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The effect of local fungicides on conidial germination of *Spilocaea oleagina* in Palestine

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Abstract: Olive leaf spot (OLS) disease or peacock disease is caused by the fungus *Spilocaea oleagina,* it is the most destructive disease on olive trees in most regions of Palestine. The disease is controlled by application of copper containing fungicides. Currently, there are more than 20 different fungicides sold in the Palestinian market. The efficacy of these fungicides was not tested on OLS in Palestine. The aim of this work was to test the efficacy of three major fungicides used against the disease. Fungicide solutions containing Fungran, Copper Antracol, and Kocide®101 were prepared by dissolving 0.5 g of each in 200 ml distilled water (DW) according to manufacturer instruction. Five leaves infected with OLS were soaked in each solution for 30 min. Control leaves were placed in 200 ml DW. Leaves were then placed in 9 cm petri dishes containing 3 ml DW to provide high humidity (> 85%). Each day, one leaf was removed, washed in DW and cut into one-cm^2 pieces. Leaf pieces holding OLS conidia were printed on olive leaf extract agar media. Results showed that after 24h of fungicide treatment, Kocide®101 was the most effective fungicide followed by Copper Antracol and Fungran with percent conidial germination 2.08, 2.9 and 25.5%, respectively. Interestingly, Fungran efficacy after 48h (2.8% germination) was higher but not significantly different than Kocide®101 and Copper Antracol. This study showed that the efficacy of the three commonly used fungicides against OLS disease in Palestine diminished after four days of treatment. Further studies are needed to test the efficacy of these fungicides under field conditions for a better control planning of peacock disease in Palestine.

***Keywords*:** *Spilocaea oleagina*, Olive trees, Peacock disease, Olive leave spot (OLS), Palestine

Introduction

Olive tree (*Olea europaea L*) is one of the oldest agricultural tree crops which are cultivated in Palestine. It is distinctly important in the economic and social life of the people; it is considered one of the main sources of income, contributing up to 13% of the annual agricultural production (Jabi, 2007). Olive trees are affected by some pests and diseases. Because the produced olive oil retains the residual chemicals, olive trees are the least sprayed crops (Dahmen and Staub, 1992). *Spilocaea oleaginea* (Cast) Huges or (*Cycloconium oleaginum*) infects olive leaves and cause olive leaf spot (OLS), olive scab, or peacock eye disease (Azeri, 1993). Yield losses are estimated up to 20% (Jabi, 2007). The principal symptoms of OLS are severe premature, defoliation and poor twig development (López-Doncel et al., 2000; Mekuria et al., 1999). OLS usually occur on the upper surface of olive leaf, as the spots expand to cover a large proportion of the leaf, leaves often senesce and are shed prematurely. OLS disease is usually more abundant on leaves of the lower parts of olive trees, of which many shoots become completely defoliated. Recurrent infections often cause poor growth and dieback of defoliated twigs (Khadari et al., 2001; Mekuria et al., 1999). Timing of the application of copper containing fungicide was reported to be critical for effective control of the disease based on geographical location and environmental conditions (e.g. long dry summers); application of fungicides is better prior to winter rains (Graniti, 2003; Macdonald et al., 2000; Teviotdale et al., 1998). The disease is considered one of most important diseases that affect olive trees in Palestinian coastal areas as well as countries of the Mediterranean basin, USA (California), and China (Teviotdale et al.,1998). In Palestine, the disease is highly severe in all olive growing regions in Palestine (Salman et al., 2011). Most of olive cultivars are susceptible to the disease (Abuamsha et al., 2013). Control of the disease is limited due to increased cost in addition to the fact that copper compounds result in disturbance of plant metabolism following Cu accumulation in the soil (Obanar et al., 2008). Most fungicides available in the Palestinian market were not evaluted for their efficacy against the disease. The aim of this work

was to test three commonly used fugicides agianst OLS under in vitro conditions.

Materials and methods

Three fungicides were used. Fungicide solutions containing Fungran (a.i 77% $Cu(OH)_2$ equivalent to 50% metallic copper), Copper Antracol (a.i 37% Copper Oxychloride equivalent to ~Ca 24% metalic copper) and Kocide®101 (a.i 77% Copper Hydroxide equivalent to 50% metallic copper) were prepared by dissolving 0.5 g of each in 200 ml distilled water (DW) according to manufacturers' instruction. After that, 5 leaves infected with OLS (collected from infected treas) were soaked in each solution for 30 min. Infected control leaves were placed in 200 ml DW. Leaves were then placed in 9 cm petri dishes containing 3 ml DW to prvide high humidity (> 85%). Each day, one leaf was removed, washed in DW and cut into one cm^2 squares . Leaf pieces holding OLS conidia were printed on Olive Leaf Extract Agar (OLEA) media containing:, 5 g glucose and 7.5 g agar made in 500 ml of olive leaf extract (prepared by boiling 10 g healthy fresh leaves in 500 ml of DW for 20 min, strained), the medium was autoclaved for 15 min. Inoculated plates were incubate at 21°C for 24h in the dark.Total spores were counted microscopically under 200X magnification and compared with germinated spores in three different fields. Each experiment contained three replicates and three repeats

