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**PROTECTIVE EFFECT OF OLIVE POLYPHENOLS ON WATERMELON AGAINST  
*FUSARIUM OXYSPORUM* F. SP. *NIVEUM* INFECTION**

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**ABSTRACT**

The inhibitory activity of polyphenolic compounds extracted from olive leaves was tested against *F. oxysporum* f. sp. *niveum* growth. Results showed phenolic and alcoholic extracts inhibited fungal growth by 70.6% and 66.75%, respectively, at 4000 ppm concentration. Olive leaves powder inhibited the growth by 47.50%. All treatments increased watermelon germination percentage after 7 days when scored 85% and 100% at 0.4% and 0.8% olive leaf powder concentrations, respectively. Alcoholic treated seeds at 4000 ppm scored 90% compared to pathogenic fungus treatment with 25% germination percent. Whereas the germination percentages were 90% and 100%, respectively for fungicide (Bltanol) and healthy control (untreated) treatments. All treatments could protect watermelon seedlings for continuous 35 days after planting. The phenolic extract showed the highest vigour index values when scored 1616.7 and 1821.6 at the concentrations of 4000 and 8000 ppm, respectively. Whereas, alcoholic extract and olive leaf powder at scored 1332 and 1102.5 at concentration 8000 ppm and 0/8% respectively, compared to the pathogenic fungal and the fungicidal treatments with 360.5 and 896.8, respectively. These results indicated the effectiveness of the olive leaf extract in fungal growth inhibition. This study confirmed the presence of antifungal active substances in olive leaves against pathogenic fungi.

**Keywords:** Anti-fungal activity, olive extracts, *Fusarium oxysporum* f. sp. *niveum*, Watermelon.

**INTRODUCTION**

Watermelon *Citrullus lanatus* L. is one of cucurbit crops grown worldwide (including Iraq) due to its economic importance. In 2016, the estimated global production of watermelon fruits was 117,022,560 tones, whereas, the Iraqi production of watermelon was 192,013 tones (FAO 2016).

The olive *Olea europaea* L. is an important evergreen fruit tree worldwide, especially in the Mediterranean Basin area. It is grown due to the high nutritional, industrial and health values of olive fruits (Huang *et al*, 2003). Olive leaf extract contains phenolic compounds which are effective against bacteria, mycoplasma and yeasts. They have been used to treat fever and malaria

and to reduce blood pressure in animals (Friedman 2007; Proestos *et al*, 2005). In the medical field, phenolic compounds found in olive leaves, particularly oleuropein and hydroxytyrosol, have widely been used to treat blood pressure and influenza (Friedman *et al*, 2007; Michele and Andrew, 2002). Phenolic compounds are known for their inhibitory activity against bacteria, fungi and viruses (Bisignano *et al*, 1999; Lee-Huang *et al*. 2007). Soni *et al*, (2006) reported that olive leaves inhibited gram-positive and -negative bacteria as well as fungi. *Fusarium* wilting disease caused by the fungus *Fusarium oxysporum* f. sp. *niveum* is one of the devastating cucurbit diseases. It impacts watermelon causing significant losses (Kurowski, *et al*, 2015). It can cause mortality to infected plants grown in open fields and greenhouses as well, especially in warm areas. Managing this disease is so difficult due to the nature of pathogen growth (Bowers *et al*, 2000).

Due to the importance of *Fusarium* wilting disease in Iraq, this study investigated the antifungal activity of

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multiple phenols from olive leaves against *F. oxysporum* f. sp. *niveum*, infecting watermelon plants.

#### MATERIALS AND METHODS

**Fungal isolation and identification:** The pathogenic fungus was isolated from the stem of a watermelon plant exhibiting canker and wilt symptoms. The infected stem was cut into small parts of about 0.5 cm, sterilised with NaCl solution (1% Cl-free), rinsed with distilled sterilised water and dried on a sterile towel paper. Then, these small pieces were transferred to Petri dishes containing potato sucrose agar (PSA), then incubated for 5 days at  $25 \pm 2$  °C. Afterwards, they were examined and investigated for the pathogenic fungus causing the disease and then genus and species were identified based on morphological and microscopic characteristics following the method described by Parmete and Whitney (1970).

