseae*, *F. geosporus*, *Gigaspora rosea*, *Gi. decipiens*, *Glomus macrocarpum*, *Rhizophagus aggregatus*, and *R. intraradices*. The inoculum comes from the soil of tropical fruit crops, mainly pineapple, from Veracruz, Mexico. This inoculum was propagated in *Brachiaria decumbens* grass according to the technique proposed by Sieverding [17].

AM fungal species in the consortium were identified from spores by Dr. Lucía Varela and five more international experts. The vouchers of the spores were deposited at the Instituto de Ecología IE-XAL herbarium in Mexico. The 12 species and quality control of the consortium are monitored every six months.

Pineapple suckers, 40-cm height, were planted, and 20 days later the mycorrhizal inoculum was applied by placing it on the top roots and then covering with a layer of soil. The inoculum contained fresh roots of *Brachiaria decumbens* (80% of colonization) in sand culture (2597 spores in 50 g), and 15 g were applied per plant.

2.2. Experimental Site and Agroecological Characteristics

The experiment was established in a plot located in the main pineapple production region of Mexico in the municipality of Loma Bonita, Oaxaca (18°06′25″ N, 95°53′50″ W at 30 m.a.s.l.). The experimental field has 5000 m² and a cropping history of 10 years of sugar cane, 4 years of pineapple, and 1 year of maize.

The soil of the field was a humic acrisol (according to FAO classification [18]) with sandy texture (63% sand, 12% clay, 23% silt), 2.4% soil organic matter, and pH 4.6 (2:1 soil:water). The soil contained 19 mg kg⁻¹ N, 38 P, 48 K, 48 Ca, 19 Mg, 49 Fe, 3.2 Zn, Mn 3.2, and exchangeable-Al 0.54 meq 100 g⁻¹. It is considered to be poor soil, and no AMF spores were found in the experimental field before planting.

The site has a mean annual temperature of 25 °C and a mean annual precipitation of 1845 mm, with heavy rains during the summer and a dry season between March and May. It is located in a transition strip between AW2 and AM climates.

2.3. Treatments, Plot Size, and Experimental Design

The treatments were established in a randomized block design with 4 replicates. The treatments were: (1) farmers method without AMF inoculation and a full dose of synthetic fertilizer (FM); (2) AMF inoculation without fertilizer (M); (3) AMF inoculation

plus 25% of the full fertilizer dose (M + 25%); (4) AMF inoculation plus 50% of the full fertilizer dose (M + 50%); (5) AMF inoculation plus 75% of the full fertilizer dose (M + 75%); and (6) AMF inoculation plus 100% of the full fertilizer dose (M + 100%). We used an experimental plot of 30 plants located in two rows 70-cm apart and 30-cm spacing between plants in a row. Only 20 plants from the experimental unit were used for measuring the fruit variables. The density was 55,000 plants per ha.

Two fertilization schemes were applied, one directly to the soil with 26 gr plant⁻¹ of 16-16-16 (NPK) every 30 days, and the other a foliar fertilization with the formulation 12-18-12 (N-P-K) by dissolving 10 kg in 200 L of water. Every 15 days, the solution (50 mL) was applied per plant during the crop cycle (18 months).

Crop management during the cycle included the application of agrochemicals to protect the plants from weeds and pests. We applied Diuron 80% PH, 3 Kg ha⁻¹; Bromacil, 1 Kg ha⁻¹; Carbofuran 5 L ha⁻¹, Dimethoate 1 L ha⁻¹; Chlorpyrifos 5 L ha⁻¹, Carbaryl 5% 15–30 kg ha⁻¹; Agromil (hormones) 2 L ha⁻¹.

2.4. Evaluation of Variables

Nine months after the inoculation, we measured D-leaf dry weight according to Sideris and Krauss [19]. The D-leaf is used as a nutritional status indicator for the plant as it is the largest leaf and, therefore, is easy to identify, and it is also a young leaf that is physiologically mature. It has thus been used to estimate yields without harvesting the whole plants [15]. We harvested the fruit 18 months after planting and measured root colonization and yield variables. Roots were cleared and stained as in Philips and Hayman [20], and mycorrhizal intraradical colonization percentage was quantified with the McGonigle and Fitter [21] method. Fruit mass and size were measured as total fruit weight, crown weight, fruit weight without crown, fruit length, fruit diameter, and fruit peduncle diameter. The organoleptic properties of the fruit were measured as Brix degrees, fruit acidity, and vitamin C content.

