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Full Length Research Paper

Survey of *Spilocaea oleagina*, causal agent of olive leaf spot, in North of Iran

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Olive leaf spot or peacock spot, caused by the fungal pathogen *Spilocaea oleagina*, can cause reduced growth and yield in olive trees (*Olea eurapaea*). Investigations were carried out during 2007-2010 to measure the prevalence and severity of olive leaf spot in the northern olive growing regions of Iran. The susceptibility of ten cultivars (Amygdalifolia, Blaidy, Koronakei, Mary, Manzanillo, Mission, Rooghany, Valatolina, Wild olive, Zard) to the disease was assessed. Olive scab was found in all study areas and with the worst affected in high relative humidity (Y= -21.058 + 0.794X, p<0.001 r=0.952, Y= disease percent and X= Annual rainfall Mean monthly) and appeared to be particularly severe on trees that were growing in sheltered parts of an olive grove. Severity increased with age and a correlation between prevalence and severity was found. Native olive (Rooghany, Zard and Mary) and Manzanilla were the worst affected cultivars while Valatolina and Wild olive were least affected. The rate of conidial germ tube elongation on leaves affected by temperature and olive cultivars.

Key words: Peacock spot, *Spilocaea oleagina,* disease prevalence, disease severity, Iran.

INTRODUCTION

Olive (*Olea europaea* L.) is the most important and traditionol woody crop that cultivated over a large areas in Iran. Olive cultivation has expanded during the last decade especially in Golestan province, the northern of Iran (Figure 1). In this province nearly 10,000 ha of olive orchards are present, which represents about 20% of total national olive area (Anonymous, 2007). 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., 2004). Commercial cultivars of olive are planted in Iran but wild olive are the important genetical sources of olive, that residue of them can be seen in the East of Golestan province (Sanei et al., 2003, 2005).

Olive production in Iran is affected by two widely distributed diseases, olive leaf spot and Verticillium wilt (Sanei et al., 2010). The leaf spot disease or olive scab, also known as peacock spot, is caused by the fungus *Spilocaea oleagina* (Cast.) Hughes which lives parasitically on olive leaves, but on susceptible cultivars, fruit and fruit peduncles may also be infected (Graniti, 1993). The disease is widespread in Mediterranean region as well as in other temperate an subtropical of the world, where olive is grown. The disease was first recorded in Iran by Esfandiary in 1964 and Sharif and Ershad in 1966 from northwest of Iran in Roodbar (Gilan province) (Sanei et al., 2010). Since then the disease have increasing spread in olive hectarage and now occur throughout Iran's olive growing regions (Razavi and Jahany, 2009). Crop losses arise mostly from defoliation of infected trees, but recurrent infections cause poor growth and dieback of defoliated branches, and reducing fruit yield. Heavy defoliation may also cause a delay in ripening and decrease in oil yield (Azeri 1993; Graniti 1993; Razavi and Jahany, 2009).

Infection to olive leaf spot generally occurs during wet periods from autumn through winter to spring when inoculum levels from lesions is highest (Viruega and Trapero, 1999; Obanor et al., 2008) and wet remain or in a nearly saturated atmosphere for 1-2 days, depending on the temperature (Graniti 1993). Infections then become evident in spring with new lesions developing and causing premature leaf drop. In hot, dry weather