**Preparation of olive extracts:** Olive leaves were collected, rinsed with running water and cut into small parts. Samples were spread and left to dry. The dried leaf pieces were ground by an electrical grinder and finely sieved. A total of 100 mg plant powder was weighted and placed in a 500 mL glass flask. Alcoholic extract was prepared by adding 200 ml of 80% ethanol to plant powder. For phenolic extraction, 200 ml of 80%

methanol was added. The flasks were sealed with lids and components were mixed well for 24 h by an electrical shaker. The extracts were passed through filter papers (Whitman No.1) in a Buchner funnel with vacuum. Extracted process was repeated twice and the final extracts were collected and concentrated by a rotary evaporator device at 40 °C (Nasir *et al*, 2008). The extracts were stored in dim glass bottles -20 °C to be used later on.

**Antifungal activity of olive leaf extracts in Potato sucrose agar (PSA) medium:** The activity of olive leaf extracts was tested according to the food poisoning method described by Carling and Leine (1986). Petri dishes containing PSA culture were prepared. The concentrations of alcoholic, phenolic extracts and powder of olive leaves were adjusted to 1000, 2000 and 4000 ppm, respectively. Then 0.5 cm diameter pieces sliced from 7 days of growth fungal colony were transferred onto PSA plates and incubated at  $25 \pm 2$  °C. A control treatment of PSA culture containing the pathogenic fungus only were used. When the fungal growth in the control treatment reached the plate edge, the growth diameters of colonies were measured. The inhibition percentage was calculated using the following equation:

$$\text{Inhibition \%} = \frac{(\text{fungal growth average at the control} - \text{growth average at the treatment})}{\text{growth average at the control}} \times 100$$

**Antifungal activity of olive leaf extracts in the greenhouse:** The experiment was carried out in a greenhouse at Plant Protection Department, College of Agricultural Engineering Sciences/University of Baghdad from 2015 to 2016. One kg capacity plastic pots were filled with sterilised soil, then, contaminated with  $10^4$  diluted spores of the pathogenic fungus at 20 ml/pot. Uncontaminated control treatment was included. Four replicates were used for each treatment. Three days later, the soil was mixed with the following treatments:

- 1-Olive leaf powder at 0.4%
- 2-Olive leaf powder at 0.8%
- 3-Alcoholic extract of 4000 ppm at 20 mL/pot
- 4-Alcoholic extract of 8000 ppm at 20 mL/pot
- 5-Phenolic extract of 4000 ppm at 20 mL/pot
- 6-Phenolic extract of 8000 ppm at 20 mL/pot
- 7-Fungicide Bltanol of 1.5 ml/L at 20 mL/pot
- 8-Nothing was added on the control treatment

Pots were covered with polyethylene bags, left for 7 days and 5 watermelon seeds per pot were sowed for each treatment. The germination percentage was calculated 7

and 35 days of sowing. Plant length, root length, plant fresh and dry weights were measured. The vigour index was calculated using the following equation (Christopher *et al*, 2010):

Vigour index = (stem length average + root length average) × germination percentage. Statistical analysis was performed using SAS software (2012). Completely randomised design method (CRD) was used for data analyses.

#### RESULTS AND DISCUSSION

**Pathogen isolation and identification:** Results showed the pathogenic fungus isolated from symptomatic watermelon plants exhibiting vascular wilting formed pink colonies and dense hypha on PDA medium. Microscopic traits confirmed the detection when fungal parts, such as crescent macroconidia, oval or spherical microconidia and spherical chlamydospores that occurred in single, pairs or clusters were observed. These characteristic features indicated the fungus associated with the infected watermelon sample is *F. oxysporum* f. sp. *niveum*, as described by Summerell, *et al*, (2003).

**Antifungal activity of olive leaf extracts in PSA plates:**

The anti-fungal activity of the alcoholic, phenolic extracts and powder of olive leaves, could reduce the hypha growth of *F. oxysporum* f. sp. *niveum* at all three concentrations (Table 1). At 4000 ppm, each phenolic and alcoholic extracts and olive leaf powder scored the highest antifungal activities when inhibited the fungal growth by 70.6%, 66.75% and 47.50%, respectively. The presence of antifungal active substances, namely, phenolic compounds against pathogenic fungi in olive leaves have been reported (Michele and Andrew, 2002).