2.5. Fruit Quality Evaluation

The analyses were made in the food laboratory of the Institute of Science of the Universidad Veracruzana. Five fruits randomly selected from each treatment were used to measure the juice properties. We sampled 200 g from the middle of each fruit excluding the heart and froze the samples for later analysis. The evaluated variables were color, pH, total soluble solids, titratable acidity, and ascorbic acid.

All variables were analyzed with the STATISTICA 6 for Windows with a one-way ANOVA followed by a Tukey test ($p < 0.05$).

3. Results

3.1. Mycorrhizal Root Colonization Percentage

We found statistical differences in the root intraradical colonization percentages. The inoculated treatments with 0%, 25%, and 50% fertilizer had similar intraradical colonization (41–43%), but mycorrhizal root colonization decreased progressively in treatments with 75% and 100% fertilizer (Figure 1). The farmers method (FM) with a 100% fertilizer dose did not show root mycorrhizal colonization (Figure 1).

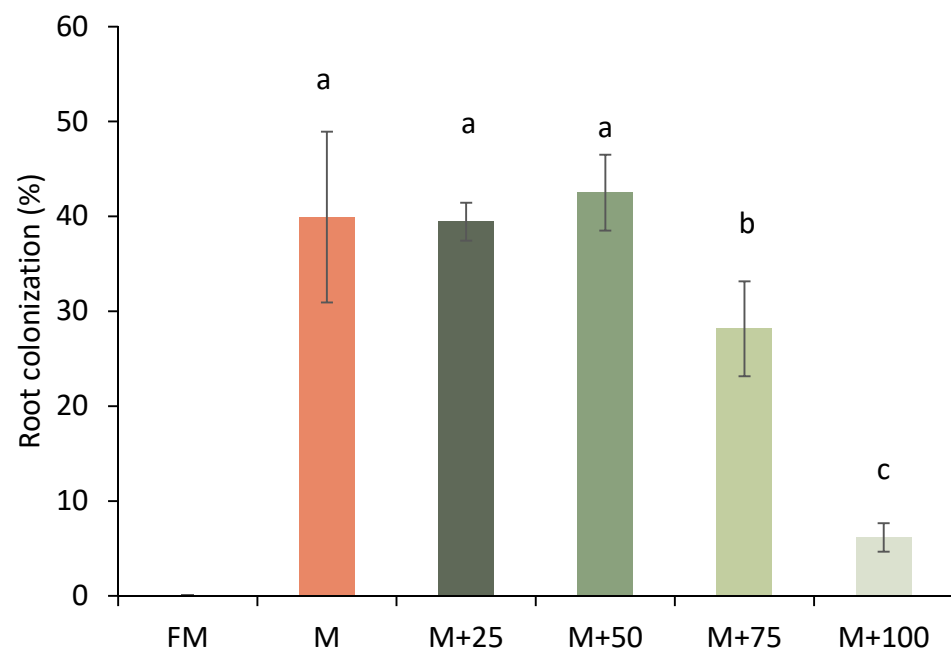


Figure 1. Root mycorrhizal intraradical colonization percentage in treatments combining AMF inoculation and/or synthetic fertilizer application. Different letters indicate significant differences (Tukey, $p < 0.05$). $n = 20$.

3.2. Dry Weight of D-Leaf (DWDL)

The D-leaf dry weight 270 days after inoculation differed significantly between treatments and was highest in the M + 50% treatment and lowest in the FM treatment (Figure 2). The rest of the treatments with AMF inoculation had intermediate values.

