Characterization of *Nattrassia mangiferae* (Nattrass 1933) isolates from different sources

A dissertation Submitted in Partial Fulfillments of the Requirements for BSc- (Honours) in Botany

By

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DEDICATION...

TO MY WONDERFUL GREAT MOTHER AND FATHER
BROTHERS AND SISTERS...
TO ALL MY FRIENDS....
Acknowledgements

I wish to thank the teaching staff at the Botany Department, for their patience, advising, and inspiration. Their gentle but firm directions have been mostly appreciated. Dr. Rashid Ibrahim M. A. Mobukker, my supervisor, was particularly extremely helpful in guiding me towards a progressively improved research methodology and for his confidence in my abilities, not only to complete the dissertation and achieve my B.Sc. degree, but to complete it with excellence.

I have found my course-time to be stimulating, thoughtful, and inspiring providing me with the tools with which I can explore both past, present, and future ideas and issues.

May Allah bless them all...
ABSTRACT

Results of the survey which were summarized in Table 1, show that, branch wilt disease caused by Nattrassia mangiferae is geographically widely distributed covering almost all the area of Khartoum State and a considerable portion in Wad Madani town in the Gezira State. The disease occurs within a wide range of host plants covering various species located under different families of flowering plants. Thus over 10 families were found to comprise as many as 19 species were affected. These families include Moraceae, Meliaceae, Fabaceae, Anacardiaceae, Myrtaceae, Combretaceae, Bombaceae, Mimosaceae, Bignoniaceae, Balanitaceae, Caesalpinaceae, and Rutaceae. Among these families, Moraceae proved to have the highest frequency of infection especially within the genus, Ficus, e.g. F. bengalensis, F. nitida, F. religiosa and F. benjamina. The family Meliaceae comes second in frequency of infection especially within K. sengalensis and A. indica. The frequency of epidemic on Rutaceae and Anacardiaceae was also quite high.

On the other hand, no symptoms of branch wilt disease were detected on some tree species, namely Eugenia jambolana (Myrtaceae), Tamarindus indica (Caesalpinaceae), Cordia africana (Boraginaceae), and Peltophorum inerme (Caesalpinaceae). These were in fact found to be highly resistant, almost immune, when tested under glass house conditions (see appropriate section).
مستخلص الأطروحة

نسبة لأرض العذول الفرعي (الموت الرجعي) في الأشجار المتسبب عن الفطر ن. mangiferae الجزيرة، فإن الاهتمام بـ "قد تزيد من قبل الباحثين على مستوى السودان وخارجية وعلية في اطار ال从来不 الاهتمام اجري يرا البحث للمساهمة في الجهود المبذولة لدراسة هذا الوباء بغرض محاصرته والقضاء عليه. وقد شمل البحث المحاور التالية:

- مسح عينات عشوانية من النباتات التي اظهرت اعراض المرض (الذبول الفرعي).
- تشغيل اللحاء، وجود طبقة سوداء تحت اللحاء تمثل جراحي الفطر المسبب للمرض.

العينات هي شجرة تلادي وشجرة من المشتل Adansonia digitata Moringa peregrina Millitia pinnata هي من شارع النيل، وشجرة من المشتل.

ويتم تنمية الفطر في وسط من PDA وبعد عملية التزريع يتم متابعة نموه في عزله من الفطر وسجل زاك في شكل قراءات لعدد من الأيام ولوحظ سرعة النمو والانتشار لكل عزله من الفطر كان واضح أن هناك اختلاف في سرعة النمو والانتشار بين عزلات الفطر المبرمج.

 وتشجع عينات عشوانية من النباتات التي اظهرت اعراض المرض (الذبول الفرعي).
- تشغيل اللحاء، وجود طبقة سوداء تحت اللحاء تمثل جراحي الفطر المسبب للمرض.

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1. Introduction and Literature Review

The Sudan covers an area of about one million square miles, about 250 million acres. One third of this area, i.e. about 84 million acres, is suitable for agriculture. This area represents about 46% of the cultivable area in the Arab world. Of this area, only about 10% is actually under cultivation, in addition to about 72 million acres as forests and natural pastures. On the other hand, water resources in the Sudan are quite diversified, including under-ground water, lakes, and rivers. Rains remain the most important resource, providing about 1000 milliard cubic meters, which are feeding rivers and their branches in the country. These facts, if seriously considered, clearly reflect the promising future for the Sudan in the field of conservation and development of crop and tree plantations.

