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CROP PRODUCTION

Arbuscular mycorrhizae in mango orchards in a Brazilian semi-arid region: influence of phenology, management and plant variety

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ABSTRACT: Arbuscular mycorrhizal fungi (AMF) increase the absorption surface of roots, providing greater absorption of water and nutrients from the soil by plants. Thus, it is important to know how arbuscular mycorrhizae are affected by factors such as phenology, management and varieties in mango (*Mangifera indica)* orchards in the irrigated semi-arid region. With this objective, soil and root samples were collected during the vegetative, flowering and fruiting stages, and in the water stress period before flowering, always on the same trees. The study was carried out in mango orchards of the Keitt and Palmer varieties, in agricultural farms located in Juazeiro - BA and Petrolina - PE, both under conventional management (with fertilizers and paclobutazol). The soil was used to quantify the number of AMF spores and phosphorus content. Mycorrhizal colonization in roots was evaluated in qualitative terms (presence of hyphae, vesicles and arbuscles) and quantitative terms (% of colonization). Total mycorrhizal colonization was high, with averages above 65%, and higher values in the fruiting period. Colonization by vesicles and arbuscles was on average above 45% and 17%, respectively, with higher means also during fruiting. The number of AMF spores in the soil averaged over 130 spores in 50 g of soil, with higher values in the period of water stress. The phosphorus content in the soil did not influence mycorrhizal colonization and the number of spores in the soil. The association of AMF with mango trees was generally affected by management and phenology, regardless of the cultivated varieties.
Key words: arbuscular mycorrhizal fungi, *Mangifera indica*, fruit trees, soil management.

Micorrizas arbusculares em pomares de mangueira no semiárido brasileiro: influência da fenologia, do manejo e da variedade

RESUMO: Os fungos micorrízicos arbusculares (FMAs) aumentam a superfície de absorção das raízes, proporcionando maior absorção de água e nutrientes do solo pelas plantas. Assim, é importante conhecer como as micorrizas arbusculares são afetadas por fatores como fenologia, manejo e variedades em pomares de mangueira (*Mangifera indica* L.) no semiárido irrigado. Com esse objetivo, foram coletadas amostras de solo e raízes durante os estádios vegetativo, floração e frutificação, e no período de estresse hídrico antes da floração, sempre nas mesmas árvores. O estudo foi realizado em pomares de mangueira das variedades Keitt e Palmer, em fazendas agrícolas localizadas em Juazeiro - BA e Petrolina – PE, ambas sob manejo convencional (fertilizantes e paclobutazol). O solo foi utilizado para a quantificação do número de esporos de FMAs e teor de fósforo. Nas raízes foi avaliada a colonização micorrízica em termos qualitativos (presença de hifas, vesículas e arbúsculos) e quantitativos (% de colonização). A colonização micorrízica total foi alta, com médias acima de 65%, e maiores valores no período da frutificação. A colonização por vesículas e arbúsculos foi, em média, acima de 45% e 17%, respectivamente, com maiores médias também durante a frutificação. O número de esporos de FMAs no solo apresentou médias acima de 130 esporos em 50 g de solo, com maiores valores no período de estresse hídrico. O teor de fósforo no solo não influenciou a colonização micorrízica e o número de esporos no solo. De modo geral, a associação dos FMAs com mangueiras foi afetada pelo manejo e fenologia, independentemente das variedades cultivadas.
Palavras-chave: fungos micorrízicos arbusculares, *Mangifera indica*, fruteiras arbóreas, manejo do solo.

INTRODUCTION

Arbuscular mycorrhizae (AM) are formed by the association between arbuscular mycorrhizal fungi (AMF) and plant roots, and represent one of the most important ecological soil associations. This association is the oldest type of mycorrhiza existing in nature, and is established with more than 80% of vascular plants, whether in natural or cultivated ecosystems (BAUER et al., 2015; BRUNDRETT & TEDERSOO, 2018).

