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Effect of certain essential oils and biocides on controlling marjoram root rot and wilt diseases

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Abstract

The aim of this study was to determine the impact of certain essential oils (eucalyptus and thyme essential oils) and biocides (Plant Guard and Rhizo-N) as fungicidal alternatives for the control of root rot and wilt diseases of marjoram (*Majorana hortensis* L.) caused by several fungi. Marjoram plants with root rot and wilt symptoms were obtained from Giza, Beni-Suief, and Minia governorates, Egypt. Pathogenicity tests showed that all isolated fungi (*Fusarium semitectum*, *F. solani*, *F. oxysporum*, *F. roseum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*) had the ability to infect plants and seedlings. *Fusarium oxysporum*, followed by *R. solani*, was the most pathogenic fungus on the marjoram seedlings, resulting in pre- and post-emergence damping-off. Additionally, the most significant disease incidence percentages on marjoram plants after transplanting were caused by *F. oxysporum* and *F. semitectum*. *In vitro* investigations were performed utilizing eucalyptus and thyme essential oils indicated that the growth of the investigated fungi (*F. oxysporum*, *F. semitectum*, and *R. solani*) was significantly inhibited. However, thyme was the most efficient treatment, especially at a concentration of 6000 ppm, which completely inhibited mycelial growth of *R. solani* and *F. oxysporum*. The effectiveness of eucalyptus and thyme essential oils, Plant Guard, Rhizo-N, and Actamyl 70% wp was determined for the control of target diseases under greenhouse conditions. The results showed that all tested treatments significantly reduced the disease incidence caused by the investigated fungi. Actamyl was the most efficient treatment. Thyme oil was an effective treatment against *R. solani* and *F. oxysporum* in the second order following Actamyl, whereas Plant Guard was effective against *F. semitectum* and *F. oxysporum*. Generally, Plant Guard was the most successful treatment for enhancing plant growth of marjoram plants. These findings demonstrate the potential of applying Plant Guard as an alternate fungicide against wilt and root rot diseases of marjoram plants.

Keywords: *Majorana hortensis*, *Fusarium oxysporum*, *Fusarium semitectum*, *Rhizoctonia solani*, Plant Guard, Actamyl 70% wp, thyme oil.

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1. Introduction

Marjoram, or sweet marjoram (*Majorana hortensis* L.), serves as one of the most valuable medicinal as well as aromatic crops in Egypt. Plantations of marjoram have expanded in recent years in Egypt. Marjoram is a perennial plant, usually planted in a nursery, and seedlings (60 days old) are transplanted in the field. Ordinarily, plants are cut twice per year for a period of three years. The dried leaves are extensively used in the food industry. Marjoram essential oil has various applications in pharmaceutical preparations. It is used as a stimulating and antibacterial component in toothpaste, as well as in drugs against whooping cough. Additionally, it is used as a carminative in the case of gastrointestinal disorders (Bellakkadar et al., 1988). Wilt and root rot diseases were recorded in Egyptian marjoram plantations, which had a negative effect on vegetative growth and plant standing (El-Gebaly, 1998; Hilal & Helmy, 1998; Hilal et al., 1990). The incidence of these diseases often rises year after year. A lack of suitable agricultural practices, as well as poor sanitation, may be contributing factors. The present research aimed to evaluate the efficacy of different fungicidal alternatives in controlling root rot and wilt diseases of marjoram caused by various fungi. Specifically, the impact of eucalyptus and thyme essential oils, as well as biocides such as Plant Guard and Rhizo-N, compared to the fungicide Actamyl 70%, was assessed.