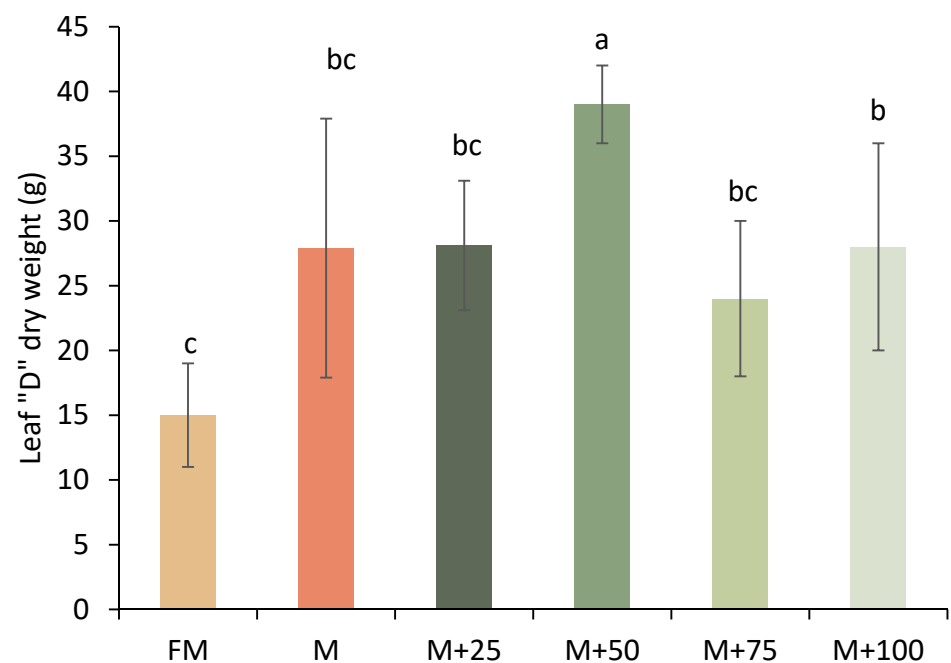


Figure 2. D-leaf dry weight 9 months after inoculation in treatments combining AMF inoculation and/or synthetic fertilizer application. Different letters indicate significant differences ($p < 0.05$). $n = 20$.

3.3. Fruit Weight, Size, and Quality

At harvest, the treatment with the highest fresh weight of the fruit was M + 50% (Table 1), and treatment M had the lowest weight. The rest had similar and intermediate weights. We observed a similar trend in most of the yield variables, including the organoleptic characteristics of the fruit (Table 2).

Table 1. Means $\pm$ Standard deviation ($n = 20$) for fruit yield variables in the fertilizer doses and the mycorrhizal inoculation combinations.

Treatments	Fruit Weight (kg)	Crownless Fruit Weight (kg)	Fruit Length (cm)	Fruit Diameter (cm)	Peduncle Diameter (cm)
FM + 100%	2.18 (0.28) b	1.98 (0.27) b	19.06 (1.94) b	11.9.79 (12.89) ab	0.34 (3.48) a
M	1.65 (0.20) c	1.43 (0.22) c	16.08 (1.92) c	11.3.92 (15.58) c	0.35 (1.71) a
M + 25%	2.20 (0.20) b	2.02 (0.21) b	18.96 (1.18) b	11.8.23 (5.43) abc	0.34 (1.85) a
M + 50%	2.70 (0.32) a	2.49 (0.32) a	21.46 (1.89) a	12.9.20 (4.80) a	0.29 (3.02) b
M + 75%	2.09 (0.36) b	1.89 (0.35) b	18.03 (2.06) bc	11.6.95 (6.21) bc	0.28 (2.63) b
M + 100%	1.99 (0.30) b	1.81 (0.27) b	17.63 (1.94) bc	11.6.57 (6.28) c	0.25 (1.26) c

Different letters indicate significant differences among treatments.

Table 2. Means $\pm$ Standard deviation ($n = 5$) for organoleptic fruit variables in the fertilizer doses and mycorrhizal inoculation combinations.

Treatments	°Brix	pH	Titrateable Acidity (% Citric Acid)	Ascorbic Acid (mg in 100 mL)
FM + 100%	7.86 (2.67) c	4.06 (0.25) a	0.44 (0.05) a	4.84 (3.08) c
M	10.99 (0.84) b	3.84 (0.03) a	0.41 (0.03) a	11.77 (4.92) c
M + 25%	12.61 (0.75) b	3.97 (0.03) a	0.37 (0.10) a	7.01 (2.14) bc
M + 50%	15.97 (0.78) a	3.28 (0.34) b	0.33 (0.066) b	18.87 (1.22) a
M + 75%	11.76 (1.23) b	3.90 (0.10) a	0.39 (0.15) a	7.99 (1.32) b
M + 100%	11.57 (0.86) b	3.99 (0.22) a	0.34 (0.11) a	5.09 (3.41) bc

Different letters indicate significant differences among treatments.

4. Discussion

The results of this study showed that the application of a mycorrhizal inoculant in a soil devoid of mycorrhizal propagules (spores) could halve the chemical fertilizer inputs required and even increase the yield and quality of pineapple in comparison with the farmers method using full fertilization.