The association of AMFs with cultivated plants can provide an increase in productivity and profits in agricultural systems because they increase the soil volume explored by the roots, which results in increased water and nutrient absorption from the soil. With this, plants can allocate more photosyntates in their shoots such as leaves and fruits, and consequently increase their growth rate and fruit production. This interaction can have a different impact during the different phenological stages of plants (CHEN et al., 2016; BOWLES et al., 2017; OEHL & KOCH, 2018).

These beneficial effects may be especially important in the Brazilian semi-arid region, even under irrigated management, as there are adverse abiotic factors for plants such as high temperatures,

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water stress and low fertility, for which there are records that arbuscular mycorrhizae can favor plant development (LIMA et al., 2007; PEREIRA et al., 2021; SILVA et al., 2021; SANTANA et al., 2023).

The establishment of irrigation projects in the Sub-middle São Francisco Valley regularized water availability for agricultural areas and enabled implementing the fruit growing pole, which has contributed to strengthening and expanding agriculture in the Brazilian semi-arid region (SIMÕES et al., 2020). Sustainable technologies and innovations can contribute to increased productivity and product quality (BALOTA, 2017; OLIVEIRA & LIMA, 2021; PRATES et al., 2021). Thus, the importance of studying the influence of AMF in irrigated fruit culture is highlighted, since they are important biotechnological tools with the potential to contribute to the region's agroindustry, favoring economic, social and environmental development.

The presence of native AMFs in agricultural ecosystems is generally not taken into account to define management in agriculture, but this does not mean that they are not present and contributing to productivity and conferring sustainability aspects to this activity, even in conventional agricultural systems (RILLIG et al., 2018).

Mango is one of the fruits produced in the fruit growing center of the Sub-middle São Francisco Valley (in Petrolina, PE and Juazeiro, BA), being most exported in terms of value and volume by Brazil, having a large share in the country's agriculture, as it produces from about 85% of the total exported in 2019. Thus, mango cultivation in the semi-arid region stands out nationally due to the large extension of cultivated area, production volume and fruit quality, with economic and social impacts in the region (SIMÕES et al., 2020; OLIVEIRA & LIMA, 2021).

Arbuscular mycorrhizae were evaluated in mango trees (*Mangifera indica* L. 'Kent') in a field experiment in the Brazilian semi-arid region using mixtures of plants for green manure, applied with or without soil tillage, on arbuscular mycorrhizae. As a result, the number of native AMF spores in the soil decreased in the fruiting stage in the management incorporating green manure by soil tillage. Mycorrhizal colonization was similar between the manure systems and the mixtures of plants for fertilization within each phonological stage (PEREIRA et al., 2021).

Considering the known benefits of AM association, the presence of native AMFs and their application in industrial agriculture in the irrigated semi-arid region, especially in fruit growing, CHARACTERIZING the dynamics of AM in mango (*Mangifera indica* L.) orchards in industrialized agriculture and the effect of factors such as cultivated variety, conventional management used and phenological stages of mango is important, and constituted the main objective of this study.

MATERIALS AND METHODS

The study was conducted in mango orchards cultivated in conventional systems in the Brazilian semi-arid region under irrigation on three farms: two belonging to the agricultural company PRITAM Fruit Exportação (Casa Nova, BA), and one belonging to the agricultural company FRUTECER (Petrolina, PE). PRITAM's orchards were cultivated with the Palmer variety, and kept under the same management conditions. The management of the orchards did not include tilling the soil in the rows or between the rows, with this system being characterized as conventional in industrialized agriculture.

The FRUTECER company's orchard was cultivated with the Palmer and Keitt varieties, kept in adjacent areas under a conventional management system, with soil tilling between the rows during the cleaning process (removal of leaves and herbs) between the end of the fruiting period (harvest) and the beginning of the vegetative period at each new beginning of the cycle. The revolved material was accumulated in the cultivation lines.

