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By-products of Piper aduncum in the control of fusariosis in black pepper plant

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ABSTRACT

Fusariosis (*Fusarium solani* f.sp. *piperis*) is one of the most damaging diseases of black pepper crop in the Amazon zone and the application of organic materials to the soil may be an alternative to control this disease. Leaf residues of *Piper aduncum* were evaluated before and after the extraction of essential oil, as additives to the soil inoculated with *Fusarium solani*, in the survival and morphophysiological behavior of black pepper seedlings. The experiments were conducted at Embrapa Eastern Amazônia, Belém, PA. The effects of the by-products of the distillation of the essential oil of *P. aduncum* on the survival to fusariosis in black pepper seedlings and the effect of dry and crushed leaves of *P. aduncum*, preincubated in the soil, in the incidence of fusariosis and in the behavior of black pepper seedlings were evaluated. The addition of solid residues from *P. aduncum* oil extraction to the soil increased the survival of seedlings by 80% and in the presence of dry and crushed leaves of *P. aduncum* the survival was 83%. The net photosynthesis of the seedlings increased in the presence of residues of *P. aduncum*. The residues from the extraction of the essential oil of *P. aduncum* and its dry and crushed leaves, without oil extraction, have potential for use in the control of fusariosis in black pepper plants.

Key Words: *Fusarium Solani* f. sp. *Piperis*; *Piper Nigrum*; Residues From Essential oil Extraction.

INTRODUCTION

Root rot and branch drying in black pepper plants, both denominated fusariosis, are caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *piperis* Albuq. (FSP), and its teleomorph, *Nectria haematococca* f.sp. *piperis* F.C. Albuq. & Ferraz, an ascomycete of the Nectriaceae family (Albuquerque, 1964). The fungus *F. solani* f. sp. *piperis* affects the root system of the plant, causing root rot and leaf fall, and ultimate death (Silva & Souza, 2009).

Root rot disease in Brazil has been responsible for the elimination of entire plantations of *Piper nigrum* L., with economic losses of millions of dollars per year and there is no efficient control measure against the pathogen and no disease-resistant cultivars (D'Addazio *et al*., 2016). The genetic variation between pepper genotypes in Brazil and the degree of susceptibility of currently available cultivars to the disease remain unknown, which makes it difficult to perform genetic control of fusariosis (Castro *et al*., 2016). As for chemical control, although the application of cupric fungicides can keep fusariosis of black pepper under control in propagators (Tremacoldi, 2010), there are restrictions on the use of fungicides due to environmental contamination and residues that may persist in the unprocessed products (Marín *et al*., 2008).

As an alternative to phytopathogen control, essential oils, secondary metabolic products of plants, can be used in various parts of the plant, such as leaves and stems (Scherer *et al*., 2009). These oils may contain secondary compounds with biological activity with potential action against plant pathogens (Schwan-Estrada, 2009).

Venturoso *et al.* (2011), observed fungistatic activity of clove extract on the development of *Fusarium solani, Aspergillus sp., Penicillium sp., Colletotrichum sp., Cercospora kikuchii* and *Phomopsis sp*., highlighting the use of natural products in the control of phytopathogens as a measure of preserving the environment. Seixas *et al.* (2011) observed a fungitoxic effect of 15, 20 and 25 µL of the citronella grass essential oil with 100% mycelial inhibition of *Fusarium subglutinans*, an etiological agent of fusariosis in pineapple crop (*Ananas comosus*), also stating that the phytosanitary control based on essential oils can be an effective and low-environmental impact-method in combating disease-causing pathogens in different plant species.

The objective of this work was to test the effect of foliar residues of *P. aduncum*, before and after the extraction of its essential oil, as additives to soil inoculated with *F. solani* f. sp. *piperis*, on the survival and morphophysiological behavior of young black pepper plants.

MATERIAL AND METHODS

Effect of by-products of the distillation of *Piper aduncum* essential oil on the survival of red pepper seedlings to fusariosis.

The experiments were conducted in the Experimental Field of Embrapa Eastern Amazônia (01°28´ S; 4827´ W), in Belém, PA, in a semi-controlled environment, with 50% interception of sunlight, with temperature varying 25-35°C and relative air humidity ranging between 80-95%.

The liquid residues (slurry) and the solid residues from the extraction of the essential oil of *P. aduncum* used in the experiments were provided by the Executive Committee of the Cocoa Plan (CEPLAC/SUPOR). The slurry was kept under refrigeration (8 ± 2°C) until use. The solid was dried in a forced air circulation oven (75°C) for three days and ground to the powder form immediately prior to use in the experiment.