We observed optimal conditions to establish the AMF inoculated in the experimental plot. AM root intraradical colonization showed an active symbiosis, and all of the inoculated treatments showed evidence of AM hyphae, vesicles, and arbuscules in the roots except for the non-inoculated treatment (FM + 100%, conventional management). The null mycorrhizal colonization in the conventional management could be attributed to two factors: monoculture and agrochemicals. The uninterrupted sugarcane monoculture for several years plus burning at the end of the cycle to facilitate harvesting appear to have had an effect. Having only one host over many years may reduce AMF richness [22–24]. Trejo et al. [24] showed that, under greenhouse conditions and 8 consecutive years with the same host, AM fungal diversity could be reduced from 27 to 1 species. In the field, Sasvári et al. [25] found that AM fungal root colonization and diversity decreased under crop monoculture. According to Johnson et al., [26], monocultures are characterized by hosting a reduced AMF diversity, and they are not efficient at promoting host development. The excessive and frequent application of agrochemicals and fertilizers in the farmers method included the application of herbicides, fungicides, and nematicides besides fertilizers. It is known that high levels of N and P accumulated in the agroecosystems negatively affect the AMF symbiosis, reducing AMF species diversity and limiting the benefits that the symbionts provide to their hosts [16,22,27–30]. The constant application of the fungicide fosetyl-A could also be affecting AMF by reducing the propagules and colonization [31,32].

Sreenivasa and Bagyaraj [33] evaluated the effect of the insecticides carbofuran, endosulfan, and quinalphos over the AMF efficiency and found that 5 and 10 kg per ha⁻¹, inhibited the AMF colonization. Likewise, the use of Dimethoate (5 mg L⁻¹) in soybean plants reduced mycorrhizal colonization [34]. Although it has not been documented, herbicides could also affect the AMF activity due to their obligate symbiotic nature, so it is not advisable to use herbicides excessively where AMF are applied [27]. For example, it is known that the herbicide bromacil inhibits microbial activity in the soil [35]. The presence of nematodes and the constant application of nematicides in the previous crop might have contributed to the reduction of native AMF populations. Some studies have shown that chronically nematode-infected soils [36] and nematicides also reduced AMF colonization and spore production [37].

The reduction of synthetic fertilizer application plus the addition of AMF produced fruits with the weight required by commercial standards. The best results were obtained by reducing 50% of the fertilizer in combination with the symbionts as the fruit weight increased 23% compared to the FM. This result is relevant considering the yield increase obtained when reducing the fertilizer by 25–50%, but it is also important to consider that each hectare used requires an investment of roughly 35,000 pesos (1750 USD) in fertilization. In some cases, more than 1500 kg of fertilizers are applied per hectare [38]. The results presented in the present study not only show the economic benefits but also the ecological benefits. AMF could mobilize and supply nutrients in pineapple to achieve the best results in fruit size and quality with half of the full fertilizer dose; thus, they could reduce soil and groundwater pollution and greenhouse gas emissions. In addition to the profound toxicity to biota, chemical synthetic fertilizers are known to deteriorate the lithosphere, hydrosphere, and atmosphere. They can also damage soil fertility mechanisms in the long-term [39].

Similar results have been reported for sugar cane [40], where the AMF inoculation and the application of half of the fertilizer dose promoted the highest values in leaf area, height, and diameter. An increase in cereal yield was reported by Mathimaran et al. [41], who also showed that the significant reduction of fertilizers in combination with biofertilizers could reduce soil degradation.

Regarding the effect of the treatments on the fruit quality, total soluble solids (°Brix) were on average 15.97 in the M + 50% treatment. These values are above the minimum required (12 °Brix) by the CODEX ALIMENTARIUS (FAO, Rome, Italy, 1993). Moreover, the treatment with the highest ascorbic acid was M + 50% (19.87 mg), much higher than the FM + 100% with 4.84 mg. These results can be considered positive for a pineapple producer as total soluble solids define the sale of the product [42] and also show the importance of incorporating AMF in pineapple production and reducing the fertilizer regime.

5. Conclusions

The results of this experiment show that the inoculation of beneficial organisms to the soil, such as AMF, might significantly reduce the use of synthetic fertilizers, maintaining and even increasing fruit quality. These results, if confirmed in further experiments, could lead to economic and environmental benefits, and even impact positively on human health, thus increasing the sustainability of the pineapple agroecosystems.

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