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Antifungal activity of essential oils emulsions, their bioactive compounds, and biological control of *Fusarium* wilt of *Majorana hortensis*

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Abstract

This investigation was conducted to evaluate the activity of some essential oils emulsions and a biocide (Bio-Cure F) for controlling *Fusarium* wilt disease of marjoram (*Majorana hortensis*) caused by *Fusarium oxysporum*. The fungal filtrate of *F. oxysporum* isolated from the infected marjoram plants significantly decreased the marjoram seed germination and caused wilting of its seedlings. The essential oil emulsions of thyme; basil, and marjoram, inhibited the *in vitro* fungal growth of *F. oxysporum*, and significantly suppressed its sporulation and spore germination. However, thyme oil was the most effective one, which completely inhibited the fungal growth at a concentration of 2000 µg/ ml, and completely prevented its sporulation and spore germination. The tested essential oils were analysed for their bioactive components using Gas Chromatography (GC), which were recorded as follows: linalool and eugenol were detected in basil; terpinen-4-ol, β-phyllandrene, and sabinene in marjoram oil, and thymol, 1.8-cineole, ρ-cymene, and α-terpinene in thyme oil. In the greenhouse experiments, Actamyl 70 % wp (wettable powder) and Bio Cure F were the most effective treatments, which significantly decreased the disease incidence and increased the plant growth parameters, compared with the untreated control. On the other hand, all treatments increased the peroxidase and polyphenol oxidase antioxidant enzyme activities, compared with the control; however, Actamyl 70 % (wp) and Bio Cure F had the superiority; recording 1.27 and 1.23 (units/ ml enzyme) for peroxidase, and 0.57 and 0.51 (units/ ml enzyme) for polyphenol oxidase, respectively. The results of the present study indicated the strong antifungal potential of the thyme essential oil. Meanwhile, Bio-Cur F biocide can be effectively used as a potent biofungicide to controlling the marjoram wilt incited by *F. oxysporum*.

Keywords: Marjoram, Bio-Cur F, Thyme essential oil, *Fusarium oxysporum*

1. Introduction

Marjoram (*Majorana hortensis* L.), which belongs to the family *Labiatae*, has many different uses, and is considered as one of the most important medicinal and aromatic crops in Egypt. It is used in the food and pharmaceutical industries, such as tooth pastes and cough medicine, and as a carminative in the case of digestive disorders (Bellakhdar *et al*., 1988). *F. oxysporum* caused wilt and root-rot in the Egyptian marjoram plantations; affecting the plant stand and growth (Hilal *et al*., 1990; El-Gebaly, 1998). The most dangerous fungus; *F. oxysporum*, affect the marjoram seedlings and cause pre- and post-emergence damping-off, in addition to wilt and root rot disease in several plants (Hassanin, 2007). A previous study conducted by El-Garhy, (1994) reported that the fungal filtrate of *F. oxysporum* made the veins of lentil leaves brown; thus causing necrotic spots.

In the open fields, the fungal pathogens have developed resistance to the chemical fungicides; as a result of the long-term application of these fungicides (Brent and Hollomon, 1998). There are several adverse environmental effects associated with the intensive application of fungicides, including teratogens and carcinogens; high toxicity, negative effects on the humans, and pollution of the environment (Pathak *et al*., 2022). Thus, there is an urgent need for the development of ecofriendly antifungal agents.

Among the benefits of the essential oils are that they are less toxic to the humans and the environment, as well as that they contain a variety of antimicrobial compounds (Hammer *et al*., 2003). ρ-cymene; 1,8-cineole, and other thymol constituents are primarily responsible for the anti-fungal activity of thyme essential oil against some fungal species, including *Aspergillus*; *Penicillium*, *Alternaria*, *Cladosporium*, and *Rhizopus* (Šegvić Klarić *et al*., 2007). The bio-active components such as pinene; limonene, cineole, eugenol, terpineol, geraniol, thymol, menthol, and terpinene are among the bioactive compounds recorded in the plant essential oils (Singh *et al*., 2007; Wang *et al*., 2009). These plant-derived compounds have antibacterial and antifungal potentials (Koga *et al*., 1999; Ahmad *et al*., 2010). Thyme essential oil and the water extract of licorice have high inhibitory efficacy against *F. oxysporum*; *Colletotrichum capsici*, and *Pythium aphanidermatum*, which infect *Capsicum annuum* L. (Arora *et al*., 2023). Moreover, thyme essential oil has inhibited the germination of spores and mycelial growth of *Alternaria linariae*; the causal agent of the early blight disease of tomato (Saltos-Rezabala *et al*., 2022).

