Plant Archives Vol. 19, Supplement 1, 2019 pp. 31-38 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

OCCURRENCE, IDENTIFICATION, PATHOGENICITY AND CONTROL OF *NEOSCYTALIDIUM DIMIDIATUM* FUNGUS, THE CAUSAL AGENT OF SOOTY CANKER ON *EUCALYPTUS CAMALDULENSIS* IN KERBALA PROVINCE OF IRAQ

Zeinab A. M. Al-Tememe[1], Adnan A. Lahuf[1], Rajaa G. Abdalmoohsin[1] and Alaa T. Al-Amirry[1]

[1]Department of Plant protection, University of Kerbala, Kerbala, Iraq.

Corresponding Author: Adnan A. Lahuf

Email: adnan.lahuf@yahoo.com

Abstract

The aims of this study were to conduct a survey related to sooty canker disease of *Eucalyptus camaldulensis* trees in Kerbala province, identify the causal agent of this disease and evaluate some of chemical control approaches to combat it. The disease survey conducted in different regions of Kerbala province and the pathogen was isolated and identified based on its morphological and molecular characterizations and pathogenicity. Two chemical material (Beltanol-L fungicide and Salicylic acid) were evaluated against the pathogen in laboratory and plastic house. Results of the disease survey showed that all eucalyptus trees in the surveyed area were infected with different disease incidence and severity. The laboratory results of isolation, identification and pathogenicity of the causative agent indicated that the fungus associated constantly with diseased Eucalyptus trees was *Neoscytalidium dimidiatum*. This identification is the first record of this fungus affecting eucalyptus trees in Kerbala province of Iraq. Furthermore, the fungicide Beltanol-L (1 ml/ 1l) and Salicylic acid(1000 mg/ 1l) showed an effective role in full inhibition ofthe pathogen growth in PDA. These treatments withsame concentrations also reduced significantly the length of canker lesions on Eucalyptus seedlings tested.

***Key words*:** *Neoscytalidium dimidiatum* fungus, sooty canker disease, *Eucalyptus camaldulensis*, Iraq

Introduction

Eucalyptus genius is an evergreen tree belonging to the family *Myrtaceae* and has 700 species, most native to Australia (Doughty, 2000). This tree is one of the world's most commonly cultivated hardwood trees due to a numerous of useful characteristics and benefits. It is for example rapid growing, producing profuse seeds and possessing wide adaptability to grow in ecologically diverse soils and climates. Furthermore, it produces timber, cellulose, paper and perfume (Foelkel, 2009; Nahal, 2003; Pirralhoa *et al*., 2014). In addition to these benefits, the volatile oils extracted of eucalyptus leaves have antimicrobial properties that issued successfully in human health care (Boland *et al*., 1991; Bajaj, 1995; Bachir and Benali, 2012; Akin *et al.*, 2010). These oils are also applied in food preservation and plant protection of fungi, bacteria and insects (Amit *et al.*, 2014; Batish *et al.*, 2008). In Iraq, the Eucalyptus tree has been adopted for afforestation parks, gardens, streets, forest and around cities as windbreaks and for protection of sand storms. Many species of Eucalyptus are cultivated, however, *Eucalyptus camaldulensis* Dehn. and *E. microtheca* Muell are the most common planted species in Iraq (Al-Iryani, 1998).Unfortunately, the eucalyptus trees suffer from a number of pathogens, especially belonging to fungi that cause different serious diseases such as rot of roots and stems, shoot blight and leaf spots and sooty canker (Old *et al.*, 2003).

The sooty canker disease is a common stem disease of eucalyptus occurring in most regions of Iraq and causing significant annual loss. This disease is also the most common diseases that result destructive damages on eucalyptus tree worldwide (Old *et al*., 2003). It is caused by *Neoscytalidium dimidiatum* (Penz.) Crouse and Slippers (Syn. = *Nattrassia mangiferae*) fungus, that has wide range of hosts such as cypress, poplar, willow, oak and citrusmulberry, ash, walnut, fig, sycamore and apple trees (Pasha, 2007; Johnson *et al*., 2002; Hassan *et al*., 2009).

