Growth and nutrient accumulation in mycorrhized papaya seedlings cultivated in a phosphorus-fertilized substrate

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ABSTRACT

The indiscriminate use of mineral fertilizers in papaya orchards has increased production costs, and the use of arbuscular mycorrhizal fungi is a promising alternative to reduce such expenses. Therefore, the present research aimed at studying the efficiency of arbuscular mycorrhizal fungi (AMF) on dry matter and nutrient accumulation in Sunrise Solo papaya seedlings, by applying doses of P_2O_5 (triple superphosphate) that are harmful to the symbiosis. The experiment was carried out in a protected environment and was set up in a randomized block design with four replications, and consisted of four P_2O_5 doses (0, 672, 1386 and 2100 mg dm^{-3}), three mycorrhizal fungi species (*Gigaspora margarita*, *Entrophospora colombiana* and *Scutellospora heterogama*) and the control treatment (mycorrhiza-free). Shoot and root dry matter as well as nitrogen, phosphorus and potassium contents in leaf and root tissues were assessed. Mycorrhizal inoculation promoted a 30% increase in shoot dry matter in relation to the control treatment. Mycorrhizal fungi promoted increases in leaf and root nitrogen content up to 672 mg dm^{-3} P_2O_5. Inoculation of *E. colombiana* favored the highest gains in root and shoot dry matter. P_2O_5 fertilization increased foliar and root phosphorus content.

Key words: *Carica papaya*, mycorrhizae, triple superphosphate.

RESUMO

Crescimento e acúmulo de nutrientes em mudas micorrizadas de mamoeiro em substrato adubado com fósforo

O uso indiscriminado de fertilizantes minerais na cultura do mamoeiro tem elevado os custos de produção, sendo o uso de fungos micorrízicos arbusculares uma alternativa promissora para diminuir os custos. Esta pesquisa teve como objetivo avaliar a eficiência dos fungos micorrízicos arbusculares (FMA), no acúmulo de massa da matéria seca e de nutrientes, em mudas de mamoeiro Sunrise Solo, perante a aplicação de doses de superfosfato triplo, consideradas prejudiciais à simbiose. O experimento foi conduzido em ambiente protegido, no delineamento em blocos casualizados, com quatro repetições, no esquema 4x3+1: quatro doses (0,0; 672; 1386 e 2100 mg dm^{-3}) de P_2O_5 (superfosfato triplo), três espécies de fungos micorrízicos: *Gigaspora margarita*, *Entrophospora colombiana* e *Scutellospora heterogama* e o tratamento controle (sem inoculação e sem P_2O_5). Foram quantificadas as massas das matérias secas das partes aérea e radicular, além dos teores de nitrogênio, fósforo e potássio dos tecidos vegetais (folha e raiz). A inoculação com fungos micorrízicos aumentou em 30% a massa da matéria seca da parte aérea, em comparação com a do tratamento controle. Os fungos micorrízicos aumentaram os teores foliar e radicular de nitrogênio até a dose de 672 mg dm^{-3} de

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P_2O_5. A inoculação do fungo *Entrophospora colombiana* promoveu maiores ganhos de biomassa. A adubação com P_2O_5 aumentou os teores de fósforo foliar e radicular.

Palavras-chaves: *Carica papaya*, micorrizas, superfosfato triplo.

INTRODUCTION

Brazil has favorable soil and climatic conditions for the cultivation of tropical fruit species and is among the major world producers of fruit and fruit juice. Papaya (*Carica papaya* L.) is one of the most harvested fruits in the country and its annual production is approximately two billion tons (Agrianual 2011).

One of the limitations in the papaya production chain is the production of high quality seedlings due to the elevated plant density per hectare and the need for orchard renewals every three years. Thus, seedling production is a fundamental step in the implementation of a productive orchard.