Biological control of the fungal plant diseases is considered as an effective alternative strategy to the chemical control (El-Rafai *et al*., 2003). The efficacy of the biocontrol agents against the plant pathogens has been reported by many researchers (Lebeda and Cohen, 2011). Several studies attributed the protection effects of the biocontrol agents to antibiosis in the infection courts (Matei and Matei, 2008). Among the most common mechanisms used by the biocontrol agents to controlling the plant pathogenic fungi are mycoparasitism; competition for nutrients and space, stimulating the plant's defense, and the release of bioactive compounds (De Curtis *et al*., 2010). In a recent study conducted by Rahman *et al*., (2023), the tested isolates of *Trichoderma harzianum* inhibited the bacterial wilt disease; reduced the disease incidence, and enhanced the tomato plant growth.

The objective of the present work was to evaluate the effect of some essential oils emulsions and a biocide (Bio-Cure F) on controlling the marjoram wilt disease incited by *F. oxysporum*.

2. Materials and methods

2.1. The pathogenic *F. oxysporum* isolate

The *F. oxysporum* isolate used in this study, which was isolated from wilted marjoram seedlings (sweet

marjoram); was kindly provided by the fungal collection of the Ornamental, Medicinal and Aromatic Plant Diseases, Plant Pathology Research Institute, ARC, Giza, Egypt.

2.2. Effect of *F. oxysporum* filtrate on marjoram seed germination

As reported by Hassanin, (2007), a five-mm disc was cut from a ten-day-old active growing culture of *F. oxysporum* using a sterile cork borer, and inoculated aseptically into a 250-ml Erlenmeyer flask containing 100 ml of Czapek's broth medium. The un-inoculated flasks served as the controls. The inoculated and control flasks were incubated at 27 °C for 15 d. After incubation, a Whatman's No.1 filter paper was used to filtrate the growing culture; followed by sterilization of the obtained filtrate using a Millipore filter (0.45 µm). Using sterile dist. water, the culture filtrate was prepared at five concentrations; mainly 20; 40, 60, 80, and 100 %. The marjoram seeds (*i.e*., sweet marjoram seeds obtained from the Medicinal and Aromatic Plants Department, Horticulture Research Institute, ARC, Giza, Egypt) were surface sterilized for 2 min. using 1% sodium hypochlorite, and then washed twice with sterile dist. water. The sterilized seeds were then placed aseptically on a sterilized filter paper in a petri plate (10 seeds/ plate), and then 5 ml of each fungal filtrate concentration/ plate was added aseptically to the seeds. The control plates involved the addition of 5 ml of sterile dist. water. Three replicate plates were used for each treatment. The plates were incubated at 27 °C for 10 d, and 3 ml/ plate of sterile dist. water was added aseptically to each plate after 5 d of incubation, to maintain a proper level of humidity (Hassanin, 2007). In all treatments, the level of marjoram seed germination (%) was recorded.

2.3. Effect of the fungal filtrate on the percentages of wilted seedlings

This experiment was carried out as reported by Hassanin, (2007). Glass vials (*i.e*., 30 ml small bottles) were used, which contained the previously prepared individual concentrations of the fungal filtrate (*i.e*., 20; 40, 60, 80, and 100 %) and the control (un-inoculated medium). Thirty-day-old healthy marjoram seedlings (10 cm in height) were planted in each vial; five seedlings/ vial and three vials were used for each treatment and for the control. All vials were kept at 28-30 °C for 7 d in the growth chamber. The seedlings were watered whenever needed. The percentages (%) of wilted seedlings were recorded 72 h after inoculation.

2.4. Preparation of essential oils emulsions

Basil; marjoram, and thyme essential oils were obtained from the Medicinal Plants Research Department, Horticultural Research Institute, ARC, Giza, Egypt. The oil emulsions were prepared as reported by Hassanin *et al*., (2017). About 5 ml of the non-ionic surfactant Tween 80 was added slowly to 10 ml of each essential oil; followed by gentle stirring until a homogeneous mixture was obtained. Sterilized dist. water was added to reach a final volume of 100 ml, and then stirred for 30 min. using a magnetic stirrer (Hotplate Magnetic Stirrer, model: JSHS-18D, JS Research company, Gongju-City, Korea).

