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## SCREENING OF SOME OLIVE CULTIVARS (*OLEA EUROPAEA* L.) FOR TOLERANCE TO *SPILOCAEA OLEAGINA*.

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### **Abstract:-**

This research aimed to evaluate the susceptibility of 23 olive (*Olea europaea* L.) cultivars (13 local and 10 Introduced) to olive leaf spot (peacock eye) disease caused by *Spilocaea oleagina*, in order to select the highest tolerant cultivars for olive breeding and genetic improvement programs. This research was carried out on 2016 In Bouka Center for Research and Plant Production, Lattakia, Syria.

Three trees of each cultivar were selected and 100 young leaves/tree, not showing any disease symptom, were collected and tested by 5% NaOH treatment. Latent and severity infection of olive leaf spot were estimated. The results were subjected to ANOVA analysis at a significant level of 5%.

Percentage of infected leaves varied between analyzed cultivars, the highest percentage of infection (92.67%) was detected in Dermlali cultivar while the lowest one (4%) was revealed in Zorzalina. The maximum percentage of infection severity (67.25%) was shown in Abo-Satl cultivar while the minimum value (1.17%) was found in the Spanish Zorzalina and Gordal cultivars. Based on tolerance level against *Spilocaea oleagina*, the cultivars were divided into five groups. The first group was classified as very tolerant and was represented by Zorzalina, Chemlal, Gordal, and Picholine cultivars, followed by tolerant (Jlot and Coratina), moderately tolerant (Frantoio cultivar), susceptible (Khdairi, Hmaisi, Klkali, Mnaikiri, Dan, Mosaabi, Konservolia and Tanche) and very susceptible (Mhati, Dermlali, Sorani, Doabli, Abo Satl, Kaisy, and Trilia). No full resistance was detected in any of the analyzed cultivars.

**Keywords:-** Olive, Olive Leaf Spot, *Spilocaea oleagina*, Latent Infection.

## **INTRODUCTION:**

The Olive tree (*Olea europaea* L.) is native to the eastern coast of the Mediterranean basin and Syria is a rich origin of olive genetic resources. The olive tree comes first among fruit trees in Syria and are cultivated in all Syrian governorates. Khdairi and Dermlali cultivars, constitute the vast majority of cultivated trees in the Syrian coast, are very susceptible to olive leaf spot (OLS) disease [1]. The major riskiness of OLS disease is the fact that most of the cultivated olive cultivars in the world do not possess resistance to this fungus [2]. Olive leaf spot (OLS) or peacock spot, caused by the fungus *Spilocaea oleagina* Cast. (Hughes) (= *Cycloconium oleagina*), which follows Dematiaceae family and Hyphales order from the class Deuteromycetes, is one of the most dangerous pests affecting the growth and production of olive trees in humid olive growing areas of the world. Severely infected trees show defoliation, poor twig and growth. As a result of infection, yield losses may reach up to 20% [3] [4].

Symptoms appear mainly on the leaves, and may affect the fruits in some cases, especially when the conditions are favorable for the fungus activity during the period of fruit growth and maturity. The infection of *Spilocaea oleagina* requires very high humidity condition for a period of 12-48 hours accompanied with temperatures in a range of 0-27°C. Temperature from 16 to 21°C with 70-80% of relative humidity is the optimal condition for OLS activity. [5] [6].

Infection of OLS have been recorded in coastal areas of all Mediterranean countries, where olive cultivation is concentrated in the world and produces more than 90% of the world's production of olive fruits [7].

The infection is spread in the wet period from the onset of rainfall in the fall and during winter to the end of spring, where the higher levels of infected spots appear on the upper surface of leaves, and the symptoms of infection are visible and clear. Infections are restricted at temperatures above 30°C and drought during the summer; OLS growth is limited or completely suspended in such conditions. [6].

The high infection of OLS is increased by increasing the age of the tree, mainly due to the increasing of canopy density. Young and small leaves are more sensitive than the old ones. [8]. The highest rates of infection were recorded at the lower level of tree canopy, on the northern side away from the sun, in the low and closed valleys where the movement of air is difficult, in the coastal areas or areas near the bodies of water, and areas near sea level in height. [9].

