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Fungi colonized the roots of seedlings in forest nurseries

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Abstract

The current study was conducted to isolate and identify fungi associated with roots of nursery plant in forest. Sampling was carried out in two main forest nurseries in Duhok province, Iraq. In Malta nursery, the most frequent fungus isolated from the roots of olive and pine seedlings was *Macrophomina phaseolina* with a percentage frequency 53% and 33%, respectively. *Rhizoctonia solani* colonized the root of both walnut (56%) and vertical cypress (39%). Roots of olive were colonized by *Trichoderma harzianum* (94%) and robenia with *Fusarium poae* (56%). In Forestry Department nursery, *Fusarium* species were the dominant fungi in almost all seedlings types. Robinia roots were colonized by *Fusarium* sp. and *M. phaseolina* with a percentage frequency of 54%, 38%, respectively. *Fusarium poae*, *Fusarium* sp. and *F. oxysporum* were prevalent on walnut, horizontal cyprees and pine roots with isolation frequency reached up to 33-39%. Other genera isolated from different host in low frequency were *Phoma, Verticillium, Aspergillus* and *Cylindrocarpon.* These results indicated that the most common genera colonized the roots of both forest seedlings belongs to different species of *Macrophomina, Fusarium* and *Rhizoctonia.*

Keywords: Forest nurseries, Soil borne fungi, Root rot pathogen, Iraq.

Introduction

Damping-off and root rot are the major diseases affecting forest seedlings of many plant species in the nurseries around the world. They are often caused by genera of soil borne fungi like *Pythium, Fusarium, Macrophomina* and *Rhizoctonia* (Sutherland and VanEerden, 1980). Species of *Pythium* and *Fusarium* were documented as the main pathogens in temperate regions (Arentz, 1991; Salerno *et al.,* 1999). Likewise, many pathogens like *Alternaria*, *Cylindrocarpon*, *Cylindrocladium*, *Fusarium*, *Trichothecium* and many species of *Mucorales* have been isolated from forest nurseries in different region of the world (Mittal and Wang, 1993). *Rhizoctonia solani* was isolated from symptomatic pine, cypress and olive seedlings in different periods during the growing season in Iran (Zakeri *et al.,* 2011; Sanei and Razavi, 2012). Soil abiotic factors like pH, electrical conductivity and organic matter considerably affected the distribution of these fungi in soil (Blagodatskaya and Anderson, 1998; Fierer and Jackson, 2006). Soil pH is reported to strongly influence carbon availability (Andersson *et al.,* 2000), nutrient availability (Aciego and Brookes, 2008) and the solubility of metals (Flis *et al.,* 1993). Substantial attempts have been made to check effect of organic matters on different pathosystem with aim to identify characteristics correlated with pathogen suppression (Janvier *et al*., 2007; Termorshuizen *et al*., 2007). However, very limited information is available on fungal populations colonized the roots of forest seedlings in Duhok province, Iraq (Yousif, 2010). Therefore, objective of this work was to determine the occurrence and identification of fungi associated with the roots of some forest seedlings in Duhok province nurseries and to assess their relevance with soil physic-chemical properties.

Materials and Methods

Soil of both nurseries were collected and analyzed for following characteristics (Table 1)

For isolation of soil fungi associated with roots of plant, seedlings of different plants grown in plastic bags in two nurseries at Malta and Forestry Department, Faculty of Agriculture, Duhok, Iraq were collected during two season winter (November–January) and Spring (February–April). Small pieces (1-2 cm long) of roots of each pine (*Pinus brutia* Ten.), olive (*Olea europaea* L.), pistachio (*Pistacia vera* L), walnut (*Juglans regia* L.), robinia (*Robinia pseudoacacia* L.), horizontal cypress (*Cupressus sempervirens* L. var. *horizontalis*), and vertical cypress

(*Cupressus sempervirens* L. var. pyramidalis) were collected. Small pieces (1-2 cm long) of root tissues were surface sterilized by placing in 2% NaOCl for 2 min and then dried by filter papers. Sterilized tissues pieces were plated onto potato dextrose agar (PDA) containing 0.25 mg mL^{-1} chloramphenicol. Hyphae growing out from the tissue pieces were cut and sub cultured onto fresh PDA plates, and incubated at 25 ± 2 °C for 7 days and isolated fungi were purified on fresh PDA. For induction of sporulation, inoculated plated were kept in darkness or under fluorescent illumination. Each plate was inoculated in triplicates and plates were kept in a completely randomized fashion. The isolates were identified using the relevant literature (Watanabe, 2002; Leslie and Summerell, 2006) and frequency of occurrence was determined as follows:

$$\text{Frequency (\%)} = \frac{\text{Colony No. of isolated fungi/plate}}{\text{Total No. of colonies/plate}} \times 100$$