The fungicides are one of the most common methods used to control diverse plant pathogens including those affecting trees. These fungicides control fungal pathogens by inhibiting or killing (Al-Dabbagh, 2012). Numerous fungicides were used to control *N. dimidiatum* fungus. For example, Mezab, Elsa and Korzet fungicides showed a significant inhibition to this fungus that causes wilt and canker diseases on cypress trees in Iraq (Murad and Al-Dabagh, 2014). However,

recently, a considerable attention of plant protection researches has drawn their attention to various agents that activate systemic acquired resistance (SAR). This type of resistance is important for plants to resist a wide range of biotic stresses and tolerant several of abiotic stresses (Esmailzadeh *et. al*., 2008). Salicylic acid(SA) which is a phenolic compound, is one of these agents that plays an important role in inducingthe host's defenses by stimulating the genes responsible for plant resistance of a large number of plant diseases (Uquillas *et al*., 2004; Jonathan *et al*., 2006). A numerous of studies related to pre-treatment of plants with SA proved a significant reduction of several disease caused by *Alternaria solani*, *A. cassiae*, *A alternata* and *Penicillium expansum* (Spletzer and Enyedi, 1999; Weete, 1992).

In spring of 2016, eucalyptus trees were subjected to severe sooty canker disease leading to significant deterioration and loss in the province of Karbala/Iraq. Thus, this study aimed to conduct a field survey about the incidence and severity of this disease, identify the pathogen and control it.

Materials and Methods

Field survey of sooty canker disease on eucalyptus in Kerbala province, Iraq

A field survey of sooty canker disease on eucalyptus trees was carried out in April of 2016 in the main four regions of Kerbala province /Iraq. Several eucalyptus tree were selected randomly in each of the four regions. These trees were evaluated to presence of the sooty canker symptoms that appeared on the bark of the stems. The percentage of symptomatic trees (disease incidence) in the surveyed area was calculated, as follows:

$$\text{Disease incidence} = \frac{\text{Number of diseased trees}}{\text{Total number of trees surveyed}} \times 100$$

The disease severity was also estimated based on a scale classified into five categories depending on the diseased part included and calculated using the following equation:

Table 1: Disease severity of sooty canker disease on eucalyptus tree

Guide of deterioration	% of diseased part of the tree
0	Healthy (no disease symptoms)
1	1-25
2	26-50
3	51-75
4	76-100

$$\text{Disease severity} = \frac{\text{Number of trees} \times \text{Category}(0) + ... + \text{number of trees} \times \text{Category}(4)}{\text{Total number of tested} \times \text{Top category index}} \times 100$$

Isolation of the fungus associated with sooty canker disease on eucalyptus trees

Samples of *E. camaldulensis* trees showing canker symptoms were randomly collected. The symptomatic parts were washed with running water for an hour. Sections (0.5 cm long) of the areas adjacent to the canker lesions were then cut with a sterile scalpel. These sections were sterilized with a 2% sodium hypochlorite for three minutes, washed three time successively with distilled water and dried using sterile filter paper (Whatman No.10). Five pieces were afterward placed in each sterile petri dishes (9 cm in diameter) containing sterile potato dextrose agar (PDA) medium with the antibiotic chloramphenicol (250 mg/1). Subsequently, all petri dishes were incubated at 25 ± 2 ° C for 3 days. The developed fungal colonies were purified using hyphal tip method. Additionally, the fungus isolated was preserved in test tubes containing slant PDA until needed for use in subsequent experiments (Burns, 2009).

Morphological and molecular Identification of the fungus associated with sooty canker disease on eucalyptus trees

Shapes, color and measurements of the fungal mycelia and conidia were characterized and compared with pervious descriptions (Farr *et al*., 2005; Crous1 *et al*., 2006; Al-Saadoon *et al*., 2012) for morphological identification.

Genomic DNA was extracted from mycelia of pure fungal colonies 7 days old cultivated on PDA using the DNeasy Plant Mini Kit (QIAGEN N.V., Hilden, Germany) by following the company's instructions. The universal primers ITS1 and ITS4 (White *et al*., 1990) were used to amplify the, the internal transcribed spacers of ribosomal DNA. The PCR amplifications were accomplished using Ready-To-Go PCR Beads kit in a total volume of 25µl solution containing the basic ingredients provided by the company as beads and 1µl of each primer (5 pmol) in addition to 2 µl (50-100 ng) of template DNA (GE Healthcare, Illinois, USA). The amplicon fragments were sequenced at Macrogen, Inc. (Seoul, South Korea) and analysed on Chromas software (https://technelysium.com.au/wp/ chromas/). Thesequences obtained were compared with other DNA sequences of fungi deposited at Gen-Bank sequence database of NCBI using a BLAST (Basic Local Alignment Search Tool) program at (http://blast.ncbi. nlm.nih.gov/Blast.cgi; Zhang *et al*., 2000). The phylogenetic analyses of the sequences data was

performed using MEGA (Molecular Evolutionary Genetics Analysis) version 6.0. (Tamura *et al.*, 2013) and consisted of neighbor-joining analyses. The generated sequences was submitted to Gen-Bank database and assigned with special an accession number.