2. Materials and methods

2.1 Isolation and identification of the causal pathogens

During the 2019 season, naturally infected marjoram plants with wilt and root rot diseases were recorded in Giza, Beni-Suief, and Minia governorates. The infected plants were at two months after transplanting (free cut), six months after transplanting (1^{st} cut), and nine months after transplanting (2^{nd} cut). Disease symptoms such as plant death, stunting, dryness, yellowing, and wilting appeared in irregular parts of the cultivation area. Samples of infected plants were collected and transferred to the laboratory. The infected stems and roots were subjected to a procedure to isolate the fungi. The procedure involved washing the infected stems and roots using tap water, cutting them into small fragments, and surface sterilizing them with 2% NaOCl for 3 minutes. Following this, the fragments were thoroughly rinsed with sterilized distilled water multiple times and then dried using sterilized filter papers. These fragments were then placed onto Petri dishes containing potato dextrose agar (PDA) medium and kept at a temperature of 27°C. Daily checks were carried out for one week to monitor any fungal development. In order to determine the prevalence of developed colonies for each fungal isolate, a calculation was performed in reference to the total isolates available. Subsequently, the isolation of these fungi was purified through the utilization of both single-spore and hyphal tip methodologies. The definitive identification of these purified fungal specimens was conducted in accordance with established protocols outlined by Gilman (1957), Barnett and Hunter (1972), and Nelson et al. (1983), with validation provided by the Department of Mycology and Plant Disease Survey at the Plant Pathology Research Institute, ARC, Giza, Egypt. Furthermore, for future investigations, pure cultures of the isolated fungi were transferred onto PDA slants and stored under controlled conditions at a temperature of 5°C.

2.2 Pathogenicity tests

This experiment was performed under greenhouse conditions. To prepare the inoculum, each fungus was grown separately on autoclaved sorghum medium (50 g of cleaned sand, 100 ml of water and 100 g of corn) in 500 ml glass bottles for 15 days at 28˚ C. A 5% formalin solution was used to sterilize clay sand soil (1:1 w/w), which was then allowed to dry for two weeks before being used. Fungal inoculums were added to the soil at a rate of 1% (w/w) and mixed thoroughly, then placed in 25-cm-diameter pots and watered one week prior to planting for fungal colonization enhancement. Seeds of marjoram were surface sterilized with 0.1% (v/v) of sodium hypochlorite for 3 minutes, and then they were rinsed with sterilized distilled water and allowed to air dry. Fifty seeds were planted per pot, and three replicates were used for each treatment. Percentages of pre- and post-emergence damping-off 15 and 60 days after sowing were recorded. Additionally, the pathogenicity of isolated fungi was tested on marjoram transplants that were 60 days old using infested pots. Five transplants were planted in each pot, and treatments were replicated three times. Infected marjoram plants were recorded as percentages at 45 and 90 days from transplanting. In order to compare with the original isolates, a new isolation of fungi from diseased plants was conducted.

2.3 Evaluation of antifungal activity of essential oils *in vitro*

To study the efficacy of volatile essential oils of eucalyptus and thyme on the mycelial growth of the tested fungi, *F. oxysporum, F. semitectum* and *R. solani*, three Petri dishes containing PDA medium were inoculated using 5 mm fungal growth discs of each fungus. The inoculated Petri dishes were inverted, and volatile oil at three concentrations (1000, 3000, and 6000 ppm) saturated 6 mm paper discs were placed at the center of plate lids as described by Maruzzella and Sicurella (1960). The dishes were incubated at 27 ˚C and checked daily for fungal development. The tested essential oils were supported by Medicinal Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Inhibition percentages in mycelial growth were calculated when control plates (without essential oils) were completely filled with the fungal growth, according to the formula of Pandey et al. (1982), as follows:

$$Growth\ inhibition\ (\%) = \frac{G1 - G2}{G1} \times 100$$

Where, G1 = growth in control, G2 = growth in treatment.

