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ABSTRACT
An experiment under greenhouse conditions was carried out to evaluate the best level of nitrogen (N) and phosphorus (P), which promote the growth of papaya (*Carica papaya* L. var. Solo) seedlings without affecting the arbuscular mycorrhizal fungi (AMF) activity. Seedlings of papaya were transplanted to pots inoculated or not with *Gigaspora margarita* spores at four levels of N (0, 50, 100, and 200 ppm) and P (0, 25, 50, and 100 ppm). P was amended at the same time of pot filling up with substrate but N was applied weekly. The experiment was harvested after 100 days and plant dry weight together with AMF colonization was measured. The major response on the plant dry weight and height was from the fertilizers (N and P) where it proportionally increased. For the dry weight, at low amounts of nutrients the effects of AMF were greater, but as much as the fertilizer level in soil increased this response decreased. The colonization decreased as the nutrient levels increased, especially the P. The best combination of N and P for plant growth and AMF colonization could be at 50 and 25 ppm, equivalent to 5 and 2.5 kg/10a, respectively.

KEYWORDS
Arbuscular mycorrhizal fungi; fertilizer; fruit; plant nutrition

Introduction

Papaya has been well grown worldwide and, in 2003, Brazil led the ranking in world production of papaya, but 10 years later was overtaken by India (FAO, 2016) due to serious problems resulting from inadequate management in the phases of production and post-harvest limited productivity. Such management problems, especially of plant health, affect the production of papaya and its insertion in the international market. Brazil reached the highest production in 2013 with 1,582,638 tons; however, India and Nigeria had the largest plantation area of 135,000 and 94,000 ha, respectively, whereas Brazil only had 31,989 ha (Agrianual, 2015). The main organisms responsible for causing diseases of economic importance in papaya (*Carica papaya* L.)

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are fungi (Agrios, 2005; Barkai-Golan, 2001), but are also the organisms that can be used to help plant productivity by symbiosis. Regarding mineral nutrition of papaya, it has three distinct development stages: (i) plant training, (ii) flowering and fructification, and (iii) production. The percentage distribution of each nutrient absorbed throughout the phenological cycle of papaya has shown that demand in each stage of development is distinct and tends to increase reaching the highest percentages during the production stage.

Before, during, and after papaya plantations establishment, leaf and soil analysis are important to understand the plant's nutritional status. This information provides the identification of nutrient levels contained in the soil-plant system and links them to nutritional deficiencies, the appropriate levels, or phyto toxicity caused by minerals. After planting establishment, soil samples should be taken from zones where the fertilizers were applied (Oliveira and Caldas, 2004).

For foliar diagnosis in papaya, the limbo analysis was more effective than the petiole for evaluating the nutritional status, in which there were significant differences of the cultivars with the N, P, K, and Cl concentrations, but not with other nutrients. Therefore, it should be used as an indicator of the nutritional status of papaya. The interpretation of leaf analysis should also consider the sampling time, since some periods are more critical for certain nutrients (Marinho et al., 2002).

It is well known that, in papaya plantation, the nitrogen and potassium fertilization induces higher productivity. Macronutrients, potassium (K), nitrogen (N), and calcium (Ca), are more absorbed as phosphorus (P) is less extracted. In the flowers it is possible to find small quantities of magnesium (Mg) and calcium (Ca) representing, respectively, rates of 12.5% and 13.5% of total absorbed (Oliveira and Caldas, 2004). The absorption of N, P, and K in papaya is higher in peak production and P was the nutrient that most accumulates in fruits (Chandra, 2014; dos Anjos et al., 2015).

