ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH FOREST TREE SEEDS AND THEIR FUNGAL FILTRATE EFFECTS ON GERMINATION RATE IN SULAIMANI GOVERNORATE

Jalal Hama Salih Ismael & Shler Hassan Mahmood
Department of Horticulture, College of Agricultural Engineering Sciences,University of Sulaimani-Kurdistan region-Iraq, Corresponding e-mail: jalal.ismael@univsul.edu.iq **&** shler.mahmood@univsul.edu.iq

ABSTRACT

This study was conducted to isolate fungi were associated with seeds of some species forest tress and effect of fungal culture filtrates on germination rate. Samples of the forest tree seeds were collected from different locations in Sulaimani Governorate including Hewary Shar and Sarchnar from Sulaimani center and Derbendikhan and Dokan and Rania out of city center. Thirteen different fungal species were isolated and identified as; *Alternaria alternate, Ascochyta* sp., *Aspergillus flavus, A. niger, Cladosporium* sp., *Fusarium oxysporum, Helminthosporium* sp., *Rhizoctonia* sp , *Rhizopus stolonifour, Penicillium* sp, *Pythium* sp., *Sclerotinia sclerotiorum, Stemphyllium* sp., and. Among these, *R. stolonifour* of isolated from Sarchnar nursery *and A. flavus* isolated of Derbendikhan nursery had the highest frequency of occurrence (46.67%) while both *Fusarium oxysporum* and *S. sclerotiorum* had the lowest frequency of occurrence (6.67%) isolated from Derbendikhan nursery. Seeds of Albizia (*Albizi alebbeck*) and Honey locust (*Gledisia triacanthos*) were soaked in culture filtrates of *A. flavus* and *Penicillium* sp. respectively and seeds of Albizia (*A. lebbeck*), Acacia (*Acacia matius*) and Persian lilac (*Melia azedarach*) were soaked in the culture filtrates of *S. sclerotiorum* and *A. alternata and Fusarium oxysporum* respectively. The result showed the lowest percentage of germination rate was after 48 hours of incubation with significant different as compare to control treatment. A total inhabitation of seed germination was also detected for *F. oxysporum*

KEYWORDS: *Albizi alebbeck, Aspergillus flavus*, Fungal filtrate, Forest tree seeds.

عزل وتشخيص الفطريات المرافقة لبذوراشجار الغابات وتأثيرراشحها على معدل نسبة الإنبات في محافظة السليمانية

جلال حمه صالح اسماعيل شليرحسن محمود
قسم البستنة/كلية علوم الهندسة الزراعية /جامعة السليمانية
البريد الألكتروني: jalal.ismael@univsul.edu.iq
shler.mahmood@univsul.edu.iq

الملخص

تم اجراءهذه الدراسة بهدف عزل الفطريات المرافقة لبذوربعض انواع أشجارالغابات وتأثير رواشحها الفطرية على معدل نسبة الإنبات.جمعت عينات من بذورمواقع مختلفة لمشاتل الغابات في محافظة السليمانية شملت و هوارى شار سرجنار و من مركز مدينة السليمانية و دربنديخان و دوكان و دربنديخان و رانية من خارج مركز المدينة.

تم عزل 13نوع فطري وشخصت بي وكانت *Alternaria alternata*،*Ascochyta* sp ، *Aspergillus flavus* ، A. *niger*،*Cladosporium* sp. ،*Fusarium oxysporum* ، *Helminthosporium* sp ، *Penicillium* sp.، *Pythium* sp ، *Rhizoctonia* sp. ، *Sclerotinia Sclerotum*، *Stemphylium* sp. ، *Rhizopus stolonifour* من بين تلك الفطريات، كان *R.* المعزول من مشتل سرجنارو *A. flavus* المعزول من مشتل دوكان أعلى نسبة ظهور (46.67 ٪) ، بينما كان الفطران *F.oxysporum* و *S. sclerotiorum* أقل نسبة ظهور(6.67 ٪) المعزولان في مشتل دربنديخان. تم غمربذورالألبيزيا (*Albizia lebbeck*) والكلاديشيا (*triacanthos*) *Gledisia* في رواشح *A. flavus* و *Penicillium* sp. وبذور الألبيزيا(*Albizia lebbeck*) والأكاسيا (*Acacia matius*) والسبحبح (*Melia azedarach*) في

رواشح الفطريات *S.sclerotiorum* و *A. alternata*و *F. oxysporum*على التوالي . أوضحت النتائج أن أقل نسبة إنبات للبذور المعاملة برواشح هذه الفطريات بعد 48 ساعة من الحضن وكانت تختلف معنويا عن معاملة المقارنة التثبيط التام لانبات البذور تم ايضا تسجيلة من قبل *mF. oxysporu.*

الكلمات المفتاحية :الالبيزيا، اسبرجلس فلافس ، رواشح الفطريات، بذور الشجرة الغابة.