The strains of *F. solani* f. sp. *piperis* (FSP) used in all experiments were obtained from the collection of phytopathogenic fungi from the Phytopathology Laboratory of Embrapa Eastern Amazônia, in Belém, PA. To obtain the soil- inoculum, the fungus was multiplied in vials with a capacity of 500 ml containing 200 g of soil medium + wheat bran (4:1 mass/ mass). Pathogen culture disks in Potato-Dextrose-Agar (PDA), with 7 mm diameter, were transferred to individual flasks and incubated for 15 days (27 ± 1°C; 12 h photoperiod). Prior to use, the inoculum was homogenized and the concentration determined by the serial dilution method.

Non-sterilized soil from forest area were put in plastic pots 2 kg capacity, with 3% of solid residues (mass/mass) or 0.35% of solid residues (mass/volume), with or without 0.5% of soil inoculum (484 spores g^{-1} soil), where two-node cuttings (one per pot) of black pepper were planted; this cuttings were obtained from ornamental branches from mother plants of Guajarina cultivar, pre-rooted for 45, 60 days in shaded beds containing charred rice straw. The survival of the plants was evaluated every 30 days during 150 days, observing the appearance of symptoms in the collection region, establishing two values (0 and 100) for the presence or absence of fusariosis. The dry matter production of the seedlings was evaluated at the end of the experiment at 240 days, by determining the dry mass of leaves, stems and roots of each plant kept in a forced circulation oven (at 75°C) for 72 hours. The leaf, stem and root mass ratios per unit mass of the whole seedling were used for the calculation of biomass allocation. The photosynthetic behavior of the plants was measured at the end of the experiment (240 days), using the newest, intact and fully developed leaf of each plant. Measurements were taken from 9am to 11am using a portable photosynthesis system (Model LI-6200, LI-COR Inc., Lincoln, NE, USA).

Effect of dry and crushed leaves of *P. aduncum*, pre- incubated on soil, on the incidence of fusariosis and on the behavior of black pepper seedlings

Green leaves of *P. aduncum* collected in the experimental field of Embrapa Western Amazônia were dried in a forced circulation oven (75°C) for 72 hours and crushed to produce powder (foliar residues). FSP inoculum was obtained by transferring 7 mm diameter discs obtained from PDA cultures with seven days of growth to Petri dishes containing the same culture medium maintained under alternating light regime (12 h light/12 h dark) for another seven days. Autoclaved forest soil (for two consecutive days) (120°C for 1 h) and natural soil (unsterilized forest soil) were used.

The foliar residues of *P. aduncum* were incorporated into the soil contained in the pots (1 kg) at concentrations of 1.5% and 3.0% (mass/mass), which were kept in a greenhouse for 15 days. After this period, 20 ml of the aqueous inoculum suspension containing 5 x 105 spores ml^{-1} were added to each pot, and the pots were maintained under the same conditions as before for another 30 days when the rooted cuttings were transplanted. The control treatment was inoculated only with sterile distilled water, without inoculum of the pathogen. Twelve treatments were tested in a completely randomized experimental design, in a 2x2x3 factorial arrangement. The evaluation was made based on plant mortality parameters at 30, 60 and 90 days, establishing two values (0 and 100), for the presence and absence of fusariosis symptoms in the plant collection, respectively.

Data corresponding to plant survival were analyzed using repeated measures ANOVA. Data on dry mass production and biomass allocation were log and arcsine $\sqrt{x}/100$ transformed, respectively, for the univariate test procedure of significance, using the Generalized Linear Models (GLM). Net photosynthetic rate data were analyzed by the univariate significance test, using GLM. The homogeneity of variances for each ANOVA was tested using the Levene test and the Tukey test was applied, when appropriate. The statistical program STATISTICA for Windows 5.5 was used to analyze the data.

RESULTS

Effect of by-products of distillation of *Piper aduncum* essential oil on the survival to fusariosis of black pepper seedlings.

