**Palestine Technical University Research Journal, 2020, 8(1), 09-13**
(http://www.ptuk.edu.ps)

# Biological control of Peacock spot disease caused by Spilocaea oleagina on Olive using bacteria.

Mazen Salman

Department of Horticulture and Agricultural Extension, Palestine Technical University-Kadoorie (PTUK), Tulkarm, State of Palestine

* Corresponding author E-mail address: salman_mazen@daad-alumni.de

**Received: 13/3/2019, Accepted: 14/4/2019, Published: 20/2/2020.**

**Abstract:** The olive leaf spot disease caused by the fungus *Spilocaea oleagina* (Cast.) Hughes (syn. *Cycloconium oleagina*) is one of the most destructive diseases on olive trees causing losses that may reach 20% of the yield. The disease is controlled by the application of chemical fungicides which is not always feasible in providing proper protection against the pathogen. In this work we report the efficacy of *Pseudomonas fluorescenc* isolate ORS3 and *Bascillus atrophaeus* isolate Bat in controlling the disease under field conditions. An Olive field in, Tulkarm governorate, Palestine was selected. The olive trees were 5-10 years old and were highly infected with the olive leaf spot. Trees were sprayed with bacteria formulated in oil. Control trees were sprayed with water. For evaluation of bacterial efficacy against the disease, olive leaves were collected before and after application of the bacteria. Germination of conidia latent infection and severity were determined. In addition to that, bacterial viability was assessed. Results of the work revealed that the bacteria were able to inhibit conidial germination of the fungus. The percent of reduction in conidial germination (85.8 and 70.2%) in the presence of *P. fluorescenc* isolate ORS and *B. atrophaeus* isolate Bat, respectively was significantly lower than that in the control or in leaves sprayed with 10% oil (69.1 and 56.1%, respectively). After two weeks of spraying, the percent of latent infectoin (figure 4) was significantly ($p<0.05$) lower on leaves sprayed with *P. fluorescenc* isolate ORS3 and *B. atrophaeus* isolate BAT (5.1 and 3.8% latent infection, respectively). However, bacterial shelf life on the surface of olive leaves was reduced after three days of spraying (i.e no bacteria were re-isolated). The results indicated that the bacteria can be used for control of the leaf spot disease. Further studies are required to evaluate the efficacy of the bacteria under different environmental conditions.

***Keywords*:** olive leaf spot, severity, biocontrol, incidence, Bacteria, spore germination

## Introduction

The Deuteromycete fungus *Spilocaea oleagina* (Cast.) Hughes (syn. *Cycloconium oleagina*) causes the olive leaf spot (OLS) or the pea cock eye disease on olives where ever the trees are grown (Gonzalez-Lamothe et al. 2002; Shabi et al. 1994). The disease is one of the most destructive diseases that might lead to severe infections resulting in about 20% yield losses (Azeri 1993; Graniti 1993). Olive leaf spot is usually more abundant on the lower parts of olive trees (Razavi and Jahany 2009; Azeri, 1993; Graniti 1993). Symptoms of the disease (Figure 1) occur usually on the upper surface of the leaves, expanding and coalescing to cover a large proportion of leaf area. The lesions form dark brown round spots (2-15 mm in diameter) which become necrotic and surrounded by concentric yellowish or pale brown haloes (Sanchez et al. 1998; Civantes 1999).

Infection with *S. oleagina* is normally associated with high humidity and winter conditions (cool and low light), where high temperatures restrict spore germination (Obanor et al. 2011; Al-Khatib et al. 2010). Growth of the fungus was found to be most prevalent in the period from late autumn to spring and of minor significance in the period from the beginning of July until the middle of November (Viruega and Trapero et al 20111999; Hajjeh et al. 2014).
In hot dry weather conditions, conidia remain viable but inactive on infected leaves and start to germinate early in winter. Conidium production is optimal at 15oC and

high humidity (100%) (Obanor et al. 2011; Graniti 1993; Guechi and Girre 1994). In Palestine, Hajjeh et al (2014) reported that the infection with OLS is minimum during summer season.

**Figure 1: Symptoms of olive leaf spot caused by *S. oleagina* on olive tree in Palestine [photograph: M. Salman, Palestine Technical University-Kadoorie]**

Due to the absence of resistant olive varieties (Abuamsha et al. 2013), the disease is controlled by application of excessive copper-containing fungicides (e.g. Bordeaux mixture, copper hydroxide, copper oxide and copper oxychlorides) that are sprayed directly after harvest (Obanor et al. 2008; Sistani et al. 2009; Salman et al. 2014). Mixtures of difenoconazole (Score 25 EC) and mineral oil (Texaco Spraytex CT774) were also used to reduce infection with the disease (Sistani et al. 2009). Unfortunately, spraying infected olive trees with fungicides is not always feasible due to human and environmental health perspectives (Carisse et al. 2000).