The conventional system adopted in the orchards included: micro-sprinkling fertigation; use of mineral inorganic fertilizers (monoammonium phosphate, iron sulfate, copper sulfate, boric acid, manganese sulfate, potassium sulfate, potassium chloride, calcium chloride), according to the recommendations for the crop; use of organic and organomineral fertilizers (urea, animal manure and plant residues); induced water stress and application of paclobutrazol (PBZ).

PBZ acts as a growth regulator (OLIVEIRA et al., 2019; RADEMACHER, 2016) which is applied in the vegetative stage and the water stress period then begins after approximately 60 days of its application. Water stress is carried out with partial and gradual suspension of irrigation for a period of 20 to 30 days, and the water volume varies according to factors such as rainfall and plant reactions in the pre-flowering period. Then, irrigation and maintenance are resumed until the end of the fruiting period. Both procedures are adopted to induce stoppage of vegetative growth and stimulate the beginning of flowering in order to standardize and increase fruit production.

Sampling was carried out in the four orchards, and 10 trees were selected for collection of rhizospheric soil and roots in each. The trees were selected at random and inside the plot to avoid the edge effect. The collections were repeated during the phenological stages (vegetative, flowering and fruiting), and also in the water stress period (before the flowering stage), always in the same trees. Soil and root samples were taken within the radius of the mango tree canopy in the 0-10 cm depth layer.

The number of spores was determined by counting spores extracted from 50 g of soil using the wet sieving and centrifugation methodologies in 2 M sucrose solution, as proposed by GERDEMANN & NICOLSON (1963) and JENKINS (1964), respectively, with modifications proposed by McKENNEY & LINDSEY (1987) (JOHNSON et al., 1999; PRATES JUNIOR et al., 2021). Sieves with meshes of 500 µm and 38 µm were used for wet sieving. Centrifugation in water was for 3 minutes at 3000 rpm and centrifugation in sucrose solution was for 1.5 minutes at 3000 rpm. The spore suspension obtained was transferred to a channeled plate for counting the spores with cellular content under a stereoscopic microscope.

The fine roots were bleached and stained with trypan blue to allow quantitative assessment of AMF colonization. The roots were processed according to the methodology of PHILLIPS & HAYMAN (1970), modified by KOSKE & GEMMA (1989) (JOHNSON et al., 1999). Root colonization was quantified using an optical microscope to observe the roots and mounted on slides using a 200x magnification according to the segment analysis method, considering the presence of different AMF structures in the roots (McGONIGLE et al., 1990; JOHNSON et al., 1999; PRATES JUNIOR et al., 2021).

Soil phosphorus content was determined using the Mehlich-1 extractor solution and spectrophotometry, according to the procedure described in the Embrapa Manual of Soil Methods and Analysis (TEIXEIRA et al., 2017). Mycorrhizal colonization data and the number of spores were submitted to analysis of variance (ANOVA), followed by the Tukey's test. The P content results were used to evaluate the correlation with the mycorrhizal parameters.

Statistical analyzes of the effects of the treatments identified on mycorrhizal colonization and number of spores were always carried out considering the different phenological stages. The following aspects were evaluated: 1) influence of soil management during the phenological stages, using data from plants of all varieties; and 2) influence of Keitt and Palmer mango varieties using data from plants kept under the same management.

RESULTS AND DISCUSSION

Effect of soil management and phenology on mycorrhizal variables

The total mycorrhizal colonization of mangoes in the two different types of management presented variations between the phenological stages, with increases in percentages throughout the cycle and maximum values in the fruiting phase being superior to 65% ($P < 0.05$) (Figure 1a). Some studies have shown that the development of AMF inside the roots can vary with environmental, seasonal and phenological conditions (RICHARDSON et al., 2013; BERRUTI et al., 2014).