Results and Discussion

Several fungi isolated from the roots of seven forest seedling (2-3 years old). The prominent genera were *Macrophomina, Fusarium* and *Rhizoctonia* and other genera isolated in low frequency were *Aspergillus, Penicillium, Alternaria, Helminthosporium* and *Verticillium* (Table 2 and 3).

In the Forestry Dept. nursery, frequency of *F. oxysporum* colonized the pine seedling was 33 % and 15 % during winter and spring. Walnut roots were found to colonize with *F. poae* (33 %) during winter. This fungus was found commonly during spring season in almost all seedlings with a high frequent in olive seedling (22 %). *M. phaseolina* found with high frequency in robinia roots (38%) during winter. In spring, this fungus was isolated from the walnut roots (100%). *R. solani* were isolated from pine roots by 13%. *Phoma* spp. were found in pine, olive, and vertical cypress during winter by 15, 17, and 19 % respectively (Table 2).

In Malta nursery, *M. phaseolina* was the dominant fungus in pine (33%), olive (58%), pistachio (19%), and vertical cypress roots (23%) during winter. *R. solani* was found in more frequently than forest nursery particularly in walnut roots (44-55%) and vertical cypress roots (34-39%) in both seasons. *F. poae* found only in spring season and isolated from almost all forest seedlings except horizontal cypress, vertical cypress and olive. Its frequency ranged from 11 to 55%. *F. oxysporum* and *Cylindrocarpon destructans* were found in horizontal cypress roots during winter by 33% and 19 %, respectively. *Phoma* spp. were found by 20% and 27% in horizontal and vertical cypress during winter and spring season respectively. *Trichoderma harzianum* was isolated in high frequent during spring season (94%) (Table 3).

Fusarium species were found in high relative densities in both nurseries. This might be ascribed to high pH value (7.0-9.0) of the soil samples with low organic matter (1.0-3.8) in both locations (Table 1). Organic matter in the soil improves the biological and physiochemical properties of soil (Weil and Magdoff, 2004), and thus influence on the crop productivity and protection from root rot pathogens (Weller *et al.*, 2002). Turkkan (2013) revealed that *F. oxysporum* can grow in wide range of soil pH (pH 6.0-9.0). Occurrence of *M. phaseolina* and *R. solani* were also recorded with high frequency in both locations during this study, these two fungi were also isolated in Duhok nurseries and reported previously (Yousif, 2010). According to Csondes *et al.* (2012), the optimal pH for this fungus varied between 4.0 and 6.0 but growth observed at pH values (3.0, 7.0, and 8.0). Foresters have long recognized *R. solani* as a primary cause of seed (pre-emergence) and seedling (post-emergence) damping off in nursery seedbeds of both conifers and hard wood (Boyce, 1961). This fungus present almost with all forest seedling during both seasons, which may be due to high tolerant of them to environmental condition. Previous studies on the variation of pH value revealed that *Trichoderma* isolates showed optimum growth and sporulation rate at pH values ranging from 2 to 7 (Bandyopadhyay *et al.*, 2003; Begoude *et al.*, 2007). Current study found this fungus with high frequency (94.4%) in forestry nursery soil during spring at pH 8.3. It could be attributed to adaptation of *T. harzianum* to the soil conditions (alkaline soils). The improvement of stress tolerance in *Trichoderma* strain could results an increasing their efficacy against plant pathogenic fungi.

More frequency of fungi recorded in Malta nursery as compared to Forestry dept. nursery might due to high value of electrical conductivity. High concentrations of salts in the soil have detrimental effects on seed germination and plant growth (Ramoliya *et al*., 2006). El-Abyad *et al*. (1988) revealed that the mycelial growth, sporulation, conidia or sclerotia germination of *R. solani*, *F. oxysporum* and *Fusarium equeseti* were stimulated when adding salts to the media.