Pathogenicity assessment of the fungus associated with sooty canker disease

The pathogenic ability of the fungus isolated was evaluated on healthy seedlings (18 month-old) of eucalyptus. Three branches of each seedlings were selected and injured by sterilized scalpels in the inner bark of the seedling branches. Each selected branch was inoculated with a mycelia disk (5 mm in diameter) collected from the edge of an actively growing pure culture of the fungus isolated. The inoculation was then covered with a sterile wet cotton and wrapped with a parafilm tape to avoid contamination. For control, similar inoculation procedure was applied on other healthy seedlings but with sterile PDA disk. All inoculated seedlings were kept in a growth chamber at 25±2°C and 80-90% relative humidity. The results calculated the average increase in the length of lesion occurring after two months of inoculation (Kepley and Jacobi, 2000).

Toxicity test of the fungus *N. dimidiatum* associated with sooty canker disease

To test the toxicity of the fungus isolated, potatoes dextrose broth medium was prepared and autoclaved. Then250-mg/l of chlorophenol antibiotic was added. The medium was then distributed in 500 ml flasks (200 ml in each flask). The inoculation, which was 5mm in diameter disk cut from the edge of the fungal colonies, was added for each flask that incubated at 25 ± 2° C for 14 days. The inoculated media were filtered using Whatman filter paper placed in Buechner funnel on the Erlenmeyer flask. The extraction was withdrawn by pressure under sterilization conditions. After that, new branches of the eucalyptus seedlings were selected with homogeneous in diameters and length (15 cm) and had four leaves. They were subsequently placed in sterilized bottles (250 ml) containing 150 ml of the fungal extractions with concentrations 50, 100%. The comparison branches were placed in bottles containing distilled water only. Each treatment consisted of three replicates. All bottles were after ward incubated at room temperature for 48 hours under fluorescent light. The results of wilt symptoms on the branches were measured according to Muhammad (1999); the wilting leaves of branches affected by the pathogenic fungus was classified into five categories as the following:

Table 2: Toxicity severity on branches of eucalyptus seedlings

Guide of deterioration	The wilting leaves of branches
0	There is no wilting branches
1	Weak wilt
2	Medium wilt
3	Clear wilt with color change to gray and brown
4	Heavy wilting accompanied by leaf wrapping from top to bottom.

Chemical control of the pathogen *N. dimidiatum* associated with sooty canker disease

In vitro

To study effect of the fungicide Beltanol-L -L (8–hydroxyquinoline) in concentrations 0.5 and 1 ml/1 and salicylic acid (2-Hydroxybenzoic acid)in also two concentrations 500 and 1000 mg/1 on mycelia growth of the pathogen *N. dimidiatum*, they were mixed thoroughly with the autoclaved PDA before solidifying and then poured in 9 cm sterile Petri dishes. Additionally, Petri dishes containing PDA only were made for control use. After that, a 5 mm in diameter disks collected from the edge of the 7-days-old pure culture of the pathogen was placed in the center of each Petri dishes made. These inoculated Petri dishes were later incubated at 25 ± 2 C. Each treatment included three replicates (Chen *et al.*, 2014).To determine whether the fungal colonies treated with the fungicide Beltanol-L and Salicyic acid were killed or inhibited only, they were examined after the fungal colonies growth reached the edge of plates in the control. This was by transferring disks of fungal growth treated to new Petri dishes containing PDA only and incubated at 25 ± 2° C. The results were calculated by averaging the measurement of two perpendicular diameter of growing fungal colony, and the following equation was applied for determining the percentage of inhibition of the pathogenicmycelia growth:

$$\text{Inhibition}(\%) = \frac{\text{Average growth of the control colony} - \text{Average growth of treatment colony}}{\text{Average growth of control colony}} \times 100$$

In vivo

Eucalyptus seedlings (18 months-old) were inoculated with the fungal pathogen as in the pathogenicity test. After three days of inoculation, Parafilm tape and cotton were removed, and the inoculated areas were treated with the fungicide Beltanol-L (1 ml/1l) and Salicyic acid (1000 mg/ 1l) separately. These two treatments were applied by

spraying the seedlings until saturation with use a barrier around them during spraying to prevent volatilization of the spray to other seedlings. The control seedlings were sprayed with distiller water only. Each treatment was replicate three times. After two months of treatments, the length of canker lesions in the stem of the treated seedlings of eucalyptus were measured. All experiments were carried out using factorial experiment in completely randomized design; the results were statistically analyzed using SAS program at a probability level of 0.05.

