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THE FIRST RECORD OF CANKER FUNGUS(*NEOSCYTALIDIUM DIMIDIATUM*) IN *MOURS ALBA* L. AND ITS DIAGNOSIS BY USING PCR IN KARBALA, IRAQ

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Abstract

The sooty canker disease was studied in some orchards of the Hosseinieh district/ Karbala province which caused by the fungus *Neoscytalidium dimidiatum*. The results of investigation revealed that all *Mours alba* L. trees were infected but in varying proportions, Pathogenicity test results showed canker on the seedlings two months after the pollination process the length of the canker was 1.43cm and significantly different from comparison treatment the pathogen was isolated and diagnosised based on their morphological, molecular, and pathogenicity. Both Zinc oxide (ZnO) alone and the Zinc oxide (ZnO) nanoparticles were evaluated against the pathogen in laboratory and plastic house. This first record of *N. dimidiatum* affecting *Mours alba* L. trees in Karbala city, Iraq. The ZnO-Nanoparticles and ZnO showed an effective role in full inhibition of the pathogen growth in PDA caused significant inhibition to mycelium growth at 5 $g.L^{-1}$ ZnO-Nanoparticles and 10 $g.L^{-1}$ ZnO Furthermore, reduce significantly to canker development on *Mours alba* L. seedlings tested which superiority of ZnO-Nanoparticles in 5 g/L^{-1} concentration, for the overall length of canker 0.39 cm, in contrast, while high significant from the ZnO which recorded a canker length 0.5 cm with a comparison among treatment and negative control which recorded 1.98 cm long.

Key Words: *Mours alba* L.¡ *Neoscytalidium dimidiatum*, Nanoparticles Zinc oxid, Iraq.

Introduction

The genus *Mours alba* L. (Moraceae). *M. alba* is one of the most urbane species in Iraq. Mulberry fruits are used fresh or dried or manufactured and desirable because they contain high nutritional value) AL-Dawoody, 1979). The fungus *Neoscytalidium dimidiatum* (Penz) attacks many of economic trees such as apples, grapes, palms, citrus fruits, bananas, casuarina and mulberry (Godoy *et al.*, 2004 and eucalyptus (AL-tememe *et al.*,2019) it one of the most important causes of disease wilt branch of poplar (AL-Murad, 2005).

The fungus *N. dimidiatum* infects the cambium causing the canker of stem because of grows under it. Finally, causes the dieback and the wilting as a result of secretion of mycotoxins(AL-tememe *et al.*, 2019).

Nanomaterials are one of the most important techniques currently used for their important role in controlling many plant diseases) Ditta, 2012.

Although the fungus was recorded on many plant families, it was not recorded on mulberry tree in Karbala province/Iraq, the symptoms were the presence of a brown-to-black space under the bark with cankers and death of the branches.

Due to the economic value of mulberry trees in the country and lack of research carried out in the area of study. Such study of this fungi is important to reduce the damage and economic loss for both commercial and industrial value. We determined to do this study to identify the source of the infection and molecularly diagnosed.

Materials and Methods

The survey of trees

A survey of degraded *Mours alba* L. trees, which

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included three orchards in Al-Husseiniya- City of Karbala, Iraq, April 2018. The disease was detected via cankers located on the stems of trees.

Infected trees are classified based on evidence of the severity of degradation according to the method (Large,1969). The severity in the trees was calculated using following equation:

Infection intensity =

$$\frac{\text{No. of trees category 1} \times \text{Repetition} + \ldots + \text{No. of trees category (5)} \times \text{Repetition}}{\text{Total Number of trees} \times \text{Top category index}}$$

The proportion of infection in the field surveyed trees use the following equation:

$$\text{Infection \%} = \frac{\text{Infection trees No.}}{\text{Total No.}} \times 100$$

Isolation

Isolated from infected *Mours alba* L trees on PDA plus of streptomycin sulfate (an antibiotic) at a ratio of 50 mg. L^{-1}. Then the dishes were put in an incubator at 25-27°C for five days.

PCR

The fungus was detected molecularly using PCR, and the nucleotide sequence determined in the Lab. of plant viruses, University of Kerbala, Faculty of Agriculture, Plant Protection Dept.

The gDNA of the fungal colony was obtained utilizing the recommended DNeasy Plant Mini Kit from QIAGEN N.V (Hilden, Germany). The 2 primers ITS1 and ITS4, were used to get ITS DNA as described by White k& el at 1990. The PCR Beads kit (ready to go), was broken in 25µl solution containing beads and 1µl of each primer (5 pmol) in adding to 2 µl (50-100 ng) of template DNA (GE Healthcare, Illinois, USA). The PCR outcome was sequenced at Macrogen, Inc. (Seoul, South Korea). The sequences result were compared with other available ITS-rDNA sequences of fungi at GenBank, NCBI applying for the BLAST program. Then, the phylogenetic analyses of the sequences were performed using MEGA6. (Tamura & *el at.,* 2013) The produced sequence was deposited to GenBank database with a unique accession number.