This interaction between plant and mycorrhizal fungus is called, in general, a mutualistic partnership comprising carbon and nutrient exchange between the symbiont (Ruotsalainen et al., 2014). A 2-year study in India of mycorrhiza in monoculture papaya found that the 18 species recorded mycorrhizal fungi as well established in *Carica papaya* and exhibited variations in the colonization of roots and spore density due to soil factors and seasonal patterns in the weather (Khade Sharda and Rodrigues, 2008). Therefore, seasonal changes can indicate different degrees of benefit of symbiosis for the plant. The mycorrhiza can improve water use and nutrient uptake, especially those with low soil mobility; P, Zn, and Cu, in parallel, can increase the plant tolerance to environmental stresses, such as heavy metal (Aguirre et al., 2011) and phytoremediation (Colodete et al., 2014).

The extent of root colonization ranges according to soil and climatic factors (Ortas et al., 2011; Soudzilovskaia et al., 2015). Mycorrhizal dependency of plant and nutrient acquisition has been shown to change with plant species and within species (Ortas et al., 2002). Host plant species characteristics, such as nutrient requirements and the translocation efficiency, can determine the efficiency of symbiosis (Ortas et al., 2002, 2011; Trindade et al., 2001). Mycorrhizal inoculation can significantly affect the fruit growth and yield since papaya is a fruit tree with mycorrhizal dependency status (Trindade et al., 2006); the inoculation can be considered a good strategy to enhance the symbiosis benefits and improve the plant mineral status (Oliveira and Caldas, 2004; Oliveira and Oliveira, 2005a; Trindade et al., 2001). Most horticultural crops, such as papaya, are grown under controlled nursery conditions before being transplanted to greenhouses or fields. The seedlings inoculation presents a crucial opportunity to assist the establishment of the symbiosis before transplanting. This step would be helpful for the plants to survive the transplant and later on increasing the performance (Ortas et al., 2008).

Considering the relation between the mycorrhiza and plant mineral nutrition, this association is very helpful to overcome problems of plant growth in areas with P and Zn deficiency, especially since these nutrients are correlated with others, such as N, Mo, and K. Several experiments have shown the ability of arbuscular mycorrhizal fungi (AMF) to absorb N, P, K, Ca, S, Cu, and Zn from soil and translocate it to associated plants (Nouri et al., 2014). This enhancement is due to the increasing of the root absorptive surface area, which might be observed by increase of dry matter and yield.

Although the AMF and fertilizers are fundamental for plant growth, an excess of some elements might decrease mycorrhizal activity, which may mask their effects on plants. These fertilizers, especially the chemical ones, should be applied in a certain level where the plants can be benefited from mycorrhiza too. Therefore, this research aimed to evaluate the best level of N and P that guarantees plant growth without affecting mycorrhizal colonization.

Material and methods

The experiment was carried out in greenhouse conditions without a temperature-controlled system. Papaya seeds were sown in trays containing vermiculite and after 24 days the seedlings, at the stage of two leaves appearing, were transplanted to 3.5-L plastic pots containing the mixture of Vermiculite:Perlite:Zeolite (2:1:1). The mycorrhizal inoculums serakinkon (Central Glass, Co., Japan) containing 30 spores per gram of the AMF *Gigaspora margarita* were used in this experiment. Each inoculated pot received 2 grams of inoculum where 60 AMF spores were added in holes at a 5-cm distance from the seedlings and in the control nothing was added.

The N was applied in four concentrations (0, 50, 100, and 200 ppm) whereas the P was applied in four concentrations (0, 25, 50, and 100 ppm), with all of them combined with each other to compose a total of 16 levels of fertilizer. Urea was used as N fertilizer and P was applied as NaH_2PO_4 $12H_2O$. The substrates were amended with P before the transplanting by mixing the solution. The N was applied weekly at dosages of 25 ppm until reaching the desired concentration for each treatment. An additional fertilizing treatment of K 100 ppm (KCl), Mg 40 ppm ($MgSO_4$), and Ca 20 ppm ($CaCl_2$) was added into each pot at once.