2.4 *In vivo* studies

Under greenhouse conditions, essential oils of eucalyptus and thyme (6 ml/l water), biocides Rhizo-N and Plant Guard, as well as fungicide Actamyl 70% wp at concentrations as mentioned in Table (1) were tested for controlling marjoram root rot and wilt diseases. In this experiment, plastic pots (25 cm diam.) were used for planting, filled with sterilized soil [clay sand soil (1:1)]. Soil infestation with the tested fungi; *F. oxysporum*, *F. semitectum*, and *R. solani*, was carried out as described in pathogenicity tests. The seedling roots were dipped for 20 minutes in the treatments or in water (as an untreated control), after which they were planted in the infested soil. Fifteen marjoram seedlings (60 days old) were used for each treatment as well as for the control (5 seedlings/ pot), and three replicates were used for each. The percentages of infection or survival plants, as well as some plant parameters such as root length, plant height, plant dry weight, and plant fresh weight, were recorded 90 days after transplanting.

Table 1: Fungicide and biocides used in the current study.

Commercial name and formulation	Common name (Active ingredients)	Application rate/L.W	Composition	Manufacture
Actamyl 70% wp (Powder)	Thiophanate methyl	1 g/l	Dimethyl [1,2 phenylenebis (iminocarbonothioyl)] bis [carbamate]	Arysta Life Science SAS, France
Plant Guard (Liquid)	*Trichoderma harzianum*	4 ml/l	*Trichoderma harzianum*; 30 × 10^6 spores/ml	El – Nasr Co. for Fertilization & Biocides, Egypt
Rhizo – N (Powder)	*Bacillus subtilis*	4 g/l	*Bacillus subtilis*; 30 × 10^6 cell/g	El – Nasr Co. for Fertilization & Biocides, Egypt

2.5 Statistical analysis

The present investigation was designed as a factorial experiment with three replicates in a completely randomized design (Snedecor and Cochran 1989). Statistical analysis was performed using MSTAT-C version (4) with the Least Significant Difference (L.S.D.) test set to 0.05.

3. Results

3.1 Isolation, identification and percentages of the isolated fungi frequency

Table (2) presents the results of isolated fungi from infected marjoram plants showing typical symptoms of root rot and wilt disease and obtained from different locations in Giza, Beni-Suief, and Minia governorates, Egypt. The study identified three genera of fungi, including *Macrophomina*, *Rhizoctonia*, and *Fusarium*, with *F. oxysporum* and *F. solani* being the most frequently isolated fungi (43.0% and 29.06%, respectively), followed by *R. solani* (11.28 %). In contrast, *F. roseum* recorded the lowest mean occurrence percentage (2.67%). *Fusarium roseum* and *F. semitectum* were isolated only from plant samples from Giza, as well as *M. phaseolina* from Minia governorate.

Table 2: Frequency (%) of fungi isolated from infected marjoram plants collected from Giza, Beni-Suief and Minia governorates, Egypt.

The isolated fungi	Location			Mean
	Giza	Beni-Suief	Minia	
Fusarium oxysporum	32.05	49.00	47.95	43.00
F. solani	37.92	41.20	8.06	29.06
Rhizoctonia solani	0.00	9.80	24.04	11.28
F. semitectum	22.03	0.00	0.00	7.34
Macrophomina phaseolina	0.00	0.00	19.95	6.65
F. roseum	8.00	0.00	0.00	2.67
Total	100	100	100	-

3.2 Pathogenicity tests

3.2.1 Percentages of pre- and post-emergence damping-off

Table (3) presents the results of the pathogenicity test of fungi isolated from collected marjoram plant samples. The test was conducted by measuring pre- and post-emergence damping-off percentages 15 and 60 days from planting, as well as healthy survival seedlings. All the tested fungi showed significant differences in percentages of pre- and post-emergence damping-off compared with the uninoculated control, subsequently decreasing healthy survival seedlings. *Fusarium oxysporum* and *R. solani* were the most pathogenic fungi, as they recorded the highest percentages of pre- (36% and 32%, respectively) and post-emergence damping-off (26% and 24%, respectively), with non-significant differences between each other in this respect. Furthermore, they recorded the highest percentage of survival seedling reductions (62% and 52%, respectively). On the contrary, *F. solani* recorded the lowest percentages of reduction in survival (26%) and pre-emergence damping-off (10%). *Fusarium oxysporum* and *F. semitectum* were the most pathogenic species and more virulent than *F. solani*.

