



Thyme (*Thymus vulgaris* L.) Root Rot/Wilt in Minia Governorate, Egypt

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Authors' contributions

This work was carried out in collaboration among all authors. Author MHI designed the study. Author MRAL wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors MMNS and HMAA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAHR/2020/v5i430060

Editor(s):

(1) Dr. Paola A. Deligios, University of Sassari, Italy.

Reviewers:

(1) Douira Allal, Ibn Tofail University, Morocco.

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(3) M. H. Niranjana, Davangere University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57047>

Original Research Article

Received 16 March 2020

Accepted 24 May 2020

Published 04 June 2020

ABSTRACT

Thyme (*Thymus vulgaris* L.) is a medicine and aromatic plant, have a great economic important due to the great income that it provides and other diversified usages. Damping-off, root rot and wilt symptoms were observed in field thyme grown in Maghagha and Beni Mazar districts, Minia Governorate, Egypt, during November to March, 2016-2017. Survey, sampling and pathogenicity tests were carried out. *Fusarium semitectum*, *F. solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* were the dominant pathogens causing thyme damping off, root rot and wilt. The inhibitory effect of two resistance inducer substances (salicylic and ascorbic acids) three fungicides (Cure M 72% WP, Rizolex Ex-T 50% WP and Rolex 50% WP) on the growth of the most pathogenic fungi *in vitro* was investigated. Salicylic acid inhibited the growth of *F. semitectum* and *R. solani* completely at 200ppm but the growth of *M. phaseolina* was completely inhibited at 400ppm. The complete inhibition for the growth of all tested pathogens was occurred at 400 ppm Ascorbic acid. *F. semitectum* and *R. solani* were more sensitive to Rizolex T, while *M. phaseolina* was more sensitive to Cure-M, (causing the lowest diameter averages The results of the present

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investigation showed the possibility to use certain antioxidants as fungicides alternatives to control plant pathogenic fungi. According to the available literature, this is a first study on thyme root rot/wilt in Egypt.

Keywords: *Thyme*; *F. semitectum*; *M. phaseolina*; *R. solani*; *Rizolex*; *Cure 50*; *antioxidants*; *rolex*.

1. INTRODUCTION

Medicinal and aromatic plants are considered to be important crops in the world. They have a great economic importance as they occupy an export priority in the first rank. These plants have an especial importance among the other traditional crops in Middle and Upper Egypt, especially Minia, Fayoum and Bani-suef Governorates, Egypt.

Thyme (*Thymus vulgaris* L.) is an aromatic perennial herb with potential uses, which can be explored for safe and effective for human benefit [1], belonging to the *Lamiaceae* family. It is native to Europe and the Mediterranean basin and adaptable to a wide range of environmental conditions [2]. Thyme is an aromatic medicinal plant of increasing economic importance in Europe, Asia, North Africa and North America. Essential oil of thyme has been reported to be one of top 10 of essential oils [1]. This herbal plant was used for many purposes as has a great importance in the pharmaceutical industry, food uses and in antimicrobial drug products, at has many advantageously uses and numerous health benefits [3]. Thyme was well known in Egypt since the ancient Egyptians. The area has cultivated with thyme in Minia Governorate at 2019-2020 reached to 309.25 hectares produce about 17425 tons of fresh foliage (Ministry of Agriculture, Department of statistics, 2019).