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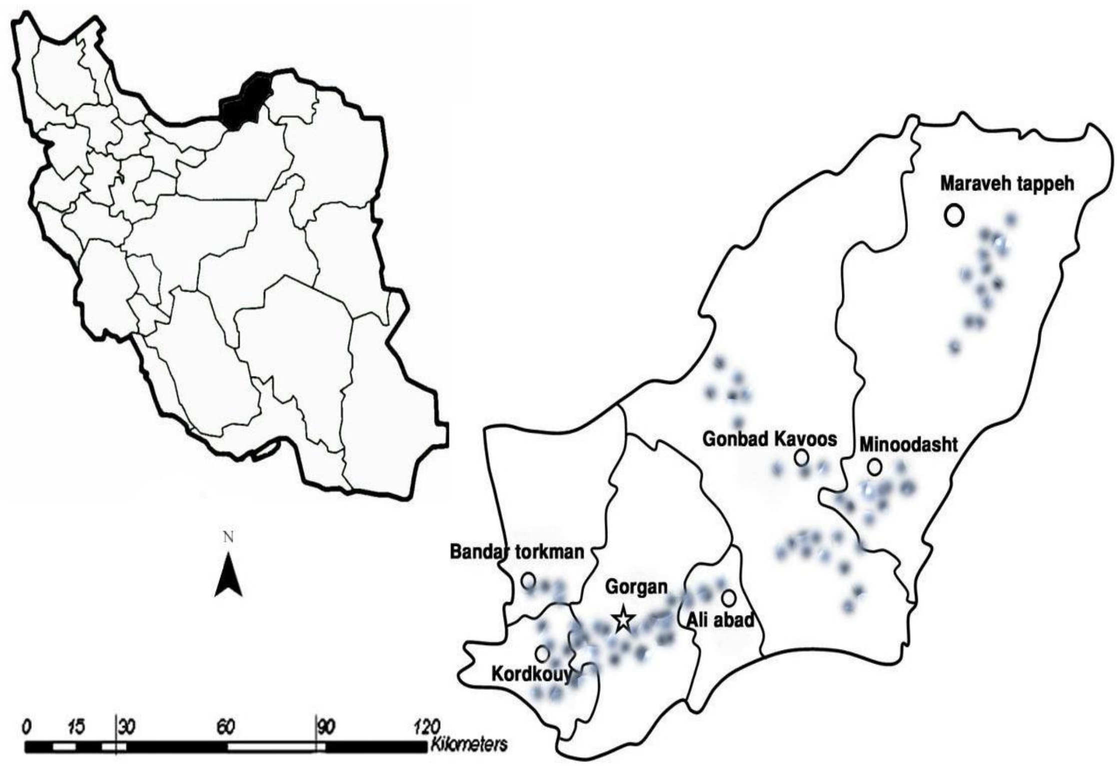

Figure 1. Iran map (left) and regional surveyed (Golestan province, right) in this study.

Table 1. Effect of region with climatological data* on trees infected with olive scab.

Region	Annual rainfall mean monthly (mm)	Mean monthly temperature (°C)	Disease severity in autumn (%)
Kordkouy	68.2	23.8	39.6±1.069**
Gorgan	53.9	26.8	23.3±0.68
Ali abad	78.6	27.0	36.7±1.054
Azadshahr	50.6	29.2	17.7±0.57
Minodasht	36.7	24.0	6.2±0.34

*Golestan meteorological station reference number, ** Mean±SE.

conditions the growth of the pathogen is limited (Graniti 1993; Saad and Masri, 1978), the disease is inactive (Teviotdale and Sibbett, 1995b) and infection seldom occurs (Laviola and Scarito, 1993). It is commonly assumed that conidia of *S. oleagina* are dispersed by rain splash or wind-borne water droplets (De Marzo et al., 1993), but Lops et al. (1990) observed significant conidial dissemination during periods without rain and suggest that wind can be an alternative factor of aerial dispersal of the fungus under high relative humidity conditions. Regardless of material, application rate or number of applications, copper containing fungicides will control olive scab when disease risk is low (Teviotdale et al., 1989a,b). However, regular annual treatment is required to prevent disease build-up in the grove, as high disease levels may be difficult to reduce (Teviotdale and Sibbett 1995a, b).

As there are limited data on olive disease in Iran, especially the susceptibility of cultivars, the objective of this study was to survey of the olive scab disease in Northern provinces with wide olive groves.

MATERIALS AND METHODS

The survey was conducted during autumn 2007/2010, because main infection periods occur during autumn and winter (Viruega and Trapero, 1999). Four to seven olive groves were selected in each of five growing regions (Table 1). Prevalence and severity of olive scab (%) was surveyed in established groves with trees preferably older than 4 years. The surveyed groves included several cultivars (Amygdalifolia, Blaidy, Koronakei, Mary, Manzanillo, Mission, Rooghany, Valatolina, Wild olive, Zard) of varying ages. Twenty randomly sampled trees of each cultivar were examined in each grove and assigned a numbered identification tag. If fewer than 20 trees of one cultivar existed in a grove then all trees were selected.

On trees smaller than 2 m, ten branches or 200 leaves were examined. Branches were randomly selected from the lower south side of the tree. Only leaves from last season's growth were assessed. Leaves with visible symptoms were collected for disease identification and severity assessments in the laboratory. Initially, symptoms are found on the upper surface of the leaf, where lesions may be inconspicuous. Leaves soaked in 5% NaOH solution for 1-2 minutes at 50-60^{o}C (Shabi et al., 1994) can enhance detection of the spots at this stage. Evaluate the severity of peacock spot was taken by visually estimating the area (%) covered with lesions and counting the number of lesions on each leaf. Severity was recorded as 5, 12.5, 25, 37.5, 50, 75, 90 and 100% area covered with the disease

In this study, the germination characteristics of the pathogen and effects of moisture were studied *in vitro*. The conidia obtained from naturally infected olive leaves, which were found to be one-celled or two-celled, were plated on water agar in petri plates and allowed to germinate at dark and 20ºC. Germinated and ungerminated conidia were counted in random fields at 100× with a light microscope. A total of 200 to 300 conidia were examined on each plate, with the higher number of conidia counted when germinability was low. A conidium was considered germinated if the length of the germ tube was greater than or equal to the length of the conidium (~20 µm). Percent germination was calculated as 100 × the number germinated/the total number of conidia examined (Figure 1). Iran map (left) and regional surveyed (Golestan province, right) in this study.