Total mycorrhizal colonization was higher in all phenological stages in undisturbed soil when compared to soil that had been disturbed (Figure 1a). Thus, conservationist management without soil disturbance has been proposed as an efficient technique to improve soil stability and AMF colonization, as it helps in ecological interactions, and can improve the response to disturbances (ZHAO et al., 2015; BOWLES et al., 2017). Similar results showing an increase in mycorrhizal colonization in plants grown under no-tillage have previously been reported (GOTTSHALL et al., 2017; KABIRI et al., 2016; DE LA CRUZ-ORTIZ et al., 2020).

There are some results regarding phenology in fruit orchards already published from studies in agricultural areas. For example, a study on apple trees in which mycorrhizal colonization was high in the fruiting phase (32%) and decreased in the post-harvest phase (26%) (LAVELY et al., 2018). In addition, mycorrhizal colonization was higher in a flowering mango orchard than in citrus species (orange, lemon and grapefruit) in the vegetative phase. These citrus species were in the same phenological phase and did not differ as to their mycorrhizal colonization degree (ABDELHALIM et al., 2014). Another study in a vine orchard in the fruit growing center of the semi-arid region of Pernambuco with areas under conventional and organic management found that mycorrhizal colonization was low even in the fruiting phase, with a difference in colonization from soil management which resulted in higher averages under organic management conditions (15.9%) than in conventional management (4.7%) (FREITAS et al., 2011).

The effect of PBZ application on AMF could thus directly inhibit the soil microbial community, and this may be related to the fact that the

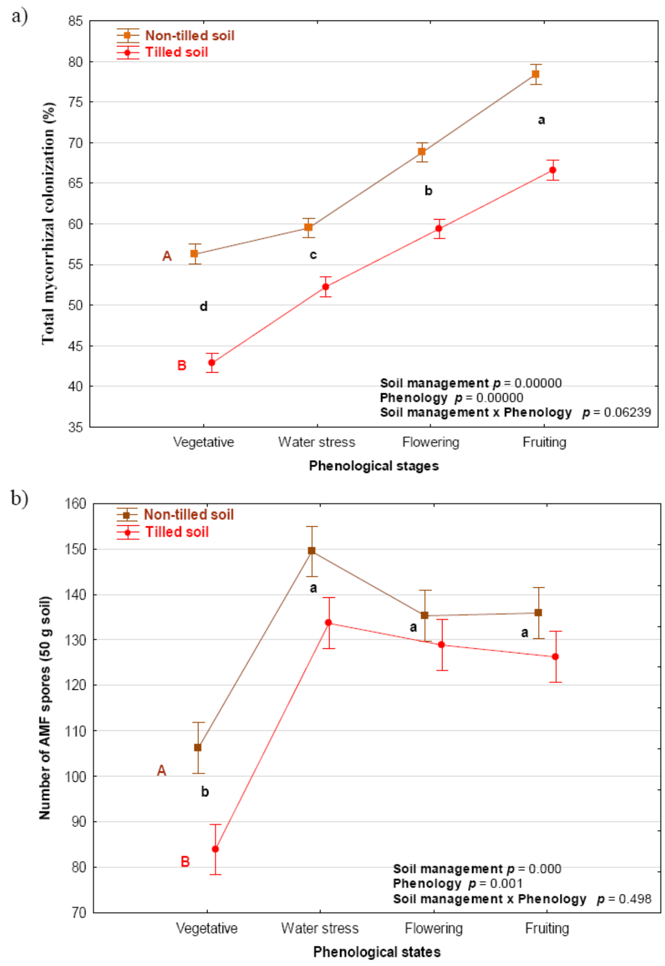

Figure 1 - Effect of soil management and mango phenology on total mycorrhizal colonization (a) and number of AMF spores (b). Means (± standard error) followed by equal letters do not differ statistically by the Tukey's test at 5% probability. Lowercase letters compare phenological stages, and capital letters compare the managements.

substance has fungicidal action. PBZ can indirectly affect the MA symbiosis, as it affects the metabolism of plants (inhibits vegetative growth and induces flowering) with which the AMF are associated (OLIVEIRA et al., 2019; RADEMACHER, 2016). As the PBZ was applied at the beginning of the cycle (vegetative period), it may be related to the lower colonization percentages (Figure 1a). SILVA et al. (2003) studied soils from irrigated mango orchards for export in Petrolina, PE and Lins, SP and observed a reduction of up to 58% in the soil microbial population with frequent application of PBZ, with the fungal community being the most affected.