The experiment was conducted during 100 days and at the harvesting, total plant biomass (shoot and root) was measured. Shoots were separated from roots 0.5 cm above the soil surface. Shoots were washed and dried at 60 °C for 48 h and submitted to sulfuric digestion for macroelements analyzing (N, P, K, Ca, and Mg). The N and P were determined by colorimetric methods after the Kjedahl digestion. The same extracts were used to analyze the Ca, Mg, and K with an atomic absorption spectrophotometer (Mizuno and Minami, 1980). Root samples were taken, washed, and stored in FAA mixture until the determination of AMF colonization from staining to percentage calculation (Ishii and Kadoya, 1994).

The experimental design composed of randomized blocks for four levels of N, four levels of P, and two mycorrhizal treatments (inoculated and control) with four replicates. The data was subjected to ANOVA analysis and the differences among treatments were compared with standard error of means at 95% significance using the computer software SPSS 9.0 (IBM Co., Armonk, NY, USA).

Results

Root colonization and plant biomass

The patterns indicate that colonization decreased at higher P levels, where at 25 ppm P the percentage was greater and 0 and 50 ppm N also showed higher values. The colonization decreased gradually with high P levels. The interesting point is that even at higher levels of P, this parameter remained the same at 30%, which is considered to be reasonable for a plant. The decreasing patterns were observed only at N 25 and 100 ppm (Figure 1).

For both AMF plants and control, N and P had a remarkable effect on dry weight (DW). The influence of P was increased at higher levels of N and vice-versa. The shoot dry weight (SDW) of AMF plants was higher at P 0 and 25 ppm, but at N 200 the curve had a saturation point in non-AMF plants, which, in fact, did not occur for the AMF ones. The inoculation of AMF significantly increased the SDW at low levels of P (Figure 2).

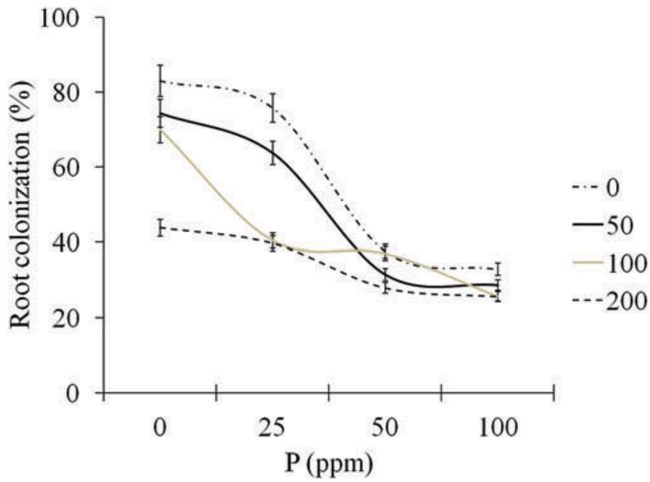

Figure 1. Arbuscular mycorrhizal fungi colonization in papaya seedlings treated with N and P. Each curve represents the N concentration (0, 50, 100, and 200 ppm).

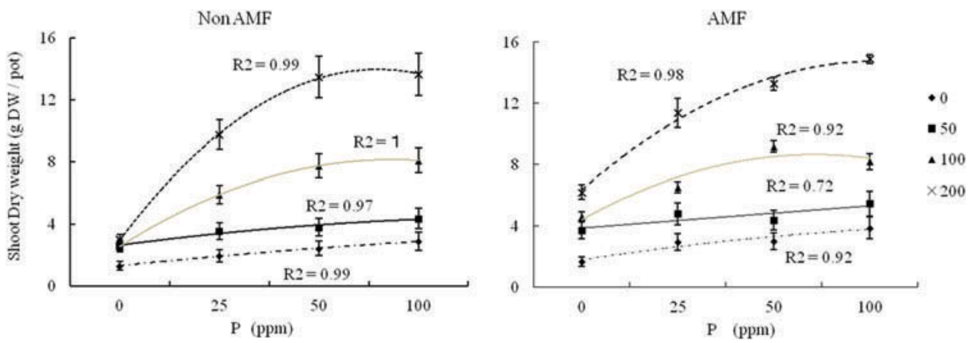

Figure 2. Shoot dry weight of papaya seedlings treated with N and P inoculated (AMF) and not (non AMF) with arbuscular mycorrhizal fungi. Each curve represents the N concentration (0, 50, 100, and 200 ppm).

