Isolation and Identification Causal Agent of *Callistemon* sp. Seedlings Root Rot

Goner A. Shaker
Iraq Natural History Museum, University of Baghdad, Baghdad-Iraq.

Abstract

Isolation and identification of the fungi which causes the root rot disease of *Callistemon* sp. trees in AL-Alaab city, Al- Zawra garden and Al- Jaderiia in Baghdad provinces reveled diagnosis of the following fungi: *Fusarium solani, F. oxysporum, F. moniliiforme, Rhizoctonia solani, Curvularia lunata, Cladosporiun herbarum, Aspergillus niger, A. flavus, Theilaviopsis,* Mycelia sterilia, The frequency percentages of the isolated fungi from root and foliar system, in the three locations ranging from 10-60 % and the highest percentages were 60 % for *F.solani* in AL-Alaab city, 40 % in AL-Jadeeria as compared to other fungi. The percentage of the disease severities were 25, 10.4, 2.1, 0 % for *R. solani, F. solani, C. lunata* and Control, respectively. The seed germination percentages which treated with *F. solani, R. solani, C. lunata* were 20, 20, 90 %, respectively, and seedling damping-off percentages were 60, 20 and 10 % for the same fungi respectively and showed significant differences ($p \leq 0.05$) among treatments. This is the first record of the disease on *Callistemon* sp. trees in Iraq.

Keywords: *Callistemon* sp., root rot, *F. moniliiforme, Rhizoctonia solani, Curvularia lunata*.

Introduction

The bottle brush is the common name of *Callistemon* sp, it is a popular ornamental tree in subtropical areas, typically growing as a perennial and described as evergreen plant, bright red flower spikes, planting in container or above-ground like espalier, hedge, and parking. The genus includes 30 species of trees and shrubs belong to Myrtaceae family, they are all native to Australia [1, 2, 3, and 4]. The tree can be reproduced vegetatively using cuttings and by seeds [2]. These trees have many benefits and used of such maximum amount of nectar sugars in the flowers of bottle brush, *C. lanceolatus*, prouduced 6.044 mg/flower sugar 72 hrs after flower opening, whereas the 24 hrs sugar value was only 0.44 mg. *Apis mellifera* harvested ≈ 90% of the sugar produced by the flowers [5]. The aqueous extracts of *Eucalyptus lanceolatus* and *C. citrinus* plants showed strong antifungal activity against *Chaetomium* sp., *Alternaria alternata* and *Aspergillus niger* fungi [6]. The aqueous and alcoholic extracts of different parts of bottle brushes *Callistemon* were found to have various pharmacological activities. For example, antifungal, antioxidant, antithrombin, anti-inflammatory, antidiabetic, antimicrobial and herbicidal activities [7]. These trees are susceptible to harmful diseases and fungal infections that can kill it, or at least severely affect its growing during a recent survey in Tunisia[8], a number of *Calonectria* spp. were showed symptoms of leaf spot, crown and root rot on seedlings of *Callistemon* spp. The damping-off and leaf spot caused by *Cylindrocladium scoparium* on bottle brush cuttings in Italy in May of 2006, has been observed [9]. Also, *Phytophthora* root rot is a disease that infects *Callistemon* sp. plants, the fungus infects the root systems and causing yellowing of leaves, early defoliation, reddish-brown roots, the dieback of branches and the discolouration of branches [2]. This study was designed to determine the causal fungal root rot to *Callistemon* sp. seedlings.

Materials and Methods

1-Sampling, isolation and diagnosis

Survey was conducted on three sites included AL-Alaab city, Al- Zawra garden and Al- Jaderiia in Baghdad province. The bottle brush *Callistemon* sp. trees showed symptoms of dieback, discolouration of branches, decline and death in nurseries. A survey was conducted and the roots were collected from a depth of 30-50 cm. Samples were kept in polyethylene bags, brought to the laboratory, placed under running water for an hour and cut to a small pieces (0.5 cm) and sterilized with 1.5% solution of sodium hypochlorite for 2-3 minutes and washed with sterile distilled water then dried by filter papers, 3-4 pieces per Petri- dish were transferred into PDA and

placed inside an incubator at 25 ° C ± 1 until fungal growth emergence. Slides were prepared from isolated fungi and diagnosed to the level of species according to taxonomic keys [10,11]. The percentage of appearance frequency for each fungus was also calculated by using the following equation:

The percentage of frequency

$$= \frac{\text{No.pieces colonized by the fungus} \times 100}{\text{Total no.cultured pieces}}$$

2-Pathogenicity test

To reveal pathogenicity, most frequent fungi were used *Rhizoctonia solani*, *Fusarium solani*, *Curvularia lunata* and as follows:-

Inoculum preparation: suspension solution of conidiospores *Fusarium solani* and *Curvularia lunata* was prepared at concentration of spores 1×10^5 Spore / ml of pure culture 7 days old on Potato Dextrose Agar which was estimated by Haemocytometer.