Table 3: Percentages of pre- and post-emergence damping-off caused by isolated fungi in marjoram plants at 15 and 60 days after planting.

The tested fungi	Damping-off (%)		Survival (%)	Reduction* (%)
	Pre-emergence	Post- emergence		
Fusarium oxysporum	36.0	26.0	38.0	62.0
Rhizoctonia solani	32.0	24.0	48.0	52.0
F. semitectum	30.0	22.0	52.0	48.0
F. roseum	18.0	22.0	60.0	40.0
Macrophomina phaseolina	22.0	16.0	62.0	38.0
F. solani	10.0	16.0	74.0	26.0
Uninoculated control	0.0	0.0	100.0	-----
L.S.D. at 5%	6.9	6.5	-----	-----

*Reduction percentages in survivals relative to the control treatment.

3.2.2 Percentages of root rot or wilt incidence

Table (4) displays the results of the infection of marjoram plants by all tested fungi. The plants were transplanted in infested soil, and all tested fungi were able to infect the plants, causing wilt or root rot diseases, as opposed to the uninoculated control. Infection percentages increased as plant ages increased from 45 to 90 days from transplant. *Fusarium oxysporum*, followed by *F. semitectum*, was the most virulent fungus, as they recorded the highest percentages of disease incidence 45 days after transplanting (46.7% and 20.0%, respectively) and 90 days after transplanting (73.3% and 26.7%, respectively). Moreover, they recorded the highest percentages of survival plant reductions (73.3% and 26.7%, respectively). On the contrary, both *M. phaseolina* and *F. roseum* recorded the lowest reduction percentage of survival plants (13.3%), resulting in 6.7% disease incidence after 45 days from transplanting and 13.3% disease incidence after 90 days from transplanting.

3.3 Antifungal activity of essential oils on mycelial growth of the tested fungi *in vitro*

Table (5) presents the results of the significant inhibition of three concentrations (1000, 3000, and 6000 ppm) of thyme and eucalyptus oils on mycelial growths of all tested fungi. Thyme oil was the best treatment, especially when it was applied at a concentration of 6000 ppm, which completely suppressed the growth of *R. solani* and *F. oxysporum*. *Rhizoctonia solani* was the most affected fungus by the tested oils, followed by *F. oxysporum*. However, the percentage reduction in linear growth was increased by increasing oil concentrations. They reached 74.44% and 100% at 6000 ppm in the case of eucalyptus and thyme, respectively, with *R. solani*. In contrast, *F. semitectum* was the least affected fungus, as it recorded 60% and 92.22% reduction in growth at 6000 ppm in the case of eucalyptus and thyme, respectively.

Table 4: Percentages of infected marjoram plants, 45 and 90 days from transplanting in infested soil under greenhouse conditions.

The tested fungi	Disease incidence (%)		Survival (%)	Reduction* (%)
	45 days	90 days		
Fusarium oxysporum	46.7	73.3	26.7	73.3
Rhizoctonia solani	6.7	20.0	80.0	20.0
F. semitectum	20.0	26.7	73.3	26.7
F.roseum	6.7	13.3	86.7	13.3
Macrophomina phaseolina	6.7	13.3	86.7	13.3
F. solani	6.7	20.0	80.0	20.0
Uninoculated control	0.0	0.0	100.0	-----
L.S.D. at 5%	5.8	11.4	-----	-----

*Reduction percentages in survivals relative to the control treatment.

Table 5: Effect of three concentrations of two essential oils on linear growth of the tested fungi.

