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Interaction among Organic Matter and Pathogen *Fusarium subglutinans* F. sp. in Soil Cultivated with *Ananas comosus*

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Abstract: The study aimed at evaluating the *in vitro* influence of organic matter on the development of the pathogen *F. subglutinans* F. sp. *ananas* in soil, as well as analyzing which the possible mechanism involved in this interaction. The experimental design was entirely randomized in scheme factorial 2×2×5, with 2 soil levels (normal and sterile), 2 organic matter (with and without organic matter) and 5 times (0, 5, 10, 15 and 20 days), in which were used 10 repetitions. The natural soil without organic matter had reduction in the colony numbers, whereas the sterile soil with organic matter presents strong increase in the colony numbers of the pathogen, in which it had 387.8 and 1135.7 colonies in the 0 and 20th day after the incubation, respectively. The results proved that the organic matter influenced significantly the survival of the *F. subglutinans* F. sp. *ananas*, as well as the pathogen survival was strongly maximized by the interaction of the sterile soil and organic matter.

Key words: *Ananas comosus*, *fusarium subglutinas*, pathogen, interaction, organic matter

INTRODUCTION

The species *Ananas comosus* (L.) Merr. is a monocotinedoneus that belonging to Bromeliaceae family, besides to be a perennial herbaceous (Reinhardt, 2000). It presents several characteristics of epiphyte plants, as capacity of storage water in the leaf tissue, as well as in the leaf axils, however it species has the terrestrial habitat.

The Brazil is a of the mains center of genetic diversity and the species can be cultivated in almost all the tropical and subtropical regions, because *Ananas comosus* presents greater adaptation to differents edapho-climatic conditions and also the possibility of to combine differents characteristics and plant size, season and flower induction, in which is possible the fruit production in all the months, it increase the yield and consequently the economic return.

The *Ananas comosus* is considered a of the more important tropical fruits, as well as the commercialization has presented a increase in the mains international markets, mainly in the Europe, in which this fruit is the second more exported by the Brazil, besides to be the third higher world producer of the culture. Besides of this, this fruit presents strong influence in the economy of the Pará state, localized in the Northern region of the Brazil, in which the cities with higher yield are Floresta do Araguaia, Jacundá and Salvaterra.

The pathogen *Fusarium subglutinans* F. sp. *ananas* is agent that cause the disease denominated fusariosis (Santos *et al*., 2002), with occurrence in areas cultivated in the Northern region of the Brazil (Kimati *et al*., 2005), in which it provokes losses of 30 until 80% of the yield under favorable conditions to the pathogen. Besides of this, the fungus is capable of to infect all the plant parts, in which the colonies occur since the leaf insertion until the fruits (Hidalgo *et al*., 1999). The mains infection places of the fungus are the style channel and the nectarine chambers during the anthesis (Matos *et al*., 2000).

The study aimed at evaluating the *in vitro* influence of organic matter on the development of the pathogen *F. subglutinans* F. sp. *ananas* in soil, as well as analyzing which the possible mechanism involved in this interaction, in which the soil of the study was to belonging of area with intensive cultived of *Ananas comosus*, localizated in Salvaterra city, Pará state, Brasil.

MATERIALS AND METHODS

Experiment local: The experiment was carried out in the Laboratório de Fitopatologia localizated in the Instituto de Ciências Agrárias da Universidade Federal Rural da Amazgnia (UFRA), in which the experiment was conducted during the months of May and June of 2006, Belém city, Pará state, Brazil (01°27'S and 48°26'W).

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Experiment design and soil characteristic: The experimental design was entirely randomized in scheme factorial 2×2×5, with 2 soil levels (normal and sterile), 2 organic matter (with and without organic matter) and 5 times (0, 5, 10, 15 and 20 days), in which were used 10 repetitions.

Soil characteristics: The characteristics of organic matter, pH, organic carbon and aluminum change are present in the Table 1, as well as the physic characteristics in the Table 2, in which the analysis were carried out in the Laboratório de Química do Solo of the Universidade Federal Rural da Amazgnia (UFRA). The soil sample was to belonging of area with *Ananas comosus* cultivation, localizated in Salvaterra city, Pará State, Brazil.

Organic matter: The source of organic matter used were bird residues previously fermented, in which were used 2 organic matter levels (0 and 50%), as well as the soil was submitted to 2 treatments (with and without sterilization). The soil sterilization was carried out in the temperature of 120°C at 3 atm^{-3} by 40 min.

Fungus obtaining: The pathogen was obtained through of infected *Ananas comosus* fruits to belonging of cultivation areas in the Floresta do Araguaia city, Pará state, Brazil, in which the confirmation of *F. subglutinans* F. sp. *ananas* colonies were carried out through of pathogenicity tests in the leaves, in agreement with the methodology described by Santos *et al*. (2001). After the tests, the fungus was included in the mycotec of the Laboratório de Fitopatologia of the Universidade Federal Rural da Amazgnia (UFRA).