Approximately 20% of the world’s agricultural production is lost each year. Drought, floods, wind, hail, frost, pests and diseases are all included in the causes, but at least one – fourth of the loss represents the toll taken by plant diseases, which attack crops in the field or their product in transit and storage.

Although field crop diseases have always had the priority when considering plant diseases, there are a number of forest, wood timber, orchard, and ornamental plant diseases of international importance, which have been experienced by people through different decades of man life. Bacteria, fungi, viruses, nematodes have been the major inciting agents causing widely distributed and varied types of diseases within these groups of plants. For example

Swollen shoot fires found in 1936, is a virus disease of cocoa, which is apparently restricted to West Africa. It can reach epidemic proportion and in parts of West Africa has caused abandonment of many acres of what were once
productive cocoa plantations. It has been estimated that some hundred million trees have already died, and several millions have been eradicated in an effort to control the spread of the disease (Posnett, 1947).

1.1. Fungal diseases

Diseases incited by fungi are the most widely distributed with diversified numerous types of hosts, there are about 8000 species of fungi capable of inciting serious diseases to the plants; the genus phytophthora occupies a considerable site between all other genera of fungi, due to its severe effects on its already wide range of hosts.

1.1.1. Foot rot of citrus caused by *phytophthora citrophthora*, although *P. parasitica* is frequently isolated from foot rot lesions, is a worldwide disease in distribution, causing a series damage to citrus production on an international basis. This disease is known by many other names, including gummosis and mal digomma. Pod rot of cocoa (*Theobroma cacao*) is another well-known disease caused by *Phytophthora palmivora*. This disease was first reported in Trinidad in 1727. It is common throughout the American tropics and occurs essentially elsewhere wherever cocoa is grown commercially. *P. palmivora* is also causing blight to rubber tree (*Hevea brasiliensis*). It has been reported from essentially everywhere rubber is cultivated, (Longford, 1945).

1.1.2. Dutch Elm disease, caused by *Ceratostomella ulmi* from the Ascomycetes, was first described in Holland (1921), where it rapidly decimated elm plantings. It was recorded in relatively rapid succession in many parts of Europe and Asia. It was first found in the United States in Ohio in 1931 and Canada in the province of Quebec in 1944. A large number of species of Ulmus are hosts (Walker, 1957).
There are numerous species of the homobasidomycetes, which are associated with one or another decay of trees. One of the most common and destructive heart rots is that known as white trunk rot, white spongy rot, or white rot. It is caused by *Fomes igniarius*; this disease is world wide in distribution and occurs on many species including poplar, Maple, Oak, birch, beach, apple and pear. In many regions where aspen is important in paper production, the losses sustained from white heart rot are of major significance, (Wallker, 1957).

While most of the agarics or gill fungi, are saprophytes, one species, *Armillaria mellea* is a wide spread incitant of root rot of forest and orchard trees. It is pathogenic on many other plants e.g., it can incite dry rot of potato tuber, (Wallker 1957).

1.1.3. Branch wilt disease or dieback caused by the imperfect fungus *Nattrassia mangiferae* Nattrass (syn: *Hendersonula toruloidea* Nattass 1933) is another severe disease of international importance. *N. mangiferae* was first described by Nattrass 1933 in Egypt, producing dieback to deciduous trees. Since then it has been the focus of concern in many parts of the world. It is universally widely distributed resulting in heavy losses in citruses (Calavan, Wallace, 1954), grapevines (Wangikkar, Raut and Gopalkrishna, 1934) and can therefore render their cultivation uneconomic. Beside citruses, the fungus was also universally recovered from more than 30 tree species belonging to different botanical families (Ahmed and Yassin, 1992).