INTRODUCTION

Seeds are a vital input in agriculture. tI may be called the foundation of agriculture. Among the agricultural inputs, seeds are the most important input for production. Hygienic or pathogen-free seeds are considered as the vital factor for desired plant population and good harvest (Islam *et al.,* 2009). The healthy of seeds can be affected by direct pathogen infections or through contamination of seeds by pathogenic proposals as contamination in, on or with the seeds or as concomitant contamination (Rashid et al. 2000). Forest-tree seed diseases are primarily caused by numerous species of fungi are associated with forest tree seeds (Gonthier et al. 2013). Seed-borne for example cause pathogens include reduced seed germination, increased seedling damping-off, and mortality of older seedlings in nursery beds (Gaur et al. 2020). Fungi of the genera *Aspergillus, Fusarium, Penicillium* and *Rhizoctonia* are known to produce mycotoxins (Singh et al., 1991). These mycotoxins had been reported to degrade the quality of seeds and reduce their viability (Kumar et al. 2020). Cultural filtrates of *Aspergillus* spp. for instance caused a reduction in seed germination (Jalander and Gachande, 2012).This study aimed to isolate identify the fungal species contaminating forest tree seeds and to investigate the effect of their culture filtrates on seeds germination rate.

2 -MATERIALS AND METHODS

2.1 Collection of samples

Collection of forest tree seeds were collected in Sulaimani Governorate from five nursery locations Figure 1 and Table 1. The collected seeds were put in paper bags and transferred to the laboratory for later processing.

Figure 1/ Locations of Forest Nurseries from which seeds are collected in Sulaimani Governorate (Ranya, Dokan, Hawaryshar, Sarchnar and Darbendikhan)

Table 1: Nursery sites and forest tree seed names in sulaimania province

nursery locations	Forest tree seeds	Scientific name
Sarchnar	Persian lilac	*Melia azedarach*
	Albizia	*Albizia lebbeck*
	Arizona cypress	*Cupressus arizonica*
	Honey locust	*Gledisia triacanthos*
	Turkish Pine	*Pinus brutia*
Hawary shar	Honey locust	*Gledisia triacanthos*
	Italian cypress	*Cupressus sempervirens*
	Black locust	*Robinia pseudoacacia*
	Arizonica cypress	*Cupressus arizonica*
	Persian lilac	*Melia azedarach*
Dokan	Acacia	*Acacia matius*
	Persian lilac	*Melia azedarach*
	Albizia	*Albizia lebbeck*
	Italian cypress	*Cupressus sempervirens*
Rania	Arizonica cypress	*Cupressus arizonica*
	Italian cypress	*Cupressus sempervirens*
	Thuja	*Thuja occidentalis*
	Acacia	*Acacia matius*
Darbandikhan	Thuja	*Thuja occidentalis*
	Turkish pine	*Pinus brutia*
	Albizia	*Albizia lebbeck*
	Persian lilac	*Melia azedarach*

2.2 Fungal isolation and identification

Seeds transferred to the Central Research Lab. of the College of Agricultural Engineering Sciences. The seeds were washed and surface1% sterilized in sodium hypochlorite for 5 min , washed with sterilized distilled water thrice and dried by sterilized filter papers .The sterilized seeds were transferred to Petri dishes contain Potatoes Dextrose Agar culture medium as 5 seed of each petri dish were incubated at 25±2°C for 7 days. The fungi associated with forest tree seed have been identified to the lower taxonomic level based macro-microscope observation such as conidia and conidiophores and sporangia using taxonomic keys (Barnett, 1965; Booth, 1971; Domsch *et al.,* 1980) .The fungal density was estimated and calculated for each isolate using the following formulas (Krebs; 1978): *Fungal frequency (%) = (No. of isolated colonies (for each fungus)/Total No. of all fungi) X 100*

2.3 Preparation of fungal culture filtrates:

The pure cultures of *Aspergillus flavus*, *Penicillium* sp., *Sclerotinia sclerotiurm*, *Fusarium oxysporum*, *Alternaria alternata* were prepared using hyphal tip method. Each fungus was grown in 250ml conical flask containing 100 ml of Czapex Dox broth and incubated at 25±2^{o}C for 21 days (Hajieghrari, 2010). The culture filtrates were filtered into pre sterilized conical flasks using 2-3 layers of filter paper (Whatman1) on a Buchner funnel. The filtrates were stored in a refrigerator at 4±2^{o}C.

