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Prevalence and Etiology of Branch Wilt Disease (*Nattrassia mangiferae*) on Tree Species in Wad Medani Area, Sudan

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Abstract: Branch wilt disease caused by *Nattrassia mangiferae* (Nattrass) has very wide host range. It attacks trees in forests, orchards, ornamental, and shade trees. It is one of the most hazardous diseases that spread in Wad Medani province, Gezira state, Sudan. In Sudan there is limited information about the disease occurrence. This study is therefore, conducted to study the prevalence of the branch wilt disease in Wad Madani and to study the pathogenicity and host range of the common *N. mangiferae* isolates in Wad Medani area. The results of the survey revealed that 29 tree species belonging to 15 families were found to be naturally infected. The highest magnitude of the disease was found in ficus (*Ficus nitida* Thunb.), cassia (*Cassia nodosa* Roxb.), and baobab (*Adansonia digitata* L.), where the disease incidence was 100% and the disease severity ranged from 55% to 60%.While the lowest magnitude of the disease was reported in *Acacia* spp. with 20 % disease incidence and 30% severity. The disease occurrence was confirmed in the different habitats surveyed with the highest disease incidence and severity reported from Attra with 100% and 53%, respectively. Whereas, the lowest incidence (30%-35%) and severity (20%) were recorded from Bankae forest and Hantoub. The laboratory studies revealed that there were 2 different isolates of *N. mangiferae.* The 1st one was isolated from ficus (*Ficus nitida*) and the 2nd was isolated from lime (*Citrus aurantifolia* Swingle.). In host range experiments using seedlings of 12 different tree species artificially inoculated by each isolate, the lime isolate successfully showed symptoms on 11 tree species. Only the seedling of *Balanites aegyptiaca* remained uninfected. While ficus isolate attacked only two tree species namely *F. nitida* and *Eucalyptus citriodora* Hook.

Keywords*:* Disease incidence, severity, die back, host range, pathogenecity

Introduction

Nattrassia mangiferae is a cosmopolitan, polyphagus fungus that attacks a multitude of tree flora. The earliest reports of plant diseases caused by *N. mangiferae* were from India on mango trees in 1908 and on citruses in California in 1923 (Sutton and Dyko, 1989). The fungus was originally described by Nattrass (1933) in Egypt as the main cause of die back on deciduous trees. *Nattrassia* is a polymorphic fungus that has two spore stages, the pycnidial and the arthroconidial (*Scytalidium*state). Sutton and Dyko (1989) revised the genus *Hendersonula* and created the new monotypic genus *Nattrassia*, whose type species is *N. mangiferae*. Earlier names for this fungus have been *Dothiorella*

mangiferae, *Exosporina fawcettii*, *Fusicoccum eucalypti*, *Hendersonula cypria*, *H. agathidis*, and *H. toruloidea*. The arthric syanamorph is known by the name *Scytalidium dimidiatum*, also *Torula dimidiata*, and *S. lignicola*. A sexual stage for *Nattrassia* has not been described (Elliott and Edmonds, 2004).

Different symptoms were found to be caused by this fungus depending on the part of the tree affected such as: mortality of almond, peach, plum and *Eucalyptus* spp in Iraq (Alzarari *et al.*, 1979). Jamaluddin *et al*. (1987) reported canker, leaf blight and die back of *Eucalyptus* spp. Cao and Wang (1989) indicated that *N. mangiferae* was the causal agent of the butt rot of *Zathoxylum bungeanum* in China, and top dying and mortality of *Gmelina arborea* in India (Harsh and Tiwari, 1992). The fungus also caused losses in bananas (Jones and Stover, 2000). More recently, the canker of Pacific madrone (*Arbutus menziesii*) in USA was attributed to *N. mangiferae* (Elliott, *et al*., 2002) and foliar disease of strawberry trees (*Arbutus unedo*) in Europe (Tsahouridou and Thanassoulopoulos, 2000). The fungus had caused stem of cassava in West Africa (Msikita *et al*. 1997). Conspicuously, the fungus was also reported to cause skin and nail diseases and fungal keratitis in humans [Moore, 1988].