Vesicular colonization in both managements presented means with increasing value along the phenological stages, being maximum in the fruiting phase with values above 50% ($P < 0.05$) (Figure 2a), with interaction between the phenology and management factors ($P = 0.015$). In a study carried out without considering the phenological stages, WANG et al. (2011) did not observe differences in the vesicular colonization percentage in citrus orchards under two vegetation control managements between the rows: with weeding or with herbicide application.

Arbuscular colonization in the different managements evaluated showed increasing averages during the phenological stages, being maximum in the fruiting phase, with averages higher than 18% ($P < 0.05$) (Figure 2b). There was no effect of soil management on arbuscular colonization. A greater

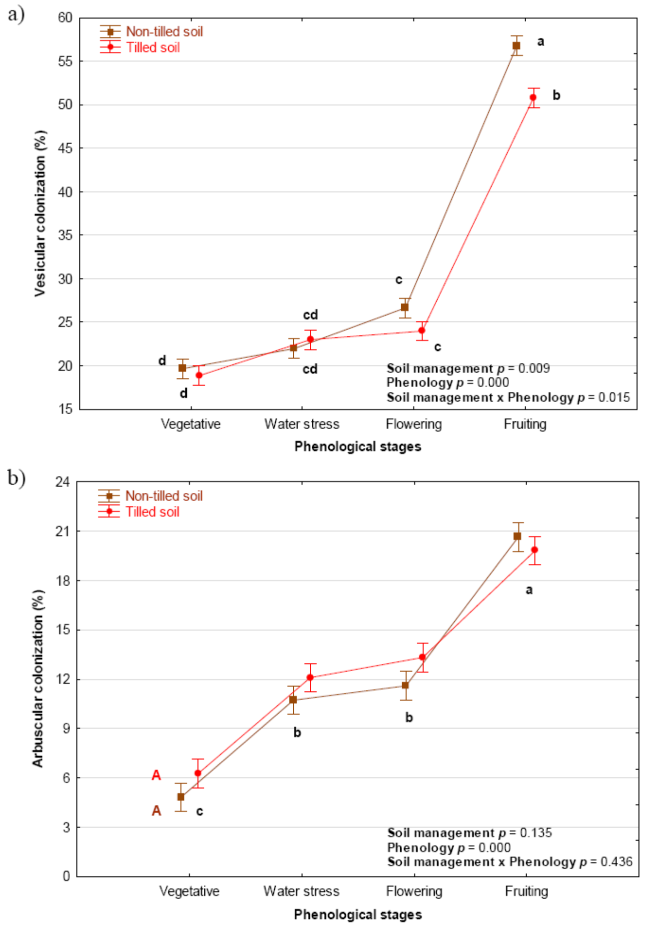

Figure 2 - Effect of soil management and mango phenology on vesicular (a) and arbuscular (b) colonization. Means (±standard error) followed by equal letters do not differ statistically by the Tukey's test at 5% probability. Lowercase letters compare the phenological stages, and the capital letters compare the managements.

presence of arbuscules in studies with fruit trees was observed in the following situations: in management with inorganic fertilization, without application of herbicide to control weeds and under the effect of seasonality, in summer (BALDERAS-ALBA et al., 2019; WANG et al., 2011; WANG et al., 2015).

The number of AMF spores was higher in the water stress stage for both managements evaluated, higher than 130 spores in 50 g of soil. Effects resulting from the phenological stage of the plants were verified with lower averages in the vegetative stage, and higher in the flowering and fruiting stages ($P < 0.05$) (Figure 1b).