**Antifungal activity of olive leaf extracts in the greenhouse:**

Protection experiments under greenhouse conditions showed a noticeable reduction in *F. oxysporum* f.sp. *niveum* infection of watermelon seeds and seedlings (Table 2). The watermelon seed germination percentage was significantly increased 7 days after olive leaf powder and extracts treatments. Olive leaf powder at 0.4% and 0.8% scored 85% and 100% highest germination percentages, respectively, followed by alcoholic treatment with 90% germination percent at 4000 ppm. Whereas seed germination percentages in fungicide (Bltanol) and control (untreated) treatments were 90% and 100%, respectively, compared to the pathogenic fungal

treatment which scored 25% seed germination percent. The ability of the tested materials to protect the watermelon seedlings continued for 35 days after planting. The seed germination percentages ranged 80 - 95% compared to pathogen only treatment (35%). Furthermore, all treatments did not show significant differences when compared to Bltanol fungicide and control (untreated) treatments. In the infectivity percentage experiment, the efficacy of the three treatments against the pathogenic fungus was quite obvious 35 days after treatment. Olive leaf powder and extract treatments could decrease the percentage of watermelon seedling infection up to 5–20% compared to the pathogenic fungus treatment which was 65%. These results confirmed the inhibitory activity of the olive leaf treatment against *F. oxysporum* f. sp. *niveum*. Thus, suppressing the disease development on the growing watermelon seedlings could be enhanced through olive leaf extract treatments. Okwu (2004) and Soni *et al*, (2006) reported the inhibitory activity of phenolic compounds from olive leaves against the growth of various micro-organisms, including fungi. Similarly, Syed (2010) stated the antimicrobial efficacy of phenolic compounds against microorganisms and their inhibitory role against bacterial growth.

Table 1. Effect of olive leaf extracts on the inhibition percentage of *F. oxysporum* f. sp. *niveum* grown on PSA at 25 °C.

Treatments	Inhibitory Percentage		
	1000ppm	2000ppm	4000ppm
Olive leaf powder	36.65	45.83	47.50
Alcoholic Extract	59.80	65.68	66.75
Phenolic extract	22.9	30.48	70.6
Control	0	0	0
L.S.D 0.05	2.37	2.85	9.54

\* Each number in the table represents the rate of four replicates

Table 2. Effect of olive leaf extracts on the growth parameters of watermelon plants infected with *F. oxysporum* f. sp. *niveum*.

Treatments	Infection %	Germination%		Growth parameters after 35 days		
		after 7 days	after 35 days	Dry weight / g	Fresh weight / g	Plant Length / cm
Control(untreated)	5	100	95	0.11	0.72	14.81
Pesticide (Bltanol)	20	90	80	0.11	0.82	11.21
Pathogenic fungus treatment	65	25	35	0.10	0.75	10.3
Leaf powder 0.4 %	5	85	95	0.09	0.75	10.93
Leaf powder 0.8%	10	100	90	0.08	0.71	12.25
Alcoholic extracted 4000ppm	20	90	80	0.08	1.00	13.49
Alcoholic extracted 8000ppm	20	70	80	0.15	0.86	16.65
Phenolic extract 4000ppm	15	70	85	0.14	1.06	19.02
Phenolic extract 8000ppm	20	70	80	0.16	0.96	20.24
L.S.D 0.05	3.57	7.56	8.08	0.32	0.75	3.35

\*Each number in the table represents the rate of four replicates

Significant differences in plant growth criteria of watermelon treated with olive leaf powder and extracts, 35 days after planting were observed. Phenolic extracts at 8000 ppm increased plant lengths up to 20.24 cm compared to 18.81 cm for the control (untreated) treatment. Whereas, plant lengths were 10.3 and 11.21 cm for pathogenic fungal and fungicidal treatments, respectively. Plant lengths were 16.65 and 12.25 cm for alcoholic extract at 8000 ppm and olive leaf powder treatment at 0.8%, respectively (Table 2). In the phenolic extract experiment, slight differences were noticed in the fresh and dry weights at 4000 and 8000 ppm, when scored 1.06 and 0.96 g fresh and 0.14 and 0.16 g dry weights, respectively. The alcoholic extracts at 4000 and

8000 ppm scored 1.00 and 0.8 g fresh weights and 0.08 and 0.15 g dry weights, respectively, compared to fresh and dry weights for the control (0.72 and 0.11g, respectively) (Table 2). Whereas, the pathogenic fungal and fungicidal treatments were slightly different from the control treatment (Table 2). These results may be attributed to the olive leaf extracts and phenolic compounds the high inhibitory activity against microorganisms' growth, including fungi (Soni *et al*, 2006). Consequently, olive extract and powder treatments increased the biological activity of treated plants compared to infected plants. Due to their organic contents, they may act as growth promoters and organic soil additives to improve and enhance watermelon seedling the plant growth.

