



Survey of Olive Fungal Disease in North of Iran

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Case Study

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ABSTRACT

New plantations of Olive tree in northern Iran are usually being severely affected by wilt or dieback and death. To determine the etiology of this problem, a study was carried out in samples of affected young trees collected in Golestan, Zanjan, Gilan and Khorasan provinces, the north of Iran during 2004-2009. Fungi that cause olive disease or associated with stem cuttings are listed. From this list *Verticillium dahliae* and *Fusicladium oleagineum* were the most common on a wide range area and on all cultivated cultivars. Several fungi were isolated from seedling rotted roots and some recorded associated with stem cuttings. Other fungal species associated with death of young olive trees in the field or in the nurseries, including *Botrytis cinerea*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Fusarium solani*, *Macrophomina phaseolina*, *Neoscytalidium dimidiatum*, *Phytophthora megasperma*, *Phytophthora nicotiana*, *Pythium aphanidermatum*, *Rhizoctonia solani* and some stem decay fungi such as species of *Ascochyta*, *Alternaria*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Diplococcium*, *Diplodia*, *Nigrospora*, *Sphaeropsis*, *Stemphyllium* and *Ulocladium*. As the fungal pathogen can affect olive production, these findings are potentially important to the future olive industry in northern Iran.

Keywords: Olive; drying syndrome; *Verticillium*; Iran; Golestan province.

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1. INTRODUCTION

Olive (*Olea europaea* L.) tree is the most important oil crop in north of Iran. This distribution area has steadily increased during the last decade especially in Golestan province, the northern of Iran (Anonymous, 2009). In this province nearly 10,000 hectare of olive orchards are present, which covers more than 20% of total national olive area (Anonymous, 2009). In the last decade most of new plantations in this region established with 'Rooghany', 'Zard' and 'Mary' cultivars, which are the native olive cultivars of Iran (Sanei et al., 2004a). Commercial cultivars of olive are planted in Iran but wild olive is the important genetical sources of olive, that residue of them can be seen in the East of Golestan province (Sanei et al., 2005).

Unfortunately, olive is subjected to be attacked with a variety of pathogens, which affect its health, yield and its oil quality (Sergeeva et al., 2005, 2008; Sanei et al., 2004a). Together with the establishment of new olive plantations, the incidence of wilting, dieback and death of young trees has also increased (Sanei et al., 2005), alarming olive farmers. A number of fungi have been reported to be associated with olive tree branch wilting and dieback or tree death. Notable among them are soilborne root rot fungi, such as *Armillaria* spp., *Rosellinia necatrix* Prill., *Macrophomina phaseolina* (Tassi) Goid., *Sclerotium rolfsii* Sacc. and *Omphalotus olearius* (DC.:Fr.) Singer, *Eutypa lata* (Pers.:Fr.) Tul. et C. Tul., *Cylindrocarpon destructans* (Zins.) Scholten and some stem decay fungi such as species of *Diplodia*, *Fomes*, *Fusarium*, *Phoma*, *Phytophthora*, *Pythium* and *Stereum* (Sanchez-Hernández et al., 1998; Moral et al., 2009; Rumbos, 1993; Zizzerini and Marte, 1976).

Comprehensive lists of plant diseases and/or associated fungi are available for Iran (Sanei et al., 2012) but there is little information available on the occurrence and distribution of olive diseases. The objective of this study was to determine the nature and pathogenicity of the biotic agents, together with the abiotic factors associated with the drying syndrome of olive trees in northern Iran. The causes of the observed disease symptoms were identified and a checklist of the plant pathogens and plant associated fungi was compiled.

2. MATERIALS AND METHODS

Short visited were made to 60 olive orchards in Golestan, Zanjan, Gilan and Khorasan provinces, the north of Iran during 2004-2009. Infected branches, leaves and roots of Olive trees and several stem cuttings collected from the orchards (4–20 years old) and seedlings from the pots of greenhouses. The samples sterilized with 0.5% sodium hypochlorite, ethanol and small pieces of them cultured on Potato-Dextrose-Agar (PDA) and Czapeck-Dox-Agar media. The plates incubated at 25±2°C and under dark conditions for 15 – 20 days. Symptoms severity were also evaluated based on a scale 0-5 (0= healthy tree and 5= dead trees) multiply number of infected trees per total number of trees.