Test of the Pathogen Capacity:

The study was done in the polyethene agriculture house, Faculty Agriculture, University of Kerbala. The healthy seedlings brought of *Mours alba* L. at age of 24 months'.

Infection was fungus growth on the PDA with of streptomycin sulfate (an antibiotic)at a ratio of 50 mg. L^{-1}. Then the dishes were put in an incubator at 25-27°C for 7 days.

The healthy seedlings were prepared of a wound in the tree bark region by cork borer of a 5 mm diameter in the tree bark region, the bark was taken out with a sterile needle (Filer, 1968). Three branches of each seedling were chosen.

Afterthat the wounds were inoculation, covered with wet cotton(sterile) closed and tied by tape (Parafilm). Each treatment contained of 3 replications. Results were analyzed by calculating the medium increase of length canker was taken after 60 days from inoculation.

Pathogenic fungus was isolated again from the seedlings after infection to confirm Koch's hypotheses.

Control : of the pathogen *N. dimidiatum*:

Effect of concentrations of ZnO and ZnO nanoparticles In the diameter development of. *N. dimidiatum* in the lab:

In the study of the impact of commercial ZnO nanoparticles were obtained from Canadian MK Nano (Mississauga, Canada) against the development of ***N. dimidiatum*** using the poisoned medium method, as described earlier (Jiseon *et al.*., 2009). after mixing the ZnO nanoparticles with the potots dextrose agar medium (PDA), used 0, 2.5,5 $g.L^{-1}$ and with concentration 0, 2.5, 5 $g.L^{-1}$ mixing enough to fully melt in the medium before hardening, was added streptomycin sulfate (antibiotic) at a rate of 50 $mg.L^{-1}$ painted on the base from the outside sterile Petri dishes by the fixed pen two orthogonal diameters after that poured PDA, each treatment included 3 replicates, all dishes were put in the an incubator at 25 -27°C.

The following percentage was determined for the inhibition of fungal spore growth:

After the pathogenic fungus reaches the edge of the dish after the pathogenic fungus reaches the edge of the dish the inhibition percentage was calculated according to the following equation:

$$\text{\% Of the growth inhibition} = \frac{\text{Average of control colony diam. - Average of treatment colony diam.}}{\text{Average of control colony diameter}} \times 100$$

Control in the polyethene agriculture house

The mulberry seedling was inoculated as in the pathogenicity test and parafilm was uninvolved after 3 days from inoculation the and the wound regions were

treated with ZnO nanoparticles at a concentration of 5 $g.L^{-1}$ and 10 $g.L^{-1}$ concentration with ZnO until full wetness sprayed the control treatment with water only, The experiment was executed using with 3 seedlings each treatment, Experiment carried out length canker in mulberry seedling was calculated after 2 months of treatment.

A simple experiment was carried out using CRD statistic method, Data were analyzed statistically. Using the method of the least significant difference, the averages were compared (LSD) test, at probability level 5%.

Results and Discussions

Survey of mulberry trees on the field:

The results of mulberry trees on the field, in Hosseinieh district in Karbala province/Iraq (Table 1) appeared high deterioration in mulberry trees by100% in the three orchards and high Severity between 91%-93%, a presence of canker was in mulberry trees, the wilting of branches (Fig. 1) The deterioration was due to tree infection by *N. dimidiatum*

Table 1: Proportion and intensity of Mulberry trees

Disease severity (%)	Disease incidence (%)	Regions
93	100	Orchard 1
90	100	Orchard 2
91	100	Orchard 3

Fig. 1: Local type of symptoms on *M.alba* L. trees.

Isolation from mulberry:

The isolation from mulberry stem appeared the frequency was 100% to *N. dimidiatum* fungus (Fig. 2). When microscopic examination was performed, conidia spores were found It is formed after the mycelium fungal is fragmented into arthrospore it has an articular phase. The diagnosis was confirmed using the nucleotide relay technique (Fig. 3).

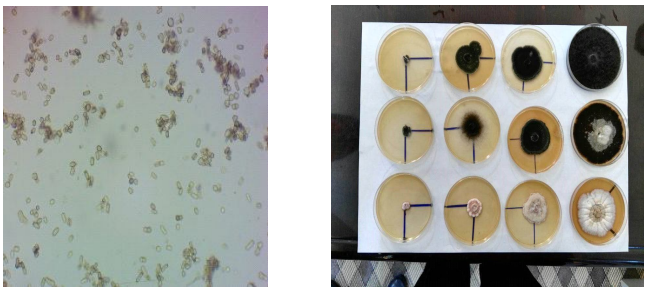

Fig. 2: 1- culture of *N. dimidiatum* on medium.
2- the spores.