Mycorrhized plants exhibit developmental advantages when compared to non-mycorrhized ones under nutrient-limiting conditions. Scientific investigations have shown that inoculation of arbuscular mycorrhizal fungi (AMF) promotes the formation of good quality papaya seedlings (TRINDADE *at al.* 2001b; MOHANDAS 2012). However, the benefits maximized by that symbiosis in plant nurseries, such as increase in nitrogen (MIYAUCHI *at al.* 2008), potassium and phosphorus uptake (OLIVEIRA & OLIVEIRA 2005), tolerance to root diseases (BORGES *at al.* 2007), and improved seedling growth and vigor during its formation period (LIMA *at al.* 2011), can be jeopardized under field conditions. Most cultivated areas occur in soils featuring low-fertility, low pH, high aluminum and low phosphorus contents and, consequently, are highly fertilized, primarily with phosphorus, which is an essential nutrient for plant growth and influences root formation and growth, water use efficiency, and uptake and assimilation of other nutrients (Epstein & Blomm 2006).

Therefore, in phosphorus-rich soils, the advantages of that type of symbiosis are reduced since carbohydrate consumption by the fungi does not cease and, indeed acts as a strong sink. Consequently, the performance of mycorrhized plants can be poorer than that of non-mycorrhized ones (Janos, 2007) which risks seedling survival in the field and causes a decrease in vigor and productivity.

The present research aimed at studying the efficiency of arbuscular *mycorrhizal* fungi (AMF) on dry matter and nutrient accumulation in Sunrise Solo papaya seedlings, by applying doses of P_2O_5 that are detrimental to the symbiosis.

MATERIAL AND METHODS

The experiment was carried out from January through May 2012 under a shaded environment, at the Department of Plant and Environmental Sciences, Agricultural Sciences Center, Campus II of the Federal University of Paraíba, located in the Municipality of Areia, Paraíba. The climate of the area, classified as As according to the Köppen system, is tropical, hot and humid, exhibiting a rainy season along fall and winter, with an average relative humidity of 80% (Gondim 1999). During the experiment, the average temperature and relative humidity varied from 22.5 to 23.6 ^{o}C and 60 to 80%, respectively.

The evaluated factors consisted of four P_2O_5 doses (0; 672; 1386 and 2100 mg dm^{-3}), based on Melo *at al.* (2007), and three species of mycorrhizal fungi supplied by Embrapa Agrobiology: *Gigaspora margarita*, *Entrophospora colombiana* and *Scutellospora heterogama*. The control treatment was mycorrhiza- and P_2O_5-free. The source of P_2O_5 was triple superposphate. The experiment was set up in a randomized block design in the factorial scheme, with four blocks and four seedlings per experimental unit.

Seeds of the cultivar Sunrise Solo, produced by Agristar, were sown in germination trays (60 cm x 40 cm) containing sand autoclaved at 121 ^{o}C. When seedlings reached 5.0 cm in height they were transferred to PVC tubes (25 cm length and 8 cm in diameter) containing 0.7 dm^{-3} of a substrate mix composed of 75% soil (Yellow Latosol, total sand = 506 g kg^{-1}, silt = 103 g kg^{-1}, clay = 391 g kg^{-1}), 15% sand and 10% manure. The soil was sandy clay loam and was mixed with cow manure. The mix was passed through a 4 mm mesh sieve. The physical-chemical characteristics of the mix are shown in Table 1. After autoclaving, N, K, Ca, Mg, S and B were added to the mix, whose amounts were based on its chemical analysis. At transplanting, 3.0 g of the mix containing fungal spores were added to each tube. Seedlings were irrigated twice a day with chlorine-free water [electrical conductivity – EC = 0.22 dS.m^{-1} and sodium adsorption rate – SAR = 2.41 $(mmol_c L^{-1})^{0.5}$], in order to keep the substrate under field capacity and avoid excessive water retention. Data collection was carried out 102 days after the beginning of the experiment and the following variables were analyzed: root and shoot dry matter, nitrogen, phosphorus and potassium contents of leaves and roots as well as mycorrhizal colonization.

For dry matter and plant tissue analysis, leaves and roots were washed in running water and rinsed with distilled water. Following that, they were placed in paper bags and dried in a drying oven with forced air circulation at 65 ºC until constant weight was reached. After dry matter determination, the plant material was ground in a Willey grinder and then passed through a 0.5 mm mesh sieve. For nutrient content analysis, the material was digested by use of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) in a block digester at 350 ºC. Nitrogen content was determined through distillation in 13N sodium hydroxide (NaOH) followed by titration with 0.07143N hydrochloric acid. Phosphorous was quantified by spectrophotometry upon measuring the color intensity of the phosphomolybdic complex, produced as result of the reduction of molybdate with ascorbic acid, and potassium was quantified by the flame photometry method (Embrapa 2011).