Considering the phenological stages, the number of spores was higher in undisturbed soil management than in the tilled soil. Studies show that intensive cultivation practices result in changes in soil fertility and quality, thus affecting AMF attributes such as spore density and mycorrhizal colonization of roots (FERNANDES et al., 2016; HADDAWAY et al., 2017; COTTON & ACOSTA-MARTÍNEZ, 2018; OEHL & KOCH, 2018; XIAO et al., 2019)

Some studies have proposed that the organic management of orchards without disturbing the soil (MEYER et al., 2015; VAN GEEL et al., 2015), and no-tillage systems (BOWLES et al., 2017; KABIRI et al., 2016) are efficient strategies to promote improvement in soil stability and colonization by AMFs, as the results obtained show a significant increase in mycorrhizal colonization.

Effect of mango varieties on mycorrhizal variables

When the effects of the Keitt and Palmer mango varieties were evaluated, it was found that the total AMF colonization was similar between them (Figure 3a), with increasing averages due to the phenological effect, as previously recorded (Figure 1a), and also with maximum value in the fruiting phase with values above 65%. These results are similar to those obtained by MEDDAD-HAMZA et al. (2017) in their studies with olive trees, in which changes were observed in the mycorrhizal colonization during the phenological/seasonal stages, but without differences between the varieties. MEYER et al. (2015) found changes in mycorrhizal colonization along phenological/seasonal stages, with maximums during the spring and seed production phase.

Vesicular colonization in the Keitt and Palmer varieties similarly increased throughout the phenological cycle, and was maximum in the fruiting phase, with values above 49% ($P < 0.05$) (Figure 4a). There were no differences in vesicular colonization between the Keitt and Palmer mango varieties.

Arbuscular colonization had similar trends in both varieties, increasing progressively throughout the phenological stages and was maximum in the fruiting phase, with values above 18% ($P < 0.05$) (Figure 4b). Differences were not detected between the varieties, constituting a fact also observed by GOVINDAN et al. (2020) for colonization by intraradicular hyphae, vesicles and arbuscules.

The number of AMF spores showed variations between the phenological stages with a maximum in the water stress stage for both varieties, presenting values greater than 130 spores in 50 g of soil ($P < 0.05$) (Figure 3b). In addition, the varieties showed differences between them, with the Palmer variety presenting the highest number of spores

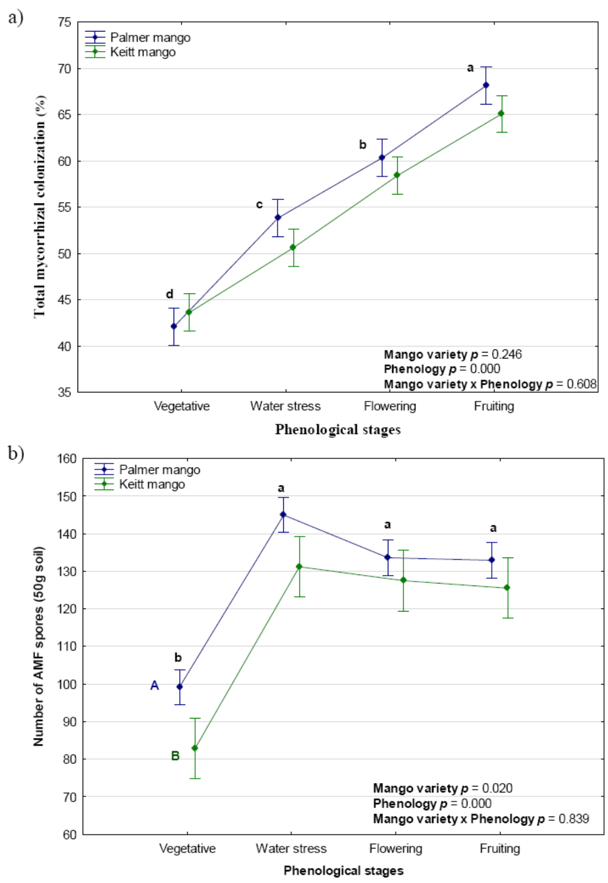

Figure 3 - (a) Effect of mango variety and phenology on total mycorrhizal colonization and (b) number of AMF spores. Means (±standard error) followed by equal letters do not differ statistically by the Tukey's test at 5% probability. Lowercase letters compare the phenological stages, and the capital letters compare the managements.