2.5. Evaluation of the *in vitro* antifungal potential of the essential oils emulsions against *F. oxysporum*

2.5.1. Effect of oil emulsions on the mycelial growth of *F. oxysporum*

The method described by Zambonelli *et al*., (1996); Hassanin *et al*., (2017) was applied with a slight modification. The oil emulsions of basil; marjoram, and thyme were added individually to potato dextrose agar (PDA) medium before solidification at five concentrations; mainly 1000; 2000, 3000, 4000, and 5000 µg/ ml (v/v). Chloramphenicol antibiotic (0.1 mg/ l) was also added to prevent bacterial contamination. Three plates were used for each treatment and for the untreated control (without oil emulsion). A single disc (5-mm) of *F. oxysporum* was added aseptically at the center of each plate and incubated at 27 °C. After incubation, the inhibition (%) of the fungal growth was calculated when the control plates were completely covered with

the fungal growth; according to the formula defined by Ahmed, (2013) as follows:

$$\text{Inhibition (\%)} = \frac{(G1-G2)}{G1} \times 100$$

Where; G1= Linear growth of the fungus in control plate, G2 = Linear growth of the fungus in the treated plate

2.5.2. Effect of the oil emulsions on the fungal sporulation

Using a sterile needle, spores of *F. oxysporum* were harvested from the colonies' edge after 7 days of incubation at 27 °C in PDA plates, which treated individually with an oil emulsion at a concentration of 3000 µg/ ml, and from the untreated control plates. These spores were inoculated into test tubes containing 10 ml of sterile dist. water. The tubes were centrifuged at 1000 rpm for 3 min. to disperse the spores, and finally a drop of the resulting spore suspension was counted using a hemocytometer, in reference to Hassanin, (2013).

2.6. Biochemical analysis of the tested essential oils using Gas Chromatography

The biochemical constituents of the tested oil emulsions were determined at the Laboratories of the National Research Centre (NRC), Giza, Egypt; using Gas Chromatography (GC) technique, as described by Elhalawany *et al*., (2019). In this method, GC was performed using a Ds Chrome 6200 Gas Chromatograph. The machine was composed of a flame ionization detector; a 5 % phenyl polysillphenylene-siloxane (30 m × 0.25 mm ID × 0.25 µm film thickness), and Column: BPX-5. The temperature program ranged from 70 to 200 °C at an average of 10 °C/ min., while the used temperature for the detector was 280 °C. Nitrogen flowed at the rate of 30 ml/ min.; hydrogen at 30 ml/ min., and air at 300 ml/ min.

2.7. Evaluation of the *in vivo* inhibitory efficacy of the essential oils emulsions and the biocide against *Fusarium* wilt of marjoram under greenhouse conditions

Thyme essential oil emulsion at a rate of 2 ml/ l; a fungicide Actamyl 70 % wp at the rate of 1 g/ l (*i.e*., Common name: Thiophanate methyl, Arysta Life Science SAS, France), and a biocide Bio-Cure F 1.15 % wp (*T. viride* 1 × 10^6 cfu/ g) (M/S.T. Stanes Company, Limit-India) at the rate of 6 g/ l, were evaluated for their abilities to controlling *Fusarium* wilt of marjoram in the greenhouse.

For preparation of the *F. oxysporum* inoculum, the fungus was grown on an autoclaved sorghum grain medium (50 g washed sand + 100 g corn + 100 ml dist. water) in 500 ml glass bottles; followed by incubation for 15 d at 27 °C. For cultivation of the marjoram seedlings, 25-cm-diameter plastic pots that were filled with formalin sterilized soil (equal amounts of sand; peat moss, and clay) were used, and these soils were infested individually with *F. oxysporum* inocula at the rate of 1 % (w/w). Throughout the experiment, 60 d old seedling roots (5 seedlings/ pot) were dipped for 20 min. in the three previously prepared treatments or in water only (as a control) and then planted in the infested soil; with 3 replicate pots for each treatment. After 90 d from planting, the percentages of disease incidence (%); survival of plants, plant height (cm), root length (cm), and plant fresh and dry weight (g) were recorded. The disease incidence percentages (%) were recorded according to the formula reported by Ahmed, (2013) as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total plant numbers}} \times 100$$

The percentages (%) of plant survival were determined by subtracting the number of living plants by the initial number of the cultivated plants. The dry weight of marjoram plants was determined by drying them in an oven (40 °C) overnight. The plants were then left to cool and weighed using a scale.

2.8. Determination of activities of the defense-related enzymes in the treated marjoram plants

To measure the activity of the peroxidase (POD) and polyphenoloxidase (PPO) antioxidant enzymes in the treated marjoram plants; approximately 72 h after each treatment, fresh samples of marjoram plants (approximately 10 g/ treatment) were collected and ground in 10 ml of 0.1 M sodium phosphate buffer (pH 6.8) using a mortar and pestle. Through four layers of cheesecloth; the samples were filtered, the filtrates were centrifuged for 20 min. at 6 ºC at 3000 rpm (Aina *et al*., 2012), and the resulting filtrates were used for enzyme analyses.