Nutrient content in leaves

The content of N in both AMF and non-AMF plants was influenced by N and P levels. In non-AMF plants the quadratic curve reached the maximum point at P 50 then started to decrease; also, no significant effect was observed for P 0 at N 0, 50, and 100. However, in AMF plants this content increased constantly with P levels. Most of AMF response occurred at N 0, 50, and 100 ppm (Figure 3).

The P in leaves increased with the application of this element in soil, where the response of N on P content in leaves also occurred after 50 ppm of P. The P content had a linear pattern for both plants where the AMF response was observed mainly at P 0 and 25 ppm. The progress rate in AMF plants was greater, and was indicated by the linear models. Even

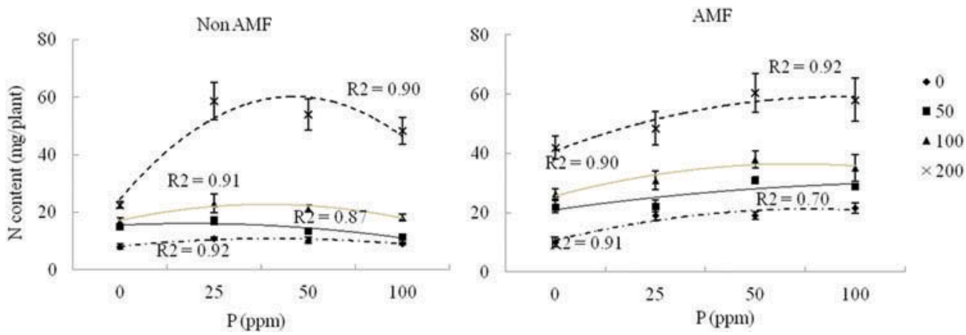

Figure 3. N content in leaves of papaya seedlings treated with N and P inoculated (AMF) and not (non AMF) with arbuscular mycorrhizal fungi. Each curve represents the N concentration (0, 50, 100 and 200 ppm).

at high levels of P (100 ppm) the response of AMF inoculation was found to be significantly different. The rate of response to P in AMF plants was higher than non-AMF, especially at 100 and 200 ppm of N; a similar pattern was observed for P content in leaves with higher values in AMF plants (Figure 4).

In reference to K content, the inoculation had no effect on this data except at P 50 and 100 ppm when some N was applied, where it increased until 50 ppm of P and then remained on the same level (Figure 5). The Ca was slightly affected by N application, but marginally increased proportionally to P application. The AMF inoculation induced higher levels after P application (Figure 6). The Mg content in AMF plants was greater at N 200 ppm after 25 ppm of P. At 0 P the Mg content increased after N application. The influence of P was found only at N 200 in both non-AMF and AMF plants, whereas this pattern was detected in AMF plants at 50, 100, and 200 ppm (Figure 7).

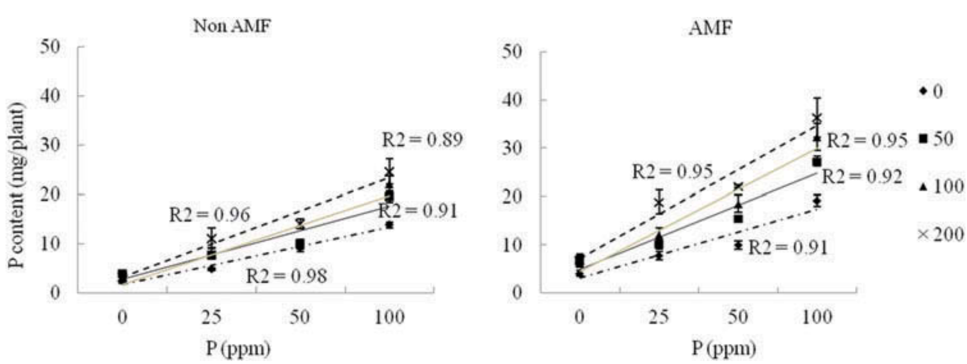

Figure 4. P content in leaves of papaya seedlings treated with N and P inoculated (AMF) and not (non AMF) with arbuscular mycorrhizal fungi. Each curve represents the N concentration (0, 50, 100, and 200 ppm).