Mycorrhizal colonization was assessed by collecting secondary roots of seedlings and immersing them in a sodium hypochlorite solution for 30 seconds followed by another 30 second immersion in 70º ethyl alcohol. Roots were then rinsed with distilled water, placed in plastic pots containing 50 mL of 70º ethyl alcohol and 3.0 mL of formalin, and kept under refrigeration at 4 ºC. For clarification and staining (Phillips & Hayman 1970) roots were immersed in a 10% KOH solution for 15 days at room temperature. After that period, they were washed, acidified in 1% HCl for 3 minutes and stained with 1 mL trypan blue. After 10 minutes, the stained roots were sliced in 1 cm pieces and uniformly spread in a 1.27 cm square Pedtri Dish with grid and analyzed in a microscope (each intersection of roots and grid) for the presence or absence of fungal structures (Giovannetti & Mosse 1980).

Analysis of variance (ANOVA) was used to test the significance of the model sources of variation. The effect of fungal species was compared by the Tukey test and that of triple superphosphate doses by a polynomial regression. The comparison with the control treatment was conducted by the Dunett test. SAS/STAT 9.3 software (SAS 2011) was used for all statistical analyses.

Table 1: Physical-chemical characteristics of the substrate mix used for growing papaya seedlings

Substrate characteristics	Unit	Value
pH (in water)	—	6.11
EC	dS m^{-1}	1.023
O.M.	%	2.4
N	%	0.14
P	mg dm^{-3}	3.7
K^+	mg dm^{-3}	1.23
Ca^{+2}	$cmol_c$ dm^{-3}	3.6
Mg^{+2}	$cmol_c$ dm^{-3}	1.65
Sand	%	62
Silt	%	12
Clay	%	26
Soil density	kg dm^{-3}	1.14
Particle density	kg dm^{-3}	2.6
Porosity	m^3 m^{-3}	0.56

EC: electrical conductivity. O.M.: organic matter. O.M.: Humid oxidation with potassium dichromate; N: sulfuric digestion/ Kjeldahl distillation; P: Mehlich-1 extractor; K^+: flame photometry; Ca^{+2} and Mg^{+2}: EDTA complexometric titration; Soil texture (sand+silt+Clay): densimeter method; Soil density: volumetric ring system; Particle density: volumetric flask; Porosity: soil density / particle density.

RESULTS AND DISCUSSION

The results revealed no difference between the mycorrhizal treatments and the control for root dry matter accumulation (Table 2), except for *E. colombiana* at the lowest phosphate dose, which was 25% lower when compared to the control. Phosphorous can alter plant growth by allocating large amounts of carbohydrates to increase root biomass. Phosphorus availability also regulates several processes such as phytomass distribution pattern. Fernandes *at al.* (2000) observed insignificant effects of phosphate fertilization on root dry matter accumulation since the substrate studied already had adequate amounts of phosphorus for seedling growth.

The fact that the control did not differ from the other treatments, in terms of root dry matter accumulation, can be related to the time the seedlings remained in the tubes containing 0.7 dm^{-3} of substrate (102 days), which was long enough for their roots to occupy the entire substrate volume. Trindade *at al.* (2000), working with the production of papaya seedlings colonized by mycorrhizal fungi, which were grown in plastic bags containing 1 dm^{-3} of substrate, also observed that 50 days after transplanting, the roots had spread throughout the whole substrate volume, because it is a fruit species with a high growth index.