Results and Discussion

Field survey of sooty canker disease on eucalyptus in Kerbala province, Iraq

The results of field survey showed that the sooty canker disease symptoms on stems of eucalyptus trees investigated were as prominent dark brown to black lesions ranged between 66-210 cm in length, 13-40 cm in width and 5-12 cm in depth. These lesions were associated with a black mass of fungal arthro conidia underneath of the barks and on the canker lesions surfaces (Figure 1). It was also noted that gummosis secretions were frequently associated with these lesions. The disease incidence in all surveyed regions was very high (Table 2), ranging between 89-100% with disease severity between 85 – 93%. This high deterioration in eucalyptus trees is attributed to mismanage of the trees and lack application of protection practices against fungal diseases including the sooty canker that appear yearly on eucalyptus trees in Kerbala province. It is worth to mention that this disease survey on eucalyptus trees is the first in Karbala province of Iraq.

Figure 1 : Different level of sooty canker symptoms on eucalyptus trees in Kerbala province of Iraq

Table 3: Incidence and severity of sooty canker disease on eucalyptus trees in Karbala province

Regions	Disease incidence (%)	Disease severity (%)
Al-Husseinia	95	88
Saed Joda	89	85
Al-Salaam	100	92
Al-Abasia	100	93

Morphological identification of the fungus associated with sooty canker disease on eucalyptus trees

The fungus *Neoscytalidium dimidiatum* was constantly isolated of almost all symptomatic tissues examined of eucalyptus trees and it initial identified based on its morphological characters. The mycelia (Figure 2 A) was fast growing filling the entire Petri dishes within 5-7 days. The structure of the fungal culture was thick and the colour was dark brown to black covered with aerial filaments in grayish black. The fungal mycelia were in chain structure (Figure 2 B) and fragmentation of these mycelia formed cylindrical or barrel like structure, brown 0-2 septa arthro conidia (Figure 2 C) (Pasha, 2007). However, the pycnidia, formed at canker lesions and beneath barks of the infected eucalyptus tree were noticed to be dark brown to black containing cylindrical conidia with also 0-2 septa translucent developed to be brown at maturity (Sigler *et al*., 1997; Farr *et al*., 2005; Crous1 *et al*., 2006; Al-Saadoon *et al*., 2012).

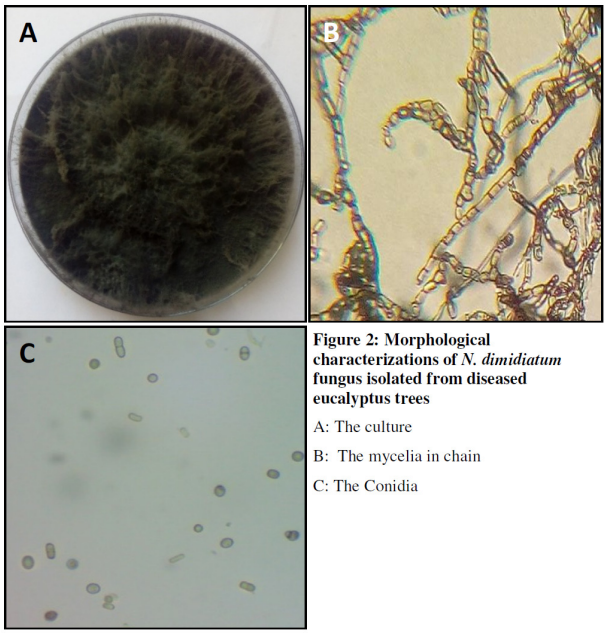

Figure 2: Morphological characterizations of *N. dimidiatum* fungus isolated from diseased eucalyptus trees

A: The culture

B: The mycelia in chain

C: The Conidia

Molecular identification of the fungus associated with sooty canker disease on eucalyptus trees