Seedlings preparing: bottle brush seedlings 50 cm long with similar external appearance in regard to size and shape, healthy were prepared, Seedlings were planted in containers (sand: peat moss) (1: 2) and sterilized by isteam (autoclave at 121°C and under 15 pound/ square inch) forth and irrigated with sterile water. Seedlings were infected with the suspension solution of the spores of *F. solani, C. lunata* and pure culture of *R. solani*, by injecting 2 ml of the suspension solution of spores *F. solani, C. lunata* on the roots crown. In crown adjacent to soil surface a wound was done by sterile knife and inoculated with small piece of *R. solani* inoculum, wrapped with cellophane paper after covering by cotton then moistened with sterile distilled water to maintain moisture. All treatments were replicated three times, the control treatment of seedlings was injected with 2 ml sterile distilled water using a piece of PDA in the control treatment of *R. solani*.

The disease severity was calculated by disease index according to the scale mentioned by [12], which consists of five degrees: 0. There is no infection, 1. 1-25% of the seedling are yellowish, 2. 26-50% of the seedling wilting and yellowing in branches, 3. 51-75% Yellowing and wilting, 4. 100% Wilting Seedling, according to the following equation mentioned by [13].

Disease index (DI) =

$$\frac{\text{No.leaves in degree0}\times 0 \ldots \text{No.leaves in degree4}\times 4}{\text{No. of leave all degrees} \times \text{Max.degree of infection}} \times 100$$

3-Seeds germination percentage treated with isolated fungi

Percentage of germinating seeds were contaminated with the fungi *R. solani, F. solani, C. lunta*, seed were planted (20 seeds/ dish) after one week from the date of soil contaminating with fungi in ceramic dishes container 25 cm diameter, the soil is composed of (sand: peat moos) (1: 2) sterilized by autoclave at 121°C under pressure 15 pounds/ square inch, for 1h and treatments were prepared as follows:

1- The seeds planted in soil contaminated with the suspension solution of spores of *F.solani* concentration of 1×10^5 Spore / ml. 2- The seeds planted in soil contaminated with the suspension solution of spores of *C. lunata* a concentration of 1×10^5 Spore / ml. 3- The seeds planted in soil contaminated with the fungus *R. solani* by 1/2 dish of pure culture. Treatments left in the field until the seeds germinated. Each treatment replicated three time and used only sterile water in control treatment, the percentage of seed germination and seedling damping off were account after 10-30 days from seed germination according to the following equations:-

$$\text{The percent of seeds germination} = \frac{\text{The no.of grown seed}}{\text{Total no.of seeds}} \times 100$$

$$\text{The percent of damping off} = \frac{\text{No.of dead seedlings}}{\text{No.of total seedlings}} \times 100$$

Results and Discussion

1-isolation and diagnosis

The isolation of the fungi causes of the root rot bottle brush seedlings in Baghdad province in the sites, Al-Alaab city, Al- Zawra garden and Al-Jaderiia yielded: *Fusarium solani, Fusariam oxysporum, Fusarium moniliiforme, Rhizoctonia solani, Curvularia lunata, Cladosporium herbarum, Aspergillus niger, Aspergillus flavus, Theilaviopsis sp*, Mycelia sterilia. The ferquency percentage of fungi in the three locations was ranging from 10-60 %. Where the highest percentage of fungi *F.solani*

was 60 % in AL-Alaab city and 30 % in Al- Jaderiia. The ferquency percentage of fungi *R.solani* 25, 25, 20 % in Al- Jaderiia, Al-Zawra garden, AL-Alaab city, respectively, The appearance percentage of fungi *F. moniliiforme, F. oxysporum* was 20 and 10 % in the AL-Alaab city, which showed that the fungi were appeared in larger proportions in isolates of AL-Alaab city, as in Table (1). There is a clear difference in the number of species of fungi isolated from the root regions for isolated from branches area, as shown in Table (2) and that is where *F. solani* appeared in the isolates roots and branches, while *R. solani* appeared in isolates roots only, as was *C. lunata* appearance in isolates branches area larger compared to isolates root regions at all locations, this could explain the role of fungi pathogens may be more power and highest impact in the case of infection by the roots and branches together compared to the case of infection by the roots or branches each separately. We infer that these fungi may infect in nurseries and then transported to the forests, field and gardens through the infected seedlings and are leading to appear symptoms on seedlings and death of these trees.

