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First record and Disease Management of PinkRot in Cocos Palm Trees in Egypt

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ABSTRACT

A new fungal disease of cocos palm (Syagrusromanzoffiana) "fungal Pink Rot of Inflorescence" was found in 15May city, Cairo in Egypt in the summer of 2014. Symptoms included chlorotic and necrotic leaves (dead tips), light-brown spots (1 to 2 mm in diameter) on the leaves and rachis, rot of the rachis, sheath, and trunk, and eventual death of infected plants. A pinkish-orange layer formed both on the surface and within the infected tissues. Morphological, biochemical and Molecular studies of fungus revealed that it belongs to the species Nalanthamalavermoeseni. The recommended concentrations were application. Three applications with Copper oxychloride, Topsin, Bio-Cure-F were used. Copper oxychloride and Topsin were highly effect than Copperoxychloride and Bio-Cure-F against the fungal pink rot.

Key words: Cocospalm tree, *Syagrusromanzoffiana*, Egypt, Pink rot, *Nalanthamalavermoeseni*, *Biofungicides*, *Fungicides*.

INTRODUCTION

The cocospalm tree, *Syagrusromanzoffiana*, is one of the most popular palms in tropical and subtropical climates because of its beautiful appearance and low maintenance[1]. This palm is very inexpensive. The cocospalms provide soft filtered sunlight perfect for shade gardens. It is also worth mentioning, that cocos Palm tree has a shallow root-base and is known for falling during high winds and hurricanes. The cocos palms attacks numbers of pests[2,3,4] .The pests that cause problems for cocos Palms are the fungus *Nalanthamalavermoeseni*(previously called *Penicilliumvermoeseni* or *Gliocladiumvermoeseni*) causes the disease pink rot. The result of a weak but opportunistic pathogen, pink rot primarily is a secondary disease that affects stressed or weakened palms. While it can attack all parts of a palm, it is most problematic in the growing tips, or apical meristem where new leaves are produced, and in newly emerged leaves.

Pink rot affects nearly all outdoor landscape and indoor palms in Egypt. A variety of symptoms characterizes pink rot, including spotting and rotting along rachis and leaves; rotting along leaf tips, leaf bases and trunks; and stunting and distortion of new leaves. Trunk rot is possible in some cases. Pinkish spore masses, from which the disease derives its name, often are present. A brownish syrupy fluid also might be present. Infected plants weaken and decline and eventually can die. In 1923, Biourge named and described the pink rot fungus as a flesh, rose, or salmon-colored fungus which in Belgium had injurious effects on Areca palms grown in greenhouses[5]. By 1924, Chevalier reported the same fungus to cause death to *Archontophoenixcunninghamiana*, *Howeaforsteriana*, *Washingtoniafilifera* and *Washingtoniarobusta*[6]. Much of this early history of the disease is summarized by [7]. Discussions of the disease lay dormant for many years until Feather and others, 1989, described pink rot as part of a disease complex along with *Fusariumoxysporum* on *Phoenix* palms. There has been little or no work on the disease since published their research[8].

The fungus causes an interesting variety of diseases. On Queen Palms (*Syagrus* spp.) it causes a trunk rot. Sometimes the spores of pink rot can be found under the pseudobark of the palm. In King Palm (*Archontophoenixcunninghamiana*), spores of the fungus can almost always be found under the clasping leaf bases. When injured (by premature removal of the leaf bases or by pruning wounds) the fungus can cause a trunk rot and death of the tree. The aim of this work was to control and evaluation the efficacy of some of the biofungicides and chemical fungicides against Pink rot.

MATERIALS AND METHODS

Collection of specimen

The experimental were carried out at 15 May city, Cairo, Egypt. All cocos trees spared at the outlet and opening of the city for beautification. The cocos trees were 4 years ago .The affected spadics and the axis were collected from the cocos palm tree and brought to the Plant Pathology laboratory, National Research Centre for further studies. Almost all the spadics present in palm trunk tree were remained unopened with light-brown spots.