For the effect of temperature on conidium germination and germ tube elongation, olive leaf lesions picked from naturally infected olive trees ('Zard') grown in a commercial grove in Kordkouy. The leaves were agitated in distilled water and the conidial suspension filtered through a double layer of cheesecloth to remove leaf debris. Inoculum suspensions were adjusted to $5×10^{4}$ conidia ml^{-1} using a haemacytometer. Six temperatures (5, 10, 15, 20, 25 and 30°C) were tested. Fully expanded leaves (4 weeks old) from different cultivars were excised from the olive plants grown in the greenhouse by cutting at the stem end of the petiole. The leaves were inoculated with three drops (10 µl) of a conidial suspension deposited on the upper leaf surface. After inoculation, the leaves were arranged randomly in the six humidity chambers (100% RH). After incubation of 12, 18, 24, 36, 48, 72, 96, 120, 144 and 168 h for determining germ tube length of 30 conidia per leaf.

RESULTS AND DISCUSSION

Olive scab is widespread disease in northern Iran olive orchards. It 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. Old spots may show necrotic areas and an annular or zonate appearance. 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 none-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, exept during hot and dry summers (Razavi and Jahany, 2009). 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. The margins of olive scab lesions expand laterally into adjacent healthy tissue, where conidia are produced. These conidia are the principle inoculum source causing subsequent infection (Shabi et al., 1994) and are dislodged and dispersed over short distances by rain (Laviola and Scarito, 1993). Lesions expand slowly, forming dark green round spots from 2-15 mm in diameter. Spots from adjacent infections may coalesce. The spots turn dark brown and become necrotic with age, often surrounded by concentric yellowish or pale brown haloes, hence the name peacock spot. Olive scab is more serious in humid olive growing areas of the world where extended periods of wet weather favour disease development (Teviotdale and Sibbett, 1995a).

Germination of conidia commenced after 10h, with emergence of germ tube(s) at one or both ends of conidia. After 40 h, only the two-celled conidia had germinated. However, the germinated conidia failed to establish fungal colonies (Figure 2). Germ tube length increased with increasing incubation period. Temperature made little difference to the mean rate of germ tube elongation except that after longer incubation periods it was less at the higher temperatures (20 and 25°C) than at the other temperatures tested. The olive cultivar also influence on germ tube elongation (Table 2).

The prevalence of olive scab significantly increased with tree age (P<0.001; Figure 3). As trees age, canopy size and canopy density increase, creating more shelter and increasing the humidity within and between trees. This makes the grove more suitable for olive scab infection (Teviotdale and Sibbett, 1995a,b). Younger trees tend to have a reduced canopy density making the tree less suitable for peacock spot to become established. Younger, less cutinised leaves are more susceptible than older leaves (Lo´pez-Doncel et al., 1999). 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 aeriation in kordkouy region (Table 1). This agree with finding of obanor et al. (2005, 2008), Virugea and Trapero (2002) and Wilson and Miller (1949). From the five regions studied; East region (Kordkouy and Gorgan) showed the highest percentage of trees infected with olive scab followed by West region (azadshahr, Minodasht), (P<0.001; Table 1).

