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ORIGNAL PAPER

Evaluation Efficacy of Some Agents as Safe Alternatives to Fungicides in Management Root-rot and Wilt Diseases of *Artemisia absinthium* and *A. santonicum*

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ABSTRACT

This study was planned to evaluate biocides, elicitors and essential oils as alternative agents to fungicides against root rot and wilt diseases of wormwood plants. *Fusarium oxysporum*, *F. semitectum*, *F. solani*, *Macrophomina phaseolina*, *Pythium ultimum* and *Rhizoctonia solani* were isolated from infected plants with root and stem rot and wilt diseases. Infected plants were collected from different localities of Qaluobiya governorate. *Fusarium oxysporum*, *F. solani* and *R. solani* were the most pathogenic fungi to *A. santonicum* unrooted cuttings in pathogenicity trials, while *F. solani* and *P. ultimum* were the most pathogenic fungi to *A. absinthium* unrooted cuttings. Also, both *Artemisia* species were infected by other isolated fungi. *In vitro* study showed that garlic essential oil and elicitor (H_2O_2) were the most active treatments against all tested fungi. Under greenhouse conditions, carbendazim (fungicide) and H_2O_2 completely prevented infection of *A. santonicum* rooted and unrooted cuttings in the presence of *F. oxysporum*. Also, carbendazim (fungicide) and H_2O_2 completely prevented infection of *A. absinthium* rooted and unrooted cuttings in the presence of *P. ultimum*. Bio-Cure F and garlic essential oil were superior treatments to improve plant growth parameters (plant height, number of branches and fresh and dry weights), also, Bio-Cure F and H_2O_2 were the best treatments in improving essential oil yield of both *Artemisia* species. In conclusion, using any of Bio-Cure F (biocide) or H_2O_2 or garlic essential oil as safe alternative to fungicide agents against root rot and wilt diseases to plants of *Artemisia absinthium* and *A. santonicum* is preferable.

Keywords: *Artemisia absinthium*, *A. santonicum*, Bio-Cure F, Garlic essential oil, H_2O_2, *Fusarium oxysporum*.

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INTRODUCTION

Wormwood belonging to the genus *Artemisia* L. is among the largest and most widely distributed genera of the family Asteraceae, consisting of 522 small herb and shrub species native to the northern hemisphere, South America, southern Africa and the Pacific Islands (Wright, 2002, Erel *et al.*, 2012 and Humara *et al.*, 2014). These herbs have been used worldwide in folk medicine since ancient times (Hose, 2002 and Erel *et al.*, 2012). They have been used as traditional anthelmintics antimalarials (Baytop, 1999). There are also several reports concerning the antioxidant, cytotoxic, antipyretic, analgesic, antimicrobial, and antifungal activities of different *Artemisia* species (Tan *et al.*, 1998 and Wright, 2002). Root rot and wilt diseases were not reported on *Artemisia absinthium* L. and *Artemisia santonicum* L. in Egypt. However, the disease was recently observed in *Artemisia absinthium* and *Artemisia santonicum* during the traffic on *Artemisia* plantations in El-Qanatir El-Khayriya, Qaluobiya governorate. Many investigators reported diseases on *Artemisia* spp. around the world. Koike (2011) in California found that *F. solani* caused leaf chlorosis symptoms and wilting of shoot tips on tarragon (*A. dracunculus*) plants. Teng *et al.*, (2015) mentioned that *Fusarium oxysporum* f. sp. *cubense*, *Phytophthora* spp., and *Pythium* spp. caused root rot of *Artemisia selengensis* plant. Chen *et al.* (2015) and Chen *et al.* (2016) found that Fusarium wilt caused by *F. oxysporum* is the major disease in continuously cropped *Artemisia selengens*. The pathogen, *F. oxysporum* infects seedlings from the incision of the cutting stem and causes Fusarium wilt of *A. selengens* seedlings and decreases the survival ratio of the seedlings. Regarding management of diseases of medicinal plants in general and control of soilborne plant pathogens in particular, essential oils, biocides and elicitors methods, are being considered because conventional fungicides can result in

accumulation of harmful residues which may lead to serious ecological and health problems. The use of fungicides alternatives to control the fungal diseases was reported by several researchers (Hassanin, 2013 and Abdallah *et al.*, 2019).

The present investigation was carried out to study *A. absinthium* and *A. santonicum* root rot and wilt diseases and their causal pathogens, factors affecting growth of such fungal species belonging to different genera under Egyptian conditions and evaluation different controlling means alternative to chemical fungicides for these diseases.

MATERIALS AND METHODS

Isolation and identification of the causal pathogens:

Naturally infected *A. absinthium* and *A. santonicum* plants showing root rot, stem rot and/or wilt diseases were collected from fields located in Qaluobiya governorate during 2019 season (Fig. 1). Infected roots and stems were cut into small fragments, surface sterilized with 2.5 % v/v Clorox for two minutes, rinsed with sterilized water, dried and placed onto potato dextrose agar medium (PDA) in Petri plates, incubated at 27°C and examined during one week. Frequency of the isolated fungi grown on plant parts was recorded. Purification of the isolated fungi was carried using the hyphal tip or single spore techniques (Brown, 1924 and Hansen, 1926). Fungi were identified depending on their morphological features according to the descriptions of Booth (1971), Domsch *et al*. (1980), Niternik and Vandler (1981) and Nelson *et al*. (1983). Identification was confirmed by the staff members of Mycology, Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza, Egypt. Pure cultures were transferred into PDA slants and kept at low temperature (5°C) for further studies.

