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Research article

PATHOGENIC MORTALITY OF *FICUS* SPP.

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ABSTRACT: *Ficus nitida* Thunb and *F. benjamina* L. have been widely planted as avenue and home gardens shade trees in Khartoum State, Sudan. They are fast growing and easily pruned into several shapes. It was noticed that both species have been showing symptoms of decline with a characteristic sooty smooth black mass of spores underneath the peeled off tree barks. Therefore they were suspected of being infected with the fungus *Nattrassia mangiferae* Nattrass which has been reported also as causing nail and skin diseases in humans. In Africa, very little research was conducted on *N. mangiferae* and most of the research was too old and needs updating [19]. *N. mangiferae* was also reported as causing skin and nail diseases and fungal keratitis in humans [18] [20]. This study was undertaken with the objective of surveying the incidence of disease in two of the most widely planted avenue and house gardens shade trees in Khartoum State, Sudan. Symptoms included chlorosis and necrosis of leaves, dieback and peeled off barks. Plant and soil samples from underneath infected trees were collected and cultured on PDA. They were dominated with *N. mangiferae* and some minor saprophytic fungi. Four isolates of *N. mangiferae* were recognized in terms of spore shape and mycelium. Pathogenicity tests on *F. nitida* and *F. benjamina* proved further that the causal organism was *N. mangiferae*. Ranking of susceptibility was determined for some other tree species.

Keywords: Mortality; *Ficus* spp.; *Nattrassia mangiferae*; Infection; Dieback; Necrosis, Chlorosis

INTRODUCTION

The fungus *Nattrassia mangiferae* Syd. and P. Syd. **[1]** (formerly *Hendersonula toruloidea* Nattrass) which was described as a dematiaceous coelomycete has been reported as responsible for causing wilt and dieback of trees in the tropics and subtropics [25]. It was polymorphic and capable of producing multiloculated pycnidia in the host tissue and *Torula* stage. In culture *H. toruloidea* produces arthroconidia [24]. The arthroconidial stage was referred to as *Scytalidium* Pesante [5]. However, the pycnidial stage was renamed by Sutton and Dyko [1] as *Nattrassia mangiferae* and *Scytalidium dimidiatum* (Penz.) Sutton & Dyko for the arthroconidial synanamorph. They proposed that the fungus was identical to *Scytalidium lignicola* Pesante. *N. mangiferae* has a wide host range of taxonomically unrelated plant species. The fungus caused branch wilt of the huge Banyan trees (*Ficus beneghalensis*) which lined the main boulevards in Khartoum, Sudan [2]. It caused top dying and mortality of *Gmelina arborea* in India [3]. The fungus was reported as endemic on *Eucalyptus camaldulensis* and on citrus trees in Arizona [6] In China, the fungus was reported to cause butt rot *of Zanthoxylum bungeanum* [7]. *N. mangiferae* was responsible for the mortality of almond, peach, plum and *Eucalyptus* spp., *Populus* spp. and *Pinus* spp. in Iraq [8] [10]. Gummosis of lemon trees has been widespread and caused by *N. mangiferae* [11]. The fungus also caused losses in bananas [12] [13]. Also *N. mangiferae* caused canker of almond [14]. More recently, the canker of Pacific madrone (*Arbutus menziesii*) in USA was attributed to *N. mangiferae* [4] [15] and foliar disease of strawberry trees (*Arbutus unedo*) in Europe [16]. The fungus had caused stem and root rot of very important food crops in Africa (cassava and White Yam) [9] [17]. Strikingly, the fungus was also reported as causing skin and nail diseases and fungal keratitis in humans [18] [20].

MATERIALS AND METHODS

Survey of infection

Frequency of infection

A survey of infection by the branch wilt disease in *Ficus nitida* Thunb and *F. benjamina* L. was carried out in two locations which were characterized by heavy infection, namely Soba (20 km south of Khartoum and University of Khartoum Farm (UKF) (Khartoum North). The survey followed a 100% enumeration sampling method where all trees in the two locations were examined. Every tree showing a single and/or a combination of chlorosis, necrosis, wilt of foliage, twigs or branches, canker and presence of the characteristic black sooty layer of conidia under the bark. Was counted as infected and was further verified by isolation and identification of microflora.

Collection of plant material and soil samples

Random samples from *F. nitida* and *F. benjamina* that developed the above mentioned symptoms were collected from the foliage, bark and roots. Woody samples were taken by means of an increment borer which was inserted inside the stem and root to a depth of 5 cm after being surface sterilized by swabbing with 95% ethanol. All samples were kept inside polythene bags and kept in a fridge for further analysis. Approximately 100 g soil samples were collected at 10 cm depth beneath 20 infected trees from each location.