As the Verticillium wilt disease was seen, the soil of orchards or the pots were assayed for the measurement of the pathogens. In this method the soil sample (mixture of 40 randomly samples per orchards) were air-dried for 3-4 weeks at room temperature (23-26°C), pulverized, mixed well and passed through a 2mm screen. Inoculum density of the pathogen was estimated by wet-seiving techniques (Huisman, and Ashworth, 1974) and ethanol streptomycin agar medium (Nadakavukaren and Horner, 1959). Pathogenicity of isolated pathogens were determined by stem puncture method for *V. dahliae* isolates (Sanei et

al.,2008) and the stem and root-tape inoculation technique of Zizzerini and Tosi (1989) for other fungal isolates in 1 year old Olive shoot and root, respectively.

For olive scab, uninfected leaves on 5-year-old trees of variety 'Rooghany' growing in glasshouse at $25\pm 7^{\circ}\text{C}$ were used for pathogenicity tests. Suspensions of conidia (5×10^6 spore ml^{-1}) from diseased leaf were prepared in sterile distilled and used for spraying the leaves. The leaves on the trees were sprayed with sterile water served as controls. Both the inoculated and control leaves covered with plastic bags and left for three days. Symptoms recorded after 10-14 days were similar to those initially observed on the trees. Stem, root and fruits of 'Rooghany' cultivar were also inoculated with other isolated fungi.

3. RESULTS AND DISCUSSION

During this study several fungal disease were found with olive trees in orchards, seedlings in greenhouse and stem cuttings (Table 1).

3.1 *Verticillium* Wilt

The soil borne fungus, *Verticillium dahliae* Kleb., attacks the vascular tissue of the trees and usually causing one or more branches to wilt early in the growing season. The disease was first observed in 1996 (Sanei et al., 1996) in the Golestan province, since time the disease spread with increase in olive hectarage (Sanei et al., 2004a,b). Since olive is evergreen, symptoms of olive dieback or *Verticillium* wilt appear on infected trees at any time of year. Young trees were highly infected, they stunted, wilted or defoliated but percent of infection decreases as the trees become older. The disease also can be seen in older trees even over trees with 20 years old, but tree death is not common. New symptoms appear in early spring and develop during summer and autumn. These symptoms start on one branch of an infected tree and characterized by drying out of floescence and progressive defoliation of twigs. The leaves become yellow and start to fall from the base to the tip of the branch until the branch become bare. Infected trees decline continuously and renew the growth of trees after pruning. After pruning and removing the dried branches, the disease severity decreased and remained at lowest level. Vascular discoloration in cross section of diseased branches are not common, although in some cases the cross section of recently infected branches show a light red color. The diseased branches differ from those of healthy branches by a distinctive smell. Disease incidence was found in 100% of Golestan olive orchards. Symptoms severity of the orchards varied from 0.6 to 2.4%. A significant difference in disease incidence and severity was observed among cultivars. *Verticillium* wilt occurred in highest frequency on the 'Mission', 'Rooghany' and 'Zard' cultivars and the least rate infestation was observed on 'Kalamon', 'Koroneiki' and 'bladi' cultivars. The pathogen isolated from twigs of all ages but mostly from 2 years old branches. Seasonal changes were observed in the detection of the fungus in the trees. The number of propagules of *V. dahliae* per gram of soil ranged from 2 to 32 and average number for groves were 13.42 ± 0.504 . Cultures grew moderately fast on PDA at 23°C . Mycelia were hyaline and white to cream colored, becoming black with formation of microsclerotia. Conidiophores were hyaline and verticillately branched, with three to four phialides at each node. Conidia borne on phialides were ellipsoidal to short and cylindrical and mainly one-celled (2.5 to 8×1.4 to $3.2 \mu\text{m}$). Microsclerotia began to form in 6- to 7-day-old cultures and were dark brown to black and varied in shape and size (25×50 to $100 \mu\text{m}$ diameter).

