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Endophytes fungi in mango Haden' orchards of Maracaibo plain, Venezuela

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Abstract

The presence and distribution of endophytic fungi (asymptomatic) in four 'Haden' mango orchards located in the Maracaibo Plain were evaluated. The orchards were under different agronomic cultural practices where arid conditions prevail. The fungi were isolated employing triple sterilization specific method for endophytes fungi. In all orchards were recorded the presence of: *Fusarium decemcellulare, Lasiodiplodia theobromae*, *Colletotrichum gloesporioides*, *Alternaria alternata, Phomopsis mangiferae*, *Pestalotiopsis* sp. and *Cladosporium* sp. Most fungi were present in vegetative and reproductive organs, except *P. mangiferae* and *Pestalotiopsis* sp. which were detected in vegetative organs only. Conidia of *L. theobromae* and *Cladosporium* sp. were detected within anthers along with pollen grains. In general, it was found that the fungi distribution in the organs of the hosts' plants was continuous and systematic, without any temporal colonization. These results could indicate that the endophytic colonization is an important way for the development of diseases in mango 'Haden' orchards of Maracaibo Plain.

Key words: endophytic fungi, phytopathogens, *mangiferae indica*.

Introduction

Mangifera indica L. (Anacardiaceae) mango has been sowed for more than 4 thousand years in India, where it originated and it was brought to the America Continent by Europeans (19). The crop adapted to climatologic conditions in such a good way that nowadays it is one of the most common landscapes, also, our continent is the second mango producer (12). In Venezuela, mango occupies the fifth place in sowed surface and is one of the most important products (22). At the end of the eighties, it was observed the expansion of mango in Maracaibo's plain, establishing in this sub-region, commercial plantations with grafted varieties from where predominated "Haden". Ten years later, when

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plantations started to enter in the second cycle (5); the expectations of "Haden" mango producers in Maracaibo's plain have been frustrated by the low production levels registered in their plantations.

That is the reason that some of these plantations have been abandoned, eliminated or substituted by other products. Several fungi illnesses have also contributed to this situation, specially *Fusarium decemcellulare* Brick., *Colletotrichum gloeosporioides*, and Blight (5, 39) which are mentioned as the first phytopathologic affections that interfere negatively in the "Haden" mango production in this region.

In the last years, it has been reported that in many vegetal species lives a mycobiota that belongs to the tissues of the host plants without causing any type of the disease. This mycobiota is conformed by systemic fungi denominated "endophytes". Originally, endophytes fungi were defined as non aggressive organisms that lived in the vegetal tissues. The term has widened in order to include those fungi that at some stage of their life's cycle remain inside the host without inducing symptoms of the disease (30, 31). Intercellular spaces and apoplastic connections constitute the main niche of these fungi. Nutrients that circulate through the vascular axes provide the necessary food for their development (3, 41).

Lots of researches show that in some occasions the saprophyte condition of the endophyte fungi that live in arboreal species transmute in negative effects when the host has nutritional disorders or hydric stress making it more sensitive to the attack of it (44, 45).

In this research are presented the results of a diagnose study that allowed detecting and identifying endophyte fungi in "Haden" mango, sowed in commercial plantations of Maracaibo's plain.

Materials and methods

Agro-ecological characteristics of the area under study:

This research was carried out in four mango plantations of "Haden" variety (*Mangifera indica* L. cv. Haden) located on Cañada de Urdaneta and Mara municipality, of the Maracaibo's plain sub-region, Zulia state, Venezuela.

The plantations were "El Carrusel" farm, located at Km. 22 of Maracaibo-Perijá highway, Cañada de Urdaneta municipality; "Los tres Jagueyes" farm, "El Quinto Patio" farm and the germoplasm bank of the "Fruit Center of Zulia, located on Mara municipality. The age of these oscillated from 6-12 years and the extension varied from 10-20 ha. For the time when the essay was taking place, "Haden" mango plantation of "El Carrusel" farm had a high plant incidence with *Fusarium decemcellulare* Brick.

The studied sub-region has an average altitude of 55 msnm and corresponds to a life zone of dry tropical forest that characterizes by

presenting 500-600 mm of annual precipitation, with two rainy seasons corresponding from May-June and July-October, and annual evapotranspiration of 1.662 mm, which causes a hydric deficit in the region, annual average temperature of 28ºC, with a maximum of 34ºC and a minimum of 25ºC; and a relative humidity of 75 % (17).