Isolation of microorganisms

Plant material from infected trees was cut into smaller pieces which were surface sterilized by immersion into 1% sodium hypochlorite for 1 minute, then washed thoroughly in two changes of sterile distilled water and dried on filter paper. All samples were cultured in Petri dishes containing sterile PDA. Cultures were then incubated at 30 ± 1°C.

Soil suspensions were prepared from the collected soil samples and diluted serially with sterile distilled water to 1:10, 1:100, 1:1000 and 1 ;10000, and mixed thoroughly with sterile PDA and then poured into Petri dishes. The final concentration of each soil suspension was 50% (v/v). Four replicates of each soil dilution were obtained. All cultures were incubated at 30 ± 1 °C. Cultures from plant material or soil were examined daily under a light microscope for microbial colonies which were identified and counted. Conidia which were usually found under the bark of branches and the stems, were also cultured on PDA.

Pathogenicity tests

Preparation of inocula

Pure cultures of *N. mangiferae* were prepared on PDA. Isolate 3 was used for pathogenicity tests because it was the most vigorous. Cultures were incubated at 30 ± 1 °C for three weeks.

Preparation of seedlings

One-year old seedlings of *F. nitida* and *F. benjamina* of comparable vigor were prepared. The seedlings were obtained by aerial layering from healthy trees. Following the development of the root system, the seedlings were transferred to plastic pots (20 cm diameter) x 24 cm (height) which were filled with sterile clay: sand (2:1) soil and placed in a green house.

Inoculation of seedlings

Five mm long and two mm deep scars were made on the stems and roots of seedlings. Stem and root scars were made 5 cm from the basal part of the stem upwards and downwards, respectively. An agar slant (5 mm in diameter) containing the vigorously growing mycelium of *N. mangiferae* (isolate 3) was pressed into each scar. Control scars received only agar slants devoid of mycelium. All scars were covered with a polythene film. Seedlings of *F. nitida* and *F. benjamina* were inoculated on the stems and roots. Each treatment (2 species x 3 inoculations) was replicated ten times. All seedlings were kept in a green house and watered regularly. The development of symptoms was recorded for five weeks after which re-isolation of microflora were carried out on PDA.

Host range test

Inoculum of *N. mangiferae* (isolate 3) was prepared as described above. Healthy seedlings (one-year old) growing in sterile clay: sand (2:1) soil was inoculated in the same way as above. Ten seedlings of the following tree species were inoculated; *Khaya senegalensis* (Desr.) A. Juss.; *Eucalyptus camaldulensis* Dehn.; *Azadirachta indica* A. and *Acacia senegal* L.

RESULTS

Description of symptoms

The following symptoms were recorded during the field survey:

1. Leaf chlorosis or yellowing usually followed by rolling of leaves and dieback of *F. benjamina* in particular.
2. 2. Leaf necrosis which was characterized by dark discoloration of the leaves ranging from one third to the entire lamina. Leaf chlorosis and necrosis were observed simultaneously, especially on *F. nitida*, whereas in *F. benjamina* the leaves though necrotized, yet remained yellow but dead.
3. Leaf shedding: wilting and defoliation with various intensities. Defoliation took the form of dieback. At this stage, most of the affected trees wilted and died.
4. The bark was peeled off revealing a smooth sooty layer of black arthrospores under the bark of branches and stems characteristic of infection by *N. mangiferae*.

Isolation of microflora from plant material

Isolations from infected *F. benjamina* at Soba showed that *N. mangiferae* was the dominant microorganism, being isolated from 79.6% of the samples. *Trichoderma viride* ranked second (13.6%) and *Rhizopus nigicans* third (6.8%) (Table 1). *N. mangiferae* was also dominant in samples taken from infected *F. nitida* at UKF, having a frequency of 49.9%, *Aspergillus* spp. were second most common (32.8%), *T viride* (9.2%0, *Penicillium* spp. (3.4%), *R. nigicans* (3.1%) and unknown (1.6%). (Table2).

Isolation of microflora from soil samples

The results of microflora isolated from soil samples were summarized in Table 3. *N. mangiferae* was the dominant species being isolated from 50.3% of all soil suspension samples. Other species isolated were *R. nigicans* (26.3%), *Penicillium* (19.4%) and *T. viride* (4.3%). Nevertheless, the frequency of *N. mangiferae* decreased with the decrease in the concentration of the soil suspension being 62.5, 55.6, 50.0 and 33.3% in 1:10, 1:100, 1:1000 and 1:10000 soil dilutions, respectively.