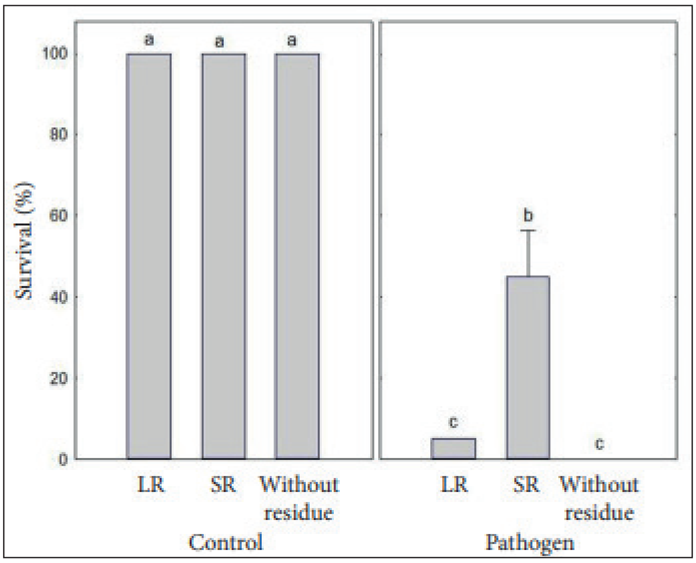
In the control treatment, the survival of black pepper seedlings reached 100%, regardless of the type of residue added to the soil. In soil inoculated with spores of the pathogen and given solid residues (SR) from the extraction of the essential oil of *P. aduncum*, the survival of the seedlings reached 45%, being significantly higher than the seedlings grown in soil without these residues, or treated soil with liquid residues (LR). The survival of the seedlings in soil with liquid residues, solid residues and without residues can be observed in Figure 1.

Seedlings cultivated in soil with *P. aduncum* residues produced significantly higher dry mass, corresponding to increases of 78 and 324% in soil with liquid and solid residues, respectively (Figures 2 and 3).

The beneficial effect of the solid foliar residues obtained after extraction of the essential oil of *P. aduncum* in the increase of the black pepper seedlings survival can be attributed to the possible antifungal action of some components present in the leaves of this Piperaceae, of the essential oil itself, which may remain in the residues, even after extraction (Navickiene *et al.*, 2006).

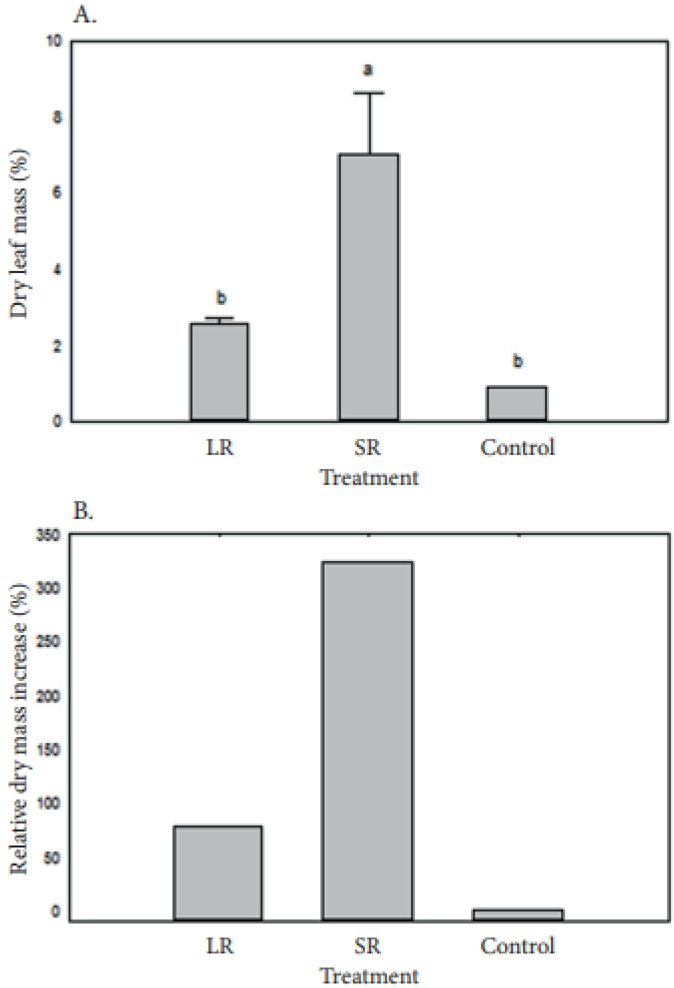
P. aduncum oil according to Silva *et al.* (2013) is composed of dilapiol, myrcene, cis-o-cimene, beta-caryophyllene and myristicin, components that may interfere with plant growth and pest and disease control. The storage and processing stages may influence the concentration of the oil, mainly environmental factors such as temperature and humidity may cause quality losses in the material, reducing or eliminating its the production of relative dry mass (B) of black pepper seedlings after 150 days of cultivation in soil with liquid (LR) or solid (SR) residues of *P. aduncum* essential oil extraction use as a source of natural dilapiol (Silva *et al.*, 2015). Thus, because solid residues are subjected to less processing than liquid residues may contain higher concentration of chemical compounds explaining the greater survival of plants.