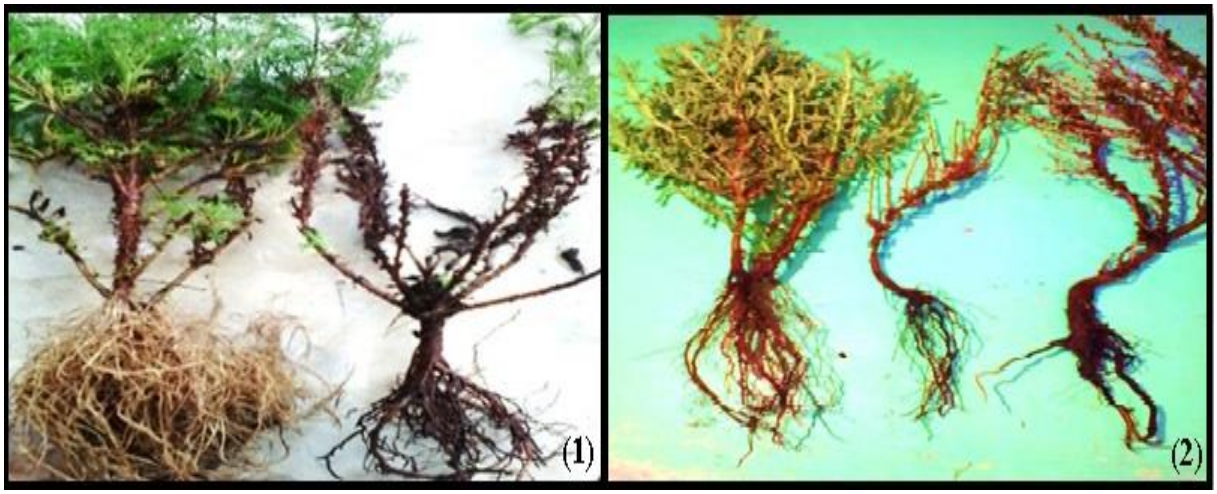

Fig. (1): Natural infection by soil-borne fungi on *A. absinthium* (1) *A. santonicum* (2) stems and roots showing dark rotted parts and black lesions. Healthy plant on the left.

Pathogenicity tests:

Pathogenicity test was conducted using all the isolated and identified fungi which are known to be the cause of root rot and wilt symptoms in diseased plants, *i.e.*, *Fusarium oxysporum*, *F. semitectum*, *F. solani*, *Macrophomina phaseolina*, *Pythium* sp. and *Rhizoctonia solani*. Soil (1 sand : 1 peatmoss : 1 clay, w:w:w) was sterilized by 5% formalin solution, then left for two weeks to get rid of formalin toxicity before use. The tested fungi were cultured on autoclaved sorghum grains medium (100 g. corn + 50 g. washed sand + 100 ml. water) and incubated at 27°C for 15 days. Soil infestation with the isolated fungi was applied at the rate of 1 % w/w, thoroughly mixed with the soil in sterilized 20 cm diam. plastic pots. Infested pots with the tested fungi, each alone, were watered one week before planting to enhance colonization of fungi. Each pot was planted with four cuttings, rooted and unrooted for each *Artemisia* species (*Artemisia absinthium* and *A. santonicum*) obtained from the nurseries of medicinal and aromatic plants at El-Qanatir El-Khayriya, Qaluobiya governorate. Each isolate was replicated three times. Disease incidence was recorded 60 and 90 day after planting.

Percentage of plants infected with root rot or wilt was recorded as disease percentage compared to the total examined plant numbers. Fungi were re-isolated from diseased plants and compared with the original isolates.

***In vitro* evaluation of four concentrations of eucalyptus and garlic essential oils on six pathogenic fungi under laboratory conditions:**

Preparation of plant essential oils:

Essential oils of air-dried eucalyptus (leaves) and fresh cloves of garlic were extracted by using steam distillation method according to Guenther (1948). The extraction was, however, done in laboratory of Ornamental, Medicinal and Aromatic Plant Diseases Research Department, Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.

Antimicrobial activity of certain essential oils:

Efficacy of volatile substances of eucalyptus (*Eucalyptus globulus* L.) and garlic (*Allium sativum*) on the fungal growth was tested using the paper disc method as described by Maruzzella and Sicurella (1960). Four PDA plates were inoculated with (5- mm-diam.) discs of each tested fungus. Paper disc (5 mm.) was impregnated with the tested essential oil at four concentrations (1000, 5000, 10000 and 15000 ppm) and placed in the center of the lid of the inoculated Petri-dish, and the plate was inverted and incubated at 25±2 °C. The radial growth was measured as cm when the growth reached its maximum (9 cm) in any plate (control).

***In vitro* evaluation of three concentrations of chitosan and H_2O_2 on six pathogenic fungi under lab. conditions:**

Two elicitors namely, chitosan (obtained from local pharmacy, Egypt) and H_2O_2 (hydrogen peroxide solution 50%, obtained from El-Nasr Pharmaceutical Chemicals Co., Egypt) were tested against the mycelial growth of the pathogenic fungi in laboratory. Effective concentrations of the materials tested against the pathogens were determined by using adequate volumes from each added to toxic liquid PDA medium. Chitosan was dissolved in 0.1% acetic acid in distilled water according to Eikemo *et al.* (2003). The medium was amended with each elicitor just before solidification. The appropriate amount of each elicitor was prepared to mix in 40 ml medium in each flask to give concentrations of 5, 10 and 15 g/L of chitosan, 0.25, 0.50 and 1 % of H_2O_2 50%. Ten ml of each toxic PDA medium were poured in each Petri dish. Discs (5 mm) of each fungus taken from seven days old cultures were used to inoculate the treated and untreated plates (without treatments) and incubated at 25±2 °C. The radial growth was measured as cm when the growth reached its maximum (9 cm) in any plate (control).

Greenhouse experiments:

Effect of various control measures on percentage of disease incidence, plant growth parameters of *A. santonicum* and *A. absinthium* grown in soil infested with the six pathogenic fungi after 90 days from planting.

In all greenhouse experiments, sterilized plastic pots (20-cm-diam.) filled with previously prepared formalin sterilized soil (1 sand: 1 peatmoss: 1 clay, w:w:w) were used for planting. A set of three pot replicates was used for each treatment, *i.e.*, garlic essential oil at 15 ml/L water prepared using Tween 80 (0.05%) in sterile distilled water compared with water and Tween 80 (0.05%) as control. The biocide, Bio-Cure F 1.15% WP (*Trichoderma viride* 1 x 10^6 cfu/g, M/S.T. Stanes Company Limit-India) was tested at the rate of 6 g/L water, also, the chemical elicitor, H_2O_2 50% was used at the rate of 1%. Also, suspension of the systemic fungicide, Carbendazim 50% WP (2 g/L) [Common name: Carbendazim, Chemical composition: 2- (Methoxy carbomylsmino) - benzimidazole) and Manufacture: Agriphar S.A., Belgium.] was used as a recommended fungicide.