Table 1. Mean frequency % of microorganisms isolated from infected *F. benjamina* in Soba campus

Sample No.	*N. mangiferae*	*R. nigircans*	*T. viride*
1	100	0	0
2	75	25	0
3	50	0	50
4	100	0	0
5	100	0	0
6	100	0	0
7	100	0	0
8	50	0	0
9	50	0	50
10	50	0	50
11	100	0	0
Mean	79.6	6.8	13.6

Description of *N. mangiferae*

On PDA, the fungus grew readily and formed a whitish mat which turned blackish within 3 days. The arthrospores were either spherical or cylindrical in shape. Subsequent attempts to produce the pycnidial stage failed. Four isolates of *N. mangiferae* were distinguished. The occurrence of each isolate was not confined to a particular *Ficus* spp. However, isolation from infected trees revealed only one type of isolate. The distinction between these isolates was based on the shape of the spores and mycelium as follows:

Isolate 1: had a brownish mycelium which was more raised in the middle of the culture. The spores were spherical with a smooth surface. On the reverse side, the colony was pale brown.

Isolate 2: The mycelium was grayish brown with a white color in the middle of the colony. The spores were spherical with a rough surface. On the reverse, the culture was pale brown.

Isolate 3: The spores were cylindrical. The mycelium was pale green initially, but turned into grayish black with a slight touch of green and finally it became black. The mycelium was submerged and reached the bottom of the Petri dish and the culture was black on the reverse side. The hyphae were thicker than those of isolate 4.
Isolate 4: The spores were cylindrical. The mycelium was dark green initially, and later turned into dark gray. It was also submerged with black septa, black on the reverse side and relatively thinner than that of isolate 3.

Table 2. Mean freqyency % of microorganisms isolated from infected *F. nitida* at UKF

Sample No.	*Penicillium* spp	*R. nigricans*	*T. viride*	*Aspergillus* spp.	*N. mangiferae*	Others
1	0	0	0	66.7	33.3	0
2	0	0	33.3	33.3	33.3	0
3	0	0	0	50	50	0
4	0	0	0	50	50	0
5	0	0	0	0	100	0
6	0	50	0	0	50	0
7	0	0	33.3	33.3	33.3	0
8	14.3	0	14.3	28.6	42.9	0
9	0	0	0	50	50	0
10	0	0	0	50	50	0
11	0	0	0	0	75	25
12	20	0	0	20	60	0
13	0	0	66.7	33.3	0	0
14	20	0	0	60	20	0
15	0	0	0	0	100	0
16	0	0	0	50	50	0
Mean	3.4	3.1	9.2	32.8	49.9	1.6

Table 3. Mean frequency % of microorganisms isolated from soil samples underneath infected trees

Soil dilution	*Penicillium spp.*	*T. viride*	*R. nigricans*	*N. mangiferae*
1:10	0	6.3	31.2	62.5
1;100	11.1	11.1	22.2	55.6
1:1000	0	0	50	50
1:10000	66.7	0	0	33.3
Mean	19.4	4.3	26.3	50.3

The frequency of infection

The frequency of infection with *N. mangiferae* was extremely high. It was much higher in *F. benjamina* than in *F. nitida* being 68% and 28%, respectively, at Soba and 90% and 75%, respectively, at UKF.

Pathogenicity test

In *F.benjamina*, symptoms started to develop five days after inoculation. First, the leaves became drooped and rolled over and then chlorosis developed in 25% of the inoculated seedlings. These symptoms were confined to stem inoculations. Three weeks later, 75% of the stem inoculated seedlings wilted completely, whereas only 25% of those with root inoculation wilted. Control plants showed no symptoms whatsoever. In *F. nitida*, leaf chlorosis and necrosis developed in the seedlings two weeks after their stems were inoculated. After four weeks, 75% of the seedlings with stem inoculations wilted completely, whereas within the same period wilt and mortality occurred in only 25% of the seedlings where roots were inoculated. Re-isolation from the stems of the infected seedlings on PDA proved that *N. mangiferae was* the dominant microorganism and the only pathogenic fungus isolated. Control plants remained free of any symptoms.

Host range

Symptoms developed in the different host plants tested were as follows:

F. beneghalensis: Chlorosis and necrosis developed on the leaves of all inoculated seedlings. five days after inoculation. Cankers of the twigs and stems was also seen, then the seedlings wilted completely and the characteristic black sooty layer of spores developed underneath the peeled off bark.

Azadirachta indica: Leaves of all inoculated seedlings became paler as compared to the very dark green leaves in control plants. Chlorosis and necrosis also developed on the foliage after one week after inoculation.

K. senegalensis: A smaller number of new foliage was produced by inoculated plants as compared to the control, and the ratio was approximately 1:3. The leaves of the former were very much stunted. Necrosis was noticed in a few leaves one week after inoculation. Control seedlings remained unaffected.