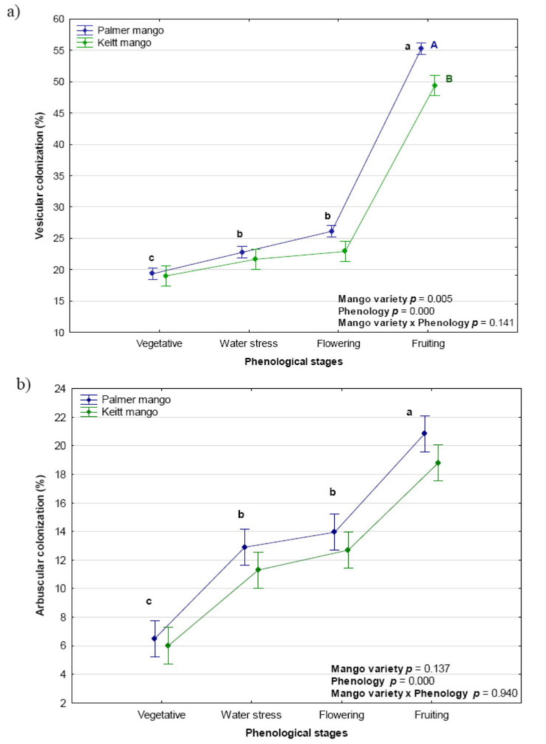

Figure 4 - Effect of mango variety and phenology on (a) arbuscular and (b) vesicular colonization. Means (±standard error) followed by equal letters do not differ statistically by the Tukey's test at 5% probability. Lowercase letters compare the phenological stages, and the capital letters compare the managements.

throughout the phenological stages ($P < 0.05$). Variations in the number of AMF spores were also observed in studies with agricultural soils of the caatinga under the influence of factors such as: plant species, seasonality (dry/rainy period), and type of management (conventional or conservationist) (LIMA et al., 2007; SANTOS et al., 2013; SOUSA et al., 2014; CARDOZO JUNIOR et al., 2018). The production of spores can be stimulated by stress situations, such as water deficit, temperature increase, soil disturbance, end of the growth cycle of annual plants or the productive cycle of perennial plants, and the high concentration of chemical substances, such as fertilizers, biocides, and plant metabolism regulators (SMITH & READ, 2008; BRUNDRETT & TEDERSOO, 2018; SALOMON et al., 2022).

Studies in fruit tree orchards of different species, plant varieties and of different ages did not show differences in mycorrhizal colonization between species (ABDELHALIM et al., 2014; ANGALESWARI & MAHALINGAM, 2014; JIANG et al., 2020; MEDDAD-HAMZA et al., 2017; MOHANDAS et al., 2012), varieties (GOVINDAN et al., 2020) or the age of the orchard (MEDDAD-HAMZA et al., 2017; MOHANDAS et al., 2012).

Phosphorus (P) content in soil on mycorrhizal variables

The P content in the orchards during the mango production cycle varied from low to high, with values between 0 and 93 mg kg^{-1} in the undisturbed soil, while it varied from 0 to 43 mg kg^{-1} in the disturbed soil, constituting a condition considered normal in industrial orchards where inorganic fertilization is used. There was no correlation between total mycorrhizal colonization and soil P content (Figure 5).

Even though many studies have highlighted negative effects of high phosphorus availability in soil on mycorrhizal colonization (SHENG et al., 2013; KRUGER et al., 2015; GAGIC et al., 2017; KONVALINKOVÁ et al., 2017; BALDERAS-ALBA et al., 2019; PEREIRA et al., 2021), there is also a record that the availability of phosphorus in the soil in irrigated crops of lemon, grapefruit and mango trees did not affect the number of AMF spores in the soil (ABDELHALIM et al., 2014; GOVINDAN et al., 2020; PEREIRA et al., 2021).