Table 1. Fungal disease was found first with olive trees from orchards, seedlings in greenhouse and stem cuttings in northern Iran

Fungal diseases	Pathogen(s)	Olive cultivar	Original location/province
Verticillium wilt	<i>Verticillium dahliae</i>	'Rooghany'	Gorgan/Golestan
Olive scab	<i>Fusicladium oleagineum</i>	'Rooghany'	Gorgan/Golestan
Anthraco nose	<i>Colletotrichum acutatum</i> and <i>C. gloeosporioides</i>	'Mission'	Gorgan/Golestan
Botrytis blight	<i>Botrytis cinerea</i>	'Mary'	Roodbar/Guilan
Nattrasia canker	<i>Neoscytalidium dimidiatum</i>	'Zard'	Guilovan/Zanjan
Shoot dieback	<i>Botryosphaeria</i> , <i>Phytophthora</i> , <i>Rhizoctonia</i> and <i>Pythium</i>	'Sevillano'	Karaj/Alborz
Root rot	<i>Fusarium solani</i> , <i>Macrophomina phaseolina</i> , <i>Phytophthora megasperma</i> , <i>Phytophthora nicotiana</i> , <i>Pythium aphanidermatum</i> and <i>Rhizoctonia solani</i>	'Mary'	Gorgan/Golestan
Fungal associated with stem cuttings	<i>Alternaria</i> spp., <i>Ascochyta</i> spp., <i>Aspergillus</i> spp., <i>Bipolaris</i> spp., <i>Cephalosporium</i> sp., <i>Chaetomium</i> sp., <i>Cladosporium</i> sp., <i>Diplococcium</i> sp., <i>Diplodia</i> sp., <i>Fusicoccum</i> sp., <i>Humicola</i> sp., <i>Nattrassia</i> sp., <i>Nigrospora</i> sp., <i>Penicillium</i> spp., <i>Phoma</i> sp., <i>Sphaeropsis</i> sp., and <i>Stemphyllium</i> spp.	'Mary'	Gorgan/Golestan
Fruit rots	<i>Colletotrichum gloeosporioides</i> , <i>Alternaria</i> spp., <i>Cladosporium</i> sp., and <i>Ulocladium</i> sp.	'Zard'	Guilovan/Zanjan

Young olive trees were highly infected and percent infection decrease as the trees become older. This result can be explain with the increase rate of recovery from disease in olive trees. The pathogen isolated from branches of all ages but mostly 2 years old branches. As the fruits produce on 2 years old branches, therefore the higher transpiration on these parts can be considered, which can describe different distribution of *V. dahliae* propagules in trees.

The several reports of dynamic populations of *V. dahliae* in cotton and potato fields in Iranian northern provinces (Sanei and Nasrollahnejad, 1995; Sanei et al., 2000) indicate that the inoculum of *V. dahliae* in soil of these regions can consider as primary of inoculum of the pathogen for olive infections. Therefore, the main contributing factor for the increase in

disease incidence and severity has been establishment of orchards in the fields that previous cropped with susceptible hosts of the pathogen especially cotton, tomato and potato. The result of this study was similar to other researchers suggest that host specificity was not common in *V. dahliae* isolates, although isolates may have exhibited different morphology, differential virulence on different hosts. The differences in the type reaction by host differential inoculation with *V. dahliae* isolates, show that the two different pathotype of *V. dahliae* in the soil of this region can attack the olive trees. The isolated of the fungus from symptomless trees indicating latent infection that could be expressed at later stage of trees development. This could be explained, also as due to less aggressive isolates of *V. dahliae* with mild–nondefoliate pathogenicity in orchard as suggested by others (Thanassouloupoulos, 1993).

With respect of isolation of *V. dahliae* from olive seedlings in nurseries, there are two reasons which may account for the incidence disease in nurseries of the area. Firstly, the nursery growers do not take nursery precautions in nursery especially with using of infected or improperly sterilized soil. Second, the selected leafy cutting from trees that already infected but were symptomless. The results suggest that with respect of inoculum density of *V. dahliae* in soils of the northern provinces, susceptibility of young trees especially the infection of young trees in nurseries and susceptibility of cultivated cultivars, an integrated control such as use fungicides, cultural method, avoiding of intercropping with other *V. dahliae* susceptible hosts, chemical control of weed, soil solarization, etc. are necessary for decreasing the disease in initial stages of the establishment of an olive orchard. Also, the use of resistant olive root sock or cultivars instead of susceptible cultivars is the best aspect *Verticillium* wilt control (Lopez-Escudero et al., 2010; Lopez-Escudero and Mercado-Blanco, 2011; Sanei et al., 2010; Thanassouloupoulos, 1993).

