# Effectiveness of some Fungicides in Controlling Vascular Wilt Disease in Tomato Caused by *Fusarium oxysporum* f. sp. *lycopersici* in Vivo

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***Abstract:***

*This research was conducted to study the effect of three fungicides from different chemical groups (thiophanate methyl, quinosol and fosetyl aluminum) in reducing the incidence of vascular wilt disease in tomato caused by Fusarium oxysporum f.sp. lycopersici in protected agriculture. The fungicides were applied by irrigation twice by 15 days intervals between them. The role of these fungicides was also studied in the stimulation of some resistance indicators such as the activity of peroxidase, polyphenol oxidase and total phenol content.*

*The tested fungicides contributed in reduction of the infection severity compared with the infected control. After 60 days, the intensity was 30.7, 35 and 27.3%, respectively, for quinosol, fosetyl Aluminum and thiophanate methyl compared with the infected control (78%). The efficacy was 67.4, 62.5 and 70.29%, respectively. The studied fungicides also contributed in stimulation of the resistance indicators. Thiophanate methyl outperformed on quinosol and fosetyl aluminum in increasing the activity of peroxidase and poly phenol oxide and the total phenol content after 30 days of treatment.*

**Keywords:** *Fusarium oxysporum* f.sp. *lycopersici*, vascular wilt, acquired resistance and fungicides.

## I. INTRODUCTION:

Tomato, (*Lycopersicon esculentum* Mill) considers one of the most common vegetables in the world, and is highly consumed due to its high nutritional value [1]. In Syria, tomato planting in greenhouses during 2016 reached to about 3007 ha. The number of greenhouses planted with tomato attained 75172, with a total production of 451032 tons [2].

Tomato is infected with many pathogens, especially the fungus *Fusarium oxysporum* Schaech f.sp *lycopersici* (Sac) W.C. Snyder & H. Hansen, causing vascular wilt disease [3]. Which is one of the most important pathogens of tomato, where the disease causes significant damage in production, especially under the protected crops conditions ([4], [5], [6]). Losses ranged from 10 to 50 % of the crop around the world [7].

The fungus *Fusarium oxysporum* belongs to the family Tubercullariaceae, ordre of Moniliales, class of Deuteromycetes [8]. Its sexual phase belongs to Ascomycetes class (currently phylum of Ascomycota) (Sordariomycetes: Hypocreales: Nectriaceae). It is a global species, affecting many important agricultural crops causing vascular wilt disease [9].

This fungus produces three forms of spores: microconidia, (which consist of one or two cells), macroconidia, (which consider the representative of this fungus and consist of 3-5 cells), and clamydospores, (spherical spores, consisting of a cell or two cells with a thick wall formed within or on the old mycillium [6].

There are many disease management strategies including biological control, agricultural cycle, solar sterilization and the prudent use of fungicides [10]. Most farmers tend to use fungicides from different chemical groups because of their ease of use and effectiveness [11].

Re. [12] noted that the fungicide Beltanol-L inhibited growth of 5 isolates of the fungus *Fusarium oxysporum* f.sp. *tuberosi* in laboratory with rate attained 30 to 43 % for all isolates when it used at the recommended concentration (500 g / ha). While in the field, Beltanol-L was effective during the first three weeks of the essay, and the disease parameter increased from 0.37 to 2.16 at the end of the experiment.

Re. [13] demonstrated that Beltanol-L reduced the severity of infection to 20% as an average of all isolates compared with the infected control, which reached to 80% as a mean of all isolates. Beltanol-L also reduces the severity of *Fusarium oxysporum* f.sp. *melonis* on melon plants after 45 days post treatment and artificial infection [14].

Re. [15] found that fosetyl aluminum significantly reduced the brown coloration caused by the fungus *Fusarium oxysporum* (62.55%) compared to the control in the field. Whereas the fungicide Mancozeb reduced the coloration by 50.19%, while carbendazim was less effective in reduction the root coloration by 37.45%.

Re. [16] observed that the fungicide Beltanol was superior to Bafastin (carbendazim) and Tashgarin (hemaxazole) when it applied as irrigation at the recommended concentration after planting. While it reduced the infection severity to 22.5%, followed by Bafastin and Tashgarin (30% and 37.5%), respectivley. Beltanol was also superior to both other fungicides when seedlings were dipped before planting. Beltanol, Bafastin and Tashgarin reduced the infection severity to 35, 40 and 40% respectively.

Systemic acquired resistance (SAR) is a promising method of controlling plant diseases. External or internal factors can significantly affect host physiology, resulting in rapid activation of the protective gene in plants, expressing susceptibility to pathogen infection [17].

