Salahuddin University - Erbil

In Vitro evaluation of some fungicide efficacy against *Fusarium oxysporum f. sp. Lycopersici* the causal agent of

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Abstract:

Tomato wilt is a serious disease caused by *Fusarium oxysporum* which cause reduction in tomato yield and mortality of tomato crops. The pathogen was isolated from infected roots of tomato. Three fungicides (Thiophanate methyl, Propamocarb And Azoxystrobin,) by three concentrations (500PPM, 750PPM, 1000PPM) were used to control *Fusarium oxysporum* in vitro. Among them, Thiophanate methyl! had the best results of inhibiting mycelial growth at a concentration of 1000PPM (76.17%). The lowest inhibition mycelial growth was recorded by Propamocarb at a 500 PPM concentration (10.42%).

1-Introduction:

The cultivated tomato, *Solanum lycopersicum L.*, is the world's most highly consumed vegetable due it is grown for multi purposes such as raw, cooked, processed food. It belongs to Solanacae family (Geisenberg and Stewart, 1986). Tomato fruit contains a large amount of water, vitamens and minerals and low quantity of protein, fats and some carbohydrates. Additionally, it has carotenes like beta-carotene and lycopene, which give the fruit its distinctive red color (OECD, 2008; Spooner, Anderson and Jansen, 1993). Tomato can be grown in a different geographical zone in open fields or greenhouses, and the fruit can be harvested by manual or mechanical means (Geisenberg and Stewart, 1986). According to FAO statistic in 2014 an estimated 5 million hectares of tomatoes were grown worldwide, yielding a production of 171 million tons. China and India are the two main tomato-producing country (FAOSTAT, 2017). Tomato has many health benefits because it contains a high level of lycopene which is an antioxidant that act affectively to slow the growth of cancerous cells and reduce prostate and stomach cancer. Also it is very useful in reducing cholesterol levels and lowering blood pressure because it contains vitamin B and potassium. In addition, the vitamin A found in tomato which improve the vision and keep hair shiny and strong (Debjit B *et al.,* 2012). Tomato crop is infected by a large number of diseases. Among them fusarium wilt of tomato is a destructive disease caused by *fusarium oxysporum lycopersici* (Ajilogba, C.F. and Babalola, O.O., 2013). The most destructive and dangerous disease of tomato is tomato wilt can cause total damage to the tomato field. A soil borne pathogen *fusarium oxysporum lycopersici* which is the causal agent of fusarium wilt of tomato car remain in the different types of soil for many years even without a host. the healthy plants get infected due to the presence of soil borne pathogen (Monda, E.O., 2002).

2- Literature review:

2-1 symptoms

The pathogen is present in most tomato-growing regions across the world and causes vascular wilt, which can have a detrimental effect on the crop (Moretti et al. 2008) crown and root rot symptoms caused by for are yellowing, wilting, and severe root rot, whereas Fusarium wilt symptoms caused by present yellowing, wilting, and vascular discoloration without root rot.

Figure (1): vascular wilt and discoloration in tomato root (Kumar, et al., 2022)

Fusarium wilt is characterized by stunting, chlorosis, and wilting, which are followed by leaf death and plant collapse. (Garibaldi et al. 2004b)

Figure (2): stunting and wilting, chlorosis, leaf death (Kumar, et al., 2022)

One of the main signs of Fusarium wilting in tomato plants is the browning of the vascular tissue. (monda et al.2002). A plant's symptoms are caused by fusarium wilt. The symptoms of fusarium wilts are similar to those of other wilts, but we can distinguish the signs of fusarium wilts in tomatoes, which include, and discolored stems. (Larkin et al.1998)

Figure (3): yellowing, wilting, dry leaves of tomato (Kumar, et al., 2022)