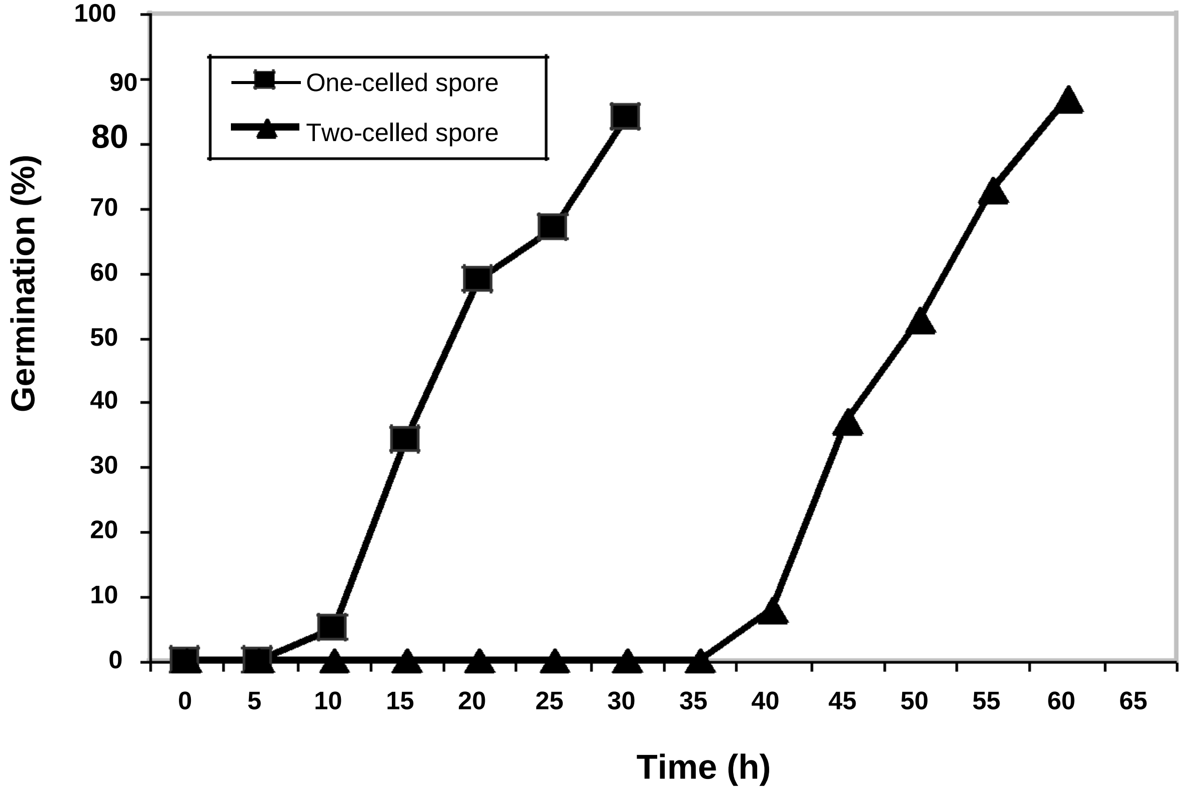

Figure 2. Germination of two types spore of *S. oleagina* conidia on PDA medium in the laboratory. The experiment was repeated three times.

Table 2. Influence of temperature, time and cultivars on germ tube elongation of *S. oleagina* on detached olive leaves.

Cultivars	Regression coefficient (probability)		R^2	Signif. for F
	Time (h)	Temperature (°C)		
Amigdalifolia	0.87 (0.007) a*	-1.73 (0.01) a	0.841	0.0001
Blaidi	0.71 (0.004) b	-1.56 (0.02) b	0.789	0.0001
Koronakei	0.45 (0.006) c	-0.87 (0.04) c	0.893	0.0001
Manzanilla	0.83 (0.007) a	-1.76 (0.01) a	0.863	0.0001
Mary	0.81 (0.006) a	-1.73 (0.01) a	0.849	0.0001
Mission	0.72 (0.007) b	-1.50 (0.02) b	0.784	0.0001
Rooghany	0.88 (0.005) a	-1.77 (0.01) a	0.877	0.0001
Valatolina	0.74 (0.006) b	-1.57 (0.05) b	0.736	0.0001
Wild olive	0.34 (0.044) d	-0.36 (0.02) d	0.881	0.0001
Zard	0.85 (0.006) a	-1.77 (0.01) a	0.855	0.0001

Of the regions studied, East region is warm, with high rainfall and humidity (Table 1). The results fitted by Y= - 21.058 + 0.794X model with $p<0.001$ $r=0.952$ (Y= disease percent and X= Annual rainfall Mean monthly). This could explain the high prevalence of olive scab in trees and on leaves. However differences in manage-ment (e.g. pruning, shelter) (Teviotdale and Sibbett, 1995a,b) could contribute to the lower tree infection on several region. The high proportion of older trees, sheltered sites and an unusually wet season in Kordkouy especially the orchards in jungles, with low aeriation, may have contributed to a relatively high severity of olive scab.