In Sudan sudden branch wilt disease was firstly reported by Giha (1975) on banyan trees (*Ficus benghalensis* L.); shade trees that line the streets of the capital Khartoum. Since then, the disease has spread all over the country, and was reported in as many as 29 different plant species that include shade, ornamental, timber and orchard trees (Ahmed and Yassin 1992; Nour 1996; Mohamed, 2000; Ahmed, 2005). The main objectives of this study were to study the occurrence and distribution of the branch wilt disease in Wad Madani province and to study pathogenicity and host range of the common *N. mangiferae* isolates.

Materials and Methods

Disease Survey:

Surveys were conducted in Wad Medani province, Gezira State, central Sudan including Hantoub, Bankae forest, and Abu Haraz on the eastern bank of the Blue Nile. Umbarona forest, Attra, and the main streets in Wad Medani city on the western bank during 2006 -2007 to determine the frequency of *Nattrassia* infection in some important tree species. Both apparently healthy and declining trees were included in the survey. Fifty plants of each species were selected randomly. Healthy characteristics of each tree were noted, as well as infected plants within each group were counted to determine the incidence of the disease as follows:

$$\text{Disease incedince\%} = \left(\frac{\text{The number of infected plants}}{\text{The total number of the survyed plants}}\right) \times 100$$

Also the numbers of wilted branches per tree were counted to determine the severity of the disease. Wilted branches were rated, using a scale of (0 to 5) where zero indicated that plant with symptomless or seems to be healthy, 1: represented 20%, 2: represented 40%, 3: represented 60%, 4: represented 80% and 5: represented 100% infection. The trees surveyed in this study were classified according to habitat to: planted forests at Umbarona and Bankae forests, orchards' trees from Attra and Hantoub, shade and ornamental trees, at the faculty of Agriculture and Natural Resources, University of Gezira, and from individual trees grown on the streets of Wad Medani city.

Samples Collection:

Random samples from different naturally infected trees showing symptoms were collected. Segments of the affected branch were collected from planted forests, orchards and shade trees plus ornamental plants in polythene bags for laboratory studies.

Isolation and Identification of the suspected causal agent:

Chipped samples 2cm each, of infected wood, were surface –sterilized in solution of sodium hypochlorite (NaOCl 0.5%) for 3-5 minutes after being thoroughly washed 3 times with sterile distilled water then dried on sterile filter papers. The disinfected specimens were placed on autoclaved sterilized medium of Potato Dextrose Agar (PDA) poured in 9 cm diameter sterilized Petri dishes, and then incubated for 3-5 days at room temperature. After incubation, the associated microorganisms growing out of the chipped wood were sub-cultured. Pure cultures of grown microorganisms were obtained using the single hyphal tip method (George, 1947). The microorganisms were identified under the microscope using main characters such as shape and color of the mycelium and spores and/or conidia. Also cultural characteristic on PDA were considered as well.

Pathogenicity and Host range experiments

Experiments for pathogenicity test were conducted in a plastic house at Abu Haraz Faculty of Agriculture and Natural Resources, University of Gezira, Sudan, to determine the virulence of the 2 isolates of *Nattrassia* spp. and to study the host range of these 2 isolates.

Pure cultures of both isolates of the fungus were made in sterilized PDA for inoculation purposes. The fungal inoculum was incubated for 10 days at room temperature before use.

Seedlings Inoculation:

Seedlings of 12 different trees 1-2 years old were selected. The seedlings were obtained from the nursery of the Faculty of Agriculture and Natural Resources, and Umbarona forest nursery.