3.2 Olive Scab

Olive scab also known as Olive leaf spot, Bird's-eye spot and Peacock spot is caused by the fungus *Fusicladium oleagineum* (Castagne) Ritschel & U. Braun (= *Spilocaea oleaginea* (Castagne) S. Hughes) is widespread disease in northern Iran olive orchards and first time reported from northwest of Iran in Roodbar (Gilan province) by Eskandari, (1964) and Scharif and Ershad (1966). The disease first appears as small sooty blotches on the leaves that later become muddy green to black, often with a yellow halo. Often the leaves drop prematurely. Initially symptoms are found on the upper surface of olive leaf. Lesions produced by the pathogen may be inconspicuous, but slowly expand to form round (3-10 mm diameter), effuse, olive-green to dark olivaceous spots. These become dark brown and lightly velvety with eruption of conidiophores and conidia. The fungus forming short (10-30×8-15 µm) ampuliform, usually unbranched, olivaceous-brown, monoblastic conidiophores. Solitary, simple conidia are formed acrogenously, and production of successive conidia is traced by distinct of the percurrent conidiogenous cell. Old spots may show necrotic areas and an annular or zonate appearance (Sanei and Razavi, 2011b). The most important effect of olive leaf spot attacks is premature defoliation which in turn has consequences on the plant's vegetative activity and yield. Yield losses may also be a consequence of non-conversion of the axillary buds of leaves shed by the disease into apices developing flowering shoots. Infection of fruits may be deleterious for table olives, and for oil cultivars it may cause a delay in ripening and a decrease in oil yield. The disease is particularly severe in densely planted orchards of susceptible olive cultivars and on northern region of trees with higher relative humidity, especially the orchards in jungles. Infections may occur throughout the year, except during hot and dry summers. Spots already formed in spring may stop growing in summer and resume their growth and sporulate in autumn. Disease severity

reached its highest level between March and June depending on rainfall, temperature and relative humidity.

With a high proportion of middle aged trees (75% of trees in the age group of 5-8 years) in Golestan, one would also expect a higher proportion of trees infected. Lack of any fungicide application may have contributed to prevalence and high severity in high wet regions especially in groves between jungle with low aeration. Also, the disease was high in North facing trees with higher relative humidity than south facing. Cultivars vary in their susceptibility to this disease and had a significant effect ($P < 0.001$) on the prevalence of disease. With 'Rooghany', 'Zard', 'Mary', 'Manzanila' and 'Amigdalifolia' showing the highest prevalence of trees infected, followed by Mission then 'Koronakei', 'Valatolina' and Wild olive. Cultivars also vary in their number of leaves infected per tree and number of lesions per leaf and these factors were very highly correlated with each other ($P < 0.001$). This correlation followed very similar patterns for age, cultivars and region as seen for tree infection. The percentage of leaves infected followed a similar trend to disease prevalence in trees. The number of lesions found on the leaves depended on cultivar. Similarly, the region where the leaves were sampled from also affected the prevalence and severity of spots on the leaves. Assessments of leaf infection indicate that 'Valatolina' and wild olive were less susceptible to disease than native olive cultivars ('Rooghany', 'Zard' and 'Mary'). This agrees with findings reported by Sanei et al. (2004a, 2005) who assessed resistance of olive cultivars to olive scab disease in Golestan province.

