

SCIENTIFIC PAPER

Fungal agents associated to disease symptoms in seedlings of Moringa oleifera Lamarck

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ABSTRACT: The objective of this study was to diagnose and identify the pathogens associated to diverse disease symptoms in *Moringa oleifera* seedlings, specifically in the provenances Supergenius and Nicaragua. The plant material (leaves and stems with spots, necrosis and leaf chlorosis and wilted seedlings) was collected at the organoponic garden of the Pasture and Forage Research Station Indio Hatuey and was disinfected, to isolate and identify the causative agents. This was done from the cultural and morphological characterization of the isolations, which were cultivated on glass Petri dishes –sterilized, of 9 cm diameter– with the media potato and dextrose agar and carnation leaf agar, and using taxonomic keys. The presumptive diagnosis allowed to identify the presence of *Colletotrichum dematium* (Pers.) Grove and *Fusarium solani* (Mart.) Sacc., in the affected seedlings. The former was mainly linked to such symptoms as chlorosis, stem necrosis and leaf spots –small and rounded–, light brown at the center and dark brown at the edge, located on the face and on the back, and spread over the leaf lamina; while the latter was related to stem spots and necrosis and wilting and death of the *M. oleifera* seedlings.

Key words: *Fusarium solani*, *Colletotrichum dematium*

INTRODUCTION

Moringa oleifera Lamarck (moringa) constitutes one of the multipurpose trees most demanded by the world population in recent years, mainly due to the wide variety of products of excellent nutritional quality it contributes, to its exceptional medicinal properties and to its use in animal and human feeding. It is also used as flocculant in water treatment and as living fence and windbreak. In addition, it is utilized to produce biodiesel, ethanol, oil and gums; as well as in the control of vectors and infections caused by microorganisms and as biopesticide (Pérez *et al.*, 2010; Ashfaq and Ashfaq, 2012; Martín *et al.*, 2013).

In Cuba, the sowing and establishment of this crop has increased remarkably, according to the scientific strategy followed at international level with such plant. This has increased the uncertainty regarding its health, because the available information about the topic is insufficient. In addition, the continuous introduction of new provenances widens the possibility of appearance of new pathogen agents, such as fungi; which have caused the following

diseases: seedling wilting and root (*Diplodia* sp.) and fruit rot (*Cochliobolus hawaiiensis*), according to the reports by Ramachandran *et al.* (1980), Kshirsagar and D'Souza (1989), Palada and Chang (2003) and Radovich (2011).

Taking the above-explained facts into consideration, the objective of this study was to diagnose and identify the main pathogens that affect *M. oleifera*, specifically the provenances Supergenius and Nicaragua.

MATERIALS AND METHODS

Plant material. In this study seedlings of the provenances Nicaragua and Supergenius, belonging to the species *M. oleifera*, with disease symptoms, were used.

The collection was done at the organoponic garden of the Pasture and Forage Research Station Indio Hatuey (EPPF-IH). The seedlings were sown at a rate of 20 per square meter, on eight plots (1,30 m wide x 23,00 m long) containing Ferralitic Red soil (Hernández *et al.*, 1999), which was fertilized with

0,4 kg of earthworm humus per square meter. Drip irrigation was applied every three days.

Sample taking. Sampling was made every 15 days, in the diagonal of the plots (CIBA-GEIGY, 1981; Suárez *et al.*, 1985) since August until October, 2011; which coincided with the appearance of the first symptoms, their development and later characterization. At the beginning of the plant health observations and the sampling, the seedlings of the provenances Nicaragua and Supergenius were 26 days old.

Procedure. The collected samples were transferred in nylon bags to the laboratory of plant protection of the EEPF-IH and to the provincial laboratory of plant health, in Matanzas, in order to describe the detected symptoms and isolate and identify the causative agents.

Twenty seedlings of each provenance with disease symptoms—leaf spots and chlorosis and stem necrosis—were randomly collected, which was initially equivalent to 10 % of the plantation. Afterwards, small leaf and stem fragments (with healthy and diseased parts) were selected, which were disinfected following these steps: washing the material with abundant tap water; its immersion in a solution of 2 % sodium hypochlorite, during 2 min, and in 70 % alcohol, for 5-10 seconds; rinsing with sterile distilled water (three successive passes); and, finally, the elimination of the moisture residues through double sheets of sterilized absorbent paper.

At the end of this process, the fragments were placed, in a septic way, in moist chamber, alternating 8 h light/16 h darkness, until the vegetative and reproductive structures of the microorganisms were observed. Then, small fractions of the somatic structures were taken and placed on glass Petri dishes—sterilized, of 9 cm diameter—containing potato dextrose agar (PDA) medium, produced by the National Center of Biopreparations (BioCen); they were incubated at $25-28\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 10 days, alternating 8 h of light and 16 h of darkness. Numerous microscopic preparations were also made, with an optical microscope (Zeiss Standard 25), to classify the agents to genus level. The pure cultures of isolated fungi were obtained through the successive transference of the vegetative structures to glass Petri dishes (9 cm of diameter), containing PDA culture medium, from which mycelia disks of 7 mm diameter were extracted; these disks were transferred to wedges with equal culture medium, in 22×165 mm glass tubes, for their conservation.