Figure 1. Survival after 150 days of black pepper seedlings in non-inoculated.



Different letters on the bars indicate differences between means, according to the Tukey test at 5% probability.
soil (control) (A) or soil inoculated with *Fusarium solani* (pathogen) spores (B), with liquid (LR) or solid (SR) residues of *P. aduncum* essential oil extraction. The data are means + standard error. A.

Figure 2. Dry matter production of leaves (A) and percentage increase in the production of relative dry mass (B) of black pepper seedlings after 150 days of cultivation in soil with liquid (LR) or solid (SR) residues of P. aduncum essential oil extraction.



Different letters on the bars indicate differences between means, according to the Tukey test at 5% probability.

Figure 3. Black pepper seedlings after 150 days of cultivation on solid (SR) and liquid (LR) residues of *P. aduncum* essential oil extraction.

A tendency of plants cultivated in the presence of solid and liquid residues to allocate carbon (biomass) preferentially to the aerial part of the plant, mainly the leaves to the detriment of the root system was observed (Figure 4A), indicating the beneficial effect of these substances in the acquisition of nutrients, probably Nitrogen, by plants. A characteristic of plants with high N supply is the increase of the biomass allocation to the aerial part, mainly to the leaves (Sims *et al.*, 2012).

The net photosynthesis of the seedlings in soil with residues of *P. aduncum* was higher than that of those cultivated in soil without residues (control), suggesting a beneficial effect on the general physiological behavior of the plants. However, no difference was observed in the net photosynthesis between the two types of residues (Figure 4B).

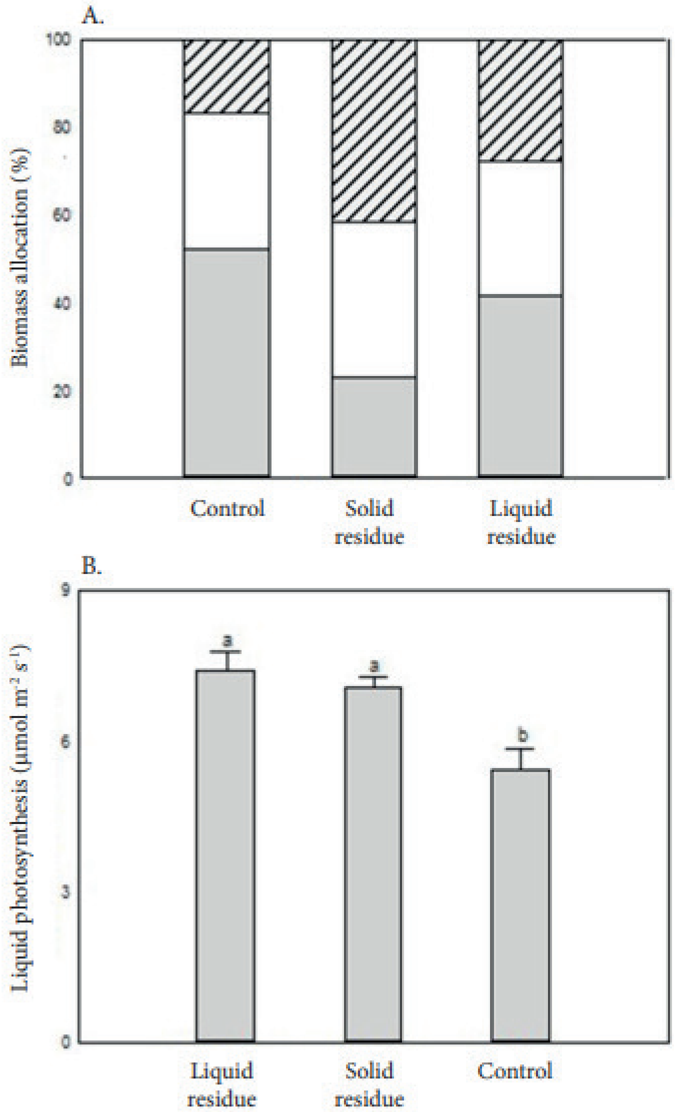
The action of the nutrients incorporated in the soil by the addition of the residues, at least in part, was responsible for the increase in the photosynthetic rate and the dry mass production of the black pepper seedlings. *P. aduncum* leaf residue, analyzed before and after the extraction of essential oil, indicate high levels of nitrogen, potassium, calcium and also some micronutrients such as Boron, Copper, Iron, Manganese and Zinc (Bastos, 2009).