The oil of thyme was reported to have antimicrobial (bacteria and fungi) [4], expectorant [5] activities, most of which are mediated by thymol and carvacrol. Antispasmodic [6] as well as antioxidant [7] activities were also reported for the alcoholic extract of thyme. Thyme oil was used to control several plant pathogenic fungi, i.e. *Phytophthora* spp. [8,9], *Ascochyta rabiei*, *Fusarium* spp., *Penicillium* spp. and *Colletotrichum* spp. [10]. Also, the fungitoxicity of aqueous extracts of thyme and other three medicinal plants was evaluated *in vitro* on development of *Alternaria alternata*, *Colletotrichum graminicola*, *Rhizoctonia solani* and *Sclerotium rolfsii* [11], who found that the extract of thyme inhibited the growth of *C. graminicola* by 97% and inhibited the growth of

other tested fungi with different degrees of inhibition. Thyme is subjected to attack by several pathogens, i.e. *Alternaria* leaf spot (caused by *Alternaria brassicicola*, *Botrytis* grey mold, *Rhizoctonia* seedling damping off, root rot and powdery mildew [3,12]. Some serious diseases were reported on thyme in certain reports to cause ashy stem rot (charcoal rot) caused by *Macrophomina phaseolina* which considered as one of fungal diseases infected cacti plants [13] as well as medicinal coleus (*Coleus forskohlii*) which firstly reported by Kamalakannan [14] causing rotting of the root, basal stem and peeling of stem bark and root epidermis. Thyme and other herb species belonging to the same family were exposed to infect with soil borne fungi causing rotted, yellowish brown to black roots and underground stems, root rot [15]. Rust, fungal leaf spots; blight, and root rots on thyme were noted by Ellis and Bradley [16].

Controlling plant diseases mainly depend on fungicide applications [17]. However, fungicidal applications widespread lead to development of pathogen resistance genes, increase environmental harmful and increase the accumulation of toxic substances in human food chain. On the other hand, using alternative disease control agents, e.g. ornamental and medical plant extracts or oils, biological control, antioxidant substances and agricultural practices, are not enough to obtain efficient results [18,19]. Therefore, eco-friendly approach treatments for management plant diseases are required [20, 21]. Among fungicides tested against *Macrophomina* charcoal rot on soybean, cotton and other crops, thiram [22] and tolclofos-methyl [23,24] increased the percentage of surviving seedlings emerged from *Macrophomina*-infected seeds. Thiram suppressed pre-emergence damping-off caused by *Fusarium oxysporum* on greenhouse-grown seedlings of Douglas-fir [25], they reported that Thiram reduced post-emergence damping-off, but not to a statistically significant level. Rizolex Ex-T 50WP is a preventative contact action, wettable powder fungicide, it was recommended for the control of *Rhizoctonia* damping off on seedlings of ornamental flowering [26], vegetable, bean and

potatoes, [27] and field crops(cotton) as well as for controlling *Corticium rolfsii* on chinchinchee bulbs and others. Saad et al. [28] reported that Rizolex Ex-T caused the complete inhibition for *A. solani* and *F. solani* mycelial growth at 200 ppm, while at 100 ppm the level of inhibition reached 95.6%,

The infection with root rot/wilt of thyme was raised in the last few years in Minia Governorate. This research aimed to survey this disease, to isolate the pathogen(s) associated with root rot/wilt, and to evaluate the inhibitory effect of some fungicides alternatives on the growth of the isolated pathogen(s) *in vitro*. The evaluated antioxidants were ascorbic and salicylic acids. Three chemical fungicides; Cure-M72%, Rizolex Ex-T 50% WP and Rolex 50% WP, were also evaluated.

2. MATERIALS AND METHODS

2.1 Survey for the Incidence and Severity of Root Rot/Wilt of Thyme in Minia Governorate, Egypt

Root rot/wilt survey of thyme was carried out in two villages, Abbad Sharouna and El Shekh Zyad, belonging to Maghagha district and a village, Al Gindiah which belonging to Beni Mazar district, Minia Governorate, where thyme is widely spread. Survey was conducted during November to March, 2016 - 2017 season in major thyme growing villages. Based on the information, in each village, two fields were selected at random, cultivated with two years old thyme plants. In each surveyed field, 100 plants were selected at five locations, four corners of the field and one at the center to record the incidence and severity of plants suffered from wilt, premature vine decline, or root rot. Percentages of disease incidence were calculated by using the following formula:

$$\text{Percent disease Incidence (DI, \%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity percent of root rot/wilt was assessed according to Rowe [29] using a rating scale of 0 to 5 on the basis of root discoloration or leaf yellowing as follows: 0, neither root discoloration nor leaf yellowing; 1, 1~25% root discoloration or one leaf yellowed; 2, 26~50% root discoloration or more than one leaf yellowed; 3, 51~75% root discoloration plus one leaf wilted; 4, up to 76% root discoloration or more than one leaf wilted; and 5, completely dead plant. For

each replicate, a disease severity index (DSI) similar to that one described by Campbell and Neher [30] was calculated as follows:

$$\text{Disease severity index (DSI)} = \frac{\sum d}{d_{\max} \times n} \times 100$$

Where,

d is the disease rating possible, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

2.2 Sampling, Isolation and Identification

Natural field root-rotted/ wilted plants, 2 years-old, of thyme collected from surveyed fields in Maghagha and Beni Mazar districts, Minia Governorate, during November 2016 to March 2017, were used for isolating the pathogen(s) associated with diseased symptoms. Portions of thyme rotted roots and based stems of wilted plants were surface sterilized by dipping in mercuric chloride solution (0.1%) for 2 minutes and washed several times in sterilized distilled water, then were cut into small pieces 2-5 mm long. Two pieces of sterilized portions were transferred onto sterilized potato dextrose agar (PDA) medium containing penicillin (40 units/plate). The inoculated plates were incubated at 25±2°C for 6 days. The developed fungal growth was sub-cultured on PDA medium. The isolated fungi were purified using hyphal tip [31] or single-spore [32] techniques. Test tubes (10ml) containing inoculated PDA slants were kept in refrigerator at 4°C as stock cultures for further studies.

The established fungi isolated were identified on the basis of morphological and microscopically characteristics according to Gilman [33], Booth [34], Barnett and Hunter [35] and Ismail et al. [36] and was further verified by Division of Fungal Taxonomy, Plant Pathology Research Institute, Agriculture Research Center, Giza. Egypt.

Frequency of fungi isolated from diseased thyme samples was made according to the following equation:

$$\text{Percent of frequency} = \frac{\text{No. of isolates/each fungus}}{\text{Total number of isolates}} \times 100$$

2.3 Pathogenicity Test

Pathogenicity test with the most frequented isolated fungi (*Fusarium semitectum*, *Rhizoctonia solani* and *Macrophomina phaseolina*) was carried out under the greenhouse conditions of Sets Research Station, Beni-Suif, ARC, using

thyme (Balady cv.) either seeds or 90 days - old transplants were purchased from private Farm in Abbad Sharona, Maghagha district, Minia Governorate. Seeds were sown in September while transplants were cultivated at two dates; either November 2016 or March 2017. Seeds or transplants were planted in clay pots (30 cm. in diameter) containing sterilized Nile loamy-clay soil (5kg/pot) previously infested, individually or in mixture, with the different three tested fungi.

Pots and soil sterilization was carried out (15 days before soil infestation) by autoclaving the soil for two hours at two kg/cm³ pressure but the pots were dipped in 5% formalin solution for 5 minutes, then soil and pots were aerated for 15 days before being infected. Inocula of the isolated fungi were prepared separately on sterilized barley grains (150 gm grains +200 ml water/500 ml Erlenmeyer flask). Inoculated flasks were kept at 25±2°C for 15 days then used for soil infestation. Soil infestation was applied 7 days before planting, by thoroughly mixing 2% grams of inoculum, representing a barley culture of one fungus or a mixture of equal portions from the barley cultures of the three tested fungi in combination, with the soil in pot. The infested soil was irrigated daily till planting. Thyme transplants (5-6 cm tall) were taken from 2 years old healthy and vigorous plants. The basal portions of thyme transplants or seeds were surface sterilized (to exclude the probability of accidental infection with saprophytic rotted bacteria or fungi by dipping in 2% sodium hypochlorite solution for two minutes then washed with several changes of sterilized water before being planted in tested pots.