Essential oils	Fungi	Linear growth (cm)						
		Control (cm)	1000 (ppm)	Reduction* (%)	3000 (ppm)	Reduction* (%)	6000 (ppm)	Reduction* (%)
Eucalyptus	*Rhizoctonia solani*	9.0	5.9	34.44	4.4	51.11	2.3	74.44
	Fusarium oxysporum	9.0	6.2	31.11	5.6	37.78	3.2	64.44
	F. semitectum	9.0	7.1	21.11	6.3	30	3.6	60
Thyme	*R. solani*	9.0	5.7	36.67	3	66.67	0	100
	F. oxysporum	9.0	6.4	28.89	3.1	65.56	0	100
	F. semitectum	9.0	6.9	23.33	3.8	57.78	0.7	92.22

*Reduction relative to the control. L.S.D.at 5 % for: Fungi (F) =1.13, Essential oils (O) = N.S., Concentrations (C) = 0.10, F × O = 0.20, F × C = 0.25, O × C = 0.13, F × O × C = 0.36.

3.4 Effect of essential oils, biocides and fungicide on disease incidence and some plant parameters under greenhouse conditions

In this experiment, the efficacy of essential oils (eucalyptus and thyme), biocides (Rhizo N and Plant Guard), and fungicide (Actamyl 70% wp) on disease incidence and some plant parameters 90 days from planting in infested soil separately with *F. oxysporum*, *F. semitectum*, and *R. solani* were investigated. These treatments were used as dipping of seedling roots for 20 minutes.

3.4.1 The impact on disease incidence 90 days from planting

Data in Table (6) indicates that percentages of disease incidence decreased, and survival plants increased with all tested treatments. Compared to the untreated control, all treatments significantly decreased disease incidence. However, Actamyl 70% wp gave the lowest percentage of disease incidence of all the tested fungi. Thyme oil came in the second order, after Actamyl with *R. solani* and *F. oxysporum*, since they caused 6.7% and 13.3% disease incidence, respectively. Subsequently, the percentages of survival plants resulting from thyme treatment with *R. solani* and *F. oxysporum* were 93.3% and 86.7%, respectively. While Plant Guard came in the second order, after Actamyl, with *F. semitectum* and *F. oxysporum*, both of which caused 13.3% disease incidence, the percentage of survival plants was 86.7%. Eucalyptus oil was the least effective treatment with *F. oxysporum*, since it gave 40% disease incidence.

Table 6: Effect of essential oils, biocides and fungicide on disease incidence (%), 90 days from planting under greenhouse conditions.

Treatments		Disease incidence (%)					
		F. oxysporum		*F. semitectum*		*R. solani*	
		Disease incidence (%)	Survival (%)	Disease incidence (%)	Survival (%)	Disease incidence (%)	Survival (%)
Essential oils	Eucalyptus	40.0	60.0	26.7	73.3	13.3	86.7
	Thyme	13.3	86.7	26.7	73.3	6.7	93.3
Biocides	Plant Guard	13.3	86.7	13.3	86.7	20.0	80.0
	Rhizo-N	33.3	66.7	26.7	73.3	20.0	80.0
Fungicide	Actamyl 70% wp	6.7	93.3	0.0	100	0.0	100
Untreated control		66.7	33.3	53.3	46.7	73.3	26.7
L.S.D. at 5%		20.2	---	21.5	---	10.2	---

3.4.2 The impact on plant height 90 days from planting

Data in Table (7) show that all treatments increased plant height more than the control treatment. The increases were significant only by using Actamyl 70% treatment with all tested fungi, Plant Guard with (*F. oxysporum* and *F. semitectum*), Rhizo-N with (*F. semitectum*) and eucalyptus with (*R. solani*). The best treatment after Actamyl which gave the superiority of increasing plant height was Plant Guard with *F. oxysporum* and *F. semitectum* (30.67% and 44.16%, respectively). However, eucalyptus was the best treatment with *R. solani* which gave a 32.87% increase in plant height.