Results and Discussion

Application of copper-based fungicides is now the main method of olive leaf spot control in Palestine and other olive growing countries. Regardless of material, the application rate or number of applications, copper containing fungicides will control OLS when disease risk is low (Teviotdal et al., 1989). Moreover, regular annual treatment is required to prevent disease build up in olive grove, as high disease levels may be difficult to reduce (Roca et al., 2007). Experimentally, as shown in figure 1, leaves treated with fungicides showed reduced conidial germination after 24h compared to untreated control infected leaves.

Interestingly, Fungran efficacy (2.8% germination) after 48h (**Figure 2)** was higher than that of Kocide®101 and Copper Antracol (7.3 and 8.1%, respectively). Our results showed that Funugran, the most used fungicide, was the least effective against conidial germination (**Table1**). Moreover, this study showed that the efficacy of the three commonly used fungicides against OLS disease e is reduced after 4 days of treatment (**Figure 2**).

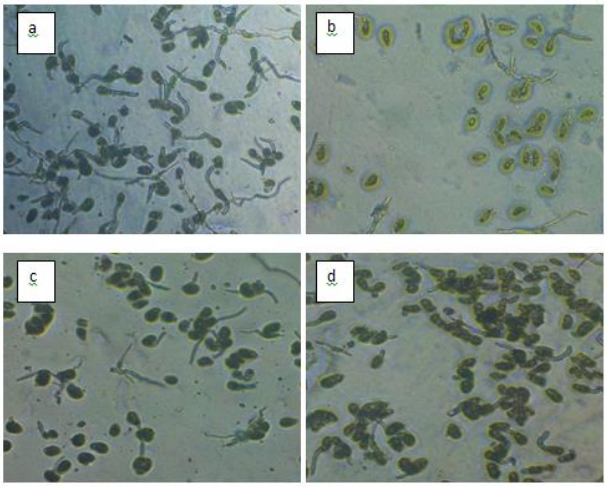

Figure 1. Conidial germination of *S. oleaginea* after 24h of treatment with fungicides, (a) control distilled water; (b) Koside; (c) Fungran and (d) Copper Antracol .All photos were taken at 200X magnification

Kocide®101 was the most effective fungicide showing 2.08% conidial germination followed by Copper Antracol (2.9%) and Fungran with 25.5% conidial germination, whereas untreated control showed 77.3% germination, (**Figure 2**).

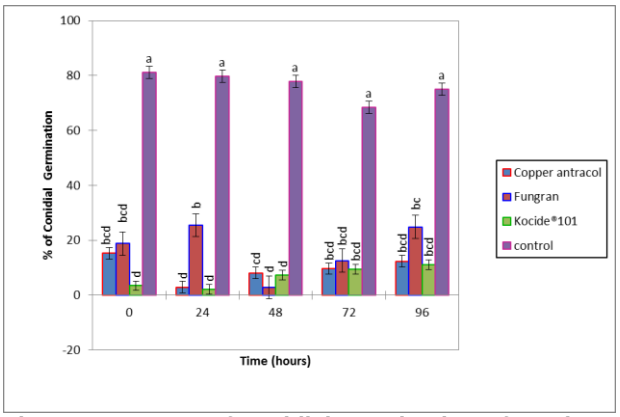

Figure 2. Percent of conidial germination of *S. oleaginea* in different fungicide treatments.

In Palestine, the efficiency of fungicide is affected by environmental conditions especially in winter and spring that promotes the outbreak of the disease and washing fungicide off leaves by rain and dew.

Application of copper-based fungicides is currently the main method of olive leaf spot control in many plant growing regions of the world. Although there is a lower risk of pathogens developing resistance to copper based fungicides since they have multi-site activity, some cases of resistance have been re-

ported in bacterial disease (Carisse et al. 2000; Vanneste et al . 2003).

Table1: Ranking based on arranging fungicides from the lowest conidial germination (1) to the highest germination (3) (1 better than 2 better than3)

Fungicide	Time (h)				
	0	24	48	72	96
Copper antracol	2	2	3	2	2
Fungran	3	3	1	3	3
Kocide®101	1	1	2	1	1

Conclusion

Considering the copper metal content; Copper Antracol can be viewed as the best choice for the control of the disease in Palestine, Further studies are needed to test the efficacy of these fungicides under field conditions for a better control planning of peacock disease in Palestine and to modify and develop fungicides active ingredients annually to obtain better understanding of the environmental effects on fungicide application and fungicides modifications.

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