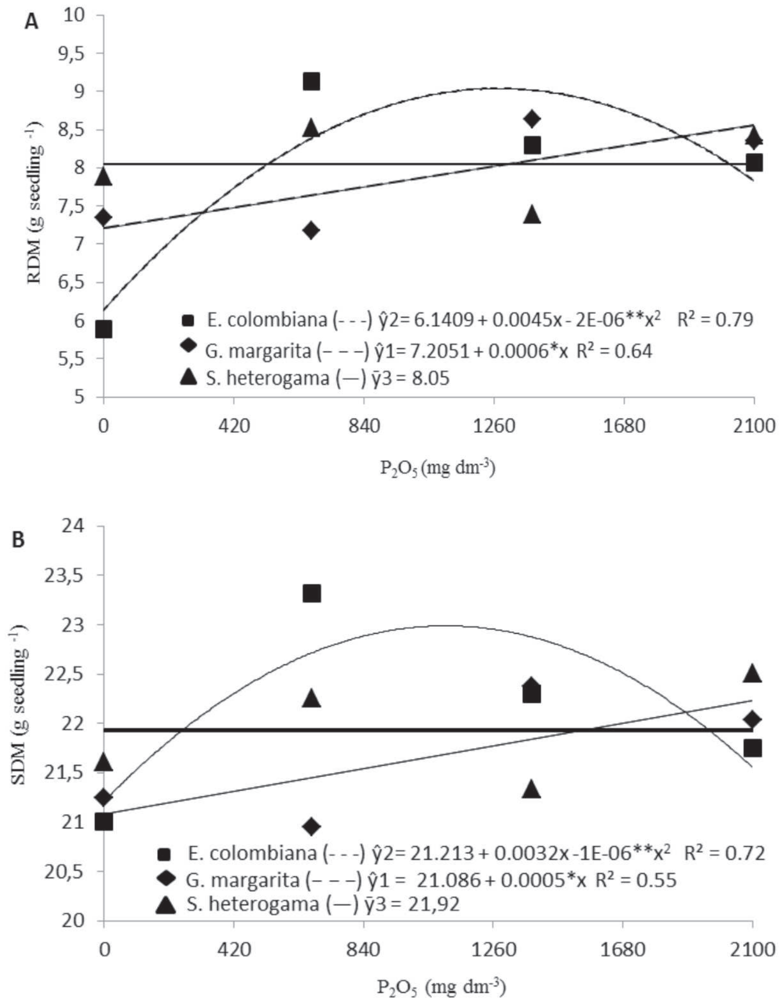
The phosphate doses had distinct effects on root and shoot dry matter, depending on the species of fungus inoculated (Figures 1 A and B, respectively). In the phosphorus-free treatment, *S. heterogama* and *G. margarita* promoted 34 and 24% increase in root dry matter accumulation, respectively, in comparison with *E. colombiana* (Table 2). Khade *at al.* (2010), studying the variation in the level of native mycorrhizal colonization in papaya varieties, detected the influence of root colonization on phosphatase activity, which increased as colonization increased.

Regarding *E. colombiana*, the maximum estimated value of root dry matter (9.03 g $seedling^{-1}$) was obtained with 1386 mg dm^{-3} P_2O_5 and the responses decreased at higher doses (Figure 1A). This behavior can be related to the inhibitory effect of phosphorus on mycorrhizal colonization, as verified with *S. heterogama* (Figure 2), while the colonization by *E. colombiana* and *G. margarita* it was not significantly affected by P_2O_5 doses. According Balzergue *at al*. (2011), elevated phosphorus concentration in the medium is accompanied by a decreased strigolactone content, decreasing the mycorrhizal colonization. Similar results were verified in studies involving mycorrhizal colonization in fruit species, such as *Hancornia speciosa* (Cardoso Filho *at al.* 2008) and *Citrus* species (Nogueira & Cardoso 2007).

Table 2: Root dry matter (g seedling^{-1}) of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5

	Mycorrhizal fungi			
P_2O_5 (mg dm^{-3})	***G. margarita***	***E. colombiana***	***S. heterogama***	**Control**
0	7.34ns A	5.88* B	7.88ns A	7.86
672	7.17ns B	9.13ns A	8.52ns A	
1386	8.63ns A	8.29ns A	7.38ns A	
2100	8.35ns A	8.06ns A	8.41ns A	

Mean values followed by the same block letter within lines are not significantly different according to the Tukey test (*P < 0.05*). ns and *: not significant and significantly different, respectively, from the control treatment, according to the Dunnett's test at (*P < 0.05*); (*P < 0.05*). Control = without fungal inoculation.