Table 7: Effect of essential oils, biocides and fungicide on plant height, 90 days from planting under greenhouse conditions.

Treatments		Plant height (cm)					
		F. oxysporum	Increase (%) *	*F. semitectum*	Increase (%) *	*R. solani*	Increase (%) *
Essential oils	Eucalyptus	18.7	24.67	16.0	3.90	19.0	32.87
	Thyme	17.8	18.67	16.0	3.90	14.8	3.50
Biocides	Plant Guard	19.6	30.67	22.2	44.16	14.5	1.40
	Rhizo-N	18.1	20.67	20.2	31.17	14.8	3.50
Fungicide	Actamyl 70% wp	20.8	38.67	24.9	61.69	18.3	27.97
Untreated control		15.0	---	15.4	---	14.3	---
L.S.D. at 5%		4.5	---	3.6	---	3.9	---

* Increases relative to control

3.4.3 The impact on root length 90 days from planting

Data in Table (8) demonstrates that all treatments resulted in root length increases compared to the untreated control (water only). However, increases were significant only in the treatment of Actamyl and thyme with all tested fungi, Plant Guard with (*F. oxysporum* and *F. semitectum*) and the percentage of increases ranged between 89.5% and 17.5%. The highest percentage of increase (89.5%) resulted from the treatment of Plant Guard with *F. semitectum*, while the lowest percentage of increase (17.5%) resulted from eucalyptus with *F. semitectum*.

3.4.4 The impact on plant fresh weight 90 days from planting

Data in Table (9) exhibit that most treatments caused increases in plant fresh weight, and the percentage ranged from 54.54% to 6.82%. Increases were significant only in the treatment of

Plant Guard with *F. oxysporum*, which recorded the highest percentage of increase (54.54%). In contrast, thyme oil, Plant Guard, and Rhizo-N with (*R. solani*) and Rhizo-N with (*F. semitectum*) had no effect on plant fresh weight since the percentage increase was 0.0%.

Table 8: Effect of essential oils, biocides and fungicide on root length, 90 days from planting under greenhouse conditions.

Treatments		Root length (cm)					
		F. oxysporum	Increase (%) *	*F. semitectum*	Increase (%) *	*R. solani*	Increase (%) *
Essential oils	Eucalyptus	7.7	28.3	6.7	17.5	7.7	28.3
	Thyme	9.3	55.0	9.3	63.16	10.3	71.7
Biocides	Plant Guard	9.3	55.0	10.8	89.5	7.2	20.0
	Rhizo-N	7.3	21.7	7.3	28.1	7.3	21.7
Fungicide	Actamyl 70% wp	9.3	55.0	9.3	63.2	9.3	55.0
Untreated control		6.0	---	5.7	---	6.0	---
L.S.D. at 5%		2.0	---	2.9	---	2.0	---

* Increases relative to control

Table 9: Effect of essential oils, biocides and fungicide on plant fresh weight, 90 days from planting under greenhouse conditions.

Treatments		Plant fresh weight (gm)					
		F. oxysporum	Increase (%) *	*F. semitectum*	Increase (%) *	*R. solani*	Increase (%) *
Essential oils	Eucalyptus	5.0	13.64	4.4	7.32	3.5	9.38
	Thyme	4.7	6.82	4.4	7.32	3.2	0.0
Biocides	Plant Guard	6.8	54.54	4.7	14.63	3.2	0.0
	Rhizo-N	4.7	6.82	4.1	0.0	3.2	0.0
Fungicide	Actamyl 70% wp	5.3	20.45	6.2	51.21	3.5	9.38
Untreated control		4.4	---	4.1	---	3.2	---
L.S.D. at 5%		1.4	---	4.5	---	1.2	---

* Increases relative to control

3.4.5 The impact on plant dry weight 90 days from planting

Data in Table (10) demonstrate that some treatments caused increases in plant dry weight. However, the increases were significant only in the treatments of Actamyl 70% wp with (*F. oxysporum* and *F. semitectum*) and Plant Guard with (*F. oxysporum*). In contrast, Rhizo-N with all tested fungi, Plant Guard with (*F. semitectum* and *R. solani*), thyme with (*F. oxysporum* and *F. semitectum*) and eucalyptus with (*F. semitectum*) had no effect on plant dry weight since the percentage increase was 0.0%.