2.4 Effects of fungal culture filtrates on seed germination:

Hundred seeds from each investigated tree were surface sterilized with 70% ethanol for three mints and then rinsed three consecutive times with sterile distilled water then one hundred seeds were soaked in each fungal filtrate for 24 and 48 hours. Twenty seeds (for small seeds) and five seeds (for large ones)

were put in sterilized Petri dishes (9 cm) containing two layered wet filter paper (Patil et. al., 2012). Control treatment were maintained by using distilled water for 24and 48 h. After seven days of incubation, percentage of seed germination was calculate using the following formula

Germination rate (%) = number of germinated seeds / Total Number investigated seeds X 100

The completely Randomized design was designed and applied for this experiment, the difference between the means determined by Duncan's Multiple Range test at the level of significance 5%.

3 RESULTS AND DISCUSSION

3.1 Isolation and identification

Thirteen fungi of species were isolated and identified as *Rhizoctonia* sp., *Rhizopus stolonifour, Ascochta* sp., *Aspergillus flavus, A. niger, Penicillium* sp., *Helminthosporium* sp., *Alternaria alternata, Fusarium oxysporum, Stemphylium* sp., *Cladosporium* sp., *Sclerotinia sclerotiurm* and *Pythium* sp. (Table 2 and 3).

3.1.1. The percentage of frequency of isolated fungi from forest seeds at sulaimani Nurseries.

The highest frequency was *Rhizopus stolonifour* (46.67%) isolated from *Melia azedarach* in Sarchnar followed by *Aspargillus flavus* (33.33%) isolated from *Albizia lebbeck* while the lowest frequency was *Rhizoctonia* sp. (10%) isolated from *Melia azedarach* (Table 2). Also the highest frequency of A. *niger* (33.33%) was isolated from *Gledisia triacanthos* and *Melia azedarach* in HaweryShar followed by *A. flavus* (20%) from both *Cupressus sempervirens* and *Robinia pseudoacacia* while the lowest frequency was *Rhizoctonia* sp. (10%) isolated from from *Gledisia triacanthos*. Similar results have been shown by Sumanth and Waghmare(2010) on seeds mycoflora, their results demonstrated that both *A.s niger* and *A. flavus* were the most dominant fungi. Our results are in agreement with those found by Askun (2006) who isolated fungi from maize and found that *Rhizopus* and *Aspergillus* were the most frequent genera isolated.

Table 2: The percentage of frequency of fungi isolated from forest tree seeds at Sulaimani nurseries.

Locations	Scientific name	Isolated fungi	Fungi of Frequency% *
Sarchnar	*Melia azedarach*	*Rhizoctonia* sp.	10.00
		Rhizopus stolonifer	46.67
		Ascochyta sp.	26.67
	Albizia lebbeck	*Aspargillus flavus*	33.33
		Rhizoctonia sp.	20.00
	Cupressus arizonica	*Aspergillus niger*	23.33
	Gledisia triacanthos	*Rhizoctona* sp.	16.67
		Penicillium sp.	23.33
	Pinus brutia	*Aspergillus niger*	13.33
		Rhizopus stolonifer	13.33
Hawaryshar	*Gledisia triacanthos*	*Aspergillus niger*	33.33
		Rhizoctonia sp.	10.00
	Cupressus sempervirens	*Aspergillus flavus*	20.00
	Robinia pseudoacacia	*Helmenthosporium* sp.	13.33
		Aspergillus flavus	20.00
	Cupressus arizonica	*Aspergillus flavus*	16.67
		Rhizoctonia sp.	13.33
	Melia azedarach	*Aspergillus niger*	33.33
		Rhizoctonia sp.	13.33

***Each number is mean of three replicates (three petri plates), for each species**

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3.1.2. The percentage of frequency of fungi isolated from forest seeds at Sulaimani nurseries