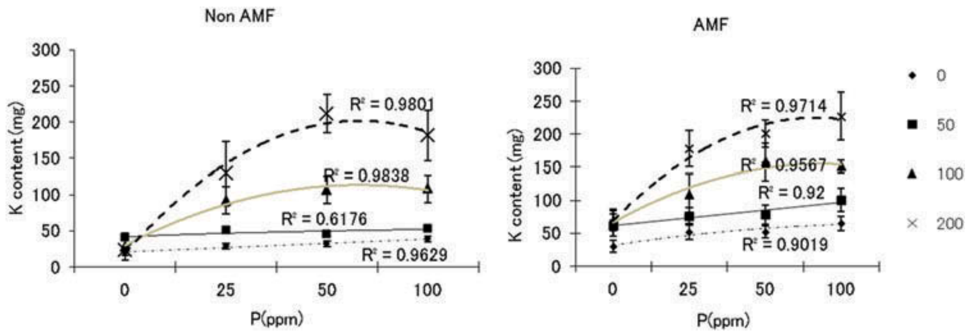

Figure 5. K content in leaves of papaya seedlings treated with N and P inoculated (AMF) and not (non AMF) with arbuscular mycorrhizal fungi. Each curve represents the N concentration (0, 50, 100, and 200 ppm).

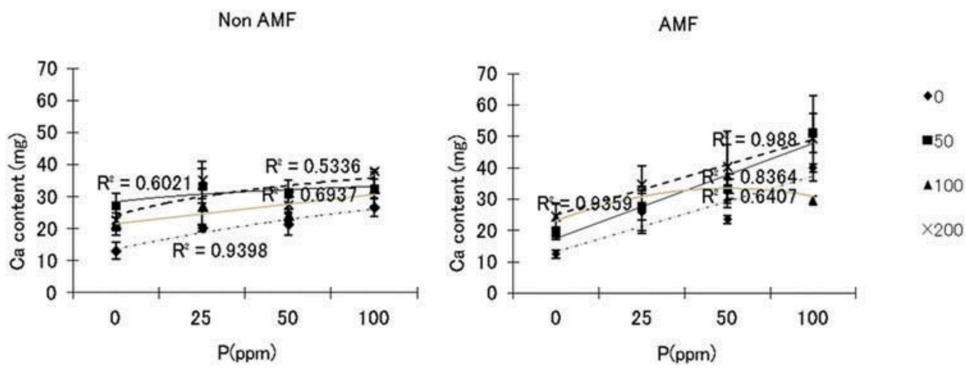

Figure 6. Ca content in leaves of papaya seedlings treated with N and P inoculated (AMF) and not (non AMF) with arbuscular mycorrhizal fungi. Each curve represents the N concentration (0, 50, 100, and 200 ppm).

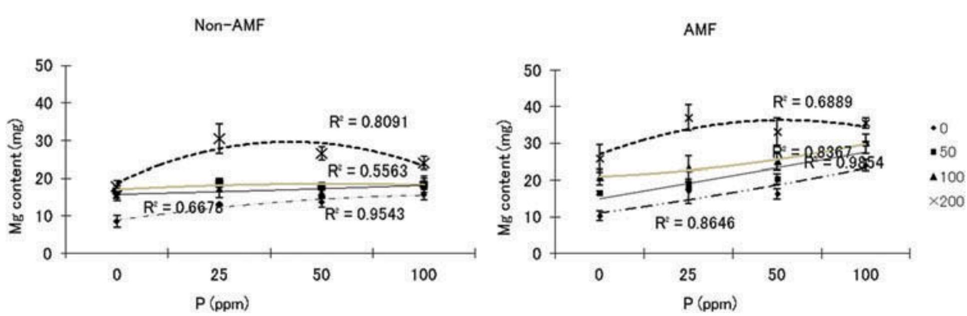

Figure 7. Ca content in leaves of papaya seedlings treated with N and P inoculated (AMF) and not (non AMF) with arbuscular mycorrhizal fungi. Each curve represents the N concentration (0, 50, 100, and 200 ppm).