Seedlings were inoculated by pressing slant of 4×4 mm solid PDA comprising spores and mycelia of the pathogen into a wound; 2×5 mm dimensions and 2mm deep on the surface of the stem using a knife, on about the middle of the stem of each seedling**.** To prevent drying and contamination, wounds were rapped with a polythene film, **(**Mohukker and Yassin, 2001). One seedling of each species served as control, inoculated with sterile PDA inserted into the wound. Ten seedlings of each tree species were used for inoculation purposes. The relative humidity was increased by covering the inoculated seedling with polyethylene bags containing moistened towel papers for 24 h. Observations were recorded weekly for symptoms appearance. Recovery of the fungal isolates was performed from the inoculated seedling to verify Koch's postulates.

Results and Discussion

Branch wilts disease survey:

The results of the survey revealed that 29 tree species belonging to 15 different families were naturally infected (Table 1).

The highest magnitude of the disease was found in ficus (*F. nitida*), *C. nodosa*, and baobab (*A. digitata*) where the disease incidence was 100% and the disease severity ranged from 55% to 60%.While the lowest magnitude of the disease was reported in *Acacia* spp. with 20 % disease incidence and 30% severity (Table 2). These results agreed with Ahmed (2005) who reported that in Gezira state the highly infected tree species were *F. nitida*, *A. lebbek*, and *A. digitata*. Similarly Nour in 1996, reported high frequency of infection by *Nattrassia* in *F. nitida* and *F. bengamina* and it ranged between 33 and 100 % in Khartoum State.

The disease occurrence was confirmed in the different habitats surveyed with the highest disease incidence and severity reported from Attra with 100% and 53%, respectively. Whereas, the lowest incidence (30% - 35%) and severity (20%) were recorded from Hantoub and Bankae forest (Table 3). The high incidence and severity of the disease might be enhanced by the environmental factors prevailing in those habitats.

It was obvious that water stress, high temperatures and wind affected the incidence and severity of the disease. In Attra, where guava trees were grown under water stress the disease incidence and severity were 100%, and 53%, respectively. Similar results were reported by Mohammed (2000), Elliott (2000) and Ahmed (2005), who found that the most predisposing factors for this disease were drought and heat. Moreover, the eucalyptus trees in the same location were highly infected due to the fact that those trees were used as wind breaks. This creates high opportunity to infection by *N. magiferae* because the fungus is wind transmitted. As indicated by Giha (1975), powdery mass of spores of *N. mangiferae* were disseminated by wind and rain. The above mentioned facts proved why the incidence and severity of the disease was the highest at Attra.

On the main streets of Wad Medani city, the trees were grown in open environments. Trees growing in open areas are more prone to sun scald, and consequently *N. mangiferae* infection being on exposed branches. Girdling of these branches creates water stress, which lower the rate of photosynthesis and respiration as the trees become defoliated due to shoot blight and dieback (Gerrish, 1993).