Hartemink & O'Sullivan (2001) studied the decomposition of leaves of species of secondary vegetation in Papua New Guinea, and showed that the leaves of *P. aduncum* decompose rapidly, being a significant source of potassium, which is one of the most important nutrients required by black pepper (Flores *et al.*, 2012). The biomass allocation pattern observed in black pepper seedlings cultivated in soil with *P. aduncun* residues suggests that this soil had high nitrogen availability (Sims *et al.*, 2012).

Effect of dry and crushed leaves of *P. aduncum* preincubated in the soil on the incidence of fusariosis and behavior of pepper seedlings.

The interaction between dry and crushed leaves of *P. aduncum* (FPAD), presence of *F. solani* f. sp. *piperis* (FSP) and evaluation period had a significant effect on the survival of black pepper seedlings to fusariosis. The presence of FPAD in the soil reduced the mortality of the seedlings by 66.7% in the concentration of 1.5% and by 83.3% in the concentration of 3% of this material, regardless of whether the soil was autoclaved or not (Figure 5).

Figure 4. A. Mean Fraction of biomass (%) allocated to roots (dark area), stems (light area) and leaves (striped area); B - Net photosynthesis of black pepper plants, after 150 days of cultivation in soil with extracts of P. aduncum essential oil.



The total dry mass production of seedlings in soil with FPAD in soil not inoculated with FSP was higher than that in soil without this material, regardless of whether the soil was autoclaved or not. The dry mass production in seedlings grown in 3.0% FPAD soil was the same both in plants grown in soil inoculated and not inoculated with the pathogen. However, in soil enriched with 1.5% of FPAD, the total dry mass production was lower in the presence of the pathogen (Figure 6).

The addition of 3.0% of FPAD to natural/non-infested, natural/infested, autoclaved/non-infested and autoclaved/ infested soils increased the dry mass production of the plants by 137, 113, 77 and 32%, respectively. The respective values of relative increase or decrease in dry mass production for the addition of 1.5% FPAD to soil were 190, 18, 71 and -27% (Figure 6B). The negative increase observed in the dry mass of the plants in soil with 1.5% of FPAD was probably due to the insufficient amount of nutrients released in the soil by this concentration of the material, together with the presence of the pathogen.

Figure 5. Survival of black pepper plants in soil infested with *F. solani* f. sp. *piperis* and with dry and crushed leaves of *P.*