Table 10: Effect of essential oils, biocides and fungicide on plant dry weight, 90 days from planting under greenhouse conditions.

Treatments		Plant dry weight (gm)					
		F. oxysporum	Increase (%) *	*F. semitectum*	Increase (%) *	*R. solani*	Increase (%) *
Essential oils	Eucalyptus	2.5	13.64	2.2	0.0	1.9	18.75
	Thyme	2.2	0.0	2.2	0.0	1.9	18.75
Biocides	Plant Guard	3.4	54.54	2.2	0.0	1.6	0.0
	Rhizo-N	2.2	0.0	2.2	0.0	1.6	0.0
Fungicide	Actamyl 70% wp	2.8	27.27	3.1	40.91	1.9	18.75
Untreated control		2.2	---	2.2	---	1.6	---
L.S.D. at 5%		0.3	---	0.4	---	0.6	---

* Increases relative to control

4. Discussion

Marjoram (*Majorana hortensis* L.) is considered one of the most important medicinal and aromatic plant crops in Egypt. Plantations of marjoram have been affected by root-rot and wilt diseases, which have resulted in reduced plant vegetative growth, plant stand, and essential oil yield (El-Gebaly, 1998; Garbagnoli & Gaetan, 1994; Hilal et al., 1990). Isolation trials from the diseased plant samples confirmed the presence of soil-borne fungi associating with the infected plant tissues. However, *Fusarium* spp. was recorded at a higher frequency compared with the other fungi. Such findings go in accordance with Hilal et al. (1990) and El-Gebaly (1998), who found soil-borne diseases of marjoram in Egypt and identified their causal fungal pathogens. The most prevalent isolate was identified as *F. oxysporum*. The same result was obtained by Garbagnoli and Gaetan (1994), who identified the causal agent of marjoram wilt as *F. oxysporum* according to symptomology, cultural, and morphological characteristics. *Fusarium semitectum*, *F. solani*, *F. roseum*, *M. phaseolina*, and *R. solani* were also identified. Some of the isolated fungi in the present investigation were formerly identified on marjoram (El-Gebaly, 1998; Hilal et al., 1990), mint and rue (El-Shazly, 1996), and rosemary (Conway et al., 1997). The pathogenicity tests of the isolated fungi showed that *F. oxysporum*, and *R. solani* were the most pathogenic fungi on marjoram seedlings, causing pre- and post-emergence damping-off, whereas in transplanted marjoram plants, *F. oxysporum* and *F. semitectum* showed the highest disease incidence. These results are somewhat similar to those obtained by El-Gebaly (1998). The inhibitory effects of eucalyptus and thyme essential oils at three concentrations (1000, 3000, and 6000 ppm) against the mycelial growth of the tested fungi were confirmed, since decreases were significant in most cases. However, thyme oil was superior in its inhibitory effect than eucalyptus oil, additionally; the high concentration (6000 ppm) was the most effective than the others. On the other hand, *R. solani* was the most sensitive fungus affected by the oils, followed by *F. oxysporum*, while *F. semitectum* was the least affected. According to Farag et al. (1989), Linskens and Jackson (1991), Chauhan and Singh (1991), Zedan et al. (1994), Zygadlo et al. (1994), and Halawa (2004), a number of essential oils, including these ones, have antifungal effects. Some compounds of thyme oil are responsible for its antifungal effects, such as Thymol and carvacrol (Agarwal et al., 1979). However, the antifungal effect of thyme may be explained by the idea that it penetrates the cell wall, causing damage to the lipoprotein cytoplasmic membrane, which allows the cytoplasm to escape (Zambonelli et al., 1996). Faghih-Imani et al. (2020) reported the highest level of antifungal effect of thyme essential oil against *F. graminearum* and *F. culmorum*, the causal pathogens of crown and root rot on wheat. The authors also mentioned that scanning the vegetative growth of pathogenic fungi under a light microscope showed destructive changes in the hyphae as a