2.8.1. Peroxidase (POD) activity

Pyrogallol's oxidation to purpurogallin in the presence of H_2O_2 was measured spectrophotometrically at 425 nm to determine the peroxidase enzyme's activity. The reaction mixture was composed of 0.3 ml of the sample filtrate; 0.1 ml of 1 % H_2O_2, 0.5 ml of 0.1 M sodium phosphate buffer solution (pH 7.0), and 0.3 ml of 0.05 M pyrogallol. The remaining 3 ml were made up with dist. water. The absorbance change per min. (Abs/ min.) was used to measure the POD enzyme activity (Aina *et al*., 2012).

2.8.2. Polyphenol oxidase (PPO) activity

The activity of the PPO enzyme was determined colorimetrically using the previous method described by Quiles *et al*., (2005). The reaction mixture consisted of 1 ml of the sample filtrate; 1 ml of 10^3 N Catechol, and 1 ml of 0.2 M sodium phosphate buffer (pH 7.0). The remaining 6 ml were made up with dist. water. The change in absorbance of the reaction mixture per min. at 495 nm was used to measure the PPO enzyme activity.

2.9. Statistical analysis

Using a total randomized design with three replicates per treatment, this study was designed as a factorial experiment (Snedecor and Cochran, 1989). Based on the Least Significant Difference (L.S.D) test at 0.05, this statistical analysis was conducted using MSTAT-C version (4) statistical package.

3. Results

3.1. *F. oxysporum* filtrate effect's on the marjoram plant

3.1.1 Effect of *F. oxysporum* filtrate on the marjoram seed germination

Data demonstrated in Fig. (1) show the effect of five concentrations of *F. oxysporum* filtrate on marjoram seed germination. The inhibition percentages for seed germination ranged from 53.3 % - 100 %, which were significantly increased by increasing the filtrate's concentration. On the other hand, complete inhibition of seed germination was obtained upon using a 60 % concentration of the fungal filtrate.

3.1.2. Effect of *F. oxysporum* filtrate on the percentages of wilted seedlings

The results presented in Fig. (1) demonstrate that the *F. oxysporum* fungal filtrate induced wilt symptoms in the marjoram seedlings after 72 h of treatment; as it raised the percentage of wilted seedlings compared with the control. The percentages of wilted seedlings were shown to be positively correlated with the fungal filtrate concentration, whereas a concentration of 100 % resulted in 100 % of wilted seedlings.

3.2. Effect of essential oils emulsions on the *in vitro* growth of *F. oxysporum*

The data presented in Table (1) proved that increasing the concentration of the essential oils was correlated with a decrease in the linear fungal growth. Compared with the control, the most effective oil emulsion against *F. oxysporum* was thyme at a concentration of 2000 µg/ ml, which completely inhibited the growth; followed by basil at a concentration of 4000 µg/ ml, while the marjoram oil completely inhibited the fungal growth at 5000 µg/ ml. Finally, the oil emulsions of basil; marjoram, and thyme completely inhibited the growth of *F. oxysporum* at a concentration of 5000 µg/ ml.

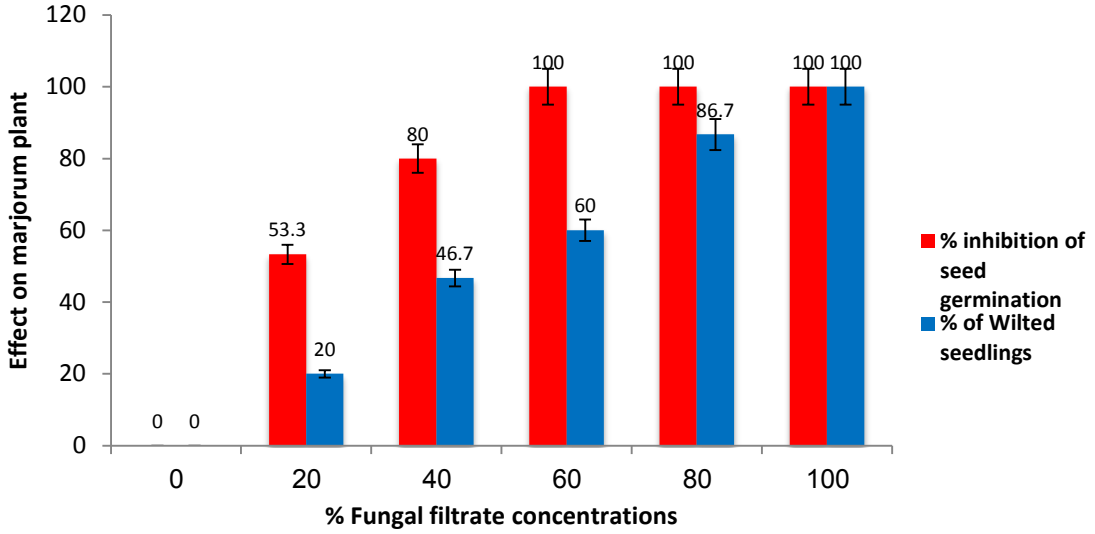

Fig. 1. Effect of *F. oxysporum* filtrate's on seed germination and wilted seedlings of marjoram, compared with the control; after 72 h of inoculation. The Error bars represent the standard deviation (SD)