The disease was first described in Sudan by Giha, producing dieback to the banyan tree (*Ficus bengalensis*). Trees appeared to collapse suddenly, with all their branches wilted and their leaves withered and shed. In banyan trees that were not quite dead, only individual branches were affected. The most characteristic
symptom of the disease appeared on branches of all sizes, but not on trunk. The bark was systematically cracked and peeled off to expose a black sooty crust beneath (Giha, 1975).

Ahmed and Yassin (1992) conducted an organized survey in Khartoum state and the central region of the Sudan in order to determine the host range of the disease among orchard, shade, wood timber and ornamental trees. Such survey specially covered a big scheme of a mango cultivation of some 200 acres a few kilometers north of Khartoum, at al Faki Hashim. Results of the survey confirmed that branch wilt is present in different hosts in several regions in the Sudan. Among 29 plant species examined during the survey, 26 were found to be susceptible to the disease and they all exhibited more or less typical symptoms. The host range found in this study is in broad agreement with that reported by other authors in different parts of the world. The percentage of infected mango trees (*Mangifera indica* L. cv. Kitchner) at all Faki Hashim Orchards, was estimated as 40%. The authors recommended that future investigations might include the following:

- Extended survey relative to tree species and locality:
- To define geographical distribution of the disease
- As well as allocating resistant tree species.
- Investigation the probability of various physiological races on the basis of extended pathogenicity and etiological tests
- Design a wide plan an integrated control strategy on the light of such biological and ecological investigation.
The objective of the present study

The objective of this investigation is mainly confined to the following:

Define possible races of *Nattrassia mangiferae* (Nattrass) in selected locations in central campus of University of Khartoum and compare that with previous findings.
2. Materials and Method

2.1. Collection of the samples:

Samples used in this study were infected bark pieces, which were heavily covered with black sooty layer representing spores of the causal pathogen. The samples were collected from different infected trees within selected locations in the central campus of the University of Khartoum, using along metal stick having a hook in one of it ends. The samples were put in polyethylene bags and kept inside the laboratory at room temperature (25 – 30 °C). collection of the samples was carried out in June 2012.

2.2. Isolation of *N. mangiferae*:

To isolate *N. mangiferae* from the collected bark samples, they were cut into small segments of about (1 – 2 cm) length each. The segments were then the surface-sterilized for three minutes using 2% solution of Clorox and then quickly washed in five changes of sterilized distilled water. The segments were dried on sterilized filter paper and plated in sterilized glass Petri dishes containing potato dextrose agar (PDA) medium, using sterilized forceps with ethanol 95%. Three Petri dishes were prepared for each bark sample.

PDA medium was prepared by suspending 39.5g of the medium in a liter of distilled water, boiled until completely dissolved and then autoclaved at 121°C under 15 lb/in for 15 minutes. The cooled medium was poured into sterilized dishes each containing 20 ml of the medium. Each Petri dish was containing 5 samples of the infected bark segments. Plates were then incubated at room temperature for 7-9 days.
2.3. Examination and identification of colonies:

The colonies of *N. mangiferae* were examined stereoscopic binocular microscope with magnification of up to 50 which helped in observation of the developing growth of the colonies on inoculation medium.

The colonies developed were identified and their frequencies were recorded. The identification was facilitated by preparing temporary and permanent slides using lactophenol as mounting medium. The slides were examined by a compound microscope. The isolations were identified according to their culture characteristics on PDA and other microscopic features using identification key of Barnett and Hunter (1972).
3. Results and Discussion

Isolation of *N. mangiferae*

*Natrassia mangiferae* was invariably associated with infected barks from different sources when cultured on PDA. Thus, a total of five isolates (races) were recorded from these sources, which were differing in their growth rate, macroscopic features and in some of their microscopic features. However, detailed investigations, 3-9 days after inoculation, revealed that the two isolates from *Mellittia pinnata* were more or less identical and thus considered as one isolate designed as race 1, and the rest were designed as race 2, race 3, and race 4 (Table 1; Plates I-IV).