The highest frequency of occurance was *Alternaria alternata* (33.33%) from *Melia azedarach* in Dokan district followed by *Stemphyllium* sp. (23.33%) of *Cupressus sempervirens* while the lowest frequency was *Fusarium oxysporum* (10%) of *Melia azedarach* and *Acacia matius* (Table 3). A dditionally, the highest frequency was *Rhizoctonia* sp. (30 %) of *Cupressus arizonica* in Rania nurseries followed by *Aspergillus flavus*(20%) of *Cupressus arizonica* and *Acacia matius*, while the lowest frequency was *Penicillium* sp. (13.33%) of *Thuja occidentalis*. The highest frequency of *A. flavus* was isolated from *Melia azedarach* (46.67%) in Derbendikhan nurseries followed by *A. niger* (43.33%) isolated from *Pinus brutia*. However the lowest frequency of *Sclerotinia sclerotiorum* (6.67%). Similar results have been recorded by Raghuwanshi and Deokar (2002) who detected that the *Aspergillus, Alternaria, Fusarium* and *Rhizopus* were predominant genera whereas the intensity of both *Penicillium* and *Sclerotinia* were the lower one. Our result were also in agreement Ismael (2010) who showed that the fungal species isolated from solanaceous seeds in Sulaimania region from tomato seeds the following fungi were detected: *Aspergillus flavus, A.niger, Penicillium spp., Pythium sp. Rhizopus spp.*, Where as the fungi detected were: *A.niger, Cladosporium sp ,Penicillium spp., Pythium sp., Rhizopus sp., Alternaria alternata, Fusarium oxysporum and Rhizoctonia sp.* with from eggplant seeds.

Table 3: The percentage of frequency of fungi isolated from forest tree seeds at Sulaimani nurseries.

Locations	Scientific name	Isolated fungi	Fungi of Frequency% *
Dokan	*Acacia matius*	*Rhizoctonia* sp.	20.00
		Fusarium oxysporum.	10.00
		Alternaria alternata	33.33
	Melia azedarach	*Aspergillus flavus*	20.00
		Penicillium sp.	16.67
		Fusarium oxysporum	10.00
	Albizia lebbeck	*Sclerotinia sclerotiorum*	16.67
	Cupressus sempervirens	*Penicillium* sp.	13.33
		Stemphyllium sp	23.33
Rania	*Cupressus arizonica*	*Rhizoctonia* sp.	30.00
		Aspergillus flavus	20.00
	Cupressus sempervirens	*Aspergillus niger*	16.67
	Thuja occidentalis	*Penicillium* sp.	13.33
	Acacia matius	*Aspergillus flavus*	20.00
Derbendikhan	*Thuja occidentalis*	*Alternaria alternata.*	33.33
		Aspergillu s flavus	26.67
		Sclerotinia sclerotiorum	10.00
	Pinus brutia	*Aspergillus niger*	43.33
		Penicillium sp.	33.33
	Albizia lebbeck	*Pythium* sp.	10.00
		Fusarium oxysporum	6.67
		Cladosporium sp.	20.00
		Ascochyta sp.	30.00
	Melia azedarach	*Aspergillus flavus*	46.67
		Sclerotinia sclerotiorum	6.67
		Rhizoctonia sp.	23.33

***Each number is mean of three replicates (three petri plates), for each species.**

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3.2 Effects of fungal filtrates of *Aspergillus flavus* and *Penicillium* sp. on the germination rate of *Albizia lebbeck* and *Gledisia triacanthos* seeds

Data presented in table 4 revealed that the percentage of germination rate of *Albizia lebbeck* seeds soaked in culture filtrate of *A. flavus* for 24h. (30%) and significantly different from the treatment for 48 h. (13%) , these treatment were reduced germination rate compared to the control treatment (93%), in this study *A. flavus* filtrates reduced the rate of germination for the two duration periods 24 and 48 hrs. if compared to the germination rate for the control treatment (distilled water only). Similarly, Jalandar and Gachande (2012) mentioned that the cultural filtrates of *Aspergillus* sp. caused reduction in seed germination. Also, *A. niger* can produce mycotoxins such as oxalic acid crystals, kojic acid and malformins depending on the growth condition and the strain of the organism (TSCA, 1997). In this study, the cultural filtrates of *Penicillium* sp. did not influence any significant difference between the two durations 24 and 48 h. on the germination of the seeds *Gledisia triacanthos.* Similarly, Khokhar *et al*. (2013) reported that *Penicillium chrysogenum* decreased the percentage of seed germination by 20.33%. *P. chrysogenum* had shown a poisoning effect on the wheat seedling at a higher percentage (90%).