Collected material:

On each of the evaluated farms 12 healthy plants per orchard were selected at random. For the microbiologic analysis, samples from the following organs were taken: leafs on the pre-flowering period, branches of the last growth flowers, inflorescences and fruits if were produced (3 organs/cardinal point/ plant). The collection of sample took place during the productive cycle from 2000-2001 years.

Microbiological analysis:

Isolation of endophytes fungi were done through the segments of the collected vegetal organs. For branches, buds, rachis and pedicels of inflorescences and peduncle, and pulp of fruits, sections from 10-20 mm of longitude were cut; for leafs, portions of apex, margins and central portions of foliar slices were cut, of 1-2 cm^2 and gynoecium (ovary, style and stigma) and anthers were dissected. 9 segments/organ were processed.

These organs segments were submitted to the triple sterilization process to eliminate superficial colonizers (30). For this, samples were immersed for 1 minute in ethanol at 95%, 10 minutes in sodium hypochlorite at 2.5% and 30 seconds in ethanol at 95%. This was followed by a rinse done with sterile water and a dry on sterile paper (30). Once sterilized, segments were incubated in Petri capsules with PDA (Potato Dextrose Agar) and/or ZDA (Carrot Dextrose Agar) modified with streptomycin sulphate (40 mm/ml) or emended with lactic acid at 25% for controlling the bacterial growth; at an environment temperature (20-25ºC) with natural light. For purifying and maintaining all isolations, fungi were transferred to other capsules and tubes with PDA, and replicated every 3 weeks. All these processes were carried out in a laminar flow chamber to minimize any risk of contamination.

For the observation of vegetative and reproductive structures of isolated fungi in pure crops, assembles with portions of the colony were prepared on slides with a drop of lactophenol cotton blue, if structures were hyalines and with lactophenol if structures were dark. Also, direct observations of the colonies were done with the stereoscopic microscope.

Likewise, micro crops were prepared as Riddel, which consisted on inoculating with a sterile dissection needle, small agar cubes (aprox. 1 cm^3) disposed on slides, later these cubes were covered with a cover-object blade, and were put under a wet and sterile filter paper inside a sterile Petri capsule, which was incubated at environment temperature and with natural light conditions from 10-15 days.

After this time, the agar tube was discharged and on a new slide a drop of cotton blue was put, which was

covered with the cover-object separated from the cube; likewise, the slide separated from the cube, was tinted with cotton blue covering it with a new cover-object (11). In this way, two assembles were obtained through a Riddel, to observe the fungi structures and to take the photography.

All prepared blades were examined with a Light microscope (100x, 400x and 1000x) and the corresponding photos were done, employing a trinocular microscope, Nixon brand. The colonies characteristics were observed with a stereoscopic microscope (Nixon brand) at 10x and 50x. Subsequently, it was proceeded to make the taxonomic descriptions of the examined specimens.

The fungi identification was done using the specialized taxonomic code (6, 8, 9, 16, 18, 27, 48) and consulting specialized mycologists (Professors T. Iturriaga of the «Simón Bolívar» University, F. Escalona, of University of Zulia and R. Castañeda, of INIFAT-Cuba).

The occurrence of a fungus was registered as positive if it was detected in at least one sample or on a segment of an organ.

For the statistical analysis, SAS System computation software was used. The statistical test that was used for analyzing all this information was the Chi squared test (non parametric test), FREQ procedure (42) was employed to analyze the relation between the variables presence or absence of each of the endophytes fungi per plantation and per organ (leaves, branches, flowers).

Results and discussion

Seven species of anamorphic fungi (hyphomycetes and coelomycetes) were recovered as endophytes after vegetative and reproductive organs, as it is shown in table 1. These species were *Fusarium decemcellulare* Brick., *Lasiodiplodia theobromae* (Pat.) Grifford & Maubl., *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Alternaria alternata* (Fr:) Keissl., *Phomopsis mangiferae* Sacc., *Pestalotiopsis* sp. Stey. and *Cladosporium* sp. Link.

These fungi prevailed in the most recent flows; finding them in young leafs and branches in the pre-flowering period, many of these surpassed the flowering, being on the inflorescences and even in the fruiting, in fruits of different ages (table 1).

These species have been reported by other authors as endophytes of the mango (21) and other woody species (13, 29, 40, 46, 52). These have also been reported as pathogen agents that cause illnesses in mango (5, 10, 20, 23, 24, 25, 26, 28, 35, 36, 38, 39, 43, 51).