Discussion

In the current experiment, the phosphorus application mainly caused an effect on growth and AMF colonization; however, the highest AMF inoculation effects were detected at 0 and 25 ppm of P, and on levels higher than those the AMF almost had no effect. The increasing of plant biomass by AMF has been observed in other experiments with *Glomus clarum* inoculation in cashew nut seedlings (Weber et al., 2004) and papaya (Trindade et al., 2001; Martins et al., 2000).

At higher P and N fertilization amounts the AMF colonization rate dropped; similarly, in papaya seedlings the decreasing of colonization rate of *Glomus etunicatum* at high fertilizer concentrations has been shown. Plants with a lower ability to absorb P may have a good AMF response (Trindade et al., 2001, 2006); some fruit cultivars are more dependent on AMF for supplying nutrients, and might have low ability of root system exploration (Habte, 2000).

Not only the P and N content were increased by AMF inoculation, other macroelements (K, Ca, and Mg) had some positive effect of AMF too. A number of researches have reported that the AMF inoculation is effective to increase the phosphorus absorption. Some accumulation of essential necessary elements (P, Zn, Cu) for plant growth by inoculation of *Glomus mosseae* in cucumber plant under pot cultivation was detected; this suggests that the fungal mycelium was able to absorb these elements and supply it to the plant (Lee and George, 2005). Furthermore, this effect was more visible at low P levels in soil. Also, some increasing on absorption of P, K, and Cu in papaya seedlings after *Gigaspora margarita* inoculation was found (Trindade et al., 2001), moreover, depending on banana variety the AMF inoculation effect could improve the P, Zn, and Mg uptake (Oliveira and Oliveira, 2005a). Also the AMF formation had some correlations with Ca, Mg, P, Cu in cupuacu (*Theobroma grandflorum*) and Ca, Fe, Zn, and Cu in guarana (*Paullinia cupana*; Oliveira and Oliveira, 2005b). Therefore, the uptaking of essential elements as affected by AMF might range according to plant species and cultivars. To reach similar plant biomass and number of fruits in papaya, only 50% of P was necessary when the plants were inoculated with AMF and helper bacterium (*Bacillus coagulans*; Mamatha et al., 2002).

The physical and chemical mechanisms of AMF on elements uptaking is well reported (Aguirre et al., 2011; Colodete et al., 2014; Soudzilovskaia et al., 2015); usually the root system can exploit a greater volume of soil by penetration in smaller soils pores not reached by root basis (Bonfante and Genre, 2010). The enhancing of nutrient uptake by AMF is often correlated to increase in plant biomass and the AMF may have biochemical properties that contribute to element supplying to roots (Habte, 2000). These results can indicate the contribution of AMF to uptake of essential elements, besides the plant growth in papaya seedlings. AMF could play a crucial role in agricultural ecosystems in which some essential elements (N, P, K, Ca, and Mg) unavailability and high costs of fertilizers could limit fruit growth. AMF would also be important in situations where it is necessary to reduce soil fertilizer applications due to environmental concerns.

As an application to the grower, the AMF inoculation could anticipate the field transplanting and help on acclimatization of these seedlings. Also, the impact of different concentrations of N and P on plant growth and AMF colonization could be observed. Probably the ideal level of N and P that could promote plant growth without damaging the AMF activity could be 50 and 25 ppm, respectively, which is equivalent to 5 and 2.5 kg per 10a, characterizing the low-input agriculture by reducing the fertilizer amount with AMF.

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