result of thyme use. The obtained results are also in agreement with Sarhan (2020) who found that the use of thyme essential oil, followed by eucalyptus, significantly inhibited the linear growth of the tested fungi; *F. moniliforme*, *F. solani*, and *R. solani*, the causal agents of soybean root rot diseases compared with the control. Among the twelve tested essential oils, thyme showed the best antifungal effect against the phytopathogenic fungi *F. oxysporum* and *Bortytis cinerea* (Palfi et al., 2019). Despite the smallest dosage applied 50 μl/10 ml of PDA agar medium, it was completely capable of inhibiting the mycelial growth of both phytopathogenic fungi. Under greenhouse conditions, using essential oils, biocides and fungicide as dipping treatments for 20 minutes on marjoram transplants significantly reduced disease incidence with all the tested fungi compared to the untreated control. However, the fungicide Actamyl 70% wp was the most effective treatment, which gave the lowest percentage of disease incidence with all fungi under study compared to the untreated control. Plant Guard had superiority after Actamyl against *F. semitectum* and *F. oxysporum*. These findings are in harmony with Sarhan (2020), who found that Plant Guard and Vitavax-200 treatments gave the highest reduction percentage of soybean root rot and damping-off under greenhouse conditions. Thyme oil came in the second order after Actamyl with *R. solani* and *F. oxysporum*. Faghih-Imani et al. (2020) revealed that thyme oil had superiority in reducing the disease incidence and severity of *Fusarium* species in inoculated wheat plants. The positive effect of these treatments may be explained by the suppression of wilt and root rot pathogens after transplantation as a result of the antifungal effects of their compounds, which reduce or inhibit the spread of infections. Abo-Elyousr et al. (2014) noticed strong antagonistic effects of four isolates of *Trichoderma harzianum* and five isolates of *T. longibrachiatum* against *Alternaria porri*, the causal pathogen of onion purple blotch. The authors also reported that microscopic scans revealed the growth of *Trichoderma* spp. over the hyphae of *A. porri* with coiling, surrounding, and lysis of the hyphae. On the other hand, the results of Hilal et al. (1990; 1994; 2003), Helmy et al. (2001), and Abo El-Ela (2003) correspond to our findings about the effectiveness of the tested treatments against the causal agents of root rot and wilt diseases of marjoram. For marjoram plant growth, Actamyl and Plant Guard were superior to other treatments in most cases for improving plant growth parameters, *i.e.*, root length, plant height, fresh and dry weight. It is possible that these treatments have beneficial effects due to their reduction in disease infection and severity, and improve nutrient absorption. Somewhat similar results were recorded on enhancing plant growth with various treatments by Nada (1997), Abo El-Ela (2003), Hilal et al. (2003), Shafie (2004), Halawa (2004), Mahmoud Amany (2004), and Hassanin (2007).

5. Conclusion

The current study evaluated the efficiency of some biocides and fungicidal alternatives in the management of marjoram wilt and root rot diseases. Actamyl 70% wp, Plant Guard and thyme oil significantly decreased disease incidence than the other treatments compared to the untreated control with all the tested fungi. Furthermore, Actamyl and Plant Guard improved plant growth parameters, *i.e.*, plant height, root length, fresh and dry weight than the other treatments in most cases. These results indicate the possibility of using Plant Guard (biocide) as a fungicidal alternative against marjoram wilt and root rot diseases.

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