Polymerase chain reaction (PCR)

Morphological Identification

To confirm the phenotypic diagnosis of the pathogen, a PCR test was performed on DNA extracted from the *N. dimidiatum* fungus. The complete multiplication of the rDNA-ITS region which is important in diagnosis (White *et al.*, 1990), the sequence was placed in the Genbank database of the National Center for Biotechnology Information (NBCI), which was given the serial code (MK567807.1).

Test of the Pathogen Capacity

seedling mulberry appeared after two months of inoculation (Fig. 4) Symptoms of dark in colour Cankers, after death and Necrotic Lesions the part of tissues, This indicates tissue death and drying of the bark then they die and exfoliate The average length of the canker 1.43 cm, re-isolated the fungus from the infection sites and pure after planting on the PDA, This fungus is recorded on many economic trees such as eucalyptus and apricot (Al-tememe *el at.,* 2019).

Control:

Effect of concentrations of ZnO and ZnO nanoparticles in inhibiting the hyphe growth of *N. dimidiatum* On the PDA:

Table 2 explain the impact of different levels of ZnO nanoparticles was tested (0,2.5,5) $g.L^{-1}$ on the growth of mycelium fungal for *N. dimidiatum* Laboratory significantly in the inhibition process the levels of 5 g. L^{-1} show a high rate of inhibition reached 100% and a