E. camaldulensis: Batches of chlorosis and necrosis were evident in 50% of the leaves of inoculated seedlings within ten days after inoculation. The leaves were also paler than those of control plants.

Acacia senegal: By the fourth week 25% of the inoculated seedlings showed very little chlorotic leaves. This chlorosis was not that obvious and needed careful examination. However, *N. mangiferae* was the only microorganism isolated from chlorotic foliage.

Re-isolation from inoculated seedlings on PDA proved that *N. mangiferae* was the only microorganism isolated.

On the basis of these results, susceptibility or otherwise resistance to *N. mangiferae* may be summarized as follows:

Highly susceptible:

Ficus spp. (*F. beneghalensis*, *F. nitida* and *F. benjamina*).

Susceptible: *Azadirachta indica* and *E. camaldulensis*.

Fairly susceptible: *K. senegalensis*.

Least susceptible: *A. senegal*.

DISCUSSION

Symptoms inflicted by *N. mangiferae* recorded in the present study agreed with those described by Giha [2] [3] [4] and Farr et al. (2005). It is clear that infection by *N. mangiferae* has reached an epidemic level, as evident from the very high frequency of infection in both *F. nitida* and *F. benjamina* in the two sires studied. This epidemic spread presents a grave threat to shade and other trees of economic value in Khartoum State (Sudan), especially that the fungus has an extremely wide host range. Some of the susceptible host species reported in the Sudan included *Ficus* spp., *Azadirachta indica*, *Albizia lebbek*, *Terminalia catapa*, *Cassia nodosa*, *Adansonia digitata*, *Acacia seyal*, *Acacia nilotica*, *Acacia senegal*, *Khaya senegalensis* and most important *Citrus* spp. (Nori, 1996). In fact, wilt by *N. mangiferae* is a grave threat to citrus industry in the Sudan [2]. It seems that the very hot conditions prevailing in Khartoum State, most of the year, were conducive to the disease development. Calavan and Wallace [21] and Paxton et al. [22] mentioned that *N. mangiferae* thrives better at high temperature (30-35 °C). The fungus produced heavy masses of loose spores under the bark of infected trees, that can readily spread the infection. It is probable that certain cultural practices might have enhanced the outbreak of the disease. For instance, in UKF it was not uncommon to find a whole row of completely infected trees. This might be attributed to the fact that irrigation of most trees was provided with a single continuous canal. Since bark fragments of infected trees which carried heavy masses of spores that had increased the possibility of dissemination of these spores from infected to healthy trees by irrigation water in addition to their being air-borne. This was confirmed by the presence of *N. mangiferae* spores in the soil samples from underneath infected trees. In addition, the use of axes in the pruning of branches might have released huge masses of spores in the air with every hit of the branch. Moreover, it is very common that the same axe was used to prune a large number of trees. The symptoms of wilt recorded in the present study were in line with those reported by several investigators. Harsh and Tiwari [3] described symptoms on plants were black necrotic lesions on leaves followed by defoliation, drying of young shoots and formation of canker and callus in twigs and stems leading to top dying and mortality of *G. arborea*. Canker, blight and dieback of *E. teriticornis*, *E. hybrid*. *E. camaldulensis* and *E. populifolia* were the symptoms reported by [23]. Al Zarari et al. [8] reported *N. mangiferae* as the causal organism of branch wilt in almond, peach, plum and *Eucalyptus* spp. It seems that *N. mangiferae* is capable of producing phytotoxic substances that lead to the development of symptoms reported in this study. When the branches were dipped in the filtrate, reduction of water uptake and wilting occurred. Unlike most research that considered *N. mangiferae* as a single isolate, four isolates of *N. mangiferae* were distinguished in the present study.

They may be divided into two groups: a group characterized by having spherical spores (isolates 1 and 2) and the other with cylindrical spores (isolates 3 and 4). Whether these isolates were different in their virulence or not requires further research. With the current great expansion in planting of *Ficus* spp. especially *F. nitida* and *F. benjamina* as avenue and home garden trees in Khartoum State, and in the light of the high levels of infection and mortality inflicted by *N. mangiferae* in these two species, it is recommended that it is high time to search for more resistant species. The fungus had caused stem and root rot of very important food crops in Africa (cassava and White Yam) [9] [17]. It is also alarming that the fungus was capable of infecting a wide range of taxonomically unrelated plant species especially that citruses were among the susceptible hosts. Furthermore, *N. mangiferae* was reported as causing skin and nail diseases and fungal keratitis in humans [18] [20]. Thus, planting of trees susceptible to *N. mangiferae* might spread more skin diseases in humans.

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