Table 1: The host range of *Nattrassia mangiferae* in Wad Madni area

Local name	English name	Scientific name	Family
Manga	Mango	*Mangifera indica* L.	Anacardiaceae
Lashouka	Ashoka tree	*Saraca indica* L.	Anacardiaceae
Damass	Thevetia	*Thevetia peruviana* (Perr.) Marr.	Apocynaceae
Wared Elhameer	Oleander	*Nerium oleander* L.	Apocynaceae
Nakheel Molokee	Royal palm	*Roystonea spp*	Areceae
Oshaar	Giant milk weed	*Calotropis procera* (Ait.) Ait. f.	Asclepiadaceae
Taballdee	Baobab	*Adansonia digitata* L.	Bombacaceae
Basaniss	Dwrypoinciana	*Poinciana pulcherrima* (L.)	Caesalpiniaceae
Cassia	Cassia	*Cassia nodosa* Roxb.	Caesalpiniaceae
Peltophroum	Peltophroum	*Peltophorum ferruginum* (Dc. Bak. Exk. Heyne)	Caesalpiniaceae
Poinciana	Flamboyant	*Delonix regia (*Hook.) Raf.	Caesalpiniaceae
Seasaban	Jerusalemthom	*Parkinsonia aculeata* L.	Caesalpiniaceae
Tamerihende	Tamarind	*Tamarindus indica* L.	Caesalpiniaceae
Brazilia	Brown terminalia	*Terminalia catappa* L.	Combretaceae
Neem	Neem tree	*Azadirachta indica* A. Juss.	Meliaceae
Mahogany	African Mahogany	*Khaya senegalensis* (Desr.) A. Juss.	Meliaceae
Degn Elbasha	Siris tree	*Albizzia lebbek* (L.) Benth.	Mimosaceae
Sount	Indian gum Arabic	*Acacia nilotica* (L.) Willd. Ex Del.	Mimosaceae
Taalih	Indian gum Arabic	*Acacia seyal* Del.	Mimosaceae
Miskeet	Iron wood	*Prosopis africana* (Guill. & Perr.) Taub.	Mimosaceae
Lapakh	Banyan tree	*Ficus benghalensis* L.	Moraceae
Ficus	Banyan tree	*Ficus nitida* Thunb.	Moraceae
Kaphore	Eucalyptus Lemon	*Eucalyptus citriodora* Hook.	Myrtaceae
Guava	Guava	*Psidium guajava* L.	Myrtaceae
Gahanameia	Bougainvillea	*Bougainvillea* spp	Nyctaginaceae
Romman	Pomegranate	*Punica granatum* L.	Punicaceae
Lemon	Lime	*Citrus aurantifolia* Swingle.	Rutaceae
Lareng	Citrus aurantium	*Citrus aurantium* L.	Rutaceae
Lantana	Lantana	*Lantana camara* L.	Verbenaceae

Naturally infected trees showed chlorosis and necrosis on the tip or leaf margins which ultimately dry out and shed, leading to the appearance of branch die-back, severely infected trees exhibit die-back of branches, and canker of trunk then the bark cracked and

peeled-off exposing a sooty layer of the fungus spores. The branch die-back in severely infected trees, extended from one branch to another until the whole tree is affected resulting in mortality of the tree.

The observed symptoms were similar to those described by Ahmed (2005) and ElTrefee (2009). Different symptoms were observed on *Eucalyptus citriodora*, family Myrtaceae. These show stunting, reduction of shoot and lesion on the lower stem portions then extended to the branches.

Similar symptoms were mentioned by Msikita *et al.* (1997) in West Africa, who described *N. mangiferae* as causal of the symptoms of stunting, reduction of shoot and root numbers, and inducing lesion up to 15 cm long on the lower stem portion of infected Cassava.

Table 2: Disease incidence and severity of branch wilt caused by *Nattrassia. mangiferae* in naturally infected tree species in Wad Medani area .

Tree species	Means of incidence %	Means of severity %
Ficus nitida	100	60
Casia nodosa	100	60
Adansonia digitata	100	55
Albizia lebbek	93	43
Thevetia peruviana	80	50
Eucalyptus citriodora	78	33
Saraca indica	74	60
Psidium guajava	70	70
Mangifera indica	70	20
Khaya senegalensis	66	33
Citrus aurantium	60	60
Citrus aurantifolia	56	20
Delonix regia	42	40
Azadirachta indica	36	40
Pletophorum ferruginum	30	40
Parkinsonia aculeate	30	50
Acacia nilotica	30	25
Acacia Arabica	20	25
Balanites aegyptiaca	00	00

Table 3: The incidence and severity of branch wilt disease caused by *Nattrassia mangiferae* at different locations.

Locations	Disease incidence %	Disease severity %
Attra	100	53
Main streets of Wad Medani	75	48
Umbarona forest	57	37
Abu Haraz	53	40
Hantoub	35	20
Bankae forest	30	20

The fungus isolates:

In the present study, 2 different isolates of *N. mangiferae* were identified; the 1st isolate was isolated from *F. nitida* and the 2nd from *C. aurantifoila.* Cultural and microscopic examinations showed significant differences between the 2 isolates in the rate of growth, shape and color of colonies and shape and color of conidia and mycelium.