Fusarium decemcellulare was detected in samples coming from all plantations. Its occurrence was detected in 79% of all analyzed samples ($P<0.1$) prevailing in 39.47% in the branches and in the buds, 34.21% in leafs and 26.32% in the flo-

Table 1. Isolated fungi as endophyte, from different organs of «Haden» mango in Maracaibo´s plain, Venezuela.

Stalk (young branches and their buds)	Leaves (foliar lamina and pedicels)	Inflorescences (rachis, peduncle, gynoecium and anthers)	Fruits (pulp)
ALternaria alternata	A. alternata	A. alternata	C. gloeosporioides
Colletotrichum gloeosporioides	C. gloeosporioides	C. gloeosporioides	F. decemcellulare
Cladosporium sp.	Cladosporium sp.	Cladosporium sp.	
Fusarium decemcellulare	F. decemcellulare	F. decemcellulare	
Lasiodiplodia theobromae	L. theobromae	L. theobromae	
Pestalotiopsis sp.			
Phomopsis mangiferae			

ral organs. These results indicate that *F. decemcellulare* occurs as the endophyte fungus with the widest distribution and with more frequency in "Haden" mango plantations studied in the Maracaibo's plain. It must be highlight the presence of *F. decemcellulare* in the pulp of mango fruits, which is important considering that this fungus has been reported as toxigenic (27).

F. decemcellulare has been recognized as a causal agent of one of the most important illnesses of mango in Venezuela, known as "agallas" or "escoba de bruja", mention in Venezuela as one of the main sources (39). Other species of the *Fusarium* genus have been identified as causal agents of this disease. Isolations done after sick tissues have allowed identifying *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, *F. moniliforme* Sheld. and *F. sacchari* S., as causal agents of Agallas" or "Escoba de bruja" (34, 47, 51).

On a research done in mago "Keitt" *F. subglutinans* was isolated as causal agent of "agallas" in the panicles (34) and it was concluded that for the development of the disease it was necessary that the fungus population on endophytic conditions would reach the infection required for the development of the symptoms. Likewise, a research done in "haden" mango plantations in Mexico, *F. subglutinans* was identified as the causal agent of "agallas" and with the capacity of remaining asymptomatically in the plants as an endophyte (28). The authors of this research also indicated the obtaining of confused results when

trying to complete the postulates by Köch, observing an influence of the environmental and physiological conditions for the development of the disease after the inoculation (28). This implies that a pathogen fungus might occur in host plants asymptomatically if these are not under the influence of stressful conditions.

Lasiodiplodia theobromae was detected in all the evaluated plantations. It was isolated after 66.66% of the analyzed samples. It turned out to be an endophyte persistent in both the vegetative and reproductive phases of the plant. On its organographical distribution, highly significant differences were found ($P<0.01$) being frequent with 64.29% in leafs, 32.14% in branches and buds; and with a low frequency 3.57% on the floral organs. Conidia of these species were detected inside the anthers along with the pollen grains. These allows to infer that there is another way of propagation of the *L. theobromae* fungus, mentioned as the causal agent of the diseased named as «regressive death» or blight in mango, in different producer countries, such as Puerto Rico (4), El Salvador (1), Malaysia (23). In Florida, is reported the same relatedness of this disease to *L. theobromae*, even though it is not exclusive but with others fungi (35). Now, on this matter Ploetz and Prakash (36) mention the impossibility of reproducing the symptoms in artificial inoculation conditions, so, it is assumed that this fungus keeps itself as endophyte and intensifies its attack when plants are weak. In India, is also recognized the transcendence of endophytic colonization of this fungus in the development of post-sowed diseases of mango (26).

Colletotrichum gloeosporioides was recovered as an endophyte in 38.38% of the analyzed samples, finding it more frequently in leafs with 50%, branches and buds 44.44% and with low frequency 5.56% in the floral organs. Significant differences ($P=0.1$) were found in this organographic distribution. The occurrence of *C. gloeosporioides* was detected only in those plantations that were not handled (SF). This fungus is definitely one of the main pathogens agents of mango worldwide, and is the one that causes anthracnose; specifically in Venezuela, since it has been reported as a mainly pathogen of fruits (11). Genetic and geographical information suggest that this pathogen was disseminated through the mango population of the world after one source, through the endophytic route (2).