2-2 Disease cycle

Fusarium oxysporum is a saprophyte and ubiquitous soil pathogen that consumes rotting and dead organic materials. Although it can live in soil detritus as a mycelium and various forms of spores, chlamydospores are the most frequently found form of the organism.There are two main ways that this virus spreads: it can travel short distances through planting equipment and water splashing, and it can travel long distances through contaminated seeds and transplants.By using mycelia or spores that germinate and penetrate the plant's root tips, root wounds, or lateral roots, *F. oxysporum* can infect a healthy plant.The mycelium penetrates the root cortex and enters the xylem intracellularly.Once inside the xylem, the mycelium generates microconidia, which are asexual spores, and stays only inside the xylem vessels. Where the sap flow pauses, the microconidia germinate. The microconidia are able to enter the sap stream and are moved upward. The plant is unable to absorb and translocate nutrients when the spores and mycelia eventually clog the vascular channels. Ultimately, the plant dies because it seems more water than it can carry, causing the stomata to close and the leaves to wilt. The fungus penetrates all plant tissues when it dies, sporulates, and spreads to nearby plants. (Li, et al., 2018)

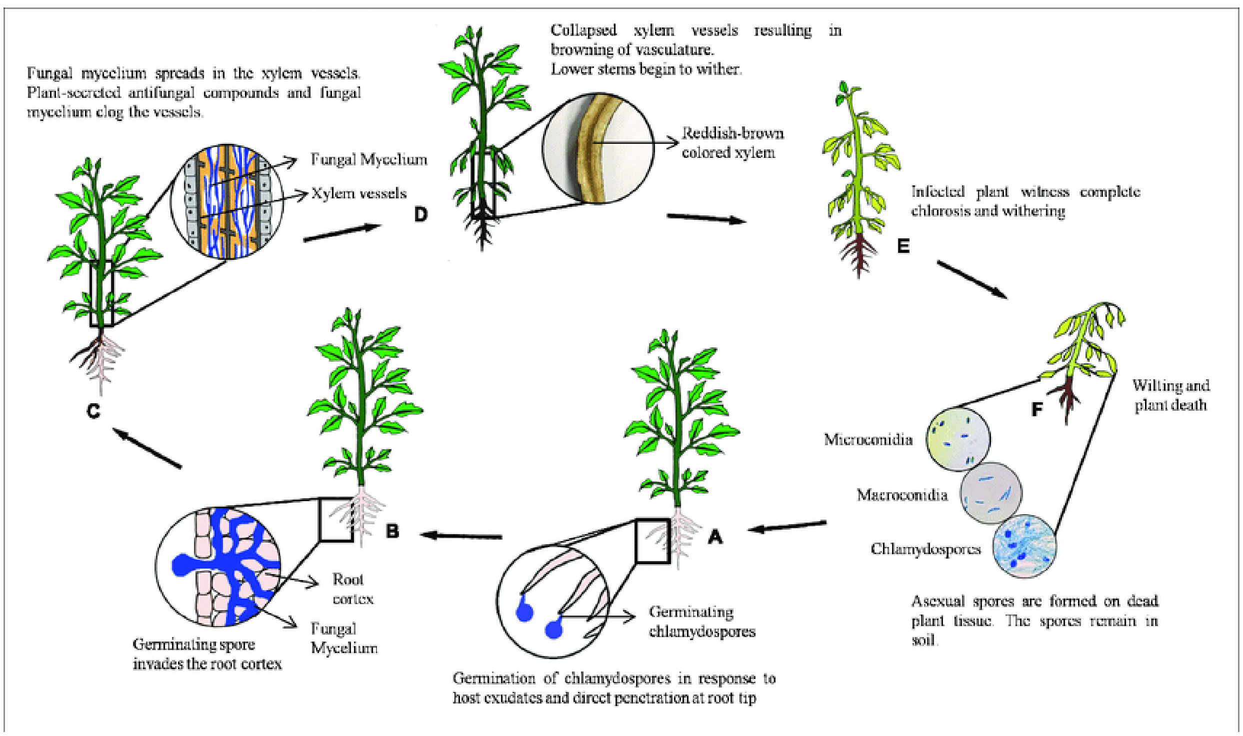


Figure (4); The Life Cycle of *Fusarium oxysporum f. sp. Lycopersici (*Khare et ai, 2017)

2-3Ethology of fungus

2-3-1Microconidia are uninucleate and have a germination efficiency of 1 to 20%, indicating poor germination (Ebbole and Sachs 1990).