Three replicates (pots), each containing ten or three surface disinfected seeds or transplants,

respectively, were used in each treatment. Sterilized and uninoculated barley medium was used in the check treatment. The pots were watered when necessary. Plants were regularly examined for disease symptoms, but final results were recorded 50 days after seed sowing for seedlings damping-off and 90 days after transplanting by recording the number of diseased plants (disease incidence) and disease severity percent of root rot/wilt as described before in survey experiment.

Re-isolation was carried out from diseased seedlings and rotted root plants to satisfy Koch's postulates.

2.4 Effect of Antioxidants and Fungicides on the Linear Growth of the Tested Pathogens

These experiments were performed under Laboratory conditions in Plant Pathology Department, Faculty of Agriculture, Minia University.

2.4.1 Preparation of the tested compounds

Two antioxidant compounds, i.e., salicylic acid (C₇H₆O₃) and ascorbic acid (C₆H₈O₆), Sigma Chemical Company, Deisenhofen, Germany and three fungicides, i.e., Cure-M 72% WP, Rizolex-Ex-T 50% WP and Rolex 50% WP (Table 1), at concentrations of 50, 100, 200 and 400 ppm, were evaluated for their effects against the linear growth of thyme root rot/wilt pathogens under laboratory condition using poisoned food technique [37].

Table 1. Trade name, active ingredient and manufacturer of the used fungicides

Trade name	Active ingredient (IUPAC name)	Manufacturer
Rizolex Ex-T 50% WP	% 30 Thiram + % 20 Tolclofos methyl Thiram: Dimethylcarbathioylsulphanyl N,N-Tolclofos methyl: 2,6-dichloro-4-methylphenol	Sumitomo Chemical Turkey Kimya SAN. ve Tic. A.Ş.
Cure-M 72% WP	Metalaxyl 8% + Mancozeb 64% (Metalaxyl): methyl-N-(2-methoxyacetyl) -N-(2-6, xylyl)-DL-alaninate (Mancozeb): Manganese ethylene bis (dithiocarbamate) (polymeric) complex with zinc salt.	Agrochem, Egypt
Rolex 50% WP	Metalaxil (150 g) Cupper oxi-chloride (350 g)	

2.4.2 Preparation of agar medium incorporated with the tested compounds

Czapek' Dox agar medium was prepared and poured in 250 ml flasks at rate of 158 ml medium then autoclaved and the concentrations of the tested compounds were mixed with the medium. Five flasks for each compound (one flask for each concentration and one for the control treatment) were prepared. Media before solidification were poured in sterilized Petri dishes (9 cm diameter), one flask poured in 16 dishes (four dishes for each fungus). Plates were inoculated with 5 mm fungal discs of each tested fungus and incubated at $25 \pm 2^\circ\text{C}$. Czapek' Dox agar medium free of compounds were inoculated and used as control. The linear growth (mm) of each fungus was measured, 7 days after inoculation, when the full growth of tested fungi was observed in a check treatment the radial growth (mm) of mycelium was measured. All the experiments were carried out in triplicates and percent reduction of mycelial growth over control (percent of growth inhibition, GI, %) was calculated according the following formula:

$$\text{Percent decrease over control (DI, \%)} = \frac{D_c - D_t}{D_c} \times 100$$

Where,

D_c = Average diameter of fungal growth in control treatment.

D_t = Average diameter of fungal growth in fungicidal treatment

2.5 Statistical Analysis

All experiment was set up in a complete randomized design. Two-way ANOVA was used to analyze differences between antagonistic inhibitor effect and linear growth of pathogenic fungi *in vitro*. Data of all experiments were analyzed by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat. Significance between means was tested by "F" test and the value of LSD ($p=0.05$) was calculated [38].

3. RESULTS

3.1 Survey for the Incidence and Severity of Seedling Damping off and Root Rot of Thyme in