Identification of the causative agents. The causative agents were identified based on the cultural and morphological characterization of the isolations, cultivated on glass Petri dishes—sterilized, of 9 cm diameter—, with the PDA and leaf carnation agar (LCA) media. For the identification of the agents to the genus level the key proposed by Barnett and Hunter (1999) was used, and in the case of the species, the taxonomic criteria expressed by Booth (1971), Booth (1977), Sutton (1980; 2004), Gerlach and Nirenberg (1982), Nelson *et al.* (1983), Seifert (2000), López (2003), Leslie and Summerell (2006).

RESULTS AND DISCUSSION

Symptomatology

From the analyzed samples the following disease symptoms were identified: leaf spot and chlorosis, stem spot and necrosis, and seedling wilting and death. They are described below, summarized in two pathologies.

Pathology No. 1. On the affected folioles, the spots were small and rounded, with light brown center and dark brown edge, located on the face and on the back and spread over the leaf lamina (fig. 1). As the disease advanced, the spots increased their size and color, and were necrotized forming irregular necrotic areas or zones. Similar symptoms are described in the crops: *Morus* sp. (Yoshida and Shirata, 1999),



Figure 1. Leaf spot by *Colletotrichum dematium* in *M. oleifera* seedlings.

Lycopersicon lycopersicum Mill. var. *pyriforme* (Dunal) L.H. Bailey (Dal, 2000), *Lablab purpureus* L. (González *et al.*, 2006), *Jatropha curcas* L. (Garcete *et al.*, 2009), *Goniolimon tataricum* (Bobev *et al.*, 2009), *Carum calvi* L. (Zalewska, 2010; Machowicz, 2010), *Singonium podophyllum* Schott (Pérez *et al.*, 2011) and *M. oleifera* Lamarck. (Castellanos *et al.*, 2012); in moringa other disease symptoms were also detected, as well as their causative agents, among which *Colletotrichum*, *Alternaria*, *Curvularia*, *Fusarium*, *Cladosporium*, *Phoma*, *Helminthosporium* and *Cercospora* are reported.

Pathology No. 2. Enlarged, irregular and light brown spots were found on the stems (fig. 2). When the infection appeared in the initial growth stage of the seedlings (20-30 days after sown), they showed thinning, change of color (from green to brown) (fig. 3a), a dryness zone at 5-10 cm from the soil (fig. 3b) and the surface cracking of that area; as well as a constriction (strangling) close to the insertion zone of the first leaves, with loss of their firmness and support capacity (fig. 4). Afterwards, total wilting of the plant, yellowing, foliage fall or premature defoliation were observed (approximately 50 % of leaf area) and death of the plant or the affected stem, which occurred between 7 and 20 days after the appearance of the disease.

When the infection occurred late, the difference in the symptomatology lied on the height at which the stem constriction originated (approximately 10 cm from the soil) and on the fact that the seedlings were taller and showed lower defoliation, chlorosis and death. Of this pathology it should be emphasized that its effects are more damaging the younger the moringa seedling is when the infection occurs, aspect that was corroborated in this research.

Similar results regarding the above-described symptomatology were reported by Escalona *et al.* (2006), Dueñas *et al.* (2007) and Herrera *et al.* (2011) in the crops *Capsicum annum* L., *Cicer*

arietinum L. and *Thevetia peruviana* (Pers.) K. Schum, respectively.

Identification of the causative agents

The four isolations akin to the description made in pathology No. 1 (isolated from the samples analyzed on ten opportunities) showed, on PDA at 25°C, radiated colonies that were initially whitish and turned gray to brown or black (in an isolation) in time (7 and 10 days, respectively, after their sowing started). In addition, they showed abundant, surface acervuli, black in color, with numerous dark setae and masses of unicellular, hyaline, falcate to fusiform conidia, with both ends rounded, from 13 to 19 µm long x 3,5-7 µm. Such characteristics coincide culturally and morphologically with those described by Sutton (1980; 2004), Dal (2000), Pérez *et al.* (2003) and Santamaría *et al.* (2011) for the fungus *Colletotrichum dematium* (Pers.) Grove; which, according to González *et al.* (2006), constitutes an important pathogen for the diverse diseases it causes, for instance: rots, leaf spots and seedling wilting.