In recent years, several studies were conducted to seek alternatives to the use chemical fungicides (Salman 2017). Biological control using antagonistic microorganisms alone, or as supplements to minimize the use of chemical pesticides in a system of integrated plant disease management, has become more important in recent years (Mao et al. 1997; Hwang et al 1993). Different microorganisms including bacteria can be used to control plant diseases. There has been a large number of literature describing potential uses of plant associated bacteria as biocontrol agents stimulating plant growth and managing soil and plant health (Blakeman and Fokkema 1982). However; little is known about the use of bacteria against olive diseases. The aim of this work is to exploite the use of bacteria as biocontrol agents against the disease under field condtions.

## Material and methods

### Bacterial isolates growth conditions and formulation

*Pseudomonas fluorescenc* isolate ORS3 and *Bascillus atrophaeus* isolate BAT obtained fom culture collection of the Biotechnology Laboratory, Palestine Technical Univeersity-kadoorie, were grown on KB liquid media on a rotary shaker at 150 rpm for 24 h at 37°C. A rifampicin mutant strains were generated to facilitate reisolation of bacteria from the sprayed olive trees. For this, the bacteria were grown on KB liquid media suplemented with increasing rifampicin concentration (0.0, 10, 25, 50, 75 and 100 µg/mL). The bacteria were maintained on KB agar media containing 100 µg/mL rifampicin and stored at 4°C until use. Bacterial suspension (~$10^9$ cfu/mL) were gown in liquid KB media supplemented with 100 µg/mL rifampicin. The cultures (50 ml aliquots) were centrifuged at 6000 rpm and the bacterial pellets were resuspended each in 50 ml Normal saline (0.9% NaCl) which were then collected in 1L Erlenmeyer flasks. Each bacterial isolate was mixed with 10% (v/v) vegetable oil.

### Field application of bacteria

A rain fed olive field (~2 donums) was chosen in Shwekeh village (32,34497°N, 35,03689°E) in Tulakrm governorate. About 5-10 years old olive trees cultivar Nabali were grown in the field. The age of the olive trees was estimated in the range of 5-10 years. The trees were highly infected with the OLS disease. Each tree was sprayed with bacterial suspension until run off. Infected and non infected olive leaves (50 leaves from each tree) were collected before and after spraying with bacteria and continued over a period of four weeks. The experiment was done in triplicates and repeated three times.

### Viability test of bacteria

Leaves were cut into 1 cm pieces; vortexed in autoclaved normal saline (0.9%) for 30 s and 100µL of each suspension was spread on KB agar media supplemented with 100 µl/mL rifampicin. The plates were incubated at 37°C for 24 h. Bacterial colonies were then counted and the cfu was calculated.

### Germination of fungal conidia

Germination test was done as mentioned by Salman (2017). Three 20 µl droplets of conidial suspension obtained from leaves with sporulating symptoms were placed separately onto glass slides. The slides were then placed on the lid of a Petri dish containing approximately 3 ml of 0.5% water agar to provide high humidity and the dishes were then incubated upside down at 18°C for 24 h. Germination of 100 randomly selected conidia in each droplet was evaluated with a compound microscope at 200 X magnification and the mean percent germination, relative to nil bacteria controls, was calculated for each treatment.

### Determination of latent infection and severity

Latent infection was determined by soaking the leaves in 5% of NaOH at 50°C for 3 min. Number of spots was then counted. Disease incidence was determined by recording the percent of infected leaves. For disease severity, the number of lesions per leaf was counted and graded; 1 (1 lesion), 2 (2 lesions), 3 (3-5 lesions), 4 (6-10 lesions) or 5 (>11 lesions) (Salman et al. 2011).