Uniform rooted and unrooted cuttings were dipped in the previously prepared materials or in water only as control (water and 0.05% Tween 80 in case of garlic essential oil) for 20 minutes and planted in the infested soil. A set of 3 pots was used for each particular treatment and control. Twelve rooted and unrooted cuttings were planted for each treatment (four cutting/pot), the potted soils, however, were drenched with each treatment at the previous rates after 15 days from planting. Disease incidence, growth parameters as plant height, number of branches and fresh and dry weight/plant as well as essential oil percentage (determined by hydro distillation according to the method described in British Pharmacopoeia, 1963) were determined 90 - days after planting as mentioned before.

Statistical analysis:

The layout of this experiment was designed as factorial experiment in a complete randomized design with three replicates (Snedecor and Cochran, 1980). Statistical analysis was done by using the computer program MS-TATEC software version (4) using the L.S.D. test at 0.05.

RESULTS AND DISCUSSION

1- Isolation, purification and identification of the causal organisms and their frequency (%):

Isolation trials on PDA yielded six fungal species belonging to four genera (Table, 1). They were identified as: *Fusarium oxysporum* Schlecht., *F. semitectum* Berk. & Rav., *F. solani* (Mart.) Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Pythium ultimum* Hesse and *Rhizoctonia solani* Kühn. These fungi were, however, isolated from all infected plant samples of *Artemisia santonicum* except of *P. ultimum* was isolated from *A. absinthium* only, Also, *R. solani* was isolated only from *A. santonicum* which was collected from Qaluobiya governorate during 2019 season. *Fusarium oxysporum* (25.74% & 30.48%) and *F. semitectum* (23.53% & 28.57%) recorded the highest frequency (%) in isolation trials from the two *Artemisia* species, respectively, followed by *R. solani* (20.59%) with *A. santonicum* and *P. ultimum* (20.95%) with *A. absinthium*. Only *F. oxysporum* was isolated from wilted and/or root rotted plants. On the other hand, frequency (%) ranged between 0.0% (*P. ultimum*) to 25.74% (*F. oxysporum*) and 0.0% (*R. solani*) to 30.48% (*F. oxysporum*) were recorded for the fungi isolated from *A. santonicum* and *A. absinthium*, respectively. According to the available literature, these fungi were isolated from these species of *Artemisia*, for the first time in Egypt. In this respect, many investigators in other countries isolated different soil-borne fungi from diseased parts of Artemisia plants. (Koike, 2011, Chen *et al.*, 2015 and Teng *et al*., 2015).

2. Pathogenicity tests:

All isolated and identified fungi (Table, 2) were able to infect stems and roots of the two *Artemisia* species, rooted or unrooted cuttings, grown in artificially infested soil. The infected plant organs were usually rotted, therefore stunting, weathering and/or complete death appeared on this wormwood rooted or unrooted cuttings. The percentage of infection was increased with increasing the growing period. Generally, study of the pathogenicity test using both *Artemisia* species indicated that both tested species were infected by each of the isolated fungi. On the other hand, the isolated fungi from each *Artemisia* species were able to infect the same species more than the other. *Fusarium oxysporum* (66.7%), *F. solani* (66.7%) and *R. solani* (66.7%) gave the highest percentages of disease incidence in case of unrooted cuttings of *A. santonicum*, while *F. solani* (83.3%) followed by *P. ultimum* (75.0%) gave the highest percentages of disease incidence in cases of rooted and unrooted cuttings of *A. absinthium* after 90 days from planting in artificially infested soil. Generally, unrooted cutting of the two species tested were more susceptible to infection by all fungi than the tested rooted cuttings after 90 days from sowing. These results might be due to some factors such as the environmental conditions, the host, and the pathogenic fungus itself which affected disease development. These results are in agreement with those reported by Chen *et al.* (2015) who found that *F. oxysporum* was the major causal pathogen for disease incidence in *Artemisia selengens* in continuously cropped soil. Also, with Koike (2011) on Tarragone (*Artemisia dracunculus*).

Table (1): Frequency and symptoms of the fungi isolated from *Artemisia santonicum* and *A. absinthium* diseased plants, collected from some plantations of Qaluobiya governorate.

Isolated fungi	*Artemisia santonicum*		*Artemisia absinthium*		Symptoms
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)	
Fusarium oxysporum	35	25.74	32	30.48	Wilt & Root rot
Fusarium semitectum	28	23.53	30	28.57	Root rot
Fusarium solani	15	11.03	12	11.43	Root rot
Macrophomina phaseolina	26	19.12	9	8.57	Root rot
Pythium ultimum	0	0.00	22	20.95	Root rot
Rhizoctonia solani	32	20.59	0	0.00	Root rot
Total	136	100	105	100	-

Table (2): Pathogenicity of the six isolated fungi after 60 and 90 days on rooted and unrooted cuttings of *Artemisia santonicum* and *A. absinthium* plants, grown in artificially infested soil under greenhouse conditions.

Fungi	*A. santonicum**				*A. absinthium***			
	% Disease incidence after:				% Disease incidence after:			
	60 days		90 days		60 days		90 days	
	Rooted cuttings	Unrooted cuttings	Rooted cuttings	Unrooted cuttings	Rooted cuttings	Unrooted cuttings	Rooted cuttings	Unrooted cuttings
*F. oxysporum**	25.0	16.7	50.0	66.7	8.3	8.3	25.0	33.3
*F. oxysporum***	25.0	8.3	41.7	50.0	16.7	16.7	41.7	50.0
*F. semitectum**	41.7	16.7	58.3	50.0	16.7	25.0	25.0	41.7
*F. semitectum***	33.3	16.7	50.0	41.7	25.0	33.3	50.0	58.3
*F. solani**	33.3	25.0	41.7	66.7	25.0	50.0	33.3	58.3
*F. solani***	25.0	16.7	33.3	41.7	41.7	58.3	66.7	83.3
*M. phaseolina**	25.0	25.0	41.7	58.3	8.3	25.0	33.3	58.3
*M. phaseolina***	16.7	8.3	25.0	25.0	33.3	50.0	58.3	66.7
*P. ultimum***	16.7	16.7	33.3	41.7	41.7	41.7	66.7	75.0
*R. solani**	41.7	16.7	50.0	66.7	25.0	8.3	33.3	16.7
Control (without fungus)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D. at 5%for:	0.6	0.8	0.8	0.7	0.6	0.5	0.7	0.8