** *: Significant at (*P<0.01*) and (*P<0.05*) respectively, according to the F test.

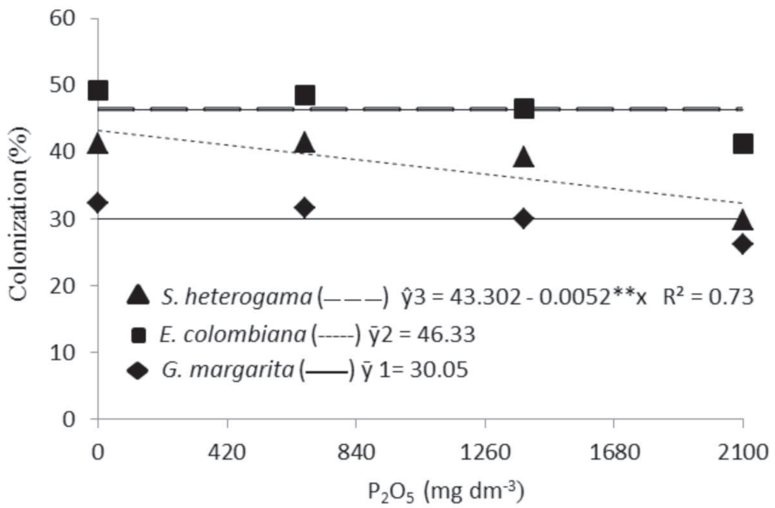
Figure 1: Root dry matter (RDM) "A" and shoot dry matter (SDM) "B" of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5.

Inoculation with *E. colombiana*, *G. margarita* and *S. heterogama* promoted a 32, 33 and 36% increase in shoot dry matter, respectively, in relation to the control treatment, but did not differ among the fungal species (Table 3). Lima *at al.* (2011) reported that papaya seedlings inoculated with *mycorrhizal* fungi exhibited higher growth rates than those not inoculated, regardless of the amount of phosphorus in the soil.

Shoot dry matter behavior was similar to that observed for root dry matter (Figure 1 B). It is worth highlighting the effects on *E. colombiana*, which exhibited quadratic growth as fertilization doses increased, reaching a maximum value of 22.98 g seedling^{-1}, which corresponds to an estimated dose of 1104 mg dm^{-3} of P_2O_5. This superiority in relation to the control (15.88 g seedling^{-1}) expresses the importance of *mycorrhizal* fungi in promoting one of the main advantages of that symbiosis: nutrient uptake. The increments in root uptake capacity, provided by hyphae development and branching, which in turn increase root-soil contact area, favor a greater absorption of nutrients such as phosphorus.

Trindade *at al.* (2001a) demonstrated that papaya cultivars can benefit from inoculation with *Glomus clarum* and *G. margarita*, reducing up to seven times the need for P fertilization so that maximum shoot growth can be achieved. Lima *at al.* (2011), evaluating growth of papaya cv Golden seedlings, showed that fungal inoculation treatments promoted an increase in seedling leaf area, with an increment of 19.3% in relation to the control treatment, which influenced dry matter accumulation but did not differ among the fungal species studied.

No differences for leaf N content of seedlings were detected among *mycorrhizal* fungi (Table 4). However, all fungi treatments exhibited increases in foliar N, whose values were 24, 23 and 21% higher than the control for *G. margarita*, *E. colombiana* and *S. heterogama*, respectively, at 672 mg dm^{-3} P_2O_5 (Table 5). Chu *at al.* (2001) also reported no differences in terms of foliar N content of *Annona muticata* seedlings when inoculated with the same fungi species assessed in the present study and using fumigated and non-fumigated soil.



** Significant at (*P<0.01*) according to the F test.

Figure 2: Mycorrhizal colonization in papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5.

Table 3: Shoot dry matter (g seedling^{-1}) of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5

	***Mycorrhizal* fungi**			
P_2O_5 (mg dm^{-3})	***G. margarita***	***E. colombiana***	***S. heterogama***	**Control**
0	21.24* A	21.00* A	21.60* A	15.88
672	20.95* B	23.31* A	22.25* A	
1386	22.37* A	22.29* A	21.33* A	
2100	22.03* A	21.74* A	22.51* A	

Mean values followed by the same block letter within lines are not significantly different according to the Tukey test (*P<0.05*). and *: significantly different, respectively, from the control treatment, according to the Dunnett's test at (*P<0.05*); (*P<0.05*). Control = without fungal inoculation.