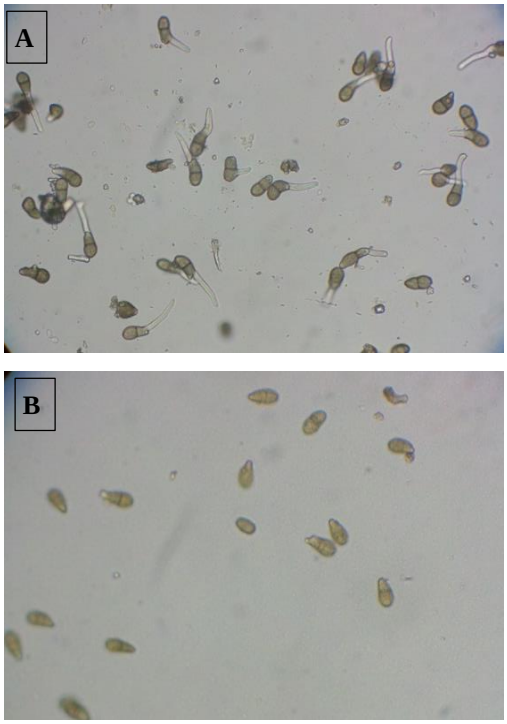
### Statistical analysis

All experiments were done in triplicate. Differences between treatments were analyzed with the Kruskal-Wallis test (Kruskal, 1952) using Xlstat software (Adinosoft, USA).

## Results and discussion

A recent study conducted by Salman (2017) showed that some bacteria isolates could inhibit the germination and growth of fungal conidia under in vitro conditions. In this work, the isolates were formulated in oil (10% v/v) and sprayed on infected olive trees. The bacteria were reisolated from the surface of the leaves after 24 h of application. After one week of spraying, bacteria were not recovered from the leaves. This might be attributed to the hot summer weather conditions which affect the survival of bacteria on leaf surfaces. However, the concentration of the reisolated bacteria was the same as that used to prepare the oil formulation (~$10^9$ cfu/mL).

It is well known that the survival shelf life of bacteria is a critical factor in controlling plant pathogens (Corrêa et al 2015). Despite the short life span of the bacteria on the surfaces of olive leaves, our isolates showed significant reduction in conidial germination. As shown in figure (2), the conidia isolated from sprayed leaves (after 24 h of spraying) showed high germination rates compared to the contol non sprayed trees. Significan ($p< 0.05$) reduction in conidial germiantion was recoreded in sprayed leaves the bacteria (Table 1). The percent of germination of the conidia in leaves sprayed wih B. atrophaeus isolate Bat and P. fluorescenc isolate ORS (20.6 % and 9.8 %, respectively) were significantly lower than that in the control or in leaves sprayed with 10% oil (69.1 and 56.1%, respectively).



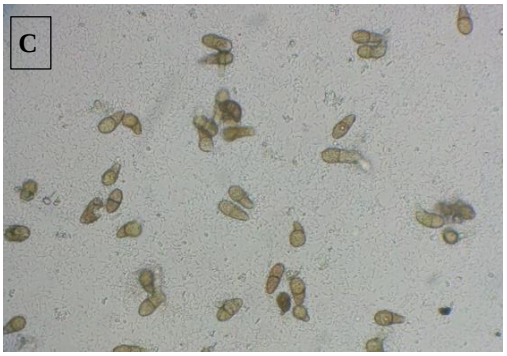



**Figure 2: Germination of S. oleaginaoleagina conidia isolated from olive leaves after 24 h of spraying with the bacteria, control (A); *P. fluorescenc* isolate ORS3 (B) and (c) *B. atrophaeus* isolate BAT.**

**Table 1: Effect of bacteria on germination of conidia after 24h of spraying.**

| Treatment | Percent of Conidial germination ± SE | % Reduction |
|---|---|---|
| Control | 69.1 ± 1.8 a | |
| OIL (10%) | 56.1 ± 8.0 a | 18.7 |
| *B. atrophaeus* isolate Bat | 20.6 ± 3.4 b | 70.2 |
| P. *fluorescenc* isolate ORS | 9.8 ± 0.96 b | 85.8 |

Data with different letters are signifcantly different at p<0.05

Interestingly, the number of conidia that were isolated from OLS spots on olive leaves was reduced significantly after 4 weeks of spraying. In fact we could not isolate the conidia from the surface of the leaves. Our results indicated that the reduction in the number of conidia on infected leaves might be attributed to the presence of bacteria. The results obtained on germination of conidia after spraying the leaves with the bacteria are in agreement with the results published by Salman (2017).

Disease severity and incidence were tested before spraying the trees with bacteria. High infection rates (> 80% disease incidence and grade 5 disease severity) were recorded on leaves before spraying with the bacteria. After two weeks of spraying, the percent of latent infection (figure 3) was significantly ($p<0.05$) lower on leaves sprayed with *P. fluorescenc* isolate ORS3 and *B. atrophaeus* isolate BAT (5.1 and 3.8% latent infection, respectively). In addition to that, the disease severity was also reduced in the presence of the bacteria (figure 4). The percent of reduction of severity was 41.6 and 63.8% on leaves sprayed with *P. fluorescenc* ORS3 and *B. atrophaeus* BAT, respectively.