To confirm the morphological identification, PCR test was conducted using the universal primers (ITS1/ ITS4) on genomic DNA extracted of *N. dimidiatum* fungus. These primers were successfully amplified the rDNA-ITS region of the fungus. The PCR product was successfully sequenced and the sequence was deposited in Gen-Bank database with accession number MH744729. This sequence was compared with fungal sequences database stored in Gen-Bank using BLAST program. This comparison showed 98-100% similarity with those sequences belonging to *N. dimidiatum*. The phylogenetic analysis showed that the ITS sequence of the represented isolate was grouped within a clade including many reference strains of the fungus *N. dimidiatum* (Figure 3).The out group was *Rhizoctonia solani* fungus isolate FX1, AG4-HG-I with accession No: HG934415.1. This result confirms the preliminary morphological identification of *N. dimidiatum*. This fungus has been recorded world wide affecting various plant hosts including citrus trees in Iran (Alizadeh *et al*., 2000), peaches trees in Egypt (Farr *et al*., 2005), almond trees in USA (Inderbitzin *et al*., 2010) and Adansonia trees in Australia (Sakalidis *et al*., 2011). It has been also reported infecting grape vine trees in Baghdad (Natour and Ahmed, 1969) and Basra (Al-Saadoon *et al*., 2012) provinces of Iraq. However, no such a report related to *N. dimidiatum* fungus affecting eucalyptus trees in Kerbala has been found. Thus, this study is first report of this fungus as causative agent of sooty canker disease on eucalyptus trees in Kerbala province, Iraq.

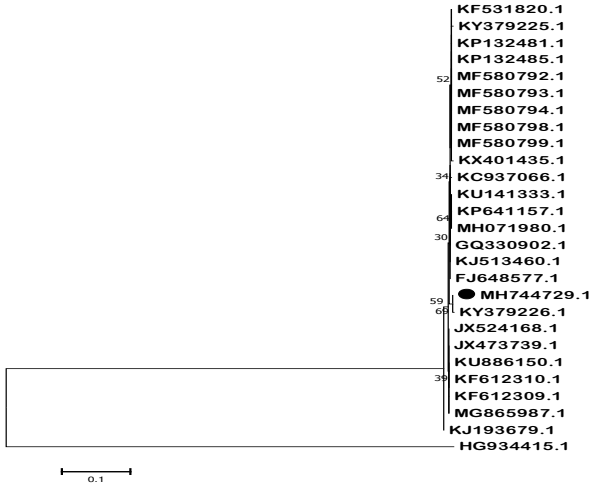

Figure 3: Phylogenetic tree constructed using ITS-rDNA sequences, presenting numerous identified *N. dimidiatum* strains stored at Gen-bank database including that was isolated from diseased eucalyptus tree (MH744729.1; indicated by a black dot). Numbers locate above of the branches denote to bootstrap values. *R. solani* (HG934415.1) was the out-group species.

Pathogenicity assessment

Pathogenicity assessment of the fungus associated with sooty canker disease

The results of the pathogenicity test of the of fungus *N. dimidiatum* indicated to pathogenic ability of this fungus by producing canker lesions averaged 2.99 cm in length on inoculated eucalyptus seedlings (Figure4). However, the control seedlings appeared to be healthy with no disease symptoms. This fungus is well known to be pathogen infecting several economic trees such as gouge, pine, gary, olive, poplar and eucalyptus causing canker diseases (Hassan *et al*., 2009; Hassan *et al*., 2011).

Figure 4 : Canker lesion covered with mycelia of *N. dimidiatum* (indicated with red dot)

Toxicity test the pathogen associated with sooty canker disease

Treatments with *N. dimiditum* extraction caused wilt symptoms on the new branches of eucalyptus seedlings tested. The results showed that 100% concentration was more effect (4 based on the toxicity severity) than 50% concentration that reached 2. The affected branches began to dry after three days of treatment. However, no disease symptoms were seen on the branches placed in the distillated water. These results are in consistent with statement of Muhammad (1999) who influence that the fungi can influence branches of trees causing wilting symptoms because of toxicity on vesicular tissues thus reflects negatively on the process of absorption of water and nutrients, and transfer capacity.

Chemical control of the pathogen *N. dimidiatum* associated with sooty canker disease

In vitro

The efficacy of the fungicide Beltanol-L (0.5 and 1 ml/1 l) and salicylic acid (500 and 1000 mg/l) on the growth of *N. dimiditum* fungus was examined. The results showed that the concentrations 1 ml/1 l of Beltanol-L and 1000 mg /l of salicylicacid inhibited completely (100%) the growth of the pathogenic fungus *N. dimiditum* Figure (6). However, the less concentration of both chemical substances were less efficiency as can be seen in Table (3) and Figure (5). The results also demonstrated that Beltanol-L killed the fungus *N. dimiditum*, while salicylic acid inhibited it only. The effectiveness of the fungicide Beltanol-L is due to its therapeutic and preventive efficiency, which gave it ability to penetrate into the host tissue and transfer to the rest of the plant in sufficient proportions to eliminate the pathogen (Al-Adel, 2006). In the other hand, the salicylic acid efficacy was attributed to its role in increasing production of hydrogen peroxide compound that inhibits the catalase enzymes of several pathogens (Al-Dabbagh, 2012).