- ✓ The most often generated spores are microconidia.
- ✓ Macroconidia are multinucleate and germinate quickly. They are generated on aerial mycelia and have an oval, elliptical, or kidney shape.

2-3-2 Macroconidia are found on sporodochia on the surface of sick plants. In culture, sporodochia may be few or non-existent. Macroconidia are three to five cells with increasingly sharp or curved edges.

2-3-3Vegetative hyphae or a thick-walled conidial cell and auxiliary spores structurally modify to produce chlamydospores (Schippers and van Eck 1981).

- ✓ Chlamydospores often form in pairs or singly, but they can also occasionally be observed in short chains or clusters.

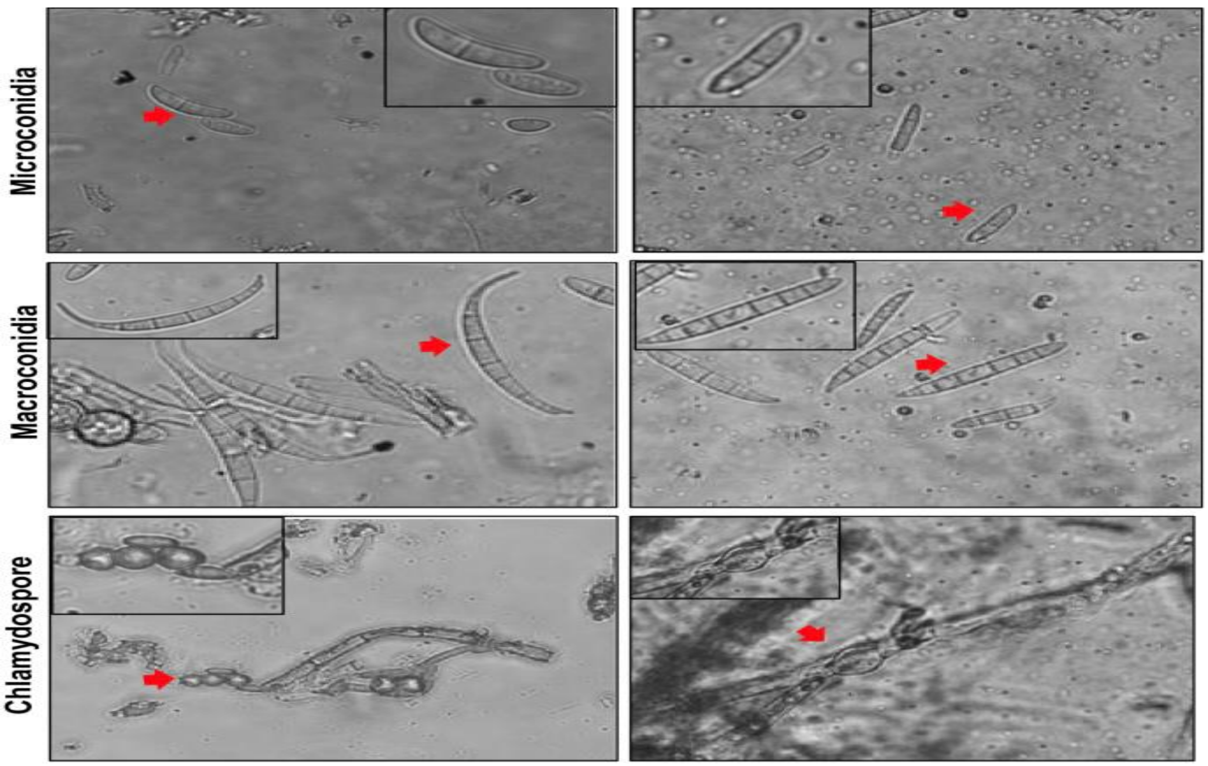


Figure (5): *Fusarium oxysporum f. sp. Lycopersici* Under the microscope (Parihar et