The ficus isolate was detected in *F. nitida and F. benghalensis* and it is characterized by: the texture of the middle of the culture was powdery; the mycelium color begins white for 2-3 days, turns into dark green in the middle after 5 days then the color changes to blackish brown in the middle and brownish grey at the edges after 7 days. Finally it changes to black in the middle and brown at the edges of the colony after 10 days of growth when grown on PDA medium (Plate 1).

Under the light microscope, the mycelium appeared to be brown in color, septated, branched or simple. Conidia were mostly in chains or single, spheroid or cylindrical, their color ranged between hyaline and pale to dark brown. They have none or with 2 septa (Plate 2).

The Lime isolate was isolated from *C. aurantifolia, C. aurantium, P. guajava, M. indica, A. lebbek, P. ferruginum, D. regia, A. indica ,C. nodosa, T. indica., E. citriodora, A. nilotica*. and *A. seyal*. The culture showed white color at the 1st day and changed to olive or green in the middle with shades of white 2-4 days. Finally it changed to grey green in the middle then turned dark brown with shade of white color after 7 days when grown in PDA medium (Plate 3).

Under the light microscope the mycelium appeared to be dark brown in color, septated, branched or simple. The conidia were ovoid or cylindrical with 0-3 septa; they showed hyaline color at first and then turned to dark brown in the center and pale brown in the edges (Plate 4). These results are in consistency with Nour (1996) and El Atta and Aref 2013 who recognized four isolates of *N. mangiferae*; infecting avenue trees in Khartoum state depending on spore shape and mycelium growth characteristic.

Host range studies using the two isolates of *N. mangiferae*:

Seedlings of ficus and eucalyptus inoculated with the ficus isolate of *N. mangiferae* showed the typical symptoms of branch wilt disease 23 and 26 days after inoculation, respectively. The seedlings of the other tree species remained uninfected. On the other hand all seedlings inoculated with the lime isolate of *N. mangiferae* were infected except *B. aegyptiaca* which remained symptomless (Table 4).

These results were in agreement with those reported by Ahmed (2005) who found that the lime isolate was more virulent than the ficus isolate, which infected successfully the artificially inoculated seedlings of tree species tested except *B. aegyptiaca*. On the other hand seedlings inoculated with ficus isolate took longer time to be affected or even not. Moreover, Ahmed (2005) found that fine paste of *B. aegyptiaca* was the most potential in controlling the pathogen under laboratory conditions. The findings of Abu Al Futuh, (1983) explained the resistance of *B. aegyptiaca* due to the fact that the tree has solid wood that contains phenolic compounds, proteins and ethylene group and sometimes formed gitogenine. In addition, Bettolo, (1998) reported that *B. aegyptiaca* showed antimicrobial, insecticidal and fungicidal potentials. The first symptoms observed were chlorosis in all infected seedlings of *F. nitida, P. guajava, E. citriodora, A. lebbek, A. seyal, A. nilotica, P.ferruginum, D. regia, A. indica, M. indica* and *C. auratifolia* after 5-12 days followed by wilting and defoliation of leaves at the end of the 4th week. The sudden wilting on guava was in accord with those reported by Mirzae *et al*. (2002) who found that the symptoms on guava appeared as sudden wilting of branches, followed by bark discoloration ranging from dark grey to black without exposure of any sooty layer of arthrospores.

In conclusion, the biodiversity of *N. mangiferae* need to be studied all over the country on the basis of molecular characteristic to differentiate between the

different isolates and to find out if they are different isolates or races. *Balanites aegyptiaca* appeared to be highly resistant to the disease in laboratory experiments, and it was not infected under natural conditions. Thus the effect of its extract from the different parts in controlling *N. mangiferae* should be investigated. Furthermore, an effective control measure/s should be developed to control this devastating disease that represent real threat to fruit, forest and ornamental trees.

Table 4: The Pathogencity of two *Nattrassia mangiferae* isolates (Lime and Ficus) on different seedlings of tree species.