Alternaria alternata was detected in all the evaluated plantations. The occurrence of this fungus was registered in 30.14% of the analyzed samples, from which 40.91% only corresponds to leafs, 31.81% to branches and buds, and 27.27% to floral tissues. Significant differences were not found in this organographic distribution ($P>0.1$). This specie has been reported as one of the dominant endophytes of woody plants such as *Quercus suber* (13) and eucalyptus (7). Lots of authors mention this specie as a causal agent of black spots on fruits, lesions in leafs and inflorescences of

mango, when it is sowed in arid environments of countries such as Egypt, Australia and South Africa, which conditions favor the development of this specie (14, 15, 37). These reports correspond to the detection of it in the Maracaibo's plain, agroecological area that characterizes by presenting semi-arid environmental conditions.

Phomopsis mangiferae was isolated as an endophyte in 23.33% of the analyzed samples; which came from the handled plantations (F). On its organographic distribution, highly significant differences were found ($P<0.001$) since it was isolated exclusively after vegetative organs such as branches and buds. Previous research mention that other species of *Phomopsis* have been isolated as endophytes after different organs such as leafs, stalks, flowers and fruits of arboreal species, citing *P. castanea* (Sacc.) Höhn in chestnut plants (52) and non identified species in beech tree (40) peach tree (49) and some *Erica* Sp. (29). Likewise, it has been isolated as a pathogen causing the weakening of branches, necrosis and death of buds, so it has been related to the syndrome of regressive death (32, 33, 49, 50, 52).

Pestalotiopsis sp. was isolated as an endophyte in 10% of the analyzed samples coming from the handled farms (F). According to its organographic distribution highly significant differences were found ($P<0.01$) since it was only isolated from the vegetative organs such as branches and buds. Non identified species of this genre have been reported as causal agents of the gray dots in leaves and branches in mango and cashew, even though this illness is not too important (24). It has been isolated as an endophyte through leaves, young branches, inflorescences and pedicels in mango plants (21). It has also been found *Pestalotiopsis guepinii* (Desm.) Steyaert. as an endophyte in pine plantations, where it produces a cancer preventive product like taxol (46).

Non identified specie of *Cladosporium sp.* was detected in all the evaluated plantations. Its occurrence was determined in 71.43% of the evaluated samples, from where 42.86% corresponded to floral organs (gynoecium and anthers), 32.14% to leaves and 25% to branches and buds; thus obtaining significant differences on this organographic distribution ($P<0.1$). It was the only fungus that prevailed on the reproductive phase. As *L. theobromae*, conidia of this fungus were detected inside anthers with pollen grains. Up to now, reports have not yet been registered of *Cladosporium* species, as important pathogens of mango, but as opportunist. In fact, it is known the presence of *Cladosporium* spp. in different organs of the superior plants and materials with a vegetal origin, as a parasite such as saprophytes (6, 16) being recognized as an important contaminant of environment and crop environment. *C. cladosporioides* (Fresen.) of Vres specie has already been reported as a mango endophyte in Australia (21).

The lather indicate the occurrence of fungi under endophyte conditions, as well as an organographic distribution that follows a systematic and continuous

colonization pattern, since the recovered or any other fungi are not isolated (except *P. mangiferae* and *Pestalotiopsis sp.*). Such observations have been registered in some deciduous species where a spatial distribution pattern extended from its endophyte has been found (40).

Knowing the crop phenology is very important to diagnose, since the sensitiveness of the culture to the damage caused by pathogens may vary according to its development phase. During the vegetative development, most of the energy of the plant goes to the foliage. During this period, the damage caused by pathogen fungi to the foliar area is not too critical, because the plant tolerates the lost of leaves and has a great capacity to recover them regenerating the photosynthetic tissue lost. However, once the reproductive phase is achieved illnesses survive, thus originating losses in the production. That is why it is so important to keep an eye on these processes throughout a considerable time scale, because these represent a key for the comprehension eco-physiologic models in the fungus-host interaction, and to determine the critical phase where host stop being endophyte to become pathogens.

Conclusions

Endophyte colonization may be considered as an important way for the development of illnesses in "Haden" mango, sowed in the Maracaibo's plain. Besides, it is recommended to have a special care on the grafting programs in greenhouses, considering that the main phytopathogen fungi of "Haden" mango, sowed in Maracaibo´s plain occur as endophyte in young branches and buds of healthy plants, and these organs are those employed for the propagation of new plants. This warning must be valid for the propagation research in vitro, where culture means are employed that favor the growth of an endophyte, which might be an obstacle for the development of propagation activities.

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