Table 4: Effect of Fungicide Beltanol-L and salicylic acid on *N. dimiditum*

Fungicide Beltanol-L			salicylic acid		
0	**0.5**	**1**	**0**	**500**	**1000**
0 %	75 %	100 %	0 %	29.1 %	100%

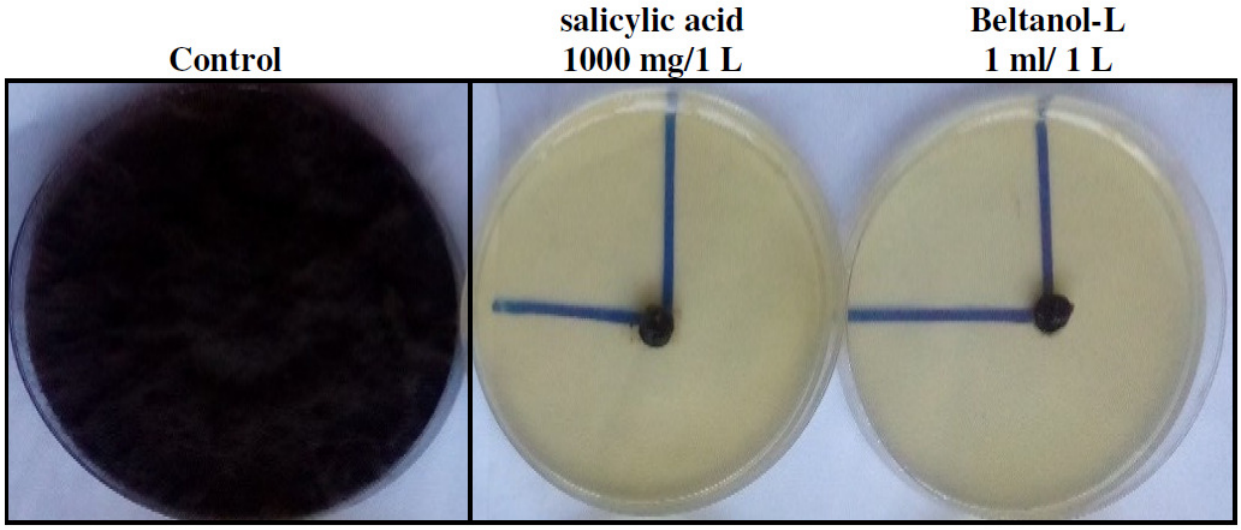

Figure 5: Effect of Fungicide Beltanol-L and salicylic acid on *N. dimiditum*

In vivo

The results demonstrated that the fungicide Beltanol-L and salicylic acid were able to reduce length of lesions caused by the fungus *N. dimiditum* after two months of inoculation. As shown in Figure (6) the average length of the lesions after treatment with Beltanol-L at 1 ml/1 l was 0.20 cm while salicylic acid at 1000 mg/1L was 0.88 cm. However, the control (inoculated with the pathogen only) reached 2.16 cm. The result of Beltanol-L -L is in agreement with previous studies that indicated to a high effectiveness ofthis fungicide against numerous plant pathogens such as *R. solani* and *F. oxysporum* that were completely inhibited (Srobarova and Kakalikova, 2007; Juber *et al*., 2008). Furthermore, it is known that the salicylic acid works on the induction of resistance against many of the fungi through stimulated genes responsible for the production of proteins that are related to resistance of pathogen (Abu Arqoub, 2002).

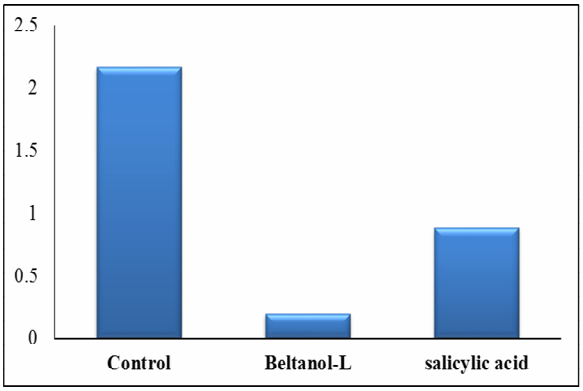

Figure 6: Effect of fungicide Beltanol-L and salicylic acid on *N. dimiditum* in plastic house

Acknowledgement

Authors would like to appreciate the administration of plant protection Department/ Agriculture College /University of Kerbala for their support in accomplishment of this study. In addition, the appreciation is extended to those undergraduate students who assisted us in performing the disease survey.