Table 4: Effects of culture filtrates of *Aspergillus flavus* and *Penicillium* sp. on the percentage seed germination of *Albizia lebbeck* and *Gledisia triacanthos* seeds in Sarchnar nurseries.

Scientific name	Fungal filtrate	Treatments (h.)	Germination rate (%)
Albizia lebbeck	*Aspergillus flavus*	24	30.00 b (67.74)
		48	13.00 c (82.02)
	Control	24	93.00 a (0.00)
		48	93.00 a (0.00)
Gledisia triacanthos	*Penicillium* sp.	24	15.00 b (81.01)
		48	15.00 b (81.01)
	Control	24	79.00 a (0.00)
		48	79.00 a (0.00)

- Different letters indicated there were significantly different according to Duncan' Multiple test (P≤0.05).

--The numbers between brackets are the percentages of reduced germination rate= % of germinated seeds in control - % of germinated seeds in treatment /% of germinated seeds in control x 100

3.3 Effects of fungal filtrates of *Sclerotinia sclerotiorum,Alternaria alternata and Fusarium oxysporum* on the germination rate of *Albizia lebbeck, Acacia matius* and *Melia azedarach* seeds

Data presented in Table 5 show that the percentage of the germination rate of *Albizia lebbeck* seeds soaked within the culture filtrate *Sclerotinia sclerotiorum* (16%) for 24 h. which was significantly different from the 48 h. and of the control treatments. This result was also supported by Sharma *et.al* (2014) that of the cultural filtrates of *S. sclerotiorum* from different geographical isolates that inhibited the seed germination rate and seedling vigor by effective producing toxic metabolites in the media in which they were grown. The cultural filtrates of *Alternaria alternata* the current study did not influence significantly for the seeds of *Acacia matius* immersed after 48 h., however

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the filtrate this fungus reduced the rate of seed germination for (24 and 48h.) compared to the control treatment. and this result was agreement with the results of Garuba *et al*., (2014) who observed that the percentage germination of the maize seeds treated with culture filtrates of *A. niger* and *P. chrysogenum* (65.33% and 79.67% respectively) was lower than the control (100%). The culture filtrates of *F. oxysporum* did not affect any significant difference neither for 24 h. nor for 48 h. on germination seeds. Finally, the filtrate *F. oxysporum* totally inhibited (100%) the seed germination of *Melia azedarach.* and this result was supported by Ibraheem *et. al.* (1987) who observed that *A. alternata A. flavus* and *A. niger* had inhibitory power to reduce seed germination.

Table 5: Influence of culture filtrates of *Sclerotinia sclerotiorum, Alternaria alternata* and *Fusarium oxysporum* on seed germination rate of *Albizia lebbeck, Acacia matius* and *Melia azedarach* seeds in Dokan nurseries.

Scientific name	Fungal filtrate	Treatments (h.)	Germination rate (%)
Albizia lebbeck	*Sclerotinia sclerotiorum*	24	16.00 b (82.60)
		48	0.00 c (100.00)
	Control	24	92.00 a (0.00)
		48	92.00 a (0.00)
Acacia matius	*Alternaria alternata*	24	20.00 b (78.02)
		48	6.00 b (93.40)
	Control	24	91.00 a (0.00)
		48	91.00 a (0.00)
Melia azedarach	*Fusarium oxysporum*	24	0.00 b (100.00)
		48	0.00 b (100.00)
	Control	24	67.00 a (0.00)
		48	67.00 a (0.00)

- Different letters indicted there were significantly different according to Duncan' Multiple test (P≤0.05).

--The numbers between brackets are the percentages of reduced germination rate= % of germinated seeds in control - % of germinated seeds in treatment /% of germinated seeds in control x 100

CONCLUSION

Current study exposed very good relationship between the seed-borne infections and germination failure of forest tree seeds. The culture filtrates of *Aspergillus flavus, Penicillium* spp *Alternaria alterna* and *Sclerotinia sclerotiorum* reduced significantly percentage of seed germination. A total inhibition of seed germination was also detected for Fusarium *oxysporum* in both treatments (24 and 48h.) and by *S. sclerotiorum* after 48 h. of seed incubation.

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