Symptom studies

The spadices containing brown spots was washed thoroughly with sterilized distilled water, surface sterilized with 0.5% sodium hypochlorite for 2 min and then rinsed with 3-4 washings of sterilized distilled water. The spathe was removed with the help of sterilized scalpel to observe the flower condition.

Isolation of the pathogen

Inoculum from infected spadics was scratched and streaked on Potato Dextrose Agar (PDA) and incubated at 25 °C. Also the pink colored mucilaginous substance was observed under microscope and it was found to be a fungus, the mucilaginous material was also streaked on Nutrient Agar (NA), incubated for 48-72 h at 30 °C. The pink colored colonies on both the media were separated and purified[9,10,11].

Pathogen identification

For morphological observations, Czapek yeast extract agar (CYA), oatmeal agar (OA), creatine sucrose agar (CREA) and MEA were used. The isolates were inoculated at three points on each plate of each medium and incubated at 25 °C in the dark for 7 days. For microscopic observations, microscopic mounts were made in sterile distilled water from PDA or MEA colonies. The species characteristics such as colony colours, the structure of conidial heads and the shapes of conidia were observed and identify according to [12].Fungal mycelium or spores were cultured on potato dextrose agar medium and used for DNA isolation. DNA was extracted using method described by [13,14].

Formulations used

Three formulations used, Copper oxychloride (300 gm/100L) spray the tree heart &Topsin(5gm/tree) soil treated and Bio-Cure-F (*Trichodermaviride*) Bio-Cure-F is a biological preparation based on the naturally occurring antagonistic fungus *Trichodermaviride*,it contains spores and mycelial fragments of *T. viride* @ 1 x 10^9 CFU's / ml .The product was Soil application: 2.5 liters in 500 kg Organic fertilizers . Each tree treated with 5.0 kg in soil.

Field experimental

Thirtycocos palm trees infestation selected were used in each treatments. Each ten trees were treated with copperoxy chloride spray and topsin or Bio- Cure – F in soil .The ten other trees leave without treatment as control . Ten replicates were carried out. (Each cocos palm tree was replicate). Three application used , each 15 days one application , sample from each tree before application and after application by 10 days for counted the fungal spores in each tree section (25x25 cm^2). The reduction in the numbers of spores was calculated with [15].

Statistical analysis

The data were statistically analyzed using one- way analysis of variance (ANOVA) and comparisons were made based on Duncan ' s new multiple range test (computer program SPSS, 11, 2011).

RESULTS AND DISCUSSION

Disease Symptoms and Pathogen identification:

Disease symptoms are pink rot , spotting and rotting and stunting and distortion of new leaves and affected most of indoor palms trees. Infected plants weaken and decline and decay (Fig.1).

Sporodochia and conidia formed by *Nalanthamala* , growth rates of colonies, pigmentation of conidial masses and pigmentation of colonies are similar characters formed by the type of *Nalanthamala* [16]. conidiophores arising

from aerial mycelium forming heads of ellipsoidal to fusiform conidia. Mean conidial dimensions were 3.5 (± 0.2) × 5.5 (± 0.2) µm .

The ITS rDNA of *N. vermoesenii* strains was identical. A BLASTn search of GenBank with sequences of rDNA ITS (JX456472 to JX456474) revealed 100% identity of three isolates to that of *Nalanthamala vermoesenii* (Biourge) Schroers, comb. nov. [syn. *Penicillium vermoesenii* Biourge; *Gliocladium vermoesenii* (Biourge) Thom] originating from several palm species in Spain, the Czech Republic, and the United States (GenBank AY554212 to AY554217). So, our tested of morphological and molecular characteristics revealed that the fungus recovered from symptomatic palm trees was *N. vermoesenii* .

Palms (Arecaceae) trees are important tree and affected by a many of pathogens in Egypt, most of which are fungi e.g. fusarium wilt (*Fusarium oxysporum* f.sp. *canariensis*) of canary island date palm (*Phoenix canariensis*), diamond scale (*Phaeochoropsis neowashingtoniae*), ganoderma butt rot (*Ganoderma zonatum*), lethal yellowing (*Candidatus* Phytoplasma palmae subgroup 16SrIV-A), and diseases caused by *Nalanthamala* (*Gliocladium*). *Nalanthamala* (*Gliocladium*)[17].