3.3 Anthracnose

Anthracnose caused by *Colletotrichum acutatum* J.H. Simmonds and *C. gloeosporioides* (Penzig.) Penzig & Saccardo, is a common and widespread fruit rot disease of olives in most olive growing regions in the world (Tjamos et al., 1993; Talhinas et al., 2005) and causing major losses in yield of olives and the quality of olive oil (Iannota et al., 1999; Talhinas et al., 2005). The occurrence of anthracnose on leaves and fruits of olives reported for the first time in Golestan province (Saneiet al., 2005). Anthracnose infection starts as small spots on the upper surface of leaves which can enlarge to form extensive dead areas. Brown spots carrying heavily sporulating colonies of *C. acutatum* and *C. gloeosporioides* were observed. The infected spots were observed mostly at the edges of the leaves but also occurred near the midrib. The spots were light to dark brown in colour and the necrotic areas had irregular shapes and were 1–3 cm in size. The acervuli on infected leaves produced salmon coloured spore masses under high relative humidity. Symptoms associated with leaf infection by *C. gloeosporioides* were similar to those caused by *C. acutatum*. Conidiomata are visible on leaves as small black dots. 3 days in a humid chamber at 25°C. *Colletotrichum acutatum* produced orange to pink coloured colonies with whitish aerial mycelium on PDA. *C. gloeosporioides* produced grey colonies with whitish aerial mycelium on PDA. Leaves showing the symptoms were surface sterilised with 1% sodium hypochlorite for 2 min followed by washing three times in sterile distilled water. The fungal isolate was cultured on PDA and grown at 25°C. Pathogenicity tests were carried out by using conidial suspensions of *C. acutatum* and *C. gloeosporioides*. No symptoms developed on the leaves sprayed with sterile water. *C. acutatum* and *C. gloeosporioides* were consistently reisolated from surface sterilised leaves of inoculated plants indicating internal infections of these leaves. The incidence of naturally occurring infection on leaves by *C. acutatum* and *C. gloeosporioides* was relatively low in the different olive varieties and locations of olive groves. However, it is very likely that the pathogens were present during the whole season. The lengthy presence of *C. acutatum* and *C. gloeosporioides* can be a continuing source of flower and fruit

infection (Sergeeva et al., 2008). Infected leaves were considered as inoculum sources for fruit anthracnose.

3.4 Botrytis Blight

Olive Botrytis blight first time report from northwest of Iran in Roodbar (Gilan province) by Majidieh-Ghasemi et al. (1995). Blight and dieback disease on shoots, buds and leaves of olive trees mostly on 'Mary' cultivar in many olive orchards from Rostamabad and Roodbar, Gilan province. The fungus *Botrytis cinerea* causes blight of buds, blossom, leaves, stems and canker formation on olive. The color of colonies of isolated fungus were grey or grayish brown. After 12-14 days black sclerotia formed on PDA medium abundantly. Conidia are ellipsoidal or obovoid, colorless to pale brown, smooth, 6-18 × 4-11 micrometer in size. Conidia are airborne, but may be carried by rain droplets. The fungus overwinter as sclerotia or as mycelium in plant debris. Optimum temperature for growth, sporulation and spore germination are 25 °C. The fungus could cause blight and dieback on olive young stems, shoots and leaves in greenhouse on 'Mary' and 'Rooghany' cultivars. The fungus can attack the plants in favor condition alone or with contribution of other fungi.

3.5 Nattrasia Canker

The fungus *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers (= *Nattrasia mangifera* (Syd. & P. Syd.) Sutton & Dyko) isolated from 'Rooghany' cultivar showing branch dieback and elongated canker on branch in Zanjan province. The fungus grew readily on PDA and forming a white colony consisting of septate mycelia that later become dark gradually and produce chains of 1-2 celled (mostly one celled) spores. The conidia were rather variable in shape and size, mostly elongated (7.5-10 × 5 µm) or spherical (5-7.5 µm). *N. dimidiatum* produced pycnia on PDA and on inoculated olive shoot-tips 30 days after incubation. Pycnidia were eustromatic, eruptive and spherical. Pycniospores were hyaline, 0-2 septate, average 5 × 12.5 µm. Disease symptoms appeared 15 days after inoculation of the pathogen on 2 years old branches of 'Rooghany' cultivar with surface sterilized with ethanol 75% and wound inoculation with 5mm disk of fungus culture.