Seedlings species	Ficus isolate	Lime isolate
Balanites aegyptiaca	–	–
Azadirachta indica	–	+
Albizzia lebbek	–	+
Peltophorum ferruginum	–	+
Delonix regia	–	+
Ficus nitida	+	+
Psidium guajava	–	+
Mangifera indica	–	+
Citrus aurantifolia	–	+
Acacia nilotica	–	+
Acacia Arabica	–	+
Eucalyptus citriodora	+	+

(+) indicated that seedlings are susceptible to infection, (-) indicated no infection.

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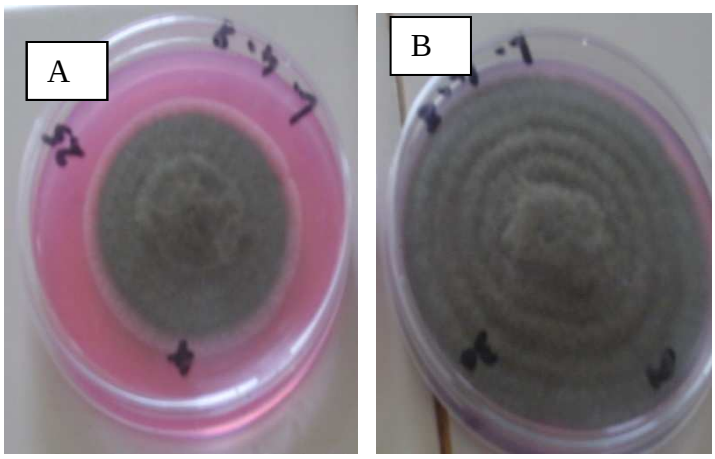

Plate. 1. Culture of *N. mangiferae* (Ficus isolate) on PDA medium, the mycelium color begins white for 2-3 days, turns into dark green in the middle after days 5 (A), changes to blackish brown in the middle and brownish grey at the edges after 7 days (B).

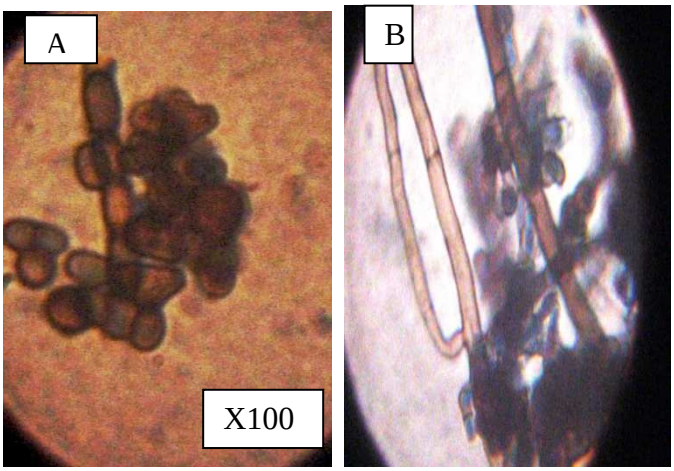

Plate 2. Morphological characteristic of N. mangiferae – Ficus isolate. A, conidia; B, mycelia (x100 magn: fication)

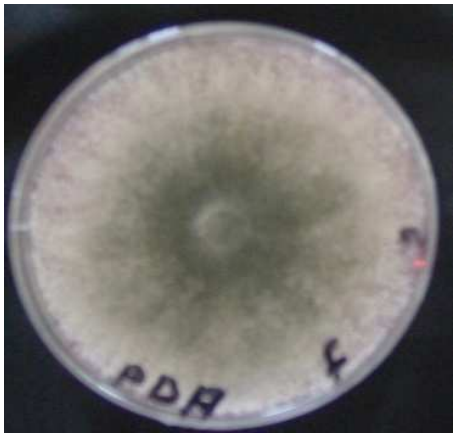

Plate 3. Culture of *N. mangiferae* (Lime isolate) on PDA medium showed white color at the first day, changed to olive or green in the middle with shades of white 2-4 days. Finally it changed to grey green in the middle with shade of white color after 7 days.