Cultivar vary in their susceptibility to this disease on the Mary, Manzanila and Amigdalifolia showing the highest prevalence of disease (Table 3). With Rooghany, Zard,

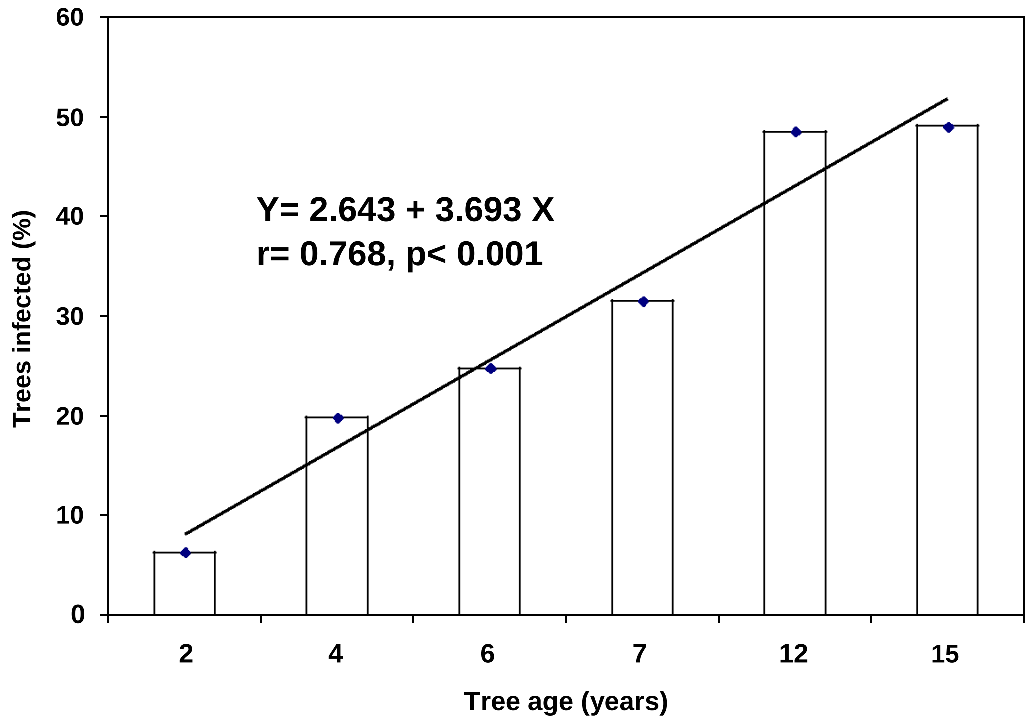

Figure 3. Effect of tree age on olive scab severity. Data was pooled across all cultivars and all sites.

Table 3. Effect of cultivar on olive scab severity and leaf infection from infected trees in Golestan olive's groves.

Cultivars	Disease severity (%)	Leaf infection (%)
Amigdalifolia	39.3 a	43.7 c
Blaidi	16.2 c	30.4 d
Koronakei	10.1 d	21.5 e
Manzanilla	36.9 b	30.5 d
Mary	34.7 b	59.3 b
Mission	15.4 c	22.9 e
Rooghany	43.5 a	67.5 b
Valatolina	16.4 c	31.5 d
Wild olive	3.4 e	4.7 f
Zard	41.3 a	80.2 a

**Within columns, values followed by a common letter do not differ significantly at P=0.01 according to Duncan′s protected least significant difference test, *Within columns, values followed by a common letter do not differ significantly at P=0.01 according to T test.

prevalence of trees infected, followed by Mission and Sillvinio, 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 (Figure 3). 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. Assess-ments 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. (2005) who assessed resistance of olive cultivars to olive scab disease in Golestan province. However, the relative resistances/ susceptibilities of olive cultivars to olive scab requires thorough evaluation for Iran environmental and growing conditions.

The main source(s) of infection and spread of olive scab disease in Iran are unknown. One source could be nurseries. Many young trees in isolated sites were infected, suggesting that the pathogen had arrived with the planting material. The nursery environment provides ideal conditions for olive scab infection (that is, overhead sprinklers and shade cloth). Epidemiological studies are required to identify and quantify inoculum sources and to assess climatic conditions for disease build-up and spread under Iran conditions. This will then enable targeted control of disease with chemical and/or tree management strategies and development of a disease forecasting system (Viruega and Trapero, 1999). The effect of olive scab infection levels on tree health, fruit yield and olive oil quality also requires further evaluation. This survey was conducted during the Iran autumn season and therefore some limitations should be mentioned because main infection periods occur during autumn and winter (Viruega and Trapero, 1999, 2002). The survey only provides a preliminary assessment of prevalence of olive scab during the autumn period. However, the survey showed that olive is widespread in Iran, with all regions and cultivars affected. Further monitoring is needed to better understand the disease incidence of the disease in Iran.

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