* Isolated fungi from *A. santonicum*
** Isolated fungi from *A. absinthium*

3. *In vitro* evaluation of four concentrations of eucalyptus and garlic essential oils on six pathogenic fungi under lab. conditions:

The inhibitory effect of four concentrations (1000, 5000, 10000 and 15000 ppm) of eucalyptus and garlic essential oils tested on linear growth of six fungi was evaluated *in vitro* (Table, 3). All concentrations of the two essential oils tested significantly reduced linear growth of all tested fungi compared with the control. Significant decrease values in the fungal growth were recorded by increasing concentrations. Garlic essential oil gave the highest reduction in linear growth compared with the eucalyptus essential oil especially with high concentration as it completely prevented the growth of the six tested fungi. Eucalyptus essential oil was the least significant effective oil in decreasing the fungi growth at all concentrations than the other oil. This may be due to the main component at different degrees in these oils. The antifungal activity of garlic essential oil is attributed to allicin, the main component of garlic (Slusarenko *et al*., 2008). The results of the present study are, to somewhat, similar to those reported by Dubey (1991) who stated that antigermination of spores might be affected by plant essential oils that may be attributed to the presence of some constituents such as linalool, eugenol, d-camphor and traces of phenols. The mode of action of the active substances in oils of medicinal and aromatic plants was interpreted by many scientists.

4. *In vitro* evaluation of three concentrations of chitosan and H_2O_2 on six pathogenic fungi *in vitro*:

Data in Table (4) indicate that all concentrations of the two elicitors tested significantly reduced radial growth of all tested fungi compared with the control. Significant decrease in the radial growth was recorded by increasing any concentration. H_2O_2 elicitor was more effective in reducing radial growth of all fungi tested than the chitosan elicitor. H_2O_2.high concentration was more effective in reducing radial growth specially in cases of *R. solani* (0.5cm), *P. ultimum* (0.7cm) *F. oxysporum* (2.6 & 3.0 cm) and *F. solani* (3.0 & 3.2 cm). On the other hand, *R. solani* was more affected by

chitosan (4.9 cm) and H_2O_2 (3.9 cm) which gave the least mean of growth compared with the other fungi tested. Chitosan is able to permeabilize the plasma membrane and kill the cells of fungi and reduce the *in vitro* growth of a number of fungi and Oomycetes. Chitosan was reported to exert an inhibitory action on the hyphal growth of numerous pathogenic fungi, including root and wilt diseases (Badawy *et al*., 2005 and Palma-Guerrero *et al*., 2008). In the present study, results indicated that H_2O_2 gave the highest reductions in linear growth of fungi tested compared with the chitosan. These results are in agreement to somewhat with Angelova *et al.* (2005) and Ali (2018) who reported that exposure of fungal mycelia or spores of 12 fungal species to hydrogen peroxide promoted oxidative stress, as evidenced by inhibition of biomass production and spore germination: accumulation of oxidative modified proteins.

Table (3): *In vitro* effect of four concentrations of eucalyptus and garlic essential oils on six pathogenic fungi measured in cm on PDA medium, incubated at 25±2°C for 7 days.

Essentia oils	Fungi	Linear growth (cm)at conc. (ppm):					Mean
		0.0	1000 ppm	5000 ppm	10000 ppm	15000 ppm	
Eucalyptus	*F. oxysporum**	9.0	7.7	6.6	5.3	4.2	6.6
	*F. oxysporum***	9.0	7.2	6.0	4.8	3.5	6.1
	*F. semitectum**	9.0	6.7	5.6	4.0	3.0	5.7
	*F. semitectum***	9.0	6.8	5.8	4.3	3.2	5.8
	*F. solani**	9.0	7.9	7.2	6.8	5.8	7.3
	*F. solani***	9.0	7.8	7.0	6.4	5.4	7.1
	*M. phaseolina**	9.0	9.0	9.0	8.2	8.0	8.6
	*M. phaseolina***	9.0	9.0	9.0	8.6	8.2	8.8
	*P. ultimum **	9.0	7.0	6.3	5.8	4.4	6.5
	*R. solani**	9.0	6.8	5.9	5.4	4.0	6.2
Mean		9.0	7.6	6.8	6.0	5.0	6.9
Garlic	*F. oxysporum**	9.0	5.0	2.4	1.3	0.0	3.5
	*F. oxysporum***	9.0	5.2	2.6	1.4	0.0	3.6
	*F. semitectum**	9.0	4.4	2.0	0.5	0.0	3.2
	*F. semitectum***	9.0	4.6	2.2	0.7	0.0	3.3
	*F. solani**	9.0	6.5	3.0	1.8	0.4	4.1
	*F. solani***	9.0	5.8	3.4	1.2	0.0	3.9
	*M. phaseolina**	9.0	2.8	1.0	0.0	0.0	2.6
	*M. phaseolina***	9.0	3.0	1.4	0.0	0.0	2.7
	*P. ultimum **	9.0	4.9	4.0	1.8	0.5	4.0
	*R. solani**	9.0	2.0	1.0	0.0	0.0	2.4
Mean		9.0	4.4	2.3	0.9	0.1	3.3
L.S.D. at 5% for:							
	Oils (A) = 0.40		Fungi (B) = 0.30		Concentrations (C) = 0.14		
	(A) × (B)=0.30		(A) × (C) =0.03		(B) × (C) = 0.23		
	(A) × (B) × (C) = 0.80						

*Fungi isolated from *Artemisia santonicum*; **Fungi isolated from *Artemisia absinthium*.

Table (4): *In vitro* effect of three concentrations of chitosan and H_2O_2 on six pathogenic fungi measured in cm on PDA medium, incubated at 25±2°C for 7 days.