Olive cultivars are significantly different in their susceptibility to peacock eye disease, and this is confirmed by many studies. A clear variation in the sensitivity against the fungus was revealed when 14 local and imported olive cultivars were subjected to 12 *Spilocaea oleagina* strains under artificial infection in the glass house [1]. The Eroni, Trilia and Zorzalina cultivars were highly tolerant, the Kaisy and Dan cultivars were tolerant. The interaction of the Succari cultivar was moderate or tolerant, and Kenino cultivar was free from visible and latent infections under normal infection using 61 cultivars tested in the Agriculture Research Center in Lattakia in 2008[1].

In the context of global studies, the analysis of some olive cultivars in Greece showed that Lianolia and Koroneiki were the least sensitive, while Chondrolia Chalkidikis was more susceptible to OLS disease [10].

In South Africa, Coratina cultivar was the most sensitive of the eight tested cultivars, while the two cultivars Manzanilla and Michen were moderately sensitive, Leccino and Mandi were moderately tolerant, and Frantoio was the most tolerant of the studied cultivars [11].

The study of [12] showed that the two cultivars Leccino and Frantoio were the most tolerant, with only 5% infection, while the incidence ranged from 20% and 30% in cultivars Parnia, Manzanilla and Nabali. Souri was the most susceptible cultivar with infection percent reached 84%.

In Tunisia, [13] studied the offspring of crossing between the French Picholine and Tunisian Miski. According to the study Miski and H17 hybrid were highly sensitive to OLS disease, while Picholine cultivar with two hybrids were fully resistant to OLS disease.

## **2- STUDY OBJECTIVES:**

The aim of the study was to evaluate the susceptibility of olive cultivars grown under the Syrian Coast conditions, to OLS infection, caused by *Spilocaea oleagina*. The findings are expected to provide a basis for recommendation of OLS- tolerant cultivars for breeding programs for commercial olive cultivars.

## **3- MATERIALS AND METHODS:**

### **3-1- Date and place of study**

The study was carried out in the spring of 2016, in an orchard of 15 acres at Bouka Center for Research and Plant Production at Tishreen University, Lattakia, Syria. The orchard is characterized by a heavy mud soil with alkaline reaction, high content of total and effective calcium carbonate, and good content of organic matter.

The area rises 36 meters above sea level. The average rainfall (by Bouka meteorological station) is 895.5 mm in 2015 and 672 mm in 2016, and the average daily temperature is 20.1°C for 2015 and 20.5°C for 2016.

### **3-2- Plant material:**

Samples were collected from 35 years-old trees for 13 local cultivars and 10 imported ones, well known in Mediterranean basin (table 1),

Three trees planted at a distance of 9x9 m, with no irrigation were chosen from each cultivar.

**Table1. Cultivar names, Origin and the purpose of cultivation of the 23 studied cultivars.**

Cultivar	Origin	Purpose	Cultivar	Origin	Purpose
Khdairi	Syria	Dual*	Chemlal de Kabali	Algeria	Oil
Hmaisi	Syria	Dual	Chemlaly	Tunisia	Oil
Mhati	Syria	Dual	Frantoio	Italy	Oil
Dermlali	Syria	Dual	Coratina	Italy	Dual
Sorani	Syria	Dual	Gordal	Spain	Table
Doaibli	Syria	Dual	Zorzalina	Spain	Dual
Klkali	Syria	Dual	Konservolia	Greece	Dual
Mnaikiri	Syria	Dual	Picholine	France	Dual
Abo-satl	Syria	Dual	Tanche	France	Dual
Dan	Syria	Dual	Trilia	Turkey	Dual
Kaisy	Syria	Table	[14] [15]		
Jlot	Syria	Dual			
Mosaabi	Syria	Table			

\*Dual means cultivar used for table and oil extraction.

**3-3- Evaluation latent infection percentage:**

A total of 100 leaves without visible symptoms were randomly collected at the first week of March, from the four sides of the tree for each sample. To evaluate latent infection, leaves without symptoms were immersed in 5% NaOH solution for 2-3 min at 50-60°C. The 5% NaOH solution causes the appearance of OLS symptoms as dark spots on the upper surface of the infected leaves, [16] [8]. Leaves that showed spots of infection were counted to determine the percentage of infected leaves from the equation:

$$P = \frac{n}{N} * 100$$

- P: the percentage of infected leaves in each sample.
- n: Number of infected leaves per sample.
- N: The total number of leaves examined in each sample.