According to these results, the most isolated fungi were known in this study as the causes of damping-off and root rot diseases. Good farming management practices could be helpful is suppression of these soil-borne fungi in nurseries.

Table 1. Soil properties of Malta and Forestry Department nurseries of Duhok Province, Iraq.

Nursery (Location)	Organic matter (%)	Potassium (ppm)	Phosphorus (ppm)	Electrical conductivity (ds m^{-1})	pH
Malta Nursery (Malta)	1.22	0.016	5.23	2.7	8.30
Forestry Dept. Nursery (Semel)	3.79	32.75	4.02	0.63	7.65

Table 2. The isolation frequency of fungal species from forest seedlings in Forestry Department nursery.

Host	Isolated fungi	Frequency (%)	
		Nov. - Jan.	Feb. – April
Pine	*Fusarium oxysporum*	32.54	15
	Macrophomina phaseolina	8.33	21.67
	Fusarium sp.	20.63	22.45
	Aspergillus niger	15.08	-
	Phoma sp.	15.08	-
	Fusarium poae	-	6.67
	Rhizoctonia solani	-	13.33
Olive	*Phoma* sp.	165.67	-
	Rhizoctonia solani	5.56	-
	Fusarium sp.	32.22	11.11
	Fusarium poae	-	22.22
Pistachio	*Fusarium* sp.	30	-
	Penicillium sp.	6.67	-
Walnut	*Fusarium* sp.	16.67	-
	Fusarium poae	33.33	-
	Macrophomina phaseolina	-	100
	Mycelia sterilia	16.67	-
Robinia	*Macrophomina phaseolina*	37.78	-
	Fusarium sp.	53.89	69.44
	Aspergillus sp.	8.33	-
	Fusarium oxysporum	-	16.67
	Alternaria sp.	-	8.33
	Aspergillus flavus	-	5.56
Horizontal cypress	*Macrophomina phaseolina*	19.44	22.22
	Fusarium spp.	34.72	38.89
	Aspergillus niger	-	11.11
	Fusarium poae	-	5.56
	Fusarium oxysporum	-	5.56
	Aspergillus flavus	-	11.11
	Emercilla sp.	-	5.56
Vertical cypress	*Aspergillus niger*	4.47	-
	Helminthosporium sp.	13.69	-
	Phoma sp.	19.05	-
	Fusarium oxysporum	4.76	6.7
	Verticillium sp.	4.17	-
	Aspergillus terreus	8.33	-
	Penicillium sp.	-	6.7
	Macrophomina phaseolina	-	20
	Fusarium poae	-	6.7
	Fusarium sp.	-	20
	Aspergillus flavus	-	6.7

Table 3. The isolation frequency of fungal species from Forest seedling in Malta nursery.

Host	Isolated fungi	Frequency (%)	
		Nov.- Jan.	Feb. – April
pine	*Macrophomina phaseolina*	33.33	33.33
	Fusarium poae	-	25
	Aspergillus terreus	8.33	-
olive	*Macrophomina phaseolina*	58.33	-
	Trichderma harzianum	-	94.4
	Aspergillus sp.	-	5.56
	Fusarium sp.	41.11	-
pistachio	*Rhizoctonia solani*	6.67	-
	Macrophomina phaseolina	19.44	-
	Fusarium oxysporum	11.11	18.89
	Aspergillus niger	40.78	29.99
walnut	*Fusarium poae*	-	28.89
	Fusarium sp.	29.17	-
	Aspergillus niger	8.33	27.78
	Rhizoctonia solani	55.56	45.56
robinia	*Fusarium oxysporum*	-	5.56
	Fusarium poae	-	11.11
	Rhizoctonia solani	22.22	-
	Fusarium sp.	66.67	-
	Aspergillus niger	11.11	-
horizontal cypress	*Fusarium poae*	-	55.56
	Aspergillus sp.	-	11.11
	Fusarium oxysporum	32.54	-
	Phoma sp.	19.84	-
	Cylindrocarpon destructans	19.44	-
	Aspergillus niger	16.67	-
vertical Cypress	*Rhizoctonia solani*	39.05	34.44
	Fusarium oxysporum	11.43	-
	Macrophomina phaseolina	23.33	-
	Fusarium sp.	25.24	38.89
	Phoma sp.	-	26.67

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