Positive correlation between levels of Polyphenol oxidase (PPO), Peroxidase (POD) and plant pathogen resistance is noted, with some evidence suggesting that activation of peroxidase and polyphenol oxidase play a critical role in biological control and plant resistance to pathogenic attack ([18], [19]). Peroxidase stimulates the formation of lignin and phenylalanine ammonia-lyase, which are incorporated into the synthesis of phenolic compounds and phytoalxine [20].

Polyphenol Oxidase is one of copper-containing enzymes that stimulate the oxidation of many phenols to *o*-quinones ([21], [22]). Re. [23] noted that the increase in phenolic content is evidence of activation of systemic resistance within the plant. The accumulation of phenols at the infection sites is associated with limiting the evolution of the pathogen as it has a toxic effect on the pathogen. It can also prevent infection by increasing the stiffness of cell walls [24].

Re. [25] observed that the highest increase in peroxidase activity was 2.4 times compared with the healthy control with fosetyl aluminum and typosonazole treatment, and 1.7 times with fentin hydroxide and iprobenfos with *Fusarium oxysporum* f.sp. *lycopersici* after 25 days, and that treatment with some pesticides contributed to increase the total phenolic content by up to 10%, including fosetyl aluminum.

The importance of research stems from the nutritional and economic importance of tomato, which is considered a strategic crop in Syrian. As well as the risk of tomato wilt in both greenhouses and field crops, causing significant economic losses. So the research goals to:

1- Field Studying the effectiveness of some fungicides in controlling tomato wilt disease causing by the fungus *Fusarium oxysporum* f.sp. *lycopersici*.

2 - The role of these pesticides in the stimulation of some indicators of resistance such as activity of peroxide and polyphenol Oxidase and the total content of phenol.

## II. MATERIALS AND METHODS:

### *A). The used fungicides:*

Fosetyl aluminum, thiophanate methyl and quinosol were used from different chemical groups (Table 1)

**Table 1: Some characteristics of used fungicides**

| Trade Name | Name and amount of the active ingredient | the group | pesticide form | the field concentration (g or ml/ l) |
|---|---|---|---|---|
| Agri Sin | thiophanate methyl 70% | Benzimidazole | watable powder WP | 1g |
| Beltanol-L | quinosol 50% | quinoline | solution SL | 2 ml |
| Aliette | fosetyl aluminum 80% | Phosphonate | granules WG | 2 g |

### *B). Studying the effectiveness of pesticides in controlling tomato wilt disease:*

Field experiment was carried out in Banias area in 2018 within a greenhouse planted with month-old tomato plants of the hybrid (Yousra) infected with vascular wilt. Where the fungus was detected in the greenhouse soil and plants through cultivation of soil and plant samples on PDA medium. The effectiveness of the studied fungicides was compared with the control. Three replicates were performed for each treatment, and each replicate contains 15 plants. Where

replicates and treatments distributed as completely randomized blocks within the greenhouse.

The treatment was treated by irrigation with the addition of the pesticide twice by 15 days intervals at the recommended concentration in the field at 0.5 liter per plant. Readings were periodically taken every 15 days and two readings were adopted after 30 and 60 days post the first irrigation with pesticides. To estimate the ratio and severity of the infection, the following ladder was used [26]:

1: no infection
2: yellowing the down leaves.
3: death the down leaves and yellowing the middle leaves.
4: yellowing the upper leaves.
5: plant death.

Infection severity was calculated depending on the following equation [27]:

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

a: number of plants in each degree.
b: degree value.
N: total plants.
K: the highest degree in infection ladder (= 5).

The effectiveness was calculated depending on equation of [28]:

$$\text{effectiveness (\%)} = 100 - \left(\frac{infection\ \ severity\ \ after\ \ treatment}{infection\ \ severity\ \ befor\ \ treatment} \times \frac{control\ \ infection\ \ severity\ \ after\ \ treatment}{control\ \ infection\ \ severity\ \ befor\ \ treatment} 100\right).$$

***C. Study the activity of peroxidase and polyphenole oxidase, and calculating the total content of phenol:***

The activity of peroxidase and polyphenols oxidase was estimated after 30 and 60 days of treatment, by grinding 1 g fresh tomato leaves in 3 mL of potassium phosphate solution (pH=7, 0.1M). Then expose the samples to centrifugation (10,000 cycles / min) for 10 minutes at 4 ° C. For peroxidase, 3.5 ml of the former potassium phosphate solution and 200 µl of the sample extract were taken and added 200 µl of guaiacol and 200 µl of oxygen water (0.1mM) to it. The enzyme activity was calculated using a spectrometer at 430 nm [29]. Peroxidase activity was estimated according to the equation:

Activity of peroxidase = (extension factor x amount of oxygen water) / (sample size x time)

While to polyphenol oxidase, 1.95 ml of the former potassium phosphate solution was taken with 1 ml of catechol solution and 50 µl of the sample extract. The enzyme activity was calculated at 410 nm ([30], [31]).