Table 1. Effect of different concentrations of the essential oils emulsions on the *in vitro* mycelial growth of *F. oxysporum*

Essential oils emulsions	Different concentrations (µg/ ml) of the oils emulsions											
	Control		1000		2000		3000		4000		5000	
	a	b	a	b	a	b	a	b	a	b	a	b
Basil	9.0	0.0	5.8	35.55	3.2	64.44	1.7	81.11	0.0	100	0.0	100
Marjoram	9.0	0.0	9.0	0.0	7.6	15.55	5.0	44.44	2.8	68.88	0.0	100
Thyme	9.0	0.0	2.8	68.88	0.0	100	0.0	100	0.0	100	0.0	100

Where; (a): represents the diameter of linear growth (cm); while (b): represents the inhibition (%) of mycelial growth. L.S.D. at 5 %: Essential oils (E) = 1, Concentrations (C) = 1.1, Essential oils (E) × Concentrations (C) = 1.8

3.3. Effect of essential oils emulsions on *F. oxysporum* sporulation *in vitro*

The results presented in Fig. (2) and Table (2) demonstrate that the tested oils emulsions significantly suppressed the *F. oxysporum* sporulation and spore germination at 3000 µg/ ml, compared with the control. However, the most effective oil on sporulation and spore germination of *F. oxysporum* was thyme, which completely prevented both of them. This was followed by basil that reduced the spore number of the fungus to 40 $\times 10^3$ spores and inhibited the spore's germination by 44.8 %, compared with the untreated control. On the other hand, marjoram was the least effective oil on the sporulation (110 $\times$ 10^3 spores) and spore germination inhibition (32.5 %) of the fungus.

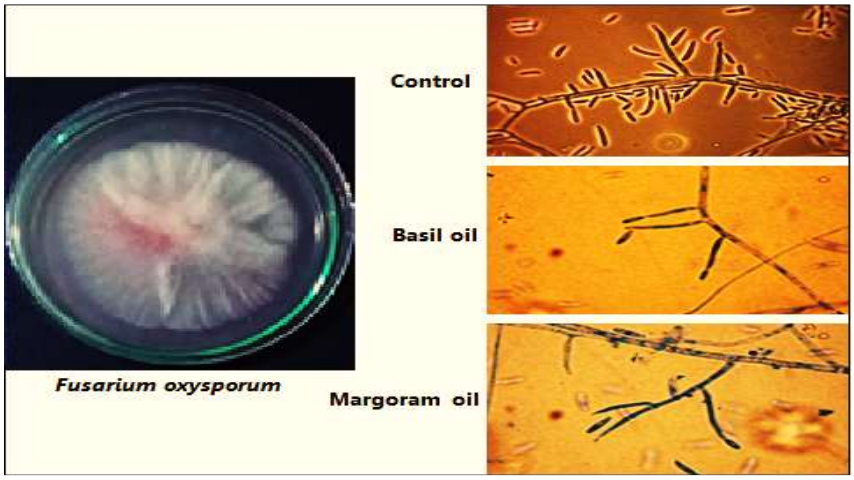

Fig. 2. Effect of essential oil emulsions of basil and marjoram at a concentration of 3000 µg/ ml on the sporulation of *F. oxysporum* after 7 d of incubation at 27 °C

Table 2. Effect of essential oils emulsions at a concentration of 3000 µg/ ml on sporulation and spore germination of *F. oxysporum* after 7 d of incubation at 27 °C

Tested essential oils emulsions	Mean sporulation ($\times 10^3$) of *F. oxysporum*	% Inhibition of spore germination
Basil	40	44.8
Marjoram	110	32.5
Thyme	0	100.0
Control	295	00.0
L.S.D. at 5 %	20.2	0.1

3.4. Chemical composition of the tested essential oils

Using GC technique, the results presented in Table (3) show that the major components of basil essential oil were linalool (46.13 %) and eugenol (18.41 %), in addition to smaller amounts of myrcene (3.94 %); α-terpineol (6.23 %), and α-pinene (4.76 %). Among thyme essential oil's main compounds, it contained thymol (27.945 %); 1.8-cineole (16.34 %), ρ-cymene (15.744 %), and α-terpinene (10.983 %). In addition, little amounts of limonene (5.729 %), myrcene (4.682 %), γ-terpineol (4.184 %), and broneol (4.179 %) were detected. The three main components of the marjoram essential oil were terpinen-4-ol (35.619 %); β-phyllandrene (12.033 %), and sabinene (11.34 7%); along with lower quantities of Thujon-4-ol (8.215 %), linalyl acetate (7.923 %), limonene (6.843 %), α-pinene (5.67 %), and other components.