Previous studies showed that the bacterial isolates were most effective in inhibiting conidial germination of the fungus under in vitro conditions (Salman 2017). Up to our knowledge, this work was the first of its kind that used bacteria as biocontrol agents against the OLS disease under field conditions. Despite the reduction in germination rates of OLS conidia and the reduced disease incidence and severity, the efficacy of the bacteria against the disease must be tested over a long period of time considering variable weather conditions (e.g winter and summer). Moreover, further studies are needed to improve the shelf life of the bacteria on the leaves of olive trees.

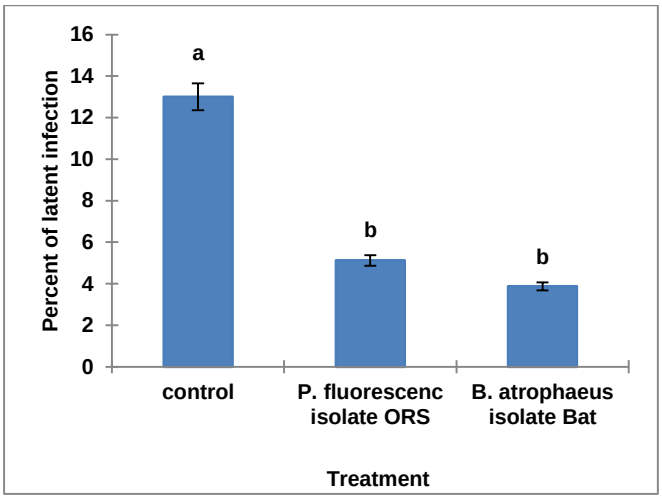



**Figure 3: Percent of latent infection after two weeks of spraying with bacteria. Data with different letters are significantly different after Tukeys HSD test using ANOVA at P < 0.05.**

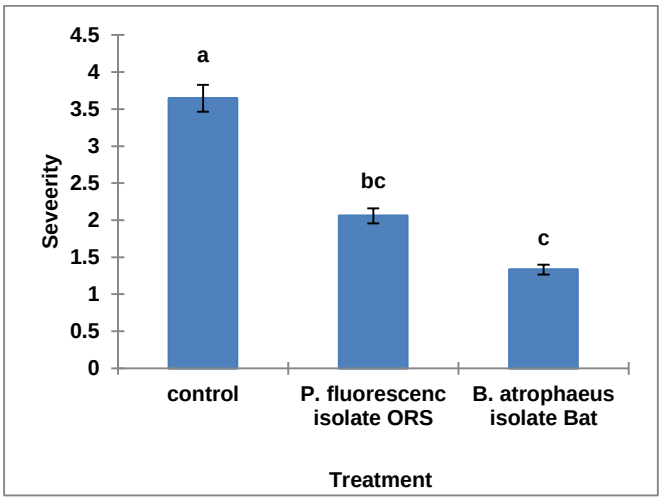


**Figure 4: Disease severity after two weeks of spraying with bacteria. Data with different letters are significantly different after Tukeys HSD test using ANOVA at P < 0.05.**

## Acknowledgements

The author would like to thank Palestine Technical University-Kadoorie for supporting this work.

## References

Abuamsha R., Abueid M., Hajjeh H. and Salman M. (2013). Evaluation of the Incidence and Severity of Olive Leaf Spot caused by *Spilocaea oleagina* in different olive cultivars in Palestine. Journal of Agriculture and Environment for International Development, 107 (2): 201-212. DOI 10.12895/jaeid.20132.138

Al-Khatib M., Alhussaen K., El-Banna N., and Zyadeh M. (2010). Biological control of olive leaf spot (peacock spot disease) caused by *Cycloconium oleaginum* (*Spilocea oleaginea*). Journal of Microbiology and Antimicrobials, 2(6): 64-67.

Azeri T. (1993). Research on olive leaf spot, olive knot and Verticillium wilt of olive in Turkey. EPPO Bulletin, 23: 437-440. doi.org/10.1111/j.1365-2338.1993.tb01349.x

Blakeman J. P. and Fokkema N. J. (1982). Potential for Biological Control of Plant Diseases on the Phylloplane. Annual Review of Phytopathology, 20:167-190. doi.org/10.1146/annurev.py.20.090182.001123

Carisse O., Ouimet A., Toussaint V. and Philion V. (2000). Evaluation of the effect of seed treatments, bactericides, and cultivars on bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians*. Plant Disease, 84(3): 295-299. doi.org/10.1094/PDIS.2000.84.3.295

Civantes M. (1999). Olive Pest and Disease Management. IOCC.