Pathogen preparation and quantification: After 7 days of cultivation in medium of culture PDA (Potato-Dextrose-Agar), the Petri plates with the dimensions (9×1 cm; diameter × height, respectively), it was add up 10 mL of Autoclaved Distilled Water (ADW) at 120°C by 20 min and using a brush of 25 mm of width, in which was prepared the spore suspension in each plate (Fig. 1a and b). In the quantification was used the Neubauer chamber, as well as the concentration was adjusted to 1.0×10^{5} conidia mL^{-1} (Matos and Cabral, 1988).

Soil humidity and soil saturation point: The samples with weight of 100 g of soil were placed in oven of forced air circulation adjusted the temperature at 35°C by 5 days, the

Table 1: Organic matter tenor, pH, organic carbon and change alumni in soil

Characteristic	Soil	Soil + Organic matter
Organic matter (g Kg^{-1} S)	11.19	76.33
pH	4.80	6.70
$C_{organic}$ (g Kg^{-1} S)	6.49	44.27
Al_{change} (cmolc dm^{-3})	1.00	0.29

Table 2: Soil physical characteristics

Soil composition	(%)
Thick sand	57.2
Fine sand	27.4
Silt	5.2
Clay	10.2

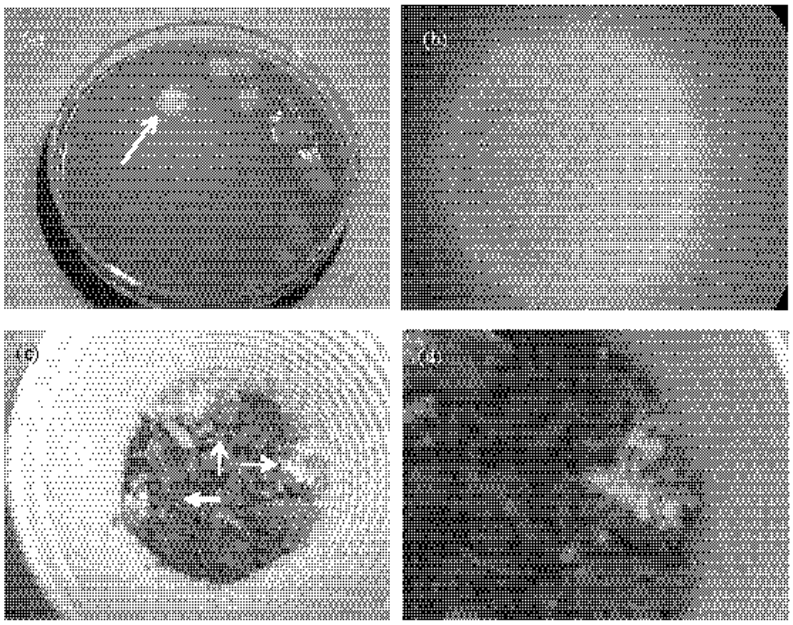

Fig. 1: Several colonies of *Fusarium subglutinans* F. sp. *ananas* (A), morphological aspects of the colony (B), fungus growth in sterile soil and in the presence of organic matter (C), detail of the colony in the soil (D)

Table 3: Humidity and water amount necessary to reach the soil saturation point

Variable	Natural soil	Sterile soil	Natural soil + organic matter	Sterile soil + organic matter
Humidity (%)	32.0	32.0	40.0	40.0
Water (mL)	10.4	16.8	18.7	25.9

dry soil was placed in a cylinder of rigid plastic with 0.75 polegad of diameter, in which in the cylinder basal part was placed a screen of 1.5 mm and the sample (cylinder + soil) was conducted to the soil hydration through of drip, in which it was used burette with precision of 0.5 mL, until the soil saturation point, when was carried out the new sample weight and evaluation of the water amount absorbed by the soil, in agreement with developed in the Laboratório de Fitopatologia of the Universidade Federal Rural da Amazgnia (UFRA) Table 3.

Soil inoculation: The samples with 50 g of dry soil were placed in containers with 200 mL capacity, in which the inoculation suspension used was previously adjusted to 1.0×10^5 conidia mL^{-1}, as well as was add up 5 mL of suspension and ADW until the soil to reach the saturation point and the containers were closed, in which were evaluated the weights of the containers with intervals of 7 days and add up ADW when necessary to keep the soil humidity in the saturation point.

Evaluation of soil-pathogen interaction: The evaluations were carried out in the periods of 0, 5, 10, 15 and 20 incubation days, in which was removed 10 g of soil of each container and add up 90 mL of ADW, as well as was makes sample agitation during 10 min. After of this dilution, it was carried out several dilutions until to reach the value of 1×10^4. Subsequently, the Petri plate preparations were conducted with 1 mL of suspension and 15 mL of culture medium of Nash and Snyder PCNB at 45°C (Tuite, 1969). The plates were incubated at 25±2°C during 8 days.

After the incubation period, the *F. subglutinans* F. sp. *ananas* colonies were counted and were showed the colonies morphological aspects (Maffia, 1980). To the colony confirmations were make pathogenicity tests in leaves removed of the plant, in agreement with the methodology described by Santos *et al.* (2001). The data were transformed to $(x + 0.5)^{0.5}$, as well as the results were analyzed through of the ESTAT software.