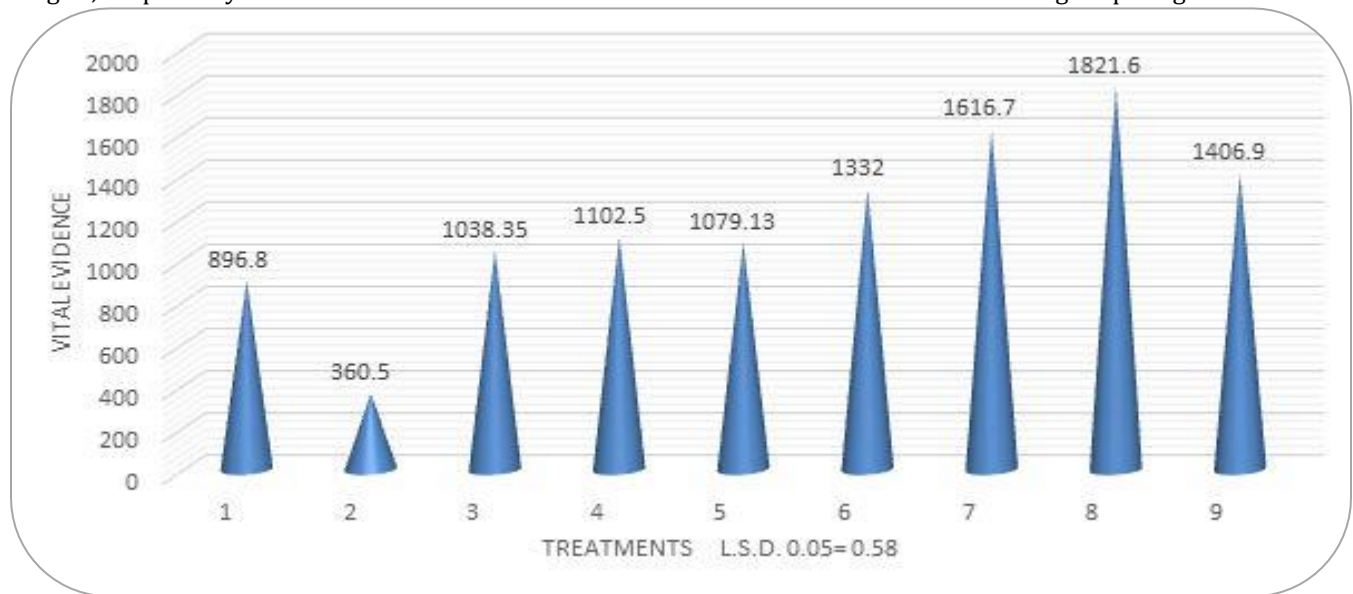


Figure 1. Effects of olive leaf extracts on watermelon. Significant differences in vigour index (Fig. 1,  $P < 0.05$ ) were scored among the experimental treatments. The phenolic extract treatment was the highest in vigour index when scored 1821.6, at 8000 ppm, (Fig 1). Whereas, the vigour index of the alcoholic extract and olive leaf powder treatments were 1332 and 1102.5, respectively, at 8000 ppm compared to the pathogen fungal treatment with only 360.5. The fungicidal treatment scored 896.8. The high vigour index values alongside those scored for growth parameters may confirm the inhibitory effects of olive extracts tested against the pathogenic fungus. The initiatory activity of olive leaf extracts may be resulted from the active substances found in leaves, especially phenolic compounds that act as antimicrobial agents against fungi (Michele & Andrew, 2002). Due to antimicrobial activity, alongside the ability to enhance growth and the high demands to discover eco-friendly pesticide alternatives (Rongai *et al*, 2015), olive leaf extract can be used as an effective and safe natural product to produce bio-fungicides against fungal diseases and fertilizers to improve plant growth as well.

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**Contribution of Authors:**

T. A. Kareem	:	Research proposal and designed experiments
Shmaael S. Mutar	:	Designed and conducted lab experiments
Eman K. A. Karim	:	Designed and conducted lab experiments
Nawres A. S. A.Kuwaiti	:	Data analysis and field experiments