3.6 Shoot Dieback

Observed on many varieties, usually young seedlings less than 2 years old, several centimetres of the main stem about 8-12cm above ground level dies. The top of the plant then also dies, but the growth below generally remains healthy. If the main stem is removed just below the dead section, the plant reshoots from the base and continues to grow. *Botryosphaeria* is the only fungus so far recovered from the dead area on the stem, but this is most likely a secondary infection. Several bacteria have also been recovered, however none are known to be plant pathogens, and are considered to be secondary infections of the dead tissue. *Phytophthora*, *Rhizoctonia* and *Pythium* have been recovered from the roots of some of the affected plants, however it is unlikely that a root pathogen would cause damage on the stem several inches above soil level. Frost has also been implicated as a possible cause, however not all samples have come from areas subject to frost. Dieback is common phenomenon in olive orchards and number of agents including fungi is responsible for this disorder (Javadi Estahbani et al., 2006). Tip dieback and shoot death can be caused indirectly by root rot from the pathogens listed above. However it is also found on plants with no apparent root problems. On young shoots, *Botrytis* has been associated with tip death.

On older trees, no organism has been associated with the shoot death, which occurs randomly over the tree.

3.7 Root Rot

Young olive especially 'Rooghany' and 'Zard' cultivars showing decline symptoms were observed in several greenhouse. This syndrome was associated with a severe root rot. Several fungal pathogens were consistently isolated from roots of symptomatic seedlings. *Fusarium solani*, *Macrophomina phaseolina*, *Phytophthora megasperma*, *Pythium aphanidermatum* and *Rhizoctonia solani* which first reported by Sanei et al. (2005). *Rhizoctonia* has been consistently recovered from browned and rotted roots of young plants. Above ground symptoms include tip death, defoliation or death. Leaf drop and tip dieback was also found on plants where *Pythium* and *Phytophthora* were recovered from the soil or roots. The plant stem was brown without any type of black spots or streaks on bark of stem, but the collar region of the stem was black. On dissecting the main root, scattered black microsclerotia of *M. phaseolina* were seen. A partial destruction of secondary and tertiary roots was common. Root systems of the plants were totally destroyed. The root tissue of diseased plants are filled with microsclerotia of the fungus, giving it a grayish appearance. Inoculum density for all nurseries varied between 4 and 9 propagules per gram of air-dried soil with average 7.61 ± 0.73 .

3.8 Fungal Associated with Stem Cuttings

Several fungal isolated from stem cuttings (2 years old branches, with 30 cm in length) on media, were as follows: *Alternaria* spp., *Ascochyta* spp., *Aspergillus* spp., *Bipolaris* spp., *Cephalosporium* sp., *Chaetomium* sp., *Cladosporium* sp., *Diplococcium* sp., *Diplodia* sp., *Fusicoccum* sp., *Humicola* sp., *Nattrassia* sp., *Nigrospora* sp., *Penicillium* spp., *Phoma* sp., *Sphaeropsis* sp, and *Stemphyllium* spp. Complimentary examinations for demonstration of pathogens in this isolates showed *Sphaeropsis* sp. and *Phoma* sp. created symptoms, brown tissue in inoculated branches.

3.9 Fruit Rots

Anthracnose, caused by the fungus *Glomerella cingulata* (anamorph: *Colletotrichum gloeosporioides*), has been identified on olive fruit for many years (Sanei and Razavi, 2011a). It causes a soft circular rot on the fruit and at high humidity produces an orange slimy masses of spores on the fruit surface. *Alternaria*, *Cladosporium*, and *Ulocladium* have been associated with the end or nose rots seen on several cultivars the fungi recovered from these including. These fungi can be both primary and secondary invaders, and further work needs to be undertaken to confirm their importance as pathogens.

4. CONCLUSION

Our results indicate that olive trees are infected with a number of fungi. Notable among them are soil borne fungi, *V. dahliae*, *M. phaseolina* and *Phytophthora* spp., associated with olive tree branch wilting and dieback or tree death. Other species such as *F. oleagineum*, *B. cinerea*, *C. acutatum* and *C. gloeosporioides*, affect aerial parts especially olive fruits. As the fungal pathogen can affect olive production, these findings are potentially important to the future olive industry in northern Iran.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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