The pink rot disease of palms is perhaps the most ubiquitous palm disease in the landscape [18]. It is caused by the fungus *Gliocladium vermoeseni*, (*Pennicillium vermoeseni)* a member of the fungi imperfecti. The perfect stage of the fungus is not known. The biology of the pink rot fungus is amazing in that it can produce billions of spores while growing on a single plant. Thus its spores are always present where palms are grown so there is no way to avoid it. The fungus is a weak pathogen in that it requires a wound or other plant stress factors that enable it to infect. Pink rot also preys on old specimens that are growing slowly and become susceptible due to reduced growth rates of the main bud. It appears to be associated with palms growing in humid coastal areas. Although it can occur in inland valleys, it is more prevalent along the coast. The host range of the fungus is large, covering several genera and species of palms.

Data in Table (1) showed thatspraying repetition with Copper oxychloride and treated soil with Topsin each fifteen daysachieved highly reduction than Copper oxychloride and treated soil with Bio – Cure – F (Bio-fungicide).The results showed that three sprays was enough to stopped the growth of spores of *Nalanthamalavermoesenii*fungi. However, the application of Copper oxychloride and treated soil with Bio – Cure – F (Bio-fungicide) was needed to more of three applications. Bio–Cure–F (Bio- fungicides) contained *Trichoderma viride*, 1 x 10^9 CFU's / ml may be need to long period because of gave highly action. The first recorded for infestation was $1x10^9$ spores /µm^2 in each cocos palm tree as mean. However, the region was contained approximately 400 cocos palm trees, widespread in the inlets and outlets of the May city, the percentage of infestation cocos palm trees with the *Nalanthamala vermoeseni* fungi recorded 70%.The cure period was begun by soil treated with Copper oxychloride and topsin as spraying at the selected trees and other 10 trees were treated with Copper oxychloride and Bio – Cure – F as spraying . The 10 trees control leaved without treated . The Copper oxychloride and topsin cocos palm trees showed that the numbers of fungi spores after the first application recorded decreasing in the numbers of fungi spores ($1x10^8$) . The Copper oxychloride and Bio – Cure – F cocos palm trees , the fungi spores reached $1x10^7$ achieving to decrease 42.0%. However, the cocos palm trees without control was increased to achieved $1x10^{12}$ fungi spores. The 2nd treated achieved to reduction, $1x10^2$ (87.6%) and $1x10^6$ (62.7%) in the Copper oxychloride and topsin cocos palm and the Copper oxychloride and Bio – Cure – F cocos palm trees, respectively. The control cocos palm trees reached the numbers of fungi spores to $1x10^{16}$. The 3rd treated indicated clearly that the Copper oxychloride and topsin was highly effect than Copper oxychloride and Bio – Cure – F. Whereas, the Copper oxychloride and topsin achieved 100% reduction (0.0 spores infection) and 73.66 % reduction as average. While, the Copper oxychloride and Bio – Cure – F achieved 75.0% reduction ($1x10^5$) and 59.9 % reduction as average. The cocos palm trees untreated reached the numbers of fungi spores $1x10^{20}$(Table 1&Fig.2).