Leaf N content exhibited a quadratic behavior as P_2O_5 doses increased (Figure 3A), reaching a maximum value of 45.38 g kg^{-1} at 336 mg dm^{-3} P_2O_5. Nitrogen uptake by *mycorrhizal* fungi becomes more efficient because of the small hyphae diameter that penetrate more easily in decomposing organic matter, competing more effectively for recently mineralized N, especially simple nitrogen organic compounds (Hodge, 2003). Contrasting results were reported by Melloni & Cardoso (1999) in *Citrus* seedlings. The increasing phosphorus doses favored linear increases in the total amount of macronutrients absorbed by the rootstocks, including nitrogen.

Phosphorus content in the seedlings increased linearly as P_2O_5 doses applied to the substrate increased (Figure 3B). Leaf phosphorus content of seedlings inoculated with *mycorrhizal* fungi also increased in relation to the control treatment at the highest P_2O_5 doses. That increase was detected at P_2O_5 doses that promoted the highest total phosphorus concentration in the seedlings, with over 80% increases at P_2O_5 doses equal and above 1386 mg dm^{-3}. The positive influence of mycorrhizal inoculation on plant growth is especially associated with the uptake of low mobility nutrients such as phosphorus, copper and zinc, which reach the roots by slow diffusion mechanisms. The fungi can access soluble forms of phosphorus that are also available for non-inoculated plants, but hyphae end up exploring the soil more thoroughly and guarantee better nutrient absorption (Kanno *at al.* 2006). The increase in leaf phosphorus content as P_2O_5 doses increased may not be related to mycorrhizal benefits at higher doses since there is a phosphorus balance between the soil and the plant tissue that controls the symbiotic association (Costa *at al.* 2001).

The effect of P_2O_5 doses on nitrogen and phosphorus content of roots are displayed in Figure 4. Root nitrogen content decreased linearly as P_2O_5 doses increased (Figure 4A). However, at lower doses the mycorrhizal effect was more distinct. According to a few authors, mycorrhizal fungi can form associations with nitrogen-fixing bacteria. The fungus plays a central role in providing phosphorus for the plant and for the bacteria in the nodules, a process that is ATP-dependent (Barea *at al.* 1992). Nitrogen

Table 4: Nutrient content (g kg^{-1}) in roots and leaves of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi

	Leaf content			Root content		
	N	P	K	N	P	K
	(g kg^{-1})					
Gigaspora margarita	43.35 a	8.05 a	44.66 a	11.08 a	10.07 a	30.23 a
Entrophospora colombiana	43.99 a	7.94 a	43.08 ab	10.78 a	8.74 a	27.22 a
Scutellospora heterogama	43.9 a	7.73 a	39.92 b	10.51 a	9.27 a	26.79 a

Mean values followed by the same letter within columns are not significantly different according to the Tukey test (P < 0.05)

Table 5: Nutrient content (g kg^{-1}) in roots and leaves of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5

P_2O_5(mg dm^{-3})	Fungus	N		P		K	
		Roots	Leaves	Roots	Leaves	Roots	Leaves
		(g kg^{-1})					
0	*G. margarita*	11.66*	44.13ns	5.52ns	5.56ns	29.04ns	44.4ns
	E. colombiana	11.2*	44.96*	4.41ns	5.24ns	26.41ns	43.34ns
	S. heterogama	11.66*	45.4*	5.61ns	5.51ns	25.88ns	39.13ns
672	*G. margarita*	10.96ns	46.93*	9.39*	7.81*	31.68ns	43.34ns
	E. colombiana	11.4*	46.36*	7.54*	7.47*	26.41ns	45.45ns
	S. heterogama	11.05*	45.46*	8.55*	6.85ns	25.36ns	40.18ns
1386	*G. margarita*	10.9ns	41.96ns	12.24*	9.08*	30.62ns	45.45ns
	E. colombiana	10.25ns	42.83ns	10.59*	8.67*	28.52ns	41.24ns
	S. heterogama	9.43ns	43.53ns	11.19*	8.83*	30.89ns	40.18ns
2100	*G. margarita*	10.8ns	40.36ns	13.13*	9.75*	29.57ns	45.45ns
	E. colombiana	10.1ns	41.8ns	13.02*	10.36*	27.99ns	42.19ns
	S. heterogama	9.8ns	41.2ns	12.93*	9.72*	26.41ns	40.18ns
	CONTROL	8.8	37.55	2.82	4.77	28.52	41.24