Phosphorus content reported in studies of fruit tree orchards varies widely, from 1.1 mg kg^{-1} in mango and lychee fertilized with manure, to 971.2 mg kg^{-1} in Japanese pear trees grown in Andosol (JIANG et al., 2020; YOSHIMURA et al., 2013), without clear correlations between this variable and the degree of plant colonization by AMFs. The relationship between the AM association and soil P may not be due to the P content in the soil, but to factors such as plant demand at each phenological stage (PEREIRA et al., 2021), or soil factors such as the P fixation ability (LIMA et al., 2020).

CONCLUSION

The mycorrhizal colonization of mango trees cut in orchards increased during the phenological stages, especially the total mycorrhizal colonization. The formation of AMF vesicles in the roots increased at the end of the mango reproductive cycle, while the colonization by arbuscules grew after the vegetative stage, remained constant between the water stress stages until flowering, and then increased again in the fruiting stage.

The number of AMF spores in the mango rhizosphere was higher in the water stress stage, regardless of the management adopted and the mango varieties. Soil phosphorus content did

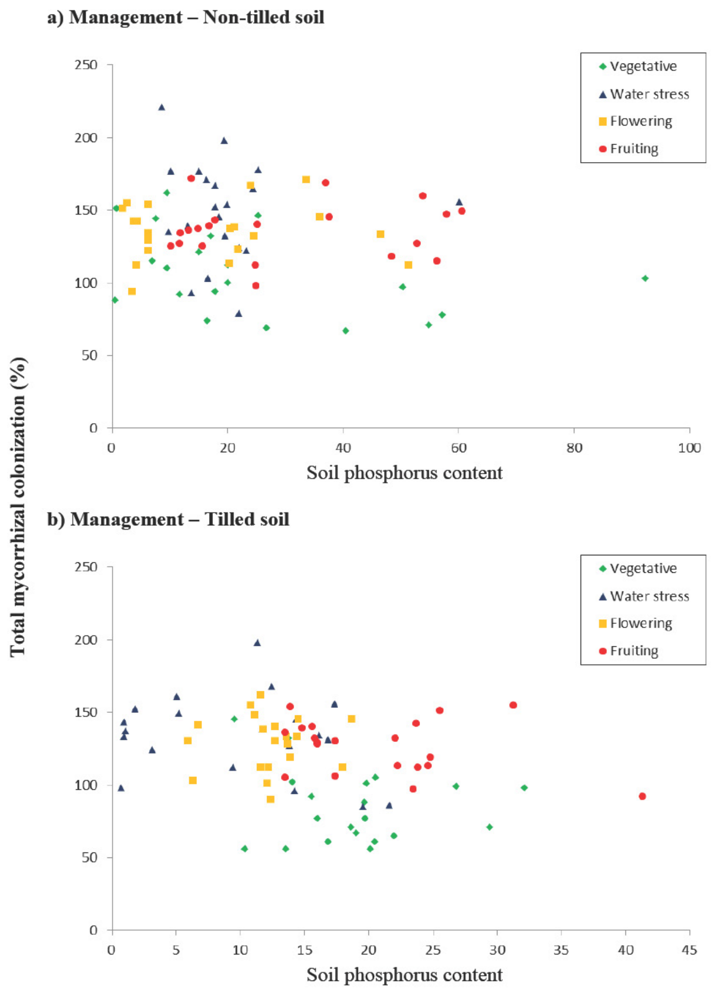

Figure 5 - Relationship between total mycorrhizal colonization (%) and phosphorus (P) content in the soil of mango orchards at different phenological stages (a) under no-tillage and (b) tilled soil managements.

not affect the number of AMF spores in the soil or the total mycorrhizal, vesicular and arbuscular colonization of mango trees.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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