Treatments	Fungi	Linear growth (cm)at conc. of:				Mean
		C	C*	C**	C***	
Chitosan	*F. oxysporum*+	9.0	8.2	6.1	5.5	7.2
	F. oxysporum++	9.0	8.0	5.8	5.2	7.0
	F. semitectum+	9.0	7.4	5.2	4.0	6.4
	F. semitectum++	9.0	7.5	5.6	4.2	6.6
	F. solani+	9.0	8.8	7.2	6.8	8.0
	F. solani++	9.0	8.6	7.0	5.7	7.6
	M. phaseolina+	9.0	9.0	9.0	9.0	9.0
	M. phaseolina++	9.0	9.0	9.0	9.0	9.0
	P. ultimum++	9.0	7.4	6.2	4.5	6.8
	R. solani+	9.0	4.3	3.7	2.5	4.9
	Mean	9.0	7.8	6.5	5.6	7.3
H_2O_2	*F. oxysporum*+	9.0	5.6	4.1	3.0	5.4
	F. oxysporum++	9.0	5.5	4.0	2.6	5.3
	F. semitectum+	9.0	5.6	4.3	3.2	5.5
	F. semitectum++	9.0	6.0	4.5	3.3	5.7
	F. solani+	9.0	6.5	5.0	3.2	5.9
	F. solani++	9.0	6.4	5.0	3.0	5.9
	M. phaseolina+	9.0	8.2	7.4	6.6	7.8
	M. phaseolina++	9.0	8.4	7.5	6.5	7.9
	P. ultimum++	9.0	4.7	2.6	0.7	4.3
	R. solani+	9.0	4.0	2.0	0.5	3.9
	Mean	9.0	6.1	4.6	3.3	5.8
L.S.D.at 5% for:						
		Treatments (A) = 0.2		Fungi (B) = 0.3		Concentrations (c) = 0.5
		(A) × (B) = 0.5		(A) × (C) = 0.4		(B) × (C) = 0.7
				(A) × (B) × (C) = 1.1		

+ Fungi isolated from *Artemisia santonicum*.; ++ Fungi isolated from *Artemisia absinthium*; *Concentrations used for each elicitor; Chitosan: (C*=5 g/L, C**= 10 g/L and C***=15 g/L); H_2O_2: (C*=0.25 %, C**= 0.50 % and C***=1 %); Control = C

5. Effect of various control measures on percentage of disease incidence of *A. santonicum* and *A. absinthium* grown in soil artificially infested with each of the six pathogenic fungi under greenhouse conditions.

The effects of essential oil of garlic, Bio-Cure F (biocide), H_2O_2 (elicitor) and carbendazim (fungicide) Table (5) applied as dipping the healthy rooted and unrooted cuttings before planting and added to the soil (as soil drench) 15 days after planting on disease incidence 90 days after transplanting in soil artificially infested with each of *F. oxysporum*, *F. semitectum*, *F. solani*, *M. phaseolina*, *P. ultimum* or *R. solani* were investigated.

Data in Table (5) indicate that percentage of fungal infection was decreased with all treatments. The fungicide (carbendazim) followed by H_2O_2 were the most effective treatments, since they gave the highest significant decreases in disease infection than the controls in case of *A. santonicum*. In this respect, the fungicide (carbendazim) followed by biocide (Bio-Cure F) and H_2O_2 were the most effective treatments tested, since they gave the highest significant decreases in disease incidence than the control in case of *A. absinthium*, however, essential oil of garlic was the least effective in decreasing disease incidence (%). Carbendazim and H_2O_2 completely prevented infection of *A. santonicum* rooted and unrooted cuttings in the presence of *F. oxysporum*. Also, carbendazim (fungicide) and H_2O_2 completely prevented infection of *A. absinthium* rooted and unrooted cuttings in the presence of *P. ultimum*.

The role of H_2O_2 in induced disease resistance may be due to activation of peroxidase, polyphenoloxidase, as well as catalase and B-1, 3- glucanase enzymes, which protect plants against pathogen infection (Morsy, 2005 and Khalifa *et al.*, 2007). Also, these results are in agreement with those obtained by Mahmoud *et al.* (2006) who found a correlation between induced resistance and some biochemical changes in root tissues like increasing the activity of oxidative enzymes

(peroxidase and polyphenoloxidase) and accumulation of phenolic compounds. They added that salicylic acid and H_2O_2 recorded the highest content of phenolic compounds and highest increase of activity of oxidative enzymes (peroxidase and polyphenoloxidase) in roots of peanut plants. Also, biological control using microorganisms, or their secretions prevented or reduced plant diseases, Also, the previous results are similar to those reported by Naglot *et al.* (2015) who reported that *Trichoderma viride* showed substantial antifungal activity against five standard phytopathogenic fungi. Culture filtrate collected from stationary growth phase of the antagonist demonstrated significantly higher degree of inhibitory activity against all the tested fungi, demonstrating the presence of an optimal blend of extracellular antifungal metabolites. Moreover, quantitative enzyme assay of exponential and stationary culture filtrates revealed that the activity of cellulase, β-1,3-glucanase, pectinase, and amylase was the highest in the exponential phase, whereas the activity of proteases and chitinase was noted highest in the stationary phase.

Also, the antifungal activity of garlic essential oil is attributed to allicin, the main component of garlic (Slusarenko *et al*., 2008). The essential oil has the ability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes, permeabilis them and finally damage mitochondrial membranes. The changes in electron flow through the electron transport system inside the mitochondria damage the lipids, proteins and nucleic acid contents of the fungal cells (Arnal-Schnebelen *et al*., 2004). The essential oils could also hassle the depolarization of the mitochondrial membranes and decreasing the membrane potential, affect Ca2+ and other ion channels, reduce the pH and also affect the proton pump and ATP pool. The change in fluidity of membranes resulted into the leakage of radicals, cytochrome, calcium ions and proteins. Thus, permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis (Yoon *et al*., 2000).

Table (5): Effect of various control measures on percentages of disease incidence on *A. santonicum* and *A. absinthium* grown in soil artificially infested with six pathogenic fungi under greenhouse conditions, 90 days after transplanting.