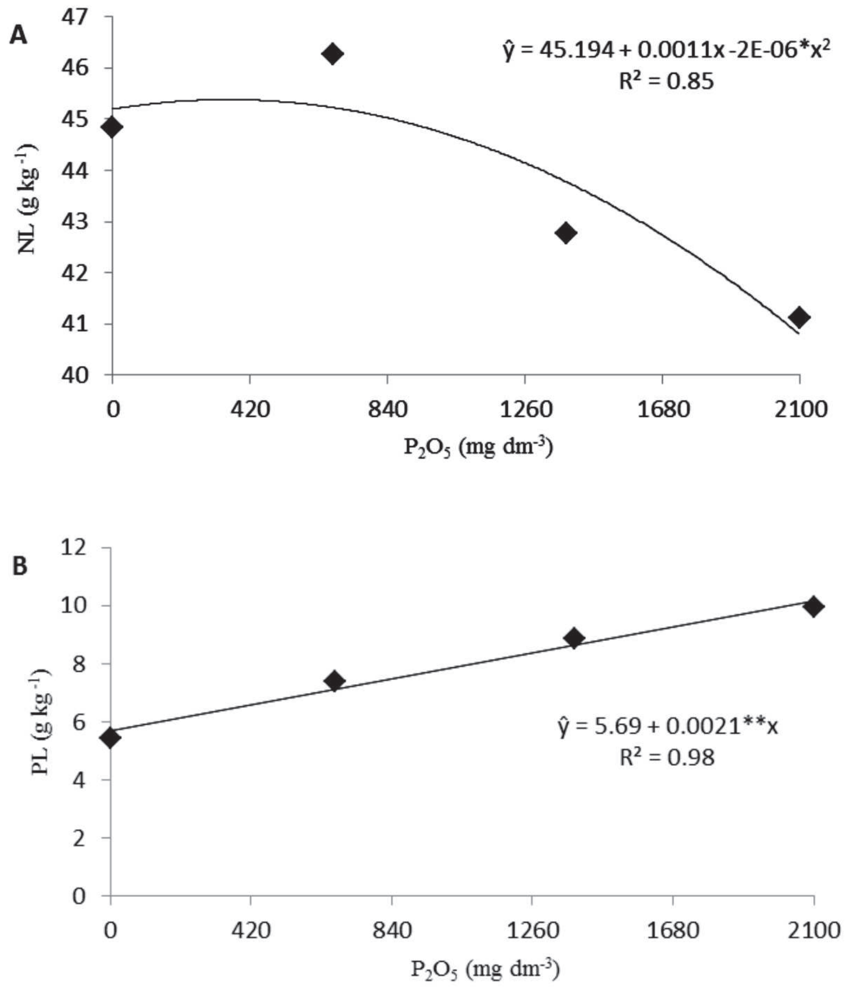
ns and *: not significant and significantly different at (P < *0.05*), respectively, in relation to the control treatment according to the Dunnett's test (P < *0.05*). Control = without fungal inoculation and fertilization.

fertilization may positively or negatively interfere with the development of plant mycorrhizal dependency. Santos *at al.* (2001) observed a decrease in the number of spores as nitrogen fertilization increased.

Root phosphorus content exhibited a quadratic tendency in response to an increase in the amount of that nutrient in the substrate (Figure 4B). Based on the studies conducted by Moreira & Siqueira (2002), it can be assumed that in conditions of high phosphorus supply, phospholipid biosynthesis is increased, and as a result, cell permeability is affected. Thus, root exudation (sugars and amino acids), infection and colonization decline, as proved by colonization of *S. heterogama* (Figure 4B). The data revealed a higher phosphorus content in the tissues of inoculated seedlings in relation to the control (Table 5), reaching the highest value (12.3 g kg^{-1}) at 1978 mg dm^{-3} P_2O_5 (Figure 4B); these results were more evident than those reported by Trindade *at al.* (2001b). This can be due to the high doses used in the present study. Differences were only detected at doses equal or higher than 672 mg dm^{-3} P_2O_5. Increments of 201, 302 and 361% were verified at 672, 1386 and 2100 mg dm^{-3} P_2O_5, respectively, when comparing fungi treatments with the control. No difference was detected among the fungi used (Table 4).