Associated to pathology No. 2, a fungal agent was isolated and identified (from the cultural and morphological characterization of five isolations) with the following distinctive aspects: fast-growing colonies on PDA at 28°C (from 6,8 to 7,5 cm of diameter on the eighth day of incubation); velvety aerial mycelium, cottony, whitish or cream in color, septate; sporodochia formed on the mycelium surface, cream in color; conidiophores that emerged laterally from the hyphae on the aerial mycelium; abundant, hyaline microconidia, with 0-1 septa, ovoid, from 7-16 x 2,8-4,2 µm, and formed in false heads; slightly curved macroconidia and less abundant than microconidia, with 5-6 septa and a size of 18-62 x 3,2-5,4 µm; and abundant, globose chlamydospores, of smooth wall, intercalated in the hyphae and with a size of 6,1-10,8 µm.

In general, these characteristics corroborate the report by Gerlach and Nirenberg (1982), Nelson

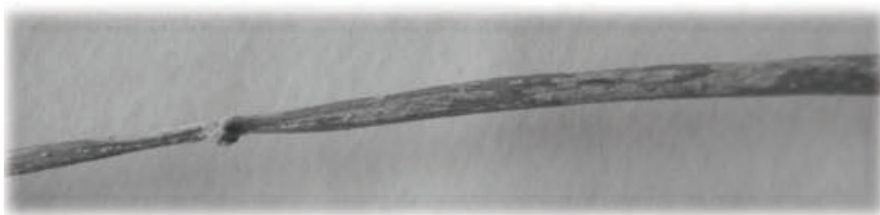


Figure 2. Spots on *M. oleifera* stems, produced by *F. solani*.

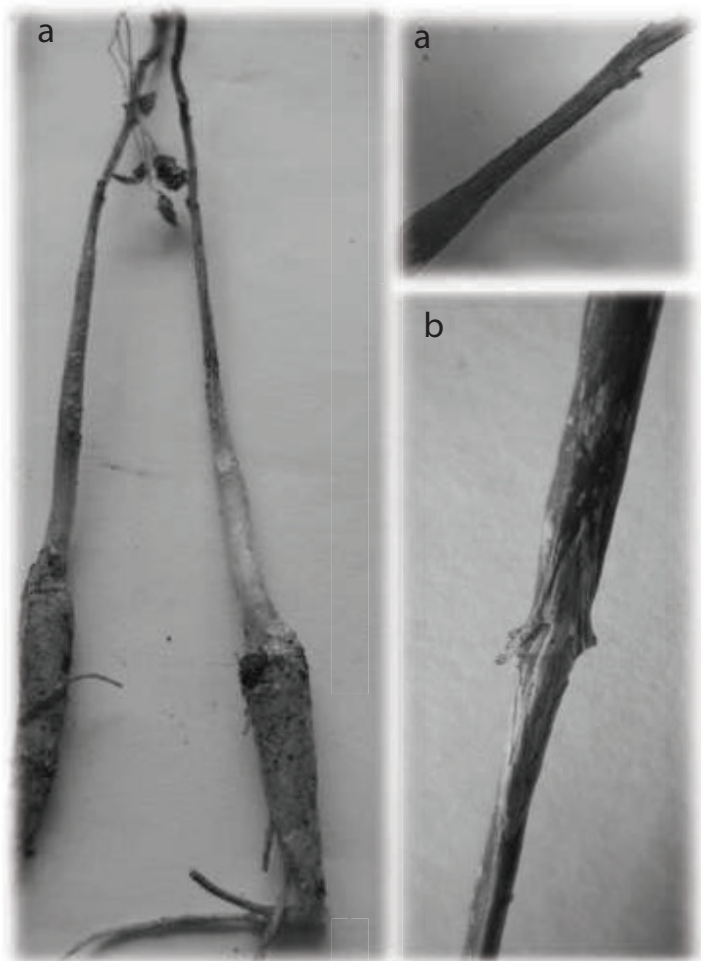


Figure 3. Thinning (a) and dryness zones (b) on *M. oleifera* stems, caused by *F. solani*.



Figure 4. Symptom of stem strangling originated by *F. solani*.

et al. (1983), Seifert (2000), López (2003), Leslie and Summerell (2006), Dueñas *et al.* (2007), Pierobom and Del Ponte (2011) and Herrera *et al.* (2011) for the species *Fusarium solani* (Mart.) Sacc. (teleomorph: *Nectria haematococca*). It is documented in literature as a pathogen associated to problems of cankers and regressive death in many legumes and tropical plants, and as causative agent of diseases in trees and other economically important crops, among which are:

Passiflora edulis, *Vaccinium corymbosum* L., *Phaseolus vulgaris* L., *Solanum tuberosum* Lin. and *Vicia* spp. (Wright *et al.*, 2007; Cubillos *et al.*, 2011; SENASA, 2012).

It is concluded that the presumptive diagnosis indicated the presence of *Colletotrichum dematium* (Pers.) Grove and *Fusarium solani* (Mart.) Sacc, associated to diverse disease symptoms (leaf spot and chlorosis, stem spot and necrosis, wilting and death) in *M. oleifera* seedlings.

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