3.5. Evaluation of the biocide and essential oils emulsions effectiveness in controlling *Fusarium* wilt of marjoram under greenhouse conditions

The results showed that all the tested treatments decreased the percentages of disease incidence and recorded higher percentages of plant survival (Fig. 3, Table 4). However, compared with the control; as they

Table 3. Chemical composition of the essential oils (EO) detected using GC

Basil oil		Thyme oil		Marjoram oil	
Component	**Area %**	**Component**	**Area %**	**Component**	**Area %**
α-pinene	4.76	α-pinene	1.356	Thujene	0.974
Myrcene	3.94	Camphene	2.187	α-pinene	5.67
β-pinene	2.76	Sabinene	3.122	Sabinene	11.347
Linalool	46.13	Myrcene	4.682	β-phyllandrene	12.033
Camphor	0.964	α-terpinene	10.983	Limonene	6.843
γ-terpineol	1.023	ρ-cymene	15.744	Linalool	2.512
Terpinen-4-ol	1.94	Limonene	5.729	Linalyl acetate	7.923
α-terpineol	6.23	1.8-cineole	16.34	Terpinen-4-ol	35.619
Methyl chavicol	2.45	γ-terpineol	4.184	α-terpineol	2.107
Fenchyl acetate	2.189	Broneol	4.179	Thujon-4-ol	8.215
Geraniol	2.776	Thymol	27.945	β-caryophyllene	3.737
Eugenol	18.41	β-caryophyllene	1.628		
β-caryophyllene	2.56				
Total	96.132	Total	98.079	Total	96.98

significantly reduced the disease incidence, Actamyl 70 % wp and Bio Cure F were the most effective evaluated treatments; recording 6.7 % and 20 % disease incidence, respectively. On the contrary, thyme essential oil was the least effective treatment in reducing the disease incidence (33.3 %). Compared with the controls, Table (4) demonstrates that all treatments increased the plant growth parameters, but Actamyl 70 % (wp) and Bio Cure F showed the highest increases in plants height (27 and 24.6 cm, respectively); roots length (12 and 9.3 cm, respectively), plants fresh weight (7.8 and 7.8 g, respectively), and in the plants dry weight (4.1 and 4.4 g, respectively). In contrast, thyme essential oil expressed the least efficacy in this respect; recording 23.7 cm, 7.7 cm, 6 g, and 3.5 g, respectively.

3.6. Effect of the tested treatments on the activity of certain defense-related enzymes

The results listed in Fig. 4 reveal that all treatments increased the activity of the POD and PPO enzymes, which ranged from 0.91 to 1.27 (units/ ml enzyme) and from 0.24 to 0.57(units/ ml enzyme); respectively, compared with the untreated control that recorded 0.38 and 0.11 (units/ ml enzyme), respectively. However, Actamyl 70 % (wp) and Bio Cure F biocide treatments resulted in the highest increase in activities of the POD, which recorded 1.27 and 1.23 (units/ ml enzyme); respectively, and PPO that recorded 0.57 and 0.51 (units/ ml enzyme), respectively.

4. Discussion

Fusarium oxysporum filtrate significantly decreased the percentage of marjoram seed germination; with a positive correlation between the fungal filtrate concentration and the percentage reduction of seed germination. Furthermore, the fungal

Fig. 3. Impact of dipping the marjoram seedling roots in various treatments on the disease incidence (%) 90 d after being planted in soil infested with pathogenic *F. oxysporum*

Table 4. Effect of different dipping treatments of marjoram seedling roots on the disease incidence (%) and some plant growth parameters 90 d after planting in soil infested with *F. oxysporum* under greenhouse conditions

Treatments	Disease incidence (%)	Survivals (%)	Plant height (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Thyme emulsion	33.3	66.7	23.7	7.7	6.0	3.5
Bio Cure F	20.0	80.0	24.6	9.3	7.8	4.4
Actamyl 70% (wp)	6.7	93.3	27.0	12.0	7.8	4.1
Untreated control	73.3	26.7	17.0	6.0	4.4	3.2
L.S.D. at 5%	19.2	---	4.4	2.0	0.5	0.1