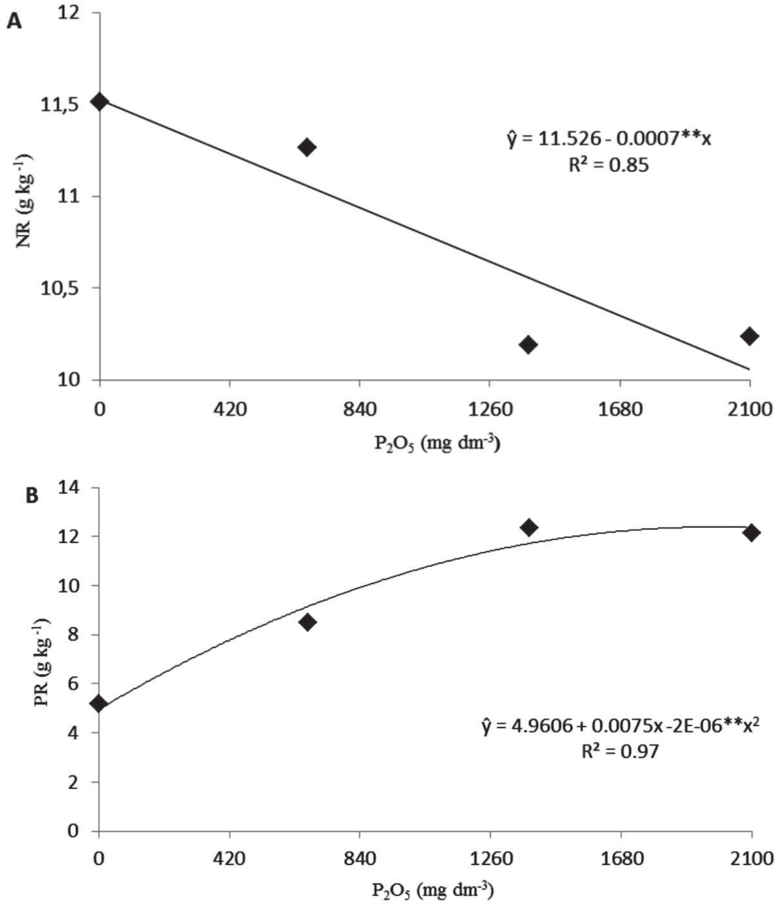
The increase in phosphorus content of roots and leaves is a result of a higher amount of P_2O_5 in the substrate. As it is a highly mobile nutrient, its concentration was higher in the tissues of those two organs. Mesquita *at al.* (2010), studying leaf mineral composition of papaya fertilized with an enriched biofertilizer, detected phosphorus increments of 4.2 g kg^{-1}, which were inferior to the results obtained in the present study.

Production of quality papaya seedlings, aiming at the enhancement of their performance after transfer to the field, makes the potential use of AMF more attractive since it is responsible for increasing the efficiency of nutrient uptake, thus reducing the use of fertilizers without affecting productivity.



** *: Significant at (*P<0.01*) and (*P<0.05*) respectively, according to the F test

Figure 3: Nitrogen (NL) “A” and phosphorus (PL) “B” contents in leaves of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5.



**: Significant at (*P<0.01*) according to the F test

Figure 4: Nitrogen (NR) "A" and phosphorus (PR) "B" contents in roots of papaya cv. Sunrise Solo seedlings inoculated with *mycorrhizal* fungi and fertilized with P_2O_5.

CONCLUSION

Mycorrhizal inoculation reduced the need for additional phosphorus fertilization in papaya seedlings, since it promoted increases of over 30% in shoot dry matter in relation to the control.

E. colombiana favored the highest gains in root and shoot dry matter, but became a sink at P_2O_5 doses above 1386 mg dm^{-3}.

The mycorrhizal fungi promoted increases in foliar and root nitrogen content up to 672 mg dm^{-3} P_2O_5 and were tolerant to amounts considered harmful to the symbiosis.

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