The total phenol content was estimated by grinding 2 g of tomato leaves in 10 mL ethanol (80%), centrifugation (10000 cycles / min) for 10 minutes at 25 ° C, then preparing a 40 µl mix of the sample extract and 200 µl of a detector (F. oxysporumin-Ciocalteu's reagent) and 3.16 ml distiller water and 600 µl of sodium carbonate (20%). These compounds were saved at dark for an hour and a half. The phenol content was estimated at a 650 nm wavelength, but in the present research the wavelength was adjusted by determining the wavelength that gave the best absorption and was 720 nm [32].

***D). Statistical Analysis:***

Statistical analysis by Genstate-12 was performed by comparing the LSD value at the 5% level. Duncan test was used to determine the significant differences between the coefficients.

## III. RESULTS AND DISCUSSION:

***A). Study the effective of the used fungicides in the control of tomato wilt disease:***

Table (2) shows the infection severity (%) of tomato plants infected by the fungus *F. oxysporum* and exposition of some fungicides under protected cultivation conditions.

**Table 2: Infection severity (%) of tomato plant infected by the fungus** *F. oxysporum* and exposition of some fungicides under protected cultivation conditions

| treatment | 30 days post treatment | 60 days post treatment |
|---|---|---|
| infected control | 69.3 b | 78.0 b |
| fosetyl aluminum | 37.3 a | 35.0 a |
| quinosol | 38.3 a | 30.7 a |
| thiophanate methyl | 33.7 a | 27.3 a |
| **L.S.D 5%** | **19.13** | **15.56** |

It is noted from Table (2) that the infection severity before the treatment was 35.3% for the control, increased during the experimental stages to 69.3% after 30 days and 78% after 60 days respectively. Infection severity of fosetyl aluminum treatment attained 37.3 and 35%, respectively, after 30 and 60 days post treatment.

Infection severity to each of quinosol and thiophanate methyl reached to 38.3 and 33.7 %, respectively, after 30 days, and attained to 30.7 and 27.3 % after 60 days. Results showed superiority of fungicides treatments compared with infected control. Thiophanate methyl exceeded on fosetyl aluminum and quinosol without significant differences.

Table (3) shows effectiveness of the used fungicides in control vascular wilt disease caused by the fungus *F. oxysporum* during the experimental period. Where it attained 55.06% and 62.55% for fosetyl aluminum respectively after 30 and 60 days. These results agreed with re. [15], where the pesticide significantly reduced roots browning by (62.55%). While the efficacy of quinosol was 54.22 and 67.44%, respectively, after 30 and 60 days of treatment. Thiophanate methyl was 58.82% after 30 days and reached to 70.29% after 60 days.

**Table (3): Effectiveness of some fungicides in controlling vascular wilt disease caused by *F. oxysporum* during experimental period**

| treatment | first reading (30 days after treatment) | second reading (60 days after treatment) |
|---|---|---|
| fosetyl aluminum | 55.06 | 62.55 |
| quinosol | 54.22 | 67.44 |
| thiophanate methyl | 58.82 | 70.29 |

***B). Study the activity of peroxidase and polyphenols oxidase and estimate the total content of phenol***

Table (4) shows the effect of the fungus *Fusarium oxysporum* f.sp. *lycopersici* and the treatment of some fungicides in the activity of peroxidase under protected farming conditions.

**Table (4): Effect of some fungicides in the activity of pyroxidase (nan omol) in tomato plant infected by the fungus *F. oxysporum* under the conditions of protected agriculture.**

| Treatment | 30 days after treatment | 60 days after treatment |
|---|---|---|
| infected control | 0.017 d | 0.120 c |
| fosetyl aluminum | 0.063 b | 0.057 d |
| quinosol | 0.042 c | 0.166 b |
| thiophanate methyl | 0.092 a | 0.263 a |
| **L.S.D 5%** | 0.001 | 0.031 |

Table (4) shows that the studied pesticides had a role in increasing the activity of peroxidase throughout the experiment period. The activity of peroxidase was 0.063, 0.042 and 0.092 nano mol respectively for fosetyl aluminum, quinosol and thiophanate methyl after 30 days. While for the infected control, it attained (0.017 nano mol).

Peroxidase activity increased after 60 days and reached to (0.120, 0.166 and 0.263 nano mol) respectively, for the infected control, quinosol and thiophanate methyl. While to fosetyl aluminum, the activity of the enzyme decreased (0.057 nano mol).

Results of the statistical analysis showed that all the studied pesticides exceeded on the infected control 30 days after the first irrigation, with significant differences between all treatments. Thiophanate methyl was the most effectiveness followed by fosetyl aluminum and then quinosol. After 60 days, thiophanate methyl was also the most effectiveness, followed by quinosol then fosetyl aluminum.

Table (5) shows the effect of *F. oxysporum* f.sp. *lycopersici* and the treatment of some fungicides in the activity of polyphenol oxidase under protected agriculture conditions.