2-4 Disease control

There are many researches that fungicides used to inhibit the growth of *Fusarium oxysporum*, a research in India who used some systemic and non-systemic fungicides like Carbendazim 50% WP, Thiophinate methyl 70% WP, Difenconazole 25% EC, Azoxystrobin 23% EC, Benomyl 50%WP, Carboxin 75% WP, Captan 75% WP, Thiram 75% WS, Copper oxychloride 50% WP, Mancozeb 75% WP, Carbendazim 25% + Mancozeb 50 % WS, Carboxin 37.5 % +Thiram 37.5% WS, the best result reported by Carbendazim 50% WP, Copper oxychloride 50% WP and Carbendazim 25% + Mancozeb 50 % WS respectively to reduce the growth of *Fusarium oxysporum* significantly (Gadhave,, *et al*., 2020). Another report who tested two potential systemic fungicides Topsin M and Carbendazim against *Fusarium oxysporum F. sp. lycopersici* at three different concentrations (50 ppm, 100 ppm and 200 ppm) and the best result showed by Carbendazim at all concentration (Sinha A *et al*., 2019). In addition, six fungicides: benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin, were evaluated for their efficacy against the disease causal agent *Fusarium oxysporum f. sp. lycopersici* in vitro and in vivo, Prochloraz and bromuconazole were the most effective fungicides against the pathogen both in vitro (Amini, J. and Sidovich, D., 2010).

3-Materials and methods

3-1 In vitro control of with fungicides   Sample collection:

The infected tomato crops were collected and taken to laboratory, the roots of infected tomato crops were cut into small pieces and washed under tap water then the small pieces of infected roots were sterilized by taken in 2% of sodium hypo chloride, after that the small pieces were put Tomato Juice Agar then incubated at 25C for 7 days. The cultured media were observed for growth of *Fusarium oxysporum.* (Offun, 1999).

3-2 Evaluation of fungicides by food poisoning techniques

Three effective fungicides (Thiophnate methyl, Azoxystrobin, Propamocarb) were used to evaluate their efficacy against *Fusarium oxysporum lycopersici* causing tomato wilt, for each fungicide three concentration (500PPM, 750PPM, 1000PPM) were used by applying food poisoning technique. The melted Tomato Juice Agar was used as basal media. The melted medium was mixed with the fungicidal suspension at the required concentrations of 500 ppm, 750 ppm, and 1000 ppm.The required amount of poisoned medium was poured aseptically in each sterilized Petri dish followed to solidify. 5mm in diameter mycelial plug of 7 old days of cultured media of the pathogen was placed on the center of petri plates. Plates without fungicides served as negative control. Each treatment was repeated four times. The results were compared with negative control. to calculate percent inhibition of mycelial growth of the fungus the Vincent's formula was used (1927).

Percent growth inhibition= $\frac{\text{growth in control} - \text{growth in treatment}}{\text{growth in control}} \times 100\%$

Table (1): The effected fungicides were used against *Fusarium oxysporum lycopersici* in vitro

Trade name	Active ingredients	Technical
Marivan	**Propamocarb**	**72.2%SL**
Zoxis	**Azoxystrobin**	**97%TC**
Mandate	**Thiophanate methyl**	**70%(w/w) (a.i)**

3-3 Result and discussion:

According the conidia and mycelium type as shown in figure (9) the isolated fungus from the roots of infected tomato was *Fusarium oxysporum lycopersici*.