RESULTS

The natural soil without organic matter had reduction in the colony numbers, in which was showed the number of 324.6, 305.5, 263.4, 206.8 and 184.7 colonies in the periods of 0, 5, 10, 15 and 20 days after the experiment implementation, respectively (Fig. 2).

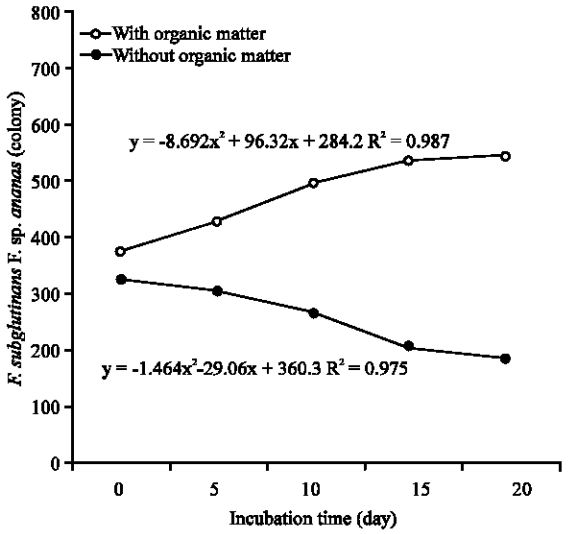

Fig. 2: Behavior of *Fusarium subglutinans* F. sp. *ananas* in natural soil

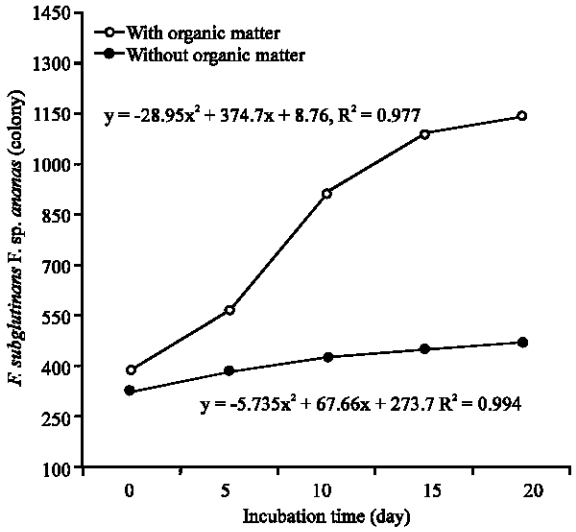

Fig. 3: Behavior of *Fusarium subglutinans* F. sp. *ananas* in sterile soil

The natural soil with addition of organic matter presented increase in the colony numbers (Fig. 2), in which was showed in the 0 and 20th incubation day the numbers of 333.1 and 470.9 colonies, respectively.

In the sterile soil without addition of organic matter was showed the increase in the colony numbers, in which present the numbers of 377.3, 430.6, 497.4, 538.9 and 544.0 colonies in the 0, 5, 10, 15 and 20 days after the incubation, respectively (Fig. 3).

The sterile soil with organic matter presents strong increase in the colony numbers of the pathogen (Fig. 3), in which it had 387.8 and 1135.7 colonies in the 0 and 20th day after the incubation, respectively.

DISCUSSION

The higher pathogen survival rate in sterile soil might be explained by the antagonist action of the microorganisms present in the natural soil, in which it not is found in the sterile soil. According Matos and Cunha (1980) the *F. subglutinans* F. sp. *ananas* pathogen can survive in sterile soils by a long period, as well as this survival period is due the thermal treatment that provokes the elimination of several microorganism present in the soil and the sufficient humidity level kept during the experiment (Stover, 1953).

The *F. subglutinans* F. sp. *ananas* presents a competitive saprophytic capacity in the soil extremely small. Besides of this, this species not haves the capacity of to form clamidispores, in which can be suggested that this fungus is a soil invader, because this not is the natural habitat, but alternative habitat (Nyvall and Kommedahl, 1970).

The organic matter provokes significant influence in the pathogen survival rate, because the benefic saprophytic action proportioned that *F. subglutinans* F. sp. *ananas* survived by more time, as well as the competitive saprophytic capacity is small. Besides of this, the increase of organic matter proportioned direct influence in the pathogen survival, as well as it was showed also in natural soil. In soils with the presence of culture residues the fungus *F. subglutinans* F. sp. *ananas* can survival by long period, because the survival rate is directly linkage to soil type and the amount of organic matter in the soil (Maffia, 1980).

The sterilization and the organic matter provoke significant influence in the pathogen survival, in which the sterile soil with organic matter had the higher indices of pathogen reproductive structures, besides to reveal that the combination of these factories induce the strongly increase of the pathogen survival in the soil and it promotes until the mycelium appearance of pathogen in this substrate (Fig. 1c and d).

The results proved that the organic matter influenced significantly the survival of the *F. subglutinans* F. sp. *ananas*, as well as the pathogen survival was strongly maximized by the interaction of the sterile soil and organic matter.

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