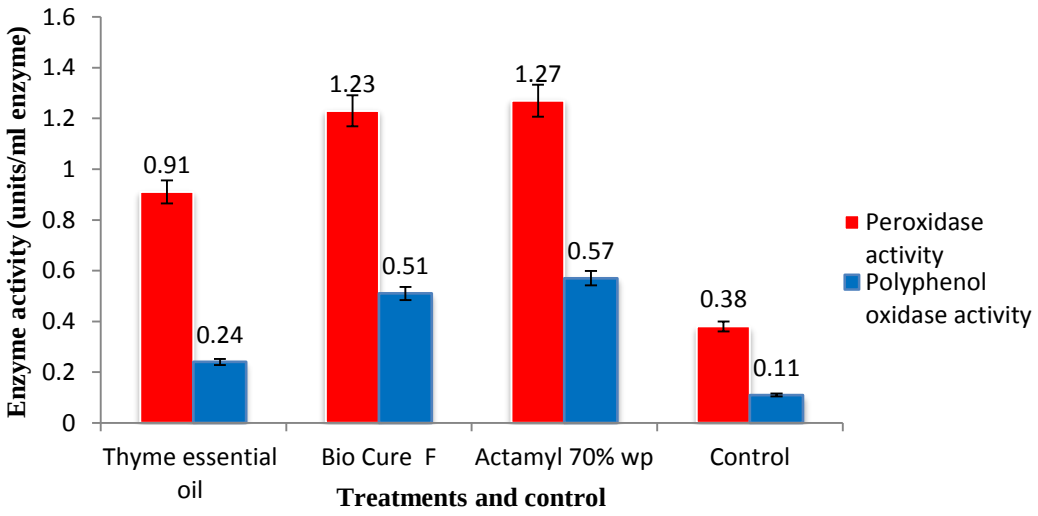

Fig. 4. Peroxidase (POD) and polyphenol oxidase (PPO) activity detected in the treated marjoram seedlings plants, compared with the control. The Error bars represent the standard deviation (SD)

filtrate enhanced the percentage of wilted seedlings compared with the control. The fungal filtrate of *F. oxysporum* caused toxic effects on the marjoram seeds and seedlings; in accordance with the study reported by Hassanin, (2007), who revealed that seed germination (%) of marjoram significantly decreased upon treatment with *F. oxysporum* filtrate, which was in line with the fungal filtrate concentration. Moreover, the author also reported that marjoram seedlings exhibited wilt symptoms as a result of growth in *F. oxysporum* fungal filtrate. Similar results were also reported by El-Garhy (1994), who demonstrated that *F. oxysporum* culture filtrate softened and browned the veins of lentil leaves; causing the development of necrotic spots.

According to the *in-vitro* assays, thyme at 2000 µg/ ml followed by basil at 4000 µg/ ml totally prevented the radial growth of *F. oxysporum*; making them the most effective essential oils against this pathogenic fungus. The tested essential oils also significantly suppressed the sporulation and spore germination of *F. oxysporum*; especially the thyme oil, which entirely prevented it. It's possible that the essential oils' phenolic compounds such as linalool in basil; terpinen-4-ol in marjoram, and thymol in thyme, in addition to their capacity to penetrate the fungal cell, are the major reasons for the fungal growth inhibition. Additionally, a rise in the permeability of the fungal cell membrane may be the cause of the observed antifungal effects of the tested essential oils. The antifungal substances present in the medical oils may inhibit the fungal sporulation by affecting the oxygen uptake of the fungal cell, as reported by Zambonelli *et al*., (1996); Šegvić Klarić *et al*., (2007). Furthermore, these authors added that the thyme oil's activity might be attributed to penetration of the chitin of the cell wall and damaging the lipoprotein of the cytoplasmic membrane; resulting in cytoplasmic escapes. Similar to our obtained results, Arora *et al*., (2023) reported that thyme essential oil and the cold extract of licorice expressed a highly antifungal potential against the tested fungal pathogens of *C. annuum* L. At 1 mg/ ml, thyme essential oil caused 100 % inhibition of the mycelial growth of *F. oxysporum*; *C. capsici*, and *Pythium aphanidermatum*. Furthermore, Saltos-Rezabala *et al*., (2022) reveled that application of the thyme essential oil caused an increase in the enzyme activity of β-1,3-glucanase; polyphenol oxidase, and peroxidase, in addition to an improvement in the accumulation of phenolic compounds and callose precipitation in the infected

plant tissue. Generally, our findings are in accordance with those of Hassanin *et al*., (2017), who reported that thyme; lemongrass, and basil essential oils completely suppressed the sporulation of *F. oxysporum* f.sp. *cumini*. Similar to the current results, Hassanin, (2013) reported that clove oil at 100 % completely inhibited the sporulation and spore germination of *F. oxysporum* and *F. semitectum*.