References

Abu Arqoub, M. M. (2002). *The antibiotics and three resistances (Acquired, Induced, Biological) and their role in plant diseases*. The Academic Library. Cairo, pp. 714.

Akin, M., A. Aktumsek and A. Nostro (2010). Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis* Dehn and Myrtuscommunis L. growing in Northern Cyprus. *African J. of Biotech.*, 9:531-535.

Al-Adel, K.M(2006). *Pesticides. Basic concepts and their role in agriculture and health fields*. Al Shams Printing and Publishing Co.Baghdad, pp. 444.

Al-Dabagh, M.N.A. (2012). A study about *Neoscytalidium dimidiatum* and *Neoscytalidium dimidiatum* on Eucalyptus in Nineveh. Master dissertation, College of Agriculture and Forestry. University of Mosul, Iraq.

Al-Iryani, A.Y. (1998). Morphological and chemical variation of *Eucalyptus camaldulensis* growing in in Nineveh. Master dissertation, College of Agriculture and Forestry, University of Mosul, Iraq.

Alizadeh, A., A. Heidarianand R.Farrokhi-Nejad (2000). Citrus branch wilt, decline and death caused by *Nattrassiamangiferae* and it other hosts in Khuzestan Province. Iran. *J. of Plant Path*., 36: 21-24.

Al-Saadoon, A.H., M.K.Ameen, M.A. Hameed, A. Al-Badranand Ali Z. (2012). First report of grapevine dieback caused by *Lasiodiplodiatheobromae* and *Neoscytalidium dimidiatum* in Basrah, Southern Iraq. *African J. of Biotech.,* 11(95): 16165-16171.

Amit, K. A.T., D. Bukvicki, D. Gottardi, G. Tabanelli, C. Montanari, A. Malik and M.G. Maria Elisabetta(2014). Eucalyptus Essential Oil as a Natural Food Preservative: In Vivo and In Vitro Antiyeast Potential. *BioMed Research International Article ID* 969143: 9.

Bachir, R.G. and M. Benali, (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific J. of Tropical Biomed*. 2(9): 739-742.

Bajaj, Y.P.S. (1995). *Medicinal and aromatic plants. Volume 8, Biotechnology in agriculture and forestry*. Springer. New York, pp. 524.

Batish, D.R., H.P. Singh, R.K. Kohli and S. Kaur (2008). Eucalyptus essential oil as a natural pesticide. *Forest Ecol. and Manag*., 256: 2166–2174.

Boland, D.J., J J. Brophy and A.D. House (1991). *Eucalyptus Oils Use: Chemistry, Distillation and Marketing*. Inkata Melbourne, pp. 252.

Burns, R. (2009). *Plant Pathology*. Techniques and Protocols. Humana Press. London, pp.453.

Chen, F., P. Han, P. Liu, N. Si, J. Junli Liu and X. Liu (2014). Activity of the novel fungicide SYP-Z048 against plant pathogens. *Nature/Scientific reports* 4: 6473 |DOI:10. 1038/srep06473.

Crous, P.W., B. Slippers, M.J. Wingfield, J. Rheeder, W.F.O. Marasas, A.J.L. Philips, A. Alves, T. Burgess, P. Barber and J. Groenewald (2006). Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in mycol.,* 55: 235–253.

Doughty, R.W. (2000). *The Eucalyptus: A Natural and Commercial History of the Gum Tree*. Johns Hopkins University Press. Maryland, pp. 560

Esmailzadeh, M., M.J. Soleimani and H. Rouhani (2008). Exogenous Applications of Salicylic Acid for Inducing Systemic Acquired Resistance Against Tomato Stem Canker Disease. *J. of Bio. Sci.,*8 (6): 1039-1044.

Farr, D.F., M. Elliott, A.Y. Rossman, and R.L. Edmonds (2005). *Fusicoccumarbuti* sp. nov. causing cankers on *Pacific madrone* in western North America with notes on *Fusicoccumdimidiatum*, the correct name for *Scytalidiumdimidiatum* and *Nattrassiamangiferae*. *Mycologia*,97: 730–741.

Foelkel, C. (2009). Papermaking Properties of Eucalyptus Trees Woods and Pulp Fibres. http://www.eucalyptus.com.br/eucaliptos/ ENG14.

Hassan, W.A., R.A. Haleem and P.H. Hassan (2011). Effect of heat-stress predisposition on the development of sooty canker caused by *Neoscytalidium dimidiatum* (Penz.) Crous& Slippers. *Actaagrobotanica*, 64 (4): 207–212.