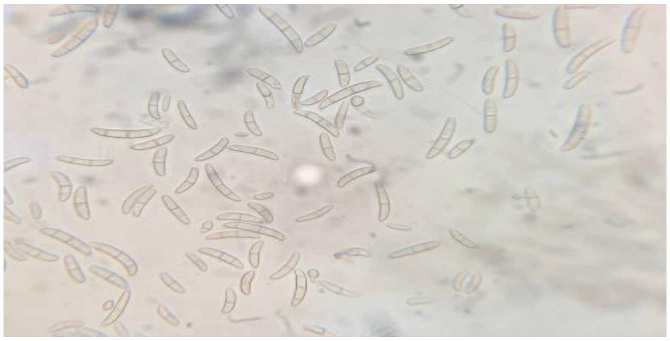


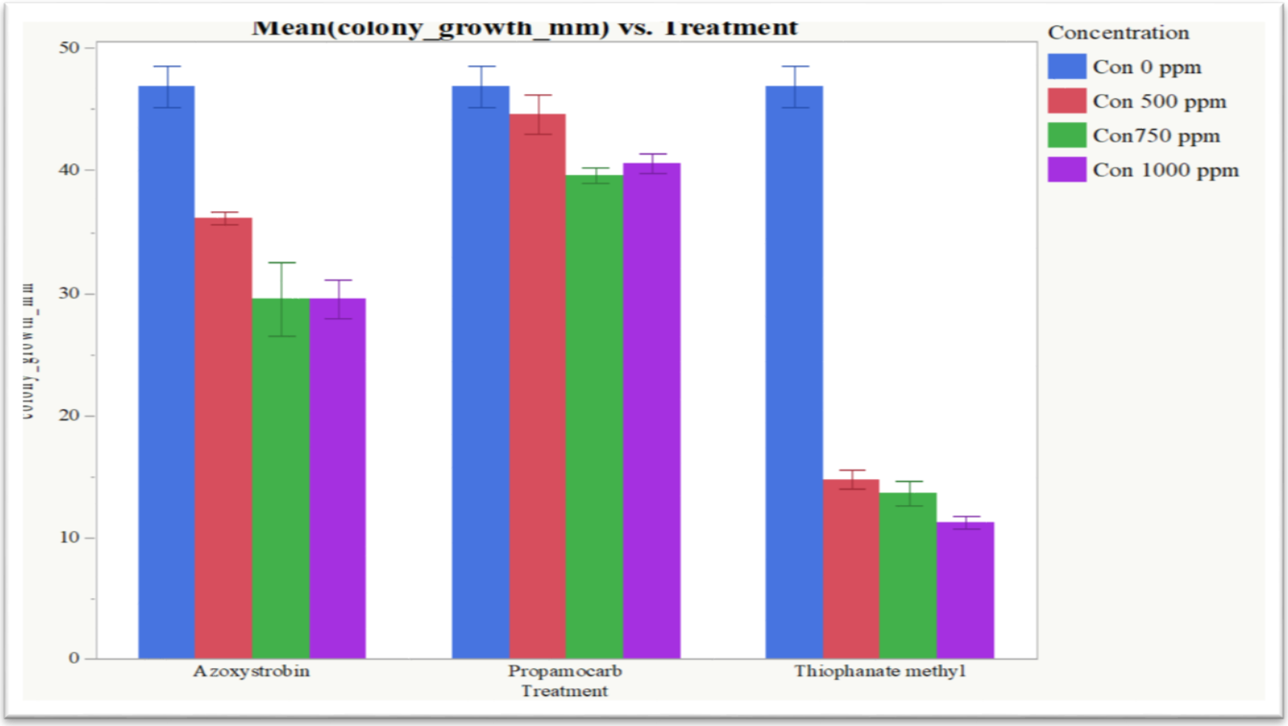
Figure (6): *Fusarium oxysporum lycopersici*. Under microscope; it is Microconidia and Macroconidia

3-4 Evaluation of different concentration of fungicides against *Fusarium oxysporum lycopersici* in vitro.