Table (1)
The percentage of frequency of fungi isolated from bottle brush Callistemon sp. trees infected with root rot in three locations.

Fungi	Fungal appearance frequency %		
	Al-Jaderiia	Al-Zawa gardn	AL-Alaab city
Fusarium soloni	30	10	60
Fusarium oxysporum	30	0	10
Fusarium moniliiforme	0	0	20
Rhizoctonia solani	25	25	20
Curvularia lunata	0	10	40
Cladosporiumherbarum	0	10	10
Theilaviopsis	0	0	5
Aspergillus niger	10	10	35
Aspergillus flavus	20	20	0
Mycelia sterilia	20	20	20

Table (2)
The presence of fungal isolates from the roots and branches of infected bottle brush Callistemon sp. trees infected with root rot.

Fungi	Al-Jaderiia		Al-Zawra garden		AL-Alaab city	
	B	R	B	R	B	R
Fusarium soloni	+	+	+	+	+	+
Rhizoctonia solani	-	+	-	+	-	+
Fusarium oxysporum	+	-	-	-	+	+
Fusarium Moniliforme	-	-	-	-	+	+
Curvularia lunata	-	-	+	-	+	+
Cladosporium herbarum	-	-	+	+	+	+
Theilaviopsis	-	-	-	-	+	-
Aspergillus niger	+	-	-	+	+	-
Aspergillus flavus	+	-	+	-	-	-
Mycelia sterilia	+	+	+	+	+	+

(+) presence of fungus, (-) No fungus. R=Roots, B=Branches

2-Pathogenicity test

Clearly, the results in Table (3) To test the pathogenicity on the ability of each of the fungi *R. solani*, *F. solani*, *C. lunata* on the occurrence of the disease, and the incidence percentages were 25, 10.4 and 2.1 %, respectively, while the control treatment showed 0 %, The appearance of the disease on the root systems and cause yellowing of leaves, early defoliation, reddish-brown roots, and change the color of the branches Fig.(1).and there were significant differences between treatments at $P \leq 0.05$ differences. It was re-isolate the fungus from the roots of seedlings inoculated. It is noticeable that there is variation in the ability of fungal pathogenicity may be due to various effects of the surrounding environmental conditions.

Table (3)
The incidence percentage of infection with fungi C. lunata, R. solani, F. solani for seedlings bottle brush Callistemon sp. trees.

Fungi	Disease index %
R.solani	25.0*
F.solani	10.4
C.lunata	02.1
Control	00.0

** LSD at 0.05 probability level =16.9*

3-Seeds germination treated with isolated fungi

The results showed that the percentage of seeds germination treated with fungi *C. lunata, R. solani, F. solani* was 90, 20, 20 % respectively, and 0 % for the control treatment, Table (4). Because *F. solani* and *R. solani* of soil fungi and they are the reason for the phenomenon of lack of seed germination and seedling death, *C. lunata* has little effect in reducing the rate of seed germination The percentage of seedling damping off for fungi *C. lunata, R. solani, F. solani* was 10, 20, and 60 %. The researchers [14,15] indicated to the fact that the infected seeds and vectors of pathogenic fungi may causes a reduction in the percentage of seed germination especially if they are not properly stored, and not collected in a timely manner and proper drying and good storage is necessary to protect the seeds from rotting and damage, If the soil is too moist, the root and crown will attacking by fungi diseases and can be a problem [1].

Table (4)
Percentages of seeds rot and seedlings damping off bottle brush Callistemon sp trees.

Treatments	*Seedlings damping off after emergence %*	*Seeds rot before emergence%*
F.solani	60	*20
R.solani	20	20
C.lunata	10	90

**** LSD at 0.05 probability level =0.14***

Fig.(1) Pathogenicity test show the symptoms on the bottle brushes Callistemon sp. treated with, 1- R. solani, 2- F. solani, 3- C. lunata, 4- control .(from left to right).

References

[1] Edward F. G. and Dennis G. W. Dwarf Bottlebrush *Callistemon citrinus 'Little John'*. Horticulture. University of Florida Equal Opportunity Institution, 2008.

[2] Bottlebrush (*Callistemon*) species. Department of Agriculture, Forestry and Fisheries. www.flowerite.co.za and www.theflowerexpert.com. 2012.