Treatments & Conc. (A)	Type of cuttings (B)	% Disease Incidence of:											
		A. santonicum						*A. absinthium*					
		F. oxysporum (C)	*F. semitectum* (C)	*F. solani* (C)	*M. phaseolina* (C)	*R. solani* (C)	Mean	*F. oxysporum* (C)	*F. semitectum* (C)	*F. solani* (C)	*M. phaseolina* (C)	*P. ultimum* (C)	Mean
Garlic oil (15 ml/L)	I	0.0	16.7	8.3	25.0	8.3	11.7	33.3	25.0	16.7	16.7	33.3	25.0
	II	16.7	33.3	33.3	33.3	25.0	28.3	33.3	41.7	33.3	33.3	41.7	36.7
	Mean	8.4	25.0	20.8	29.2	16.7	20.0	33.3	33.4	25.0	25.0	37.5	30.9
Bio Cure F (6 g/L)	I	8.3	16.7	8.3	8.3	16.7	11.7	0.0	8.3	0.0	0.0	8.3	3.3
	II	16.7	33.3	16.7	33.3	33.3	26.7	0.0	0.0	0.0	0.0	8.3	1.7
	Mean	12.5	25.0	12.5	20.8	25.0	19.2	0.0	4.2	0.0	0.0	8.3	2.5
H_2O_2 (1%)	I	8.3	0.0	0.0	0.0	0.0	1.7	8.3	8.3	8.3	16.7	0.0	8.3
	II	16.7	0.0	16.7	8.3	16.7	11.7	8.3	0.0	0.0	16.7	0.0	5.0
	Mean	12.5	0.0	8.4	4.2	8.4	6.7	8.3	4.2	4.2	16.7	0.0	6.7
Carbendazim 50%WP (2 g/L)	I	8.3	0.0	0.0	8.3	8.3	5.0	0.0	8.3	0.0	0.0	0.0	1.7
	II	0.0	0.0	8.3	16.7	33.3	11.7	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	4.2	0.0	4.2	12.5	20.8	8.4	0.0	4.2	0.0	0.0	0.0	0.9
Control	I	41.7	33.3	33.3	41.7	25.0	35.0	41.7	33.3	25.0	25.0	50.0	35.0
	II	50.0	66.7	50.0	50.0	66.7	56.7	66.7	50.0	66.7	41.7	50.0	55.0
	Mean	45.9	50.0	41.7	45.9	45.9	45.9	54.2	41.7	45.9	33.4	50.0	45.0
L.S.D. at 5 %:		Treatments (A) = 0.3			(A) × (B) = 0.4			Treatments (A) = 0.5			(A) × (B) = 0.6		
		Type of cuttings (B) = 0.5			(A) × (C) = 0.3			Type of cuttings (B) = 0.6			(A) × (C) = 0.4		
		Fungi (C) = 0.1			(B) × (C) = 0.4			Fungi (C) = 0.3			(B) × (C) = 0.4		
		(A) × (B) × (C) =0.7						(A) × (B) × (C) = 0.8					

I = Rooted cuttings, II = Unrooted cuttings

6. Effect of various control measures on plant height (cm) and number of branches/plant of *A. santonicum* and *A. absinthium* grown in soil infested with the six pathogenic fungi (each alone) under greenhouse conditions.

Data presented in Table (6) show that all treatments tested gave significant increases in plant height and number of branches per plant compared with the controls (without treatment) in soil artificially infested with each of the six pathogenic fungi, however Bio-Cure F was the superior treatment in increasing plant height and No. of branches per plant compared with the other treatments except in cases of *F. solani* and *R. solani* (*A. santonicum*) followed by essential oil of garlic with rooted and unrooted cuttings of *A. santonicum* and *A. absinthium*. In this respect, carbendazim fungicide was the least effective treatment in increasing plant height and number of branches per plant in most cases with two species tested. The improvements in plant growth parameters may be due to their efficiency in partially or completely preventing disease infection and development or may attributed to the biochemical changing in the stem base tissues. This change includes increasing the activity of peroxidase enzyme, growth hormones and phenol compounds. Somewhat similar results on various crops, under naturally or artificially infested soil were reported by Zedan *et al.* (2011) and Hassanin (2013).

Table (6): Effect of various control measures on plant height (cm) and number of branches/plant of *A. santonicum* and *A. absinthium*, grown in soil artificially infested with six pathogenic fungi (each alone) under greenhouse conditions, 90 days after transplanting.

Treatment	Plant growth parameters for:									
	Fungi	*A. santonicum*				Fungi	*A. absinthium*			
		Plant height (cm)		No. of branches/ plant			Plant height (cm)		No. of branches/ plant	
		*	**	*	**		*	**	*	**
Garlic oil	*F. oxysporum*	16.0	15.0	5.0	4.0	*F. oxysporum*	19.0	18.7	5.0	5.0
Bio Cure F		19.0	18.3	7.0	5.0		25.5	21.0	5.5	5.3
H_2O_2		16.0	14.0	5.0	3.0		19.0	15.3	3.0	2.7
Carbendazim 50%WP		14.5	12.3	5.0	3.0		16.0	11.0	4.0	2.3
Control		10.3	7.7	3.0	1.0		12.0	8.0	2.0	1.0
Mean		15.2	13.5	5.0	3.2		18.3	14.8	3.9	3.3
Garlic oil	*F. semitectum*	19.7	19.0	7.0	5.3	*F. semitectum*	14.0	12.0	5.0	3.0
Bio Cure F		25.5	20.3	9.0	6.0		14.5	13.3	5.2	3.1
H_2O_2		19.0	17.0	5.0	5.0		11.7	11.7	5.0	2.3
Carbendazim 50% WP		16.0	13.1	5.0	3.0		17.0	18.3	5.0	5.0
Control		9.0	6.6	3.0	1.0		13.0	9.0	1.0	1.0
Mean		17.8	15.2	5.8	4.1		13.9	12.9	4.2	2.9
Garlic oil	*F. solani*	14.0	13.3	5.0	3.0	*F. solani*	19.7	18.0	5.0	5.0
Bio Cure F		17.0	16.3	5.3	4.0		25.5	20.0	6.5	6.0
H_2O_2		16.5	16.0	5.0	4.7		19.0	15.4	5.0	4.3
Carbendazim 50% WP		14.0	11.1	5.5	3.0		16.0	11.0	5.0	3.0
Control		9.5	7.0	2.0	1.0		9.0	8.0	1.0	1.0
Mean		14.2	12.5	4.5	3.1		17.8	14.5	4.5	3.9
Garlic oil	*M. phaseolina*	13.1	12.3	4.0	3.0	*M. phaseolina*	16.8	13.7	6.0	4.3
Bio Cure F		17.0	14.0	4.3	3.7		18.0	14.0	6.0	4.7
H_2O_2		14.0	12.1	3.0	3.0		16.0	13.0	5.0	4.0
Carbendazim 50% WP		12.0	11.0	4.0	2.3		16.5	9.0	5.0	2.3
Control		8.7	5.3	2.0	1.0		4.0	4.0	2.0	1.0
Mean		13.0	10.9	3.5	2.6		14.3	10.7	4.8	3.3
Garlic oil	*R. solani*	17.0	15.0	6.0	4.0	*P. ultimum*	17.2	17.0	7.0	5.0
Bio Cure F		25.0	21.7	8.5	5.7		20.0	17.3	7.0	5.1
H_2O_2		17.7	15.3	5.0	4.3		20.0	18.0	7.1	5.2
Carbendazim 50% WP		16.0	14.3	6.0	3.0		17.7	10.0	6.0	2.3
Control		12.0	10.0	2.0	1.0		7.0	6.0	2.0	1.0
Mean		17.5	15.3	5.5	3.6		15.8	13.7	5.4	3.7
L.S.D. at 5 % for:										
Treatments (T) =		0.1	0.3	0.2	0.5		0.3	0.1	0.2	0.4
Fungi (F) =		0.3	0.9	0.3	0.1		0.7	0.7	0.3	0.3
F × T =		0.2	0.4	0.3	0.5		0.4	0.4	0.4	0.6