**3-4- Evaluation of latent infection severity:**

Several techniques have been developed to calculate the severity of OLS disease, or the so called (Disease Index DI%), either depending on the number of spots on the leaf upper surface[17], or depending on the area covered by infected spots from the surface of the leaf. Methods depend on the infected surface are more accurate than ones depend on the number of spots only due to the large variation in diameters of the lesions caused by OLS infection. Disease Index varies greatly between species, cultivars, regions and times.

Therefore, based on the area of the affected leaf surfaces, a five-point monitoring scale was adopted [1]:

- 0: no visible symptoms on the leaf surface.
- 1: The disease spots cover 0.1-10% of the leaf surface.
- 2: The disease spots cover 10.1-25% of the leaf surface.
- 3: The disease spots cover 25.1-50% of the leaf surface.
- 4: The disease spots cover more than 50% of the leaf surface.

The disease index was calculated for each cultivar using the equation of [18]:

$$DI(\%) = \frac{\sum(a * b)}{N * K} * 100$$

Where DI: disease index (%)

- a: Degree of injury according to the measuring scale.
  - b: Number of leaves affected by a degree of injury in each replicate.
  - N: Number of tested leaves in each replicate.
  - K: The greatest value for the measurement ladder. (= 4 In this case).
- Based on this, the studied cultivars were classified into 5 groups [1]:

Group	Disease Index DI%
Completely tolerant	0
Highly tolerant	less than 5%
Tolerant	5-10%
Moderately tolerant	10.01-25%
Susceptible	25.01-50%
Highly susceptible	more than 50%

### 3-5- Data analysis:

The analysis of variance for the obtained data was performed by GenStat program (version, 12), using analysis of variance (ANOVA) tests. Mean separation was analyzed using Duncan's multiple range test or LSR at 5% probability, because the number of studied cultivars was more than five.

## 4- RESULTS AND DISCUSSION

The 23 local and introduced cultivars varied significantly in their susceptibility to OLS infection (Table 2). Concerning the percentage of latent infection, the highest percentage was recorded in Dermlali cultivar with 92.67% of infected leaf areas while the lowest was in Zorzalina (not exceed 4% of infected leaf areas). For the severity of the infection, the highest values of the disease index was in Abo-Satl cultivar (67.25%), while the lowest values were in Zorzalina and Chemlal (about 1.17% for each cultivar).

Based on the severity of OLS infection, the studied Cultivars ranged from high tolerant such as Zorzalina, Chemlal, Gordal and Picholine, tolerant as in both Jlot and Coratina, moderately tolerant as in Frantoio, and susceptible as in Hmaisi, Klkali, Mnaikiri, Dan, Mosaabi, Konservolia, Tanche, while the remaining cultivars were highly susceptible to OLS infection, and the study did not record any fully tolerant Cultivar.

These findings confirm a study mentioning that the risk of OLS disease comes from the absence of resistance to the fungus in the most of the cultivated olive cultivars [2], this fact explains the large losses caused by the fungus in the areas planted with olives susceptible for fungus infection.

**Table2. Latent and severity infection of olive leaf spot on olive cultivars \***

Cultivars	Percentage of infection %	Disease Index %	Cultivar's susceptibility
Khdairi	65.33 fg	35.08 ef	Susceptible
Hmaisi	84.33 abcde	48.5 d	Susceptible
Mhati	87.00 abcd	54.08 cd	High susceptible
Dermlali	92.67 a	62 ab	High susceptible
Sorani	83.67 abcde	60.33 bc	High susceptible
Doaibli	89 abc	54.83 cd	High susceptible
Klkali	67.67 fg	34.58 ef	Susceptible
Mnaikiri	73 efg	36.67 ef	Susceptible
Abo-satl	90.33 ab	67.25 a	High susceptible
Dan	76.33 cdef	38.67 ef	Susceptible
Kaisy	74.67 defg	51.17 d	High susceptible
Jlot	17.33 ij	6.42 hi	Tolerant
Mosaabi	78 bedef	40.83 e	Susceptible
Chemlal	4.67 k	1.17 i	High tolerant
Chemlaly	52 h	27.83 g	Susceptible
Frantoio	21.33 i	10.08 h	Moderately tolerant
Coratina	22 i	9.83 h	Tolerant
Gordal	5.67 jk	1.92 i	High tolerant
Zorzalina	4 k	1.17 i	High tolerant
Konservolia	62.67 gh	32.50 fg	Susceptible
Picholine	8.33 jk	3.50 hi	High tolerant
Tanche	70 fg	32.08 fg	Susceptible
Trilia	91 ab	62.58 ab	High susceptible
LSD 5%	11.411	6.208	