The chemical components of the tested essential oils was detected using GC, which were recorded as follows: in the thyme oil, thymol (27.945 %); 1.8-cineole (16.34 %), ρ-cymene (15.744 %), and α-terpinene (10.983 %) were detected. Meanwhile, in the basil oil, linalool (46.13 %) and eugenol (18.41 %) were recorded; while terpinen-4-ol (35.619 %); β-phyllandrene (12.033 %), and sabinene (11.34 7%) were observed in the marjoram oil. These results are somewhat in agreement with the findings of Grigore *et al*., (2010), who performed a quantitative analysis of the thyme volatile oil of Thymus vulgaris using GC and a qualitative analysis using high performance thin layer chromatography (HPTLC). The results reported that the volatile oil of Thymus vulgaris contained p-cymene and thymol in high quantities. Pripdeevech *et al*., (2010) highlighted that methyl chavicol (81.82 %); β-(E)-ocimene (2.93 %), and α-(E)-bergamotene (2.45 %) were shown to be the primary components in the Thai basil oil, whereas basil oil largely contained linalool (43.78 %); eugenol (13.66 %), and 1,8-cineole (10.18 %). According to the previous study conducted by Kowalski *et al*., (2020), chromatographic analysis of the marjoram oil revealed the presence of terpinen-4-ol (17.61 %) and trans-sabinene hydrate (22 %). Additionally, γ-terpinene (10.29 %); α-terpinene (6.61 %), α-terpineol (5.85 %), sabinene (5.51 %), and cis-sabinene hydrate (5.39 %) were recorded in considerable amounts in the essential oil fraction. The obtained results of the current study are also in agreement with those of Šegvić Klarić *et al*., (2007); Hassanin *et al*., (2017).

In the greenhouse experiments, all the tested treatments increased the plant survival (%) and plant growth parameters, and decreased the disease incidence (%). However, the most effective treatments were of Actamyl 70 % (wp) and Bio Cure F (biocide), which significantly decreased the disease incidence, compared with the untreated control, and they displayed the highest increases in the plant growth parameters. Hassanin *et al*., (2020); Serag El-Din *et al*., (2020); El-Kaed *et al*., (2021) reported similar results on various tested crops under artificially or naturally infested soils. Using a biocide may reduce the disease incidence (%) by producing plant growth regulators, which encourage the plant tolerance to the microbial infection, as reported by Constantinescu *et al*., (2009). Furthermore, Bolar *et al*., (2000) reported that *T. harzianum* produces several destructive enzymes such as chitinase, which degrades the fungal pathogen cell wall. The biocide Bio-Cure F may also have a greater impact on the disease incidence (%) by inducing resistance in the treated marjoram plants. Another study conducted by Benhamou and Chet, (1993) reported that *Trichoderma* spp. have many inhibitory mechanisms against growth of the fungal pathogens *via* mycoparasitism. Recently, Rahman *et al*., (2023) highlighted that application of two isolates of *T. harzianum* NBG and *T. harzianum* MC2 resulted in a significant reduction in the bacterial wilt disease incidence of tomato caused by *Ralstonia solanacearum*, in addition to an increase in the tomato yield by 54.49 %. All the tested treatments increased the antioxidant enzyme activities (*i.e*., peroxidase and polyphenol oxidase); however, Actamyl 70 % (wp) and Bio-Cure F were the best treatments, compared with the untreated control. Both agents expressed the highest increase in the POD and PPO activities. These data are in agreement with those obtained by Mahmoud *et al*., (2006), who revealed that the induced resistance increases the activity of the oxidative enzymes (*i.e*., POD and PPO) in the treated root tissues; along with the phenolic compounds accumulation. In accordance, Hassanin, (2013) reported that plants grown from the black cumin seeds that were treated with some fungicide-alternative materials may produce the antioxidant enzymes as a kind of induced defense; since these treatments significantly increased the POD and PPO activities.

Conclusion

This study evaluated the effectiveness of some essential oil emulsions and a biocide (Bio-Cure F) for controlling the marjoram wilt disease incited by *F. oxysporum*. According to the obtained results; thyme essential oil has powerful antifungal properties, and the Bio-Cur F biocide may be used as an effective bio-fungicide to controlling the *Fusarium* wilt of marjoram.

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Conflict of interest

The authors declare non-existence of any conflict of interests.

Ethical approval

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Author's contributions

All authors contributed equally in all parts of this study.

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