*= Rooted cuttings; **= Unrooted cuttings

7. Effect of various control measures on fresh and dry weight (g) /plant of *A. santonicum* and *A. absinthium*, grown in soil artificially infested with six pathogenic fungi (each alone) under greenhouse conditions.

Data in Table (7) and Figs. (2&3) reveal that all the experimented treatments significantly increased fresh and dry weights of plants as well as essential oil percentages of the two species (rooted and unrooted cuttings) grown in artificially infested soil compared with these of the untreated controls. Bio-Cure F followed by essential oil of garlic were, however, the best treatments in increasing plant fresh and dry weights compared with the other treatments. Meanwhile carbendazim (fungicide) was the least effective in increasing fresh and dry weights of plants in most cases. In general, Bio-Cure F (biocide) followed by the essential oil of garlic were the superior tested treatments, whereas they were more effective in increasing fresh and dry weights than the tested fungicide and H_2O_2. On the other hand, Bio-Cure F gave the highest increase in essential oil percentages of the two species followed by H_2O_2 and essential oil of garlic. The improvements in plant growth parameters and essential oil percentages might be due to rooted and unrooted cuttings treatments and soil treatment may be attributed to biochemical changing in the stem base tissues. This change includes increasing the activity of peroxidase enzyme, growth hormones and phenol compounds. Positive efficacy of garlic essential oil, H_2O_2 elicitor, Bio-Cure F biocide and/or carbendazim fungicide tested in controlling the diseases caused by fungi or increment plant growth parameters as number of branches or fresh and dry weights and/or increasing essential oil production were to somewhat similar to those found on medicinal, aromatic and other plants by Zedan *et al.* (2011), Hassanin (2013), Nada, 2014, Hamad *et al*., 2015 and Awad, 2016.

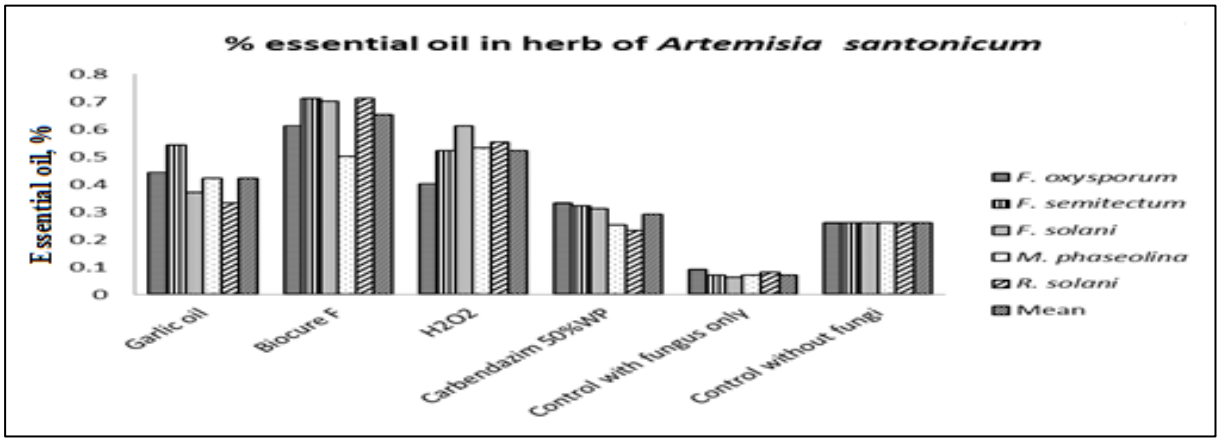

Fig (2): Effect of various control measures on essential oil percentage of *A. santonicum* plants, grown in soil artificially infested with five pathogenic fungi each alone under greenhouse conditions.