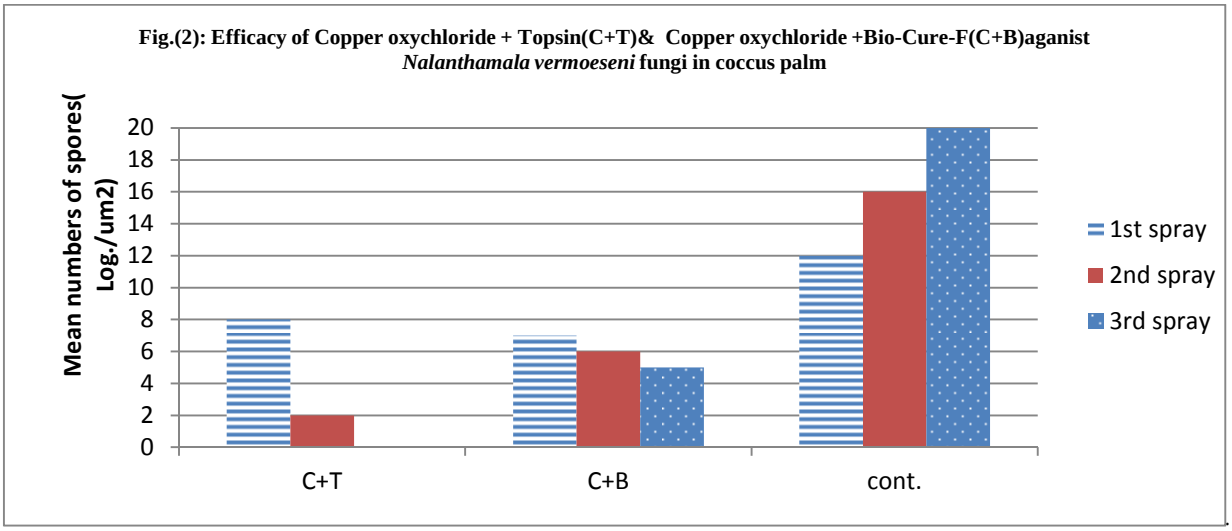
Table (1): Effect of selected formulations against *Nalanthamala vermoeseni* fungi

Formulations	Rate Of application	1st Spray			2 nd spray			3rd spray			Avg. % Reduction
		Numbers of Spores /µm² (the data correct as log.)									
		Before	After	% Red.	Before	After	% Red.	Before	After	% Reduction	
Copper oxychloride +Topsin	3.0gm/l +5.0gm/tree	$1x10^9$ (9)a	$1x10^8$ (8)ab	33.4	$1x10^5$ (5)b	$1x10^2$ (2)c	87.6	1x10 (1)c	0.0 (0.0)b	100	73.66
Copper oxychloride +Bio- Cure-F	3.0gm/l +5.0gm/tree	$1x10^9$ (9)a	$1x10^7$ (7)b	42.0	$1x10^{11}$ (11)a	$1x10^6$ (6)b	62.7	$1x10^7$ (7)b	$1x10^5$ (5)b	75.0	59.9
Cont.	0.0	$1x10^9$ (9)a	$1x10^{12}$ (12)a	-----	$1x10^{14}$ (14)a	$1x10^{16}$ (16)a	----	$1x10^{18}$ (18)a	$1x10^{20}$ (20)a	-----	-----
F		0.7un	3.4un	------	11.4un	57.7*	----	92.9*	36.1*	----	-----
LSD 0.5		4.13	4.3	-------	3.4	2.9	----	2.75	5.3	----	-----

Means within columns followed by the same letter are not significantly different.

Fig.1. Symptoms of pink rot of *cocos palm tree* caused by *Nalanthamala vermoesenii*.
Symptoms of naturally infected *Washingtonia* trees. A. B, Rachis and sheath rot with a pinkish layer on surface and interior of infected tissues. C, D.,Trunk rot and decay. E and F, Rachis decay, chlorotic and necrotic leaves. The three control plants at the left of panel G are healthy, whereas the three at right are symptomatic

Fig.(2): Efficacy of Copper oxychloride + Topsin(C+T)& Copper oxychloride +Bio-Cure-F(C+B)aganist *Nalanthamala vermoeseni* fungi in coccus palm



In general pink rot disease can attack all parts of a palm, it is most problematic in the growing tips, or apical meristem where new leaves are produced, and in newly emerged leaves. Several methods are used for diseases management[19]. The best strategy for diseases managing these diseases is an integrated pest management approach that combines prevention, appropriate species selections, and proper care in order to avoid most diseases and or to minimize pruning if possible. Biological control can be used for minimize the chemical use. The best way to disease-management system by the use of integrated disease-control management [20,21,22].

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