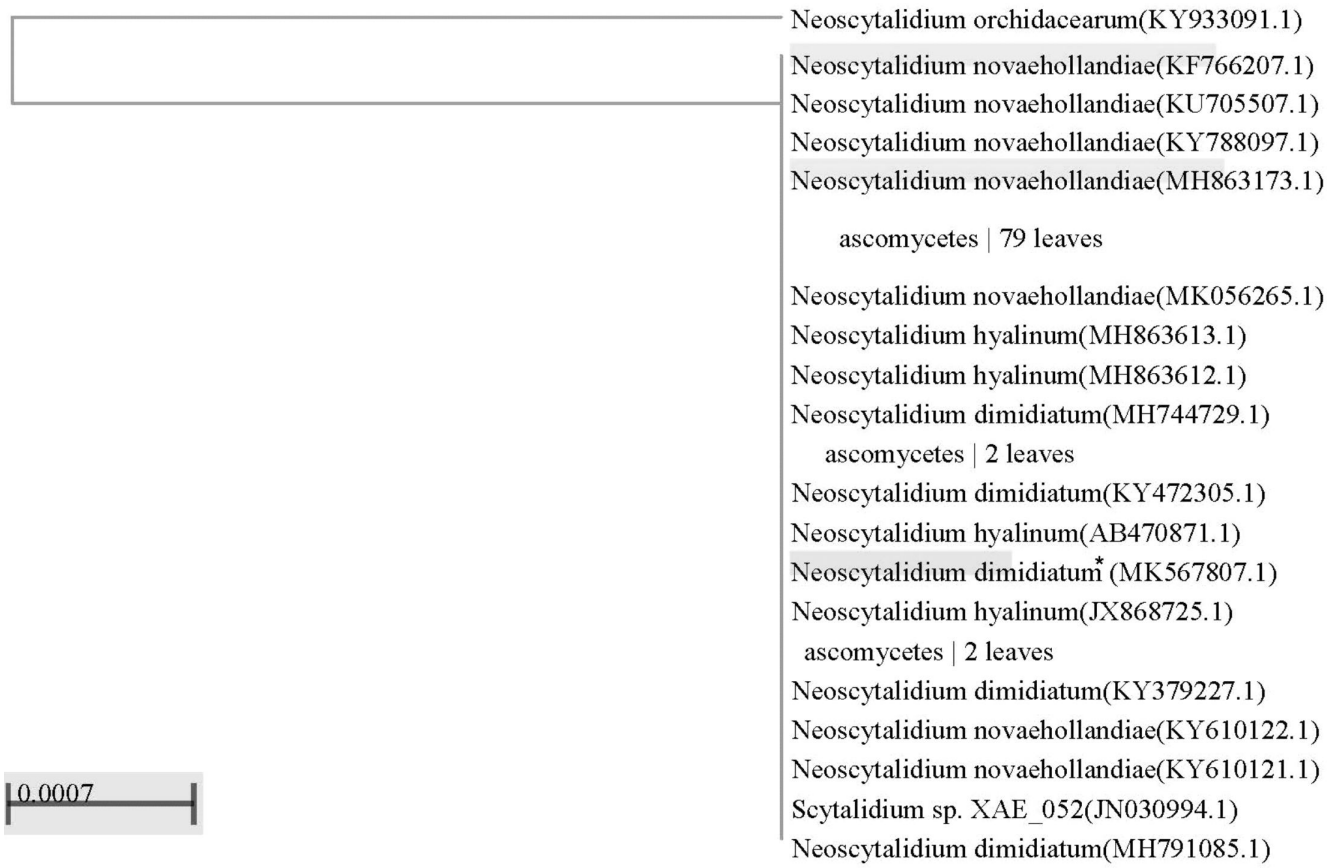

Fig. 3: The genetic tree of fungus *N. dimidiatum* using MEGA 7.

Fig. 4: Canker lesion covered with mycelia of *N. dimidiatum*

concentration of 10 g.L^{-1} ZnO record Inhibition ration reached 100% (Fig. 5).

Control in the polyethene agriculture house

Table 2: Effect of ZnO-Nanoparticles and ZnO on *N. dimiditum*

ZnO-Nanoparticles			ZnO		
0	2.5 mg. L^{-1}	5 mg. L^{-1}	0	5 mg. L^{-1}	10 mg. L^{-1}
0 %	75 %	100 %	0 %	43.1 %	100%

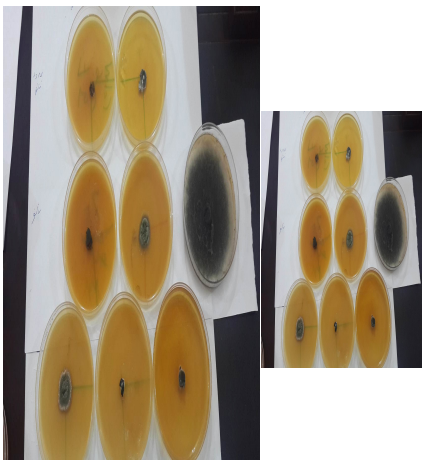

Fig. 5: Influence of ZnO-Nanoparticles mg. L^{-1} and ZnO mg. L^{-1} on *N. dimiditum*

ZnO nanoparticles showed significant superiority in reducing the damage length(canker) which was 0.39cm and deferent of ZnO which showed lower rate on canker length of 0.50cm. Control treatment registered 1.98 cm. (Fig. 6)

Appears from the results mentioned above the role of commercial zinc oxide nanoparticles in inhibition of growth and reduce the length of canker of the stems

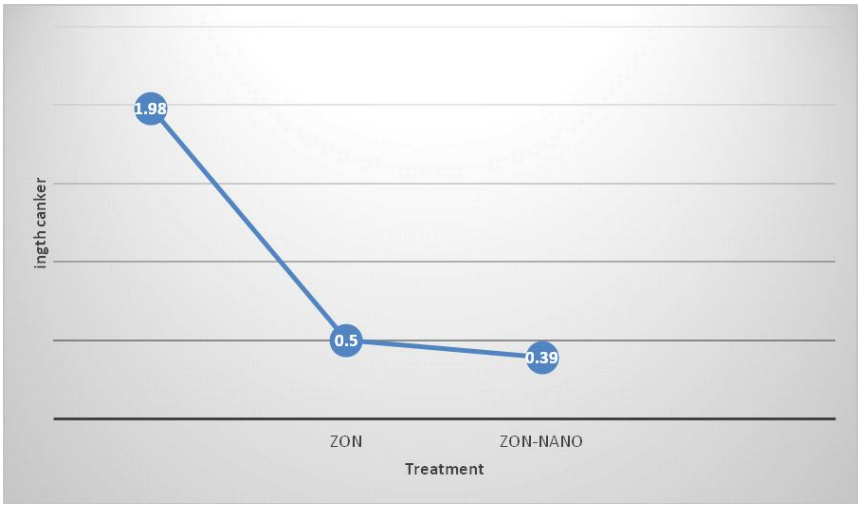

Fig. 6: Figure 5 Impact of ZnO nanoparticles and ZnO control on canker length after 60 days of treatment in the polyethylene agriculture house.

mulberry inoculated with *N. dimidiatum* Nanomaterials inhibited growth the fungi With a wide family range of plants as a result of disruption of cellular functions the result agree with (Al-tememe & *et al.*, 2019) through its role in lowering the pH of the medium by zinc ions and their role in the analysis of water molecules forming zinc oxide ZnOH and increase the concentration of free hydrogen ions responsible for increasing the acidity of the medium (Dimkpa & *et al.*, 2013).

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