**Table (5): Effect of some fungicides in the activity of polyphenol oxidase (absorption unit / min) in tomato plant infected by the fungus *F. oxysporum* under protected cultivation conditions.**

| treatment | 30 days after treatment | 60 days after treatment |
|---|---|---|
| infected control | 0.026 d | 0.032 d |
| fosetyl aluminum | 0.048 b | 0.049 a |
| quinosol | 0.040 c | 0.038 c |
| thiophanate methyl | 0.070 a | 0.045 b |
| **L.S.D 5%** | 0.004 | 0.0032 |

Table (5) shows that the studied pesticides had a role in increasing the activity of polyphenol oxidase after 30 days of treatment. The activity of the enzyme attained (0.048, 0.040 and 0.070 absorption unit / min), respectively for fosetyl aluminum, quinosol and thiophanate methyl after 30 days. As for the infected control, it reached (0.026 absorption unit). After 60 days, both fosetyl aluminum and quinosol persevere their role in stimulating enzymatic activity (0.049 and 0.038 absorption units, respectively). The enzyme activity was also reduced to 0.045 absorption units in the treatment of thiophanate methyl.

Results showed significant differences between all treatments during the experiment period. Thiophanate methyl exceeded all treatments followed by fosetyl aluminum, quinosol and infected control after 30 days of treatment. After 60 days of treatment, fosetyl aluminum was the most effectiveness followed by thiophanate methyl, quinosol, then the infected control.

Table (6) shows the effect of the fungus *F. oxysporum* f.sp. *lycopersici* and the treatment of some fungicides in total phenol content under protected farming conditions.

**Table (6): Effect of some fungicides on the total content of phenols (mg / g) in tomato plant infected by the fungus *F. oxysporum* under protected agriculture conditions.**

| treatment | 30 days post treatment | 60 days post treatment |
|---|---|---|
| infected control | 0.79 c | 0.86 d |
| fosetyl aluminum | 0.90 b | 1.16 c |
| quinosol | 0.80 c | 1.89 a |
| thiophanate methyl | 1.35 a | 1.44 b |
| L.S.D 5% | 0.072 | 0.087 |

Results shows that the studied pesticides had a role in increasing the total phenol content after 30 and 60 days of treatment. It attained (0.79, 0.90, 0.80 and 1.35 mg / g), respectively, for the infected control, fosetyl aluminum, quinosol and thiophanate methyl after 30 days (Table 5). Both of fosetyl aluminum and quinosol continued to increase total phenolic content after 60 days of treatment (1.16 and 1.89 mg / g, respectively) with value stability of the control.

Results of the statistical analysis showed significant differences between all treatments during the experiment period. Thiophanate methyl exceeded after 30 days post treatment on all studied pesticides, followed by fosetyl aluminum, then quinosol. There was also no significant difference between the treatment of quinosol and the infected control**.** While quinosol was superior to all studied pesticides after 60 days of treatment, followed by thiophanate methyl, then fosetyl aluminum.

The used pesticides had a role in stimulating resistance indicators. Whereas they increased the activity of peroxidase by 3.71 - 2.47 and 5.41 times, respectively, for fosetyl aluminum, quinosol and thiophanate methyl after 30 days compared to the infected control.

This was confirmed by [25] when they observed the maximum increase in the activity of peroxidase by 2.4 times compared with the healthy control when treated with fosetyl aluminum and fungal infection *Fusarium oxysporum* f.sp. *lycopersici* after 25 days.

Re. [33] reported that Peroxidase enters into many cellular processes such as growth, evolution, differentiation, oxidation metabolism and lignin synthesis, making the increase in the activity of peroxidase enzyme an initial indication of activation of the defense mechanism in plants.

Pesticides also played a role in increasing the activity of polyphenol oxidase enzyme at a rate of 2.69 - 1.54 and 1.85 times respectively for fosetyl aluminum, quinosol and thiophanate methyl after 30 days of treatment compared with the infected control.

Pesticides contributed to the increase of total phenol content by 34.88 - 119.77 and 67.44%, respectively, for fosetyl aluminum, quinosol and thiophanate methyl after 60 days of treatment compared to the infected control.

This ratio surpassed [25] that the treatment with some pesticides increased the phenol content by up to 10%, including fosetyl aluminum.

## IV. CONCLUSION

1. The used fungicides contributed reducing the severity of vascular wilt disease in tomato.
2. Methyl thiophene achieved the best efficacy after 60 days of treatment followed by quinosol.
3. The used fungicides have an important role in stimulating the systemic resistance of the plant. Methyl thiophene predominates over fosetyl aluminum and quinosol.

   So the present study suggests use of thiophenes Methyl, qunizol and Fostyl aluminum in control vascular wilt disease in tomato.

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