\*Means with different letters are significantly different.

Based on the results shown in Table (2), except for the only tolerant cultivar Jlot, all local cultivars were susceptible to *Spilocaea oleagina*. The level of susceptibility ranged from susceptible to highly susceptible to OLS infection.

These results are inconsistent with IOC publications on local cultivars, which indicated that the Syrian cultivars (Abo-Satl, Doaibli, Kaisy, Sorani and Zaity) are tolerant to peacock eye disease [14]. This difference between the two studies may be

explained by the fact that our study was conducted in a wet coastal environment with high rainfall, and our field is in a low area where water accumulates, all cultivars were grown in the same orchard with a permanent source of fungus infection coming from susceptible cultivars. These conditions are ideal for fungus activity. In the other side, [14] may have carried out their study on the cultivars in their natural areas of distribution, far from the sea (In drier conditions in terms of average rainfall, soil and air humidity, and away from sources of infection from sensitive cultivars) These combined factors represent unsuitable conditions for the growth and the activity of fungus causing peacock eye disease.

Regarding our results of the other local cultivars (Khdairi, Doaibli and Dermlali), they were consistent with the previous results which proved the sensitivity of these cultivars to peacock eye disease. The latent infection percentage reached 90-100% in the three cultivars in some sites in 2007 and 2008 years [1].

Concerning the introduced cultivars, our results were consistent with many previous studies, where they indicated that the Spanish Zorzalina is one of the most tolerant cultivars of peacock eye disease [14] [1], as is the case of the Frantoio [12].

A difference in reaction of some introduced cultivars (Picholine and Gordal) against OLS disease between our results and another local study [1] may due to the use of different genotypes of the cultivars. Picholine cultivar showed high tolerance in our study but was very sensitive in [1]. In the same time, our result was in accordance with other search [14] [19]. Where they confirmed that French Picholine cultivar (Picholine Languedoc) is a high tolerant cultivar to OLS disease while Picholine Marocaine is very sensitive to this disease. These results may explain the difference revealed between our results and [1]. Results which could due to a possibility that the cultivar studied by [1] could be Picholine Marocaine (Characterized by a sensitivity for fungus causing OLS disease) and our Picholine could be French Picholine cultivar (characterized by high tolerance cultivar to OLS disease).

The comparison of our results with other study draw our conclusion to the fact that the Gordal cultivar used in our study is likely Gordal Sevillana, which is one of the most tolerant cultivars to OLS disease; as the percentage of infection was 5.67% and the index of injury was 1.92%, and excluding the possibility that this cultivar is Godral de Granada, known for its high susceptibility to disease [14].

## 5- Conclusion:

- All cultivars analyzed in this study were susceptible to OLS infection at different levels, ranging from 4% in Zorzalina to 92.67% in the Dermlali cultivar.
- Local cultivars were either susceptible or highly susceptible to infection with *Spilocaea oleagina*, with the exception of Jlot cultivar which showed tolerance to OLS disease.
- Dermlali cultivar was highly susceptible to OLS disease, with the highest percentage of infection reached 92.67% and a category of high susceptibility in terms of severity, and the Khdairi was susceptible to infection, with infection percentage reached 65.33%. These two cultivars constitute the majority of cultivated cultivars in the Syrian coast.
- Some introduced cultivars (Spanish Zorzalina and Gordal, the Algerian Chemlal, and French Picholine) were characterized by high tolerance to OLS disease. □ No resistance was detected in any of the analyzed cultivars.

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