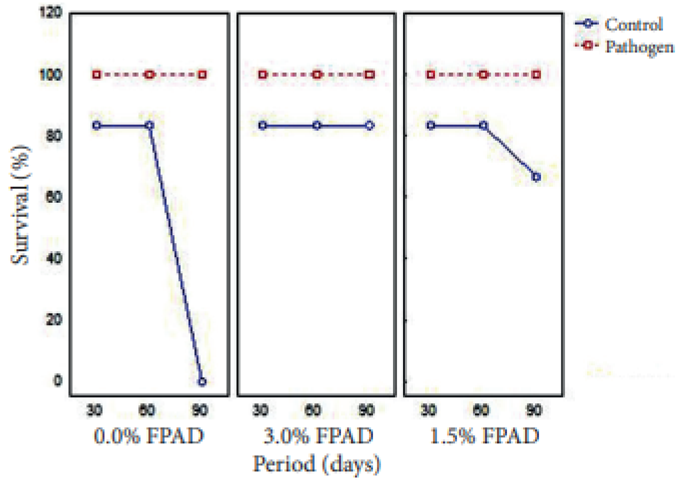
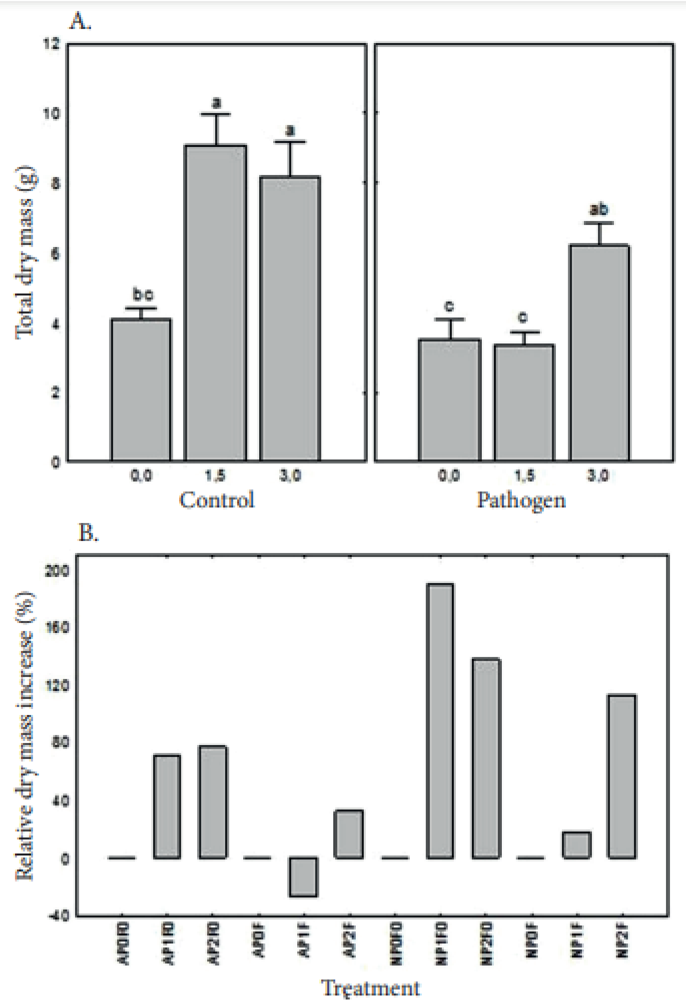


Figure 6. A- Total dry mass of black pepper plants after 90 days of cultivation in soil not inoculated (Control) or inoculated (Pathogen) with FSP spores and with dry leaves of *P. aduncum*, in three concentrations (0, 1.5 and 3%). B- Relative dry mass increase in black pepper seedlings cultivated in autoclaved or natural soil, with two concentrations of dry and crushed leaves of *P. aduncum* (FPAD).



A = autoclaved soil; N = natural soil; P0 = without FPAD; P1 = 1.5% FPAD; P2 = 3.0% FPAD; F = with pathogen; F0 = without pathogen. Data are means + standard error. Different letters on the bars indicate differences between means, according to the Tukey test at 5% probability.

Regardless of whether the soil was autoclaved or not, the seedlings tended to allocate carbon to the aerial part, even in the presence of FSP, especially on natural soil. In the plants cultivated in the presence of FPAD, the biomass allocation was higher for the leaves. The soil type x FPAD concentration x pathogen interaction was significant for the allocation of root biomass.

The incorporation of residues from *P. aduncum* essential oil extraction into the soil was effective in reducing the incidence of the witches' broom disease" of the cocoa tree in greenhouse (Bastos, 2009) and reduced the reproduction of *Meloidogyne incognita* in tomato culture (Silva *et al.*, 2007). Also, due to the broad spectrum of antimycotic action of the essential oil of *P. aduncum* "in vitro" (Navickiene *et al.*, 2006) and the fungicidal activity of the essential oil of *P. marginatum* Jacq. against *Fosarium oxysporum* (Schlecht)) "in vitro" (Santos *et al.*, 2011), it is possible that foliar residues of this piperaceae have potential for use in the control of fusariosis in black pepper.

The control of fusariosis with the use of *P. aduncum* extracts agrees with other works that have reported the efficiency of essential oils. Silva & Bastos (2007) observed fungitoxic action of the essential oils of *P. callosum, P. marginatum* var. *anisatum and P. enckea* and stated that studies for the application of oils in the control of phytopathogens results in the possibility of using natural products with low toxicity, thus less aggressive to the environment.

CONCLUSIONS

Solid residues from the extraction of the essential oil of *Piper aduncum* incorporated into the soil increased the dry mass production of black pepper seedlings.

Dried and crushed leaves of *P. aduncum* incorporated into the soil reduced the mortality of black pepper seedlings by fusariosis in up to 83.3%.

Solid residues from the extraction of the essential oil and crushed and dry leaves of *P. aduncum* have potential for use in the control of fusariosis in black pepper seedlings.

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