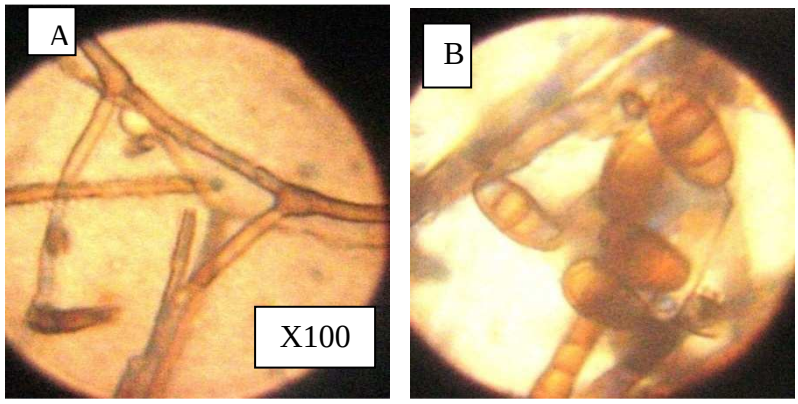

Plate 4. Morphological characteristic of *N. mangiferae* – Lime isolate. A, mycelium; B,conidia (x100 magnification).

حدوث ودراسة مسبب مرض ذبول الافرع في الاشجار (*Nattrassia mangiferae*) في منطقة ود مدني، السودان

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المستخلص:

مرض ذبول الأفرع المتسبب عن الفطر (*Nattrassia mangiferae* (Nattrass)) له مدي عائلي واسع. فهو يهاجم أشجار الغابات والبساتين وأشجار الزينة والظل. ويعتبر المرض واحد من أكثر الأمراض الخطرة التي تنتشر في محلية ودمدني ولاية الجزيرة في السودان. هنالك نقص في المعلومات حول توزيع وانتشار المرض والمدي العائلي للمسبب المرضي. لذلك أجريت هذه الدراسة بغرض تحديد حدوث وشدة الاصابة بالمرض ومعرفة المدي العائلي للمسبب المرضي. كشفت نتائج المسح أن 29 نوعاً من الأشجار التي تنتمي لـــ 15عائلة نباتية مصابة بشكل طبيعي. كانت أعلي نسبة حدوث للمرض في أشجار الفيكس (*Ficus nitida* Thunb.) والكاسيا (*Cassia nodosa* Roxb.) والتبلدي (*Adansonia digitata* L.) وبلغ معدل حدوث الاصابة بالمرض نسبة 100% وتراوحت شدة المرض من 55% الي 60%. بينما كانت أدني نسبة حدوث للمرض في أشجار الأكاشيا (*Acacia* spp.) بنسبة 20% وكانت شدة المرض 30%. أكدت نتائج البحث حدوث المرض في البيئات المختلفة التي شملها المسح وكانت أعلي نسبة لحدوث وشدة المرض في منطقة عترة حيث بلغت 100% و53% علي التوالي. في حين سجلت أدني نسبة لحدوث المرض (30%–35%) وشدة المرض (20%) في غابة بانكي ومنطقة حنتوب. كشفت الدراسات المعملية أن هنالك عزلتين مختلفتين من المسبب المرضي. تم عزل الأولي من أشجار الفيكس والثانية من أشجار الليمون (*Citrus aurantifolia* Swingle.). أوضحت تجارب المدي العائلي التي لقحت فيها شتلات من 12 نوعاً نباتياً بلقاح المسبب المرضي من العزلتين أن عزلة الليمون أظهرت الأعراض بنجاح علي 11 نوع نباتي من الشتلات، الا أن شتلات الهجليج (*Balanites aegyptiaca*) لم تصب بهذه العزلة. بينما أظهرت عزلة الفيكس الأعراض في نوعين فقط من شتلات الأشجار هما الفيكس (*F. nitida*) والكافور (*Eucalyptus citriodora* Hook.).