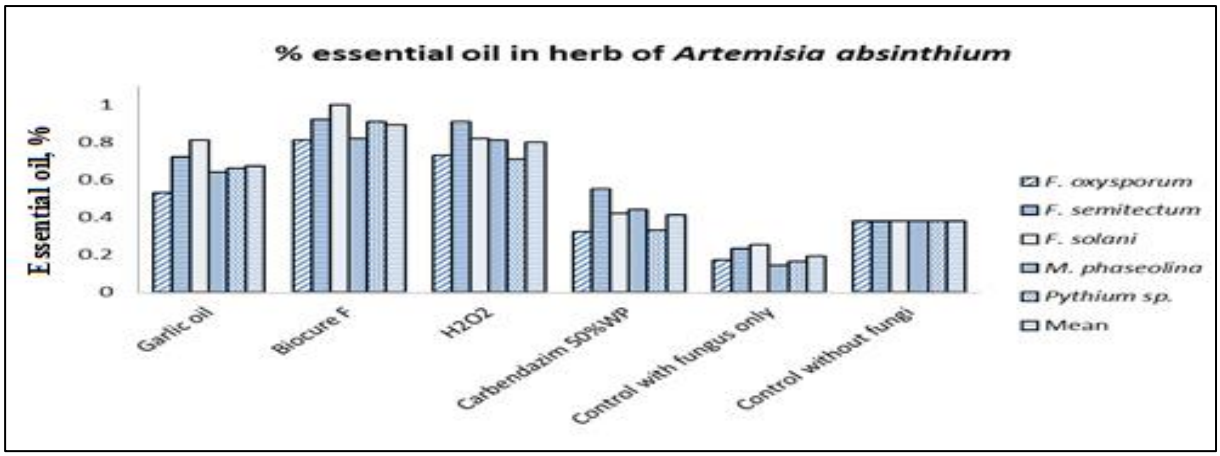

Fig (3): Effect of various control measures on essential oil percentage of *A. absinthium* plants, grown in soil artificially infested with five pathogenic fungi each alone under greenhouse conditions.

Table (7): Effect of various control measures on fresh and dry weight (g)/plant of *A. santonicum* and *A. absinthium*, grown in soil artificially infested with six pathogenic fungi (each alone) under greenhouse conditions.

Treatment	Fungi	Plant growth parameters for: *A. santonicum* Fresh weight/ plant (gm) *	**	Dry weight/ plant (gm) *	**	Fungi	*A. absinthium* Fresh weight/ plant (gm) *	**	Dry weight/ plant (gm) *	**
Garlic oil	*F. oxysporum*	21.9	18.1	9.7	6.1	*F. oxysporum*	19.0	18.8	13.3	13.1
Bio Cure F		31.8	28.5	10.4	7.2		21.1	20.0	13.5	13.2
H_2O_2		21.7	17.3	9.2	5.8		19.0	18.3	13.0	12.5
Carbendazim 50%WP		18.9	17.0	6.7	5.6		17.5	16.7	12.2	11.8
Control		10.7	10.2	3.4	3.0		12.7	11.6	8.7	8.1
Mean		21.0	18.2	7.9	5.5		17.9	17.1	12.1	11.7
Garlic oil	*F. semitectum*	21.7	19.8	9.5	5.3	*F. semitectum*	19.0	17.0	12.5	10.0
Bio Cure F		29.6	25.3	9.6	7.0		19.3	18.3	12.8	10.8
H_2O_2		21.0	19.0	9.0	5.0		18.7	17.0	12.2	10.0
Carbendazim 50%WP		18.0	16.1	6.5	5.0		17.0	15.3	10.1	9.5
Control		11.2	10.4	3.7	3.0		11.8	9.9	8.2	7.0
Mean		20.3	18.1	7.7	5.1		17.2	15.5	11.2	9.5
Garlic oil	*F. solani*	22.0	20.3	7.3	6.1	*F. solani*	19.5	18.1	9.6	9.0
Bio Cure F		27.0	24.3	9.6	8.1		20.5	18.3	9.6	9.0
H_2O_2		21.5	19.0	7.0	6.0		19.0	17.4	9.3	8.9
Carbendazim 50%WP		19.0	18.1	6.3	6.0		18.0	17.0	9.5	8.7
Control		9.9	7.7	3.3	2.6		9.8	8.4	5.3	4.9
Mean		19.9	17.9	6.7	5.8		17.4	15.8	8.7	8.1
Garlic oil	*M. phaseolina*	18.1	17.3	6.0	3.0	*M. phaseolina*	16.5	14.7	7.1	6.3
Bio Cure F		19.9	18.0	6.7	3.7		18.0	16.0	9.0	7.7
H_2O_2		18.0	17.1	6.0	3.0		16.0	14.0	7.0	6.0
Carbendazim 50%WP		17.0	15.0	5.7	2.3		16.5	15.0	8.0	7.3
Control		9.7	8.3	3.2	3.0		11.0	9.7	6.0	5.0
Mean		16.5	15.1	5.5	3.0		15.6	13.9	7.4	6.5
Garlic oil	*R. solani*	19.0	17.0	6.4	5.2	*P. ultimum*	17.6	15.0	8.0	5.0
Bio Cure F		31.5	30.0	10.6	8.8		19.9	16.3	8.2	5.0
H_2O_2		18.7	16.3	6.2	5.1		20.0	17.0	9.0	6.0
Carbendazim 50%WP		17.0	14.9	5.9	4.1		17.7	14.0	8.1	6.3
Control		10.0	8.0	3.3	2.9		8.0	6.0	5.0	2.7
Mean		19.2	17.2	6.5	5.2		16.6	14.1	7.7	5.0
L.S.D. at 5 % for:										
Treatments (T) =		0.2	0.6	0.1	0.1		0.3	0.5	0.3	0.2
Fungi (F) =		0.2	0.2	0.1	0.4		0.2	0.1	0.1	0.1
(F) × (T) =		0.3	0.7	0.2	0.5		0.4	0.5	0.4	0.3

* Rooted cuttings; ** Unrooted cuttings

CONCLUSIONS:

The present investigation evaluated efficacy of some fungicides alternative agents in management root rot and wilt diseases of two *Artemisia* species. Carbendazim (fungicide) and H_2O_2 completely prevented infection of *A. santonicum* by using rooted and unrooted cuttings in case of *F. oxysporum* and *A. absinthium* in case of *P. ultimum*. On the other hand, Bio-Cure F and garlic essential oil were the superior treatments in improving plant growth parameters and Bio-Cure F and H_2O_2 were the superior treatments in improving essential oil yield of both *Artemisia* species. Results of the present study recommend the possibility of using Bio-Cure F (biocide), H_2O_2 and garlic essential oil as alternative fungicide agents against root rot and wilt diseases of *A. absinthium* and *A. santonicum*.

AUTHOR CONTRIBUTIONS

Hassanin M.M.H. conceived and designed the experiments and collected the isolates; Hassanin M.M.H. and Halawa A.E.A. performed the experiments. Hassanin M.M.H.; Nada M.G.A. and Halawa A.E.A. carried out the data analysis, discussed the study and wrote the article. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest exists.

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