Hassan, W.A., A.A. Pasha and M.B. Mohamma (2009). Sooty canker on some thin bark trees caused by *Nattrassiamangiferae*. *Egyptian J. of Agri. Res*., 87(3): 443-456.

Inderbitzin, P., R.M. Bostock, F.P. Trouillas and T.J. Michailides (2010). A six locus phylogeny reveals high species divrsity in *Botryosphaeriaceae* from Califiornia almond. *Mycology* 102: 1350-1368.

Johonson, S., W.R. Morris and J. Mandeeki (2002). *Managing Sooty canker* .University of Nevada Cooperative Extension. Nevada, pp.1-8.

Jonathan, D., G. Jones and J.L. Dangl (2006). The plant immune system. *Nature* 444(16): 223-229.

Juber, K.S., T.A. Farhan and A.H. Rasheed (2008). Evaluation the efficiency of some biocontrol

agents and Beltanol against *Rhizoctoniasolani* and *Fusariumoxysporum* the causal of seed rot and damping off disease of watermelon. *The Iraqi J. of Agri. Sci.*, 39 (2): 68-78.

Kepley, J.B. and W.R. Jacobi (2000). Pathogenicity of *Cytospora* fungi on six hardwood species. *J. of Arboriculture*26: 326–333.

Mohammed, N.Y. (1999). The importance and resistance of some fungi associated with degraded eucalyptus trees in the Nineveh forest. PhD thesis. College of Agriculture and Forestry. University of Mosul / Iraq.

Murad, N.Y. and M.N. Al-Dabagh (2014). Evaluation some of pesticides in control of *Neoscytalidium dimidiatum* (Penz) Crous and Slippers causing wilt and canker on cypress trees in Iraq. *The Iraqi J. of Agri. Sci.* 19(2): 25-38.

Nahal, I. (2003). *Tree science*. Directorate of books and university publications. Aleppo University. Aleppo, pp. 320.

Natour, R.M. and J.M. Ahmed (1969). Control of branch wilt disease of grape. *Dis. Rep.,*53: 152-153.

Old, K.M., M.J. Wingfield and Z.Q. Yuan (2003). *A Manual of Diseases of Eucalypts in South-East Asia*. Center for International Forestry Research. Jakarta, pp. 356.

Pasha, A. A. (2007). Resistance of some wood to *Nattrassiamangiferae* fungus which causes decomposition in Nineveh. PhD thesis. College of Agriculture and Forestry. University of Mosul / Iraq.

Pirralhoa, M., Floresa, D., Sousaa, V. B., Quilhó, T., Knapica, S. and Pereira, H. (2014). Evaluation on paper making potential of nine *Eucalyptus* species based on wood anatomical features. Industrial Crops and Products 54: 327–334.

Sakalidis, M.L., G.E.S. Hardyand T.I. Burgess (2011). Endophytes as potential pathogens of baobab species *Andansoniagregorii* : a fous on *Botryosphaeriaceae*. *Fungal Eco*., 4: 1-14.

Sigler, L., R.C. Summer bell, L. Poole, M. Wieden, D.A. Sutton, M.G. Rinaldi, M. Flguirre, G.W. Estes and J.N. Galgiani (1997). Invasive *Nattrassiamangiferae* infections. Case report, literature review and therapeutic and taxonomic appraisal. *J. of Clinical Microb.* 35 (20): 433-440.

Spletzer, M.E. and A.J. Enyedi (1999). Salicylic acid induces resistance to *Alternariasolani* in hyroponically grown tomato. *Bioch. Cell Bio.*89: 722-727.

Srobarova, A. and L. Kakalikova (2007). Fungal disease of grapevines. The *European J. of Plant Sci. and Biotech*.,1 (1): 84-90.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Bio.Evo.*30: 2725–2729.

Uquillas, C., I. Letelier, F. Blanco, X. Jordana and L. Holuige (2004). NPR1. Independent Actiration of Immediate Early Salicylic acid. *Responsive Genus Society*17:34-42.

Weete, J.D. (1992). Induced systemic resistance to *Alternariacassiae* in sicklepod. *Phys. Mol. Plant path.,* 40: 437-445.

White, T.J.; Bruns, T.; Lee, S. and Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In:*PCR Protocols. a guide to methods and applications*. Eds. M.A. Gelfand, D.H. Sninsky and T.J. White. Academic Press. San Diego, pp. 315–322.

Zhang, Z., S. Schwartz, L. Wagner and W. Miller (2000). A greedy algorithm for aligning DNA sequences. *J. of Comp. Biol.,* 7(1-2): 203-214.