Corrêa E.B., Sutton J.C. and Bettiol W. (2015). Formulation of *Pseudomonas chlororaphis* strains for improved shelf life. Biological Control, 80: 50-55. doi.org/10.1016/j.biocontrol.2014.09.009

Blakeman J. P. and Fokkema B. (1982). Potential for biological control of plant diseases on the phylloplane. Annual Review of Phytopathology, 20:167-92. doi.org/10.1146/annurev.py.20.090182.001123

Gonzalez-Lamothe R., Segura R., Trapero A., Baldoni L., Botella M.A. and Valpuesta V. (2002). Phylogeny of the fungus *Spilocaea oleagina*, the causal agent of peacock leaf spot in olive. FEMS Microbiology Letters, 210: 149-155. DOI: 10.1111/j.1574-6968.2002.tb11174.x

Graniti A. (1993). Olive scab: a review. EPPO Bulletin, 23: 377-384. doi.org/10.1111/j.1365-2338.1993.tb01339.x

Guechi A. and Girre L. (1994). Sources of *Cycloconium oleaginum* (Cast.) conidia for infection of olive leaves and conditions determining leaf spot disease development in the region of Se´tif, Algeria. Mycopathologia, 125: 163-71. DOI:10.1007/BF01146522

Hajjeh H., Salman M., Abuamsha R., Abueid M., Jawabreh M, Hawamda A. and Abu Rumaileh B. (2014). Latent Infection of Olive Leaf Spot Disease on Palestinian Olives. Annual Research & Review in Biology, 4(15): 2517-2524. doi.org/10.9734/arrb/2014/9845

Hwang S.F., Chakravarty P. and Prevost D. (1993). Effects of rhizobia, metalaxyl, and VA mycorrrhizal fungi on growth, nitrogen fixation, and development of Pythium root rot of Sainfoin. Plant Disease, 77: 1093-1098. DOI: 10.1094/PD-77-1093

Mao W., Lewis J.A., Hebber P.K. and Lumsden R.D. (1997). Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of Pythium and Fusarium. Plant Disease, 81: 450-454. doi.org/10.1094/PDIS.1997.81.5.450

Obanor F.O., Jaspers M.V., Jones E.E. and Walter M. (2008). Greenhouse and field evaluation of fungicides for control of olive leaf spot in New Zealand. Crop Protection, 27: 1335-1342.

Obanor F.O., Walter M., Jones E.E. and Jaspers M.V. (2011). Effects of temperature, inoculum concentration, leaf age, and continuous and interrupted wetness on infection of olive plants by Spilocaea oleagina. Plant Pathology, 60: 190-199. doi.org/10.1111/j.1365-3059.2010.02370.x

Razavi S.E., Jahany M. (2009). Olive scab. In: olive phytopathology (S.J. Sanei, ed.) Peik-e-Reihan publication, Gorgan, pp. 147-154.

Salman M. (2017). Biological control of *Spilocaea oleagina*, the causal agent of olive leaf spot disease, using antagonistic bacteria. Journal of Plant Pathology, 99 (3): 741-744. DOI: http://dx.doi.org/10.4454/jpp.v99i3.3958

Salman M., Hawamda A., Amarni A.A., Rahil M., Hajjeh H., Natsheh B. and Abuamsha R. (2011). Evaluation of the incidence and severity of olive leaf spot caused by *Spilocaea oleagina* on olive trees in Palestine. American Journal of Plant Sciences, 2: 457-460. doi.org/10.4236/ajps.2011.23053

Salman M., Jawabreh M. and Abu Rumaileh B. (2014). The effect of local fungicides on conidial germination of *Spilocaea oleagina* in Palestine. Palestine Technical University Research Journal, 2014, 2(1): 26-28.

Sanchez M.E., Ruiz Davila A., Perez de Algaba A., Blanco Lopez M.A. and Trapero Casas A. (1998). Occurrence and a etiology of death of young olive-trees in southern Spain. European Journal of Plant Pathology, 104: 347-357. doi.org/10.1023/A:1008624929989

Shabi E., Birger R., Lavee S., Klein I. (1994). Leaf spot (*Spilocaea oleaginea*) on olive in Israel and its control. Acta Horticulturae, 356: 390-394. DOI.10.17660/ActaHortic.1994.356.83

Sistani F., Ramezanpour S.S, Nasrollanejad S. (2009). Field Evaluation of Different Fungicides Application to Control Olive Leaf Spot. Australian Journal of Basic and Applied Sciences, 3(4): 3341-3345.

Viruega J.R., Roca L.F., Moral J. and Trapero A. (2011). Factors affecting infection and disease development on olive leaves inoculated with *Fusicladium oleagineum*. Plant Disease, 95: 1139-1146. doi.org/10.1094/PDIS-02-11-0126