Race 1 (two isolates) (Botany Department Nursery)

Colonies fast growing filling the plant (9 cm) after 3 days on potato Dextrose (PDA), at a (25-30) (Figure 1), texture, floccose, begin as white mycelium change gradually to dark green from the middle to wards the edges then to dark grey-green, then to brownish-grey and finally after 7-9 days become blackish brown in the middle, changing to brownish grey at the edges (Table 1; Plate 1), septate simple or branched segmented to short or long chains of conidia conidia spheroid, ovoid, oblong-cylindrical 0-1 septate, hyaline changing to citrine to dark brown in colour when mature, some are thick walled.

Race 2 (one isolates) (Nile Avenue)

Colonies fast growing attaining 9 cm after 4 days on PDA at temperature of (25-30 ºC) (Figure 2), texture granular beginning as white then turn into dark green intermixed with white and finally after 7-9 days, become darkish (Table 1; Plate
2). Hyphae large and small septate, branched, spores sometimes very long chains containing variable shapes, conidia oblong cylindrical.

**Race 3 (one isolate) (Nile Avenue)**

Colonies very slow growing (Figure 3); texture granular; begin as white then turn into green and continue until the end of plate (Table 1; Plate 3). Hyphae septate, simple and branched, spores in chain or single oblong cylindrical septate.

**Race 4 (one isolates) (Botany Department Nursery)**

Colonies fast growing, slightly differs from race 1, filling the plate (9 cm) after 3 days on (PDA) at (25-30 °C) (Figure 4), texture, floccose, begin as white mycelium change gradually to dark green from the middle to wards the edges then to dark grey-green, then after 7-9 days become blackish (Table 1; plate 4); Hyphae septate simple or branched segmented to short or long chains of conidia; conidia spheroid, ovoid, oblong-cylindrical 0-1 septate, hyaline changing to citrine to dark brown in colour when mature.

**Table 1: Macroscopic characteristics of N. mangiferae isolates**

<table>
<thead>
<tr>
<th>Race no.</th>
<th>Source of Race</th>
<th>Location</th>
<th>Colony Texture</th>
<th>Colony Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Melittia pinnata</em></td>
<td>Nursery Bot. Dep.</td>
<td>Floccose</td>
<td>blackish</td>
</tr>
<tr>
<td>2</td>
<td><em>Adansonia digitata</em></td>
<td>Nile Avenue</td>
<td>Granular</td>
<td>dark greenish</td>
</tr>
<tr>
<td>3</td>
<td><em>Adansonia digitata</em></td>
<td>Nile Avenue</td>
<td>Granular</td>
<td>dark greenish</td>
</tr>
<tr>
<td>4</td>
<td><em>Moringa peregrina</em></td>
<td>Garden Bot. Dep.</td>
<td>Floccose</td>
<td>blackish</td>
</tr>
</tbody>
</table>
Figure 1: Growth diagram of race 1

Figure 2: Growth diagram of race 2

Figure 3: Growth diagram of race 3

Plate 1: Colony of race 1 on PDA

Plate 2: Colony of race 2 on PDA

Plate 3: Colony of race 3 on PDA

Good results
In culture isolate using PDA in the usual, *N. mangiferae* has always been the most predominant of all other fungal species isolated. It was thus invariably associated with all of the infected bark samples of various tree flora from different sources. During such isolation tests, were present the description of race, initially obtained from an infected bark of *...,* as reported herein, are in agreement with those reported by other investigator both locally and exotic e.g. Nori (1996) and Wilson (1949), respectively. The other three races were less predominant; differing from each other and from race mainly in their macroscopic characteristics e.g. shape, texture, colour of the colonies, and growth rate. The above mentioned results of such macroscopic features as regards all 4 races.

The spheroid and ovoid spores were more abundant in the middle mature growth of mycelium; whereas those oblong-cylindrical ones were more so in termini of the growing mycelium, thus it seems that those oblong and cylindrical spores change to spheroid and ovoid ones when mature. As regards colour of the spores it ranged from hyaline to subhyaline to pale-dark brown, without sharp differences between the isolates, except in isolate 3 which exhibited relatively dark colouration.

In nature spores of *N. mangifera* are produced in chains on a basal plate of fungus tissues. Though Nattrass did not follow the development of these plates, he believed that they later enlarge into stromata which produce pycnidia. Thus, two
years after isolating the fungus Nattass found its pycnidial stage in nature (Wilson, 1949).
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