[3] Elliot, W. R. and Jones, D. L. Encyclopedia of Australian Plants, 2, Lothian Publishing Company, Australia, 1982.

[4] Chenielle, D. Lois, R. Alison, N. Sylvia, M. John, L.and Mohammed A. Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. Journal of Medical and Biological sciences. 3(1), 2009.

[5] Gupta, J. K. and Kumar, J. Bottle brush *Callistemon lanceolatus* DC (Myrtaceae) nectar: amount, type of nectar sugars and honeybee foraging. Apidologie. 24 (6), 564-568, 1993.

[6] Swapna K. and Lalchand D. Antifungal Activity of Aqueous Plant Extracts against Some Book Deteriorated Fungi. International Journal of Engineering Science and Innovative Technology (IJESIT). 2(6), 2013.

[7] Sanjita, D. and Uttam, S. Therapeutic potentials of *Callistemon lanceolatus* DC, International Journal of Advances in Pharmacy, Biology and Chemistry (IJAPBC). 1(2), 206-210, 2012.

[8] Lombard, L. Polizzi, G. Guarnaccia, V. Vitale, A. and Crous, P. W. *Calonectria* spp. causing leaf spot, crown and root rot of ornamental plants in Tunisia. Persoonia: Molecular Phylogeny and Evolution of Fungi. 27: 73–79, 2011.

[9] Polizzi**,** G. Vitale, A. Aiello**,** D. and Dimartino, M. A. First Report of Damping-Off and Leaf Spot Caused by *Cylindrocladium scoparium* on Different Accessions of Bottlebrush Cuttings in Italy. The American Phytopathological Society. 91(6) 769, 2007.

[10] Barnett, H. L. Illustrated genera of imperfect fungi, second edition. Buijess Publishing com. U.S.A. 1965.

[11] Domsch, K. H., Gams, W. and Anderson, T.H. Compendium of soil fungi. Academic Press. A subsidiary of Harcourt Brace Jovanoich, Publishers .2, 1980.
[12] Abu Bakar, A. I. Nur Ain Izzati, M. S. and Umi kalsom, Y. Diversity of Fusarium species associated with post- harvest Fruit Rot Disease of Tomato. Sains Malaysiana, 42(7), 911-920, 2013.
[13] Parimala, R. D. and Marimuthu, P. Effect of Botanical Formulation of *Polygonum minus* (P-40) on control of *Alternaria solani*. Plant pathology and microbiology. 2(1), 104, 2011.
[14] Chacko, K.C. and Mohanan, C. Development of technology for collection, processing and testing seeds of important tree species of Kerala Final Technical Report (ICFRE). Kerala Forest Research Institute, Peechi, Kerala, India, 2002.
[15] Mohanan, C. chacko, K.C. chandran, A. and Varma, G. Seed health problems in tropic Forest tree seeds and their impact on seedling production. Working papers of the Finnish Forest Research Institute11. http://www.melta.Fi/ Julkaisut/ working. Papers /2005 /mwpoll. htm. 83-93, 2005.

الخلاصة

أظهرت دراسة عزل وتشخيص المسببات الفطرية لتعفن الجذور شتلات أشجار فرشة البطل *Callistemon* sp. لمواقع مدينة الألعاب ومتنزه الزوراء ومجمع الجادرية في مدينة بغداد عن تشخيص الفطريات:

Fusarium solani, Fusarium oxysporum, Fusarium moniliiforme, Rhizoctonia soloni, Curvularia lunata, Cladosporium herbarum, Aspergillus niger, Aspergillus flavus, Theilaviopsis sp, Mycelia sterilia.

التي عزلت من المجموع الجذري والخضري وكانت نسبة تكرار ظهور الفطريات في المواقع الثلاثة تتراوح ما بين 10−60 % وكانت اعلى نسبة للفطر *F.solani* وهي 60% في مدينة الألعاب و40% في مجمع الجادرية مقارنة بالفطريات الأخرى. بينما كانت النسبة المئوية لشدة ألاصابة 25 و10.4 و2.1 % للفطريات , *C.lunata, F.solani* *R.solani* على التوالي وكانت في معاملة المقارنة صفر، وسجلت نسبة أنبات البذور عند المعاملة بالفطريات *C. lunata, F. solani, R.solani* هي 90,20,20 % على التوالي. ونسب موت البادرات 10,20,60 % على التوالي وظهرت وجود فروقات معنوية ما بين عند مستوى احتمال 0.05 مابين المعاملات. وهذا يعد التسجيل الأول للمرض على شتلات أشجار فرشة البطل في العراق.