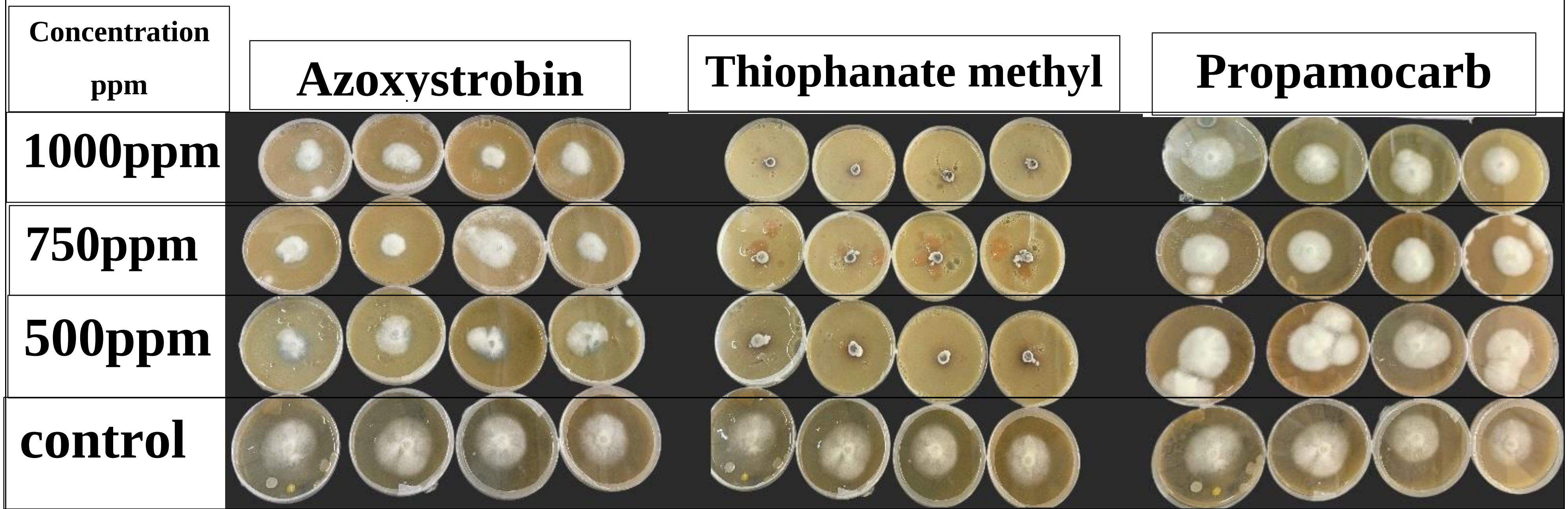
The result of effective fungicides in vitro revealed that thiophanate methyl at 1000PPM, 750PPM and 500PPM has the strongest effect on the *Fusarium oxysporum lycopersici* the causal agent of tomato wilt disease by 76.17, 71.06 and 68.72 percent inhibition respectively, in contrast Azoxystrobin and propamocarb were not effective against the pathogen as showed in (table 2).

Table (2): Percent inhibition of F. oxysporum f. sp. lycopersici in presence of different concentrations of fungicides.

Treatments	**Colony Dia.*(mm) at ppm**			**Av. Colony (mm)**	**% Inhibition* at ppm**			**Av. Inhibition (%)**
	500	750	1000		500	750	1000	
Azoxystrobin	36.10	29.5	29.5	31.70	23.19	37.23	37.23	32.55
Thiophanate methyl	14.70	13.60	11.20	13.16	68.72	71.06	76.17	71.98
Propamocarb	42.10	39.60	40.60	40.76	10.42	13.61	15.74	13.25
Control	47.00	47.00	47.00	47.00	0.00	0.00	0.00	0.00



Figures (7): the effect of three fungicides on *Fusarium oxysporum f. sp. lycopersici*.



Figures (8): culture media control of *Fusarium oxysporum f. sp. lycopersici*. By using three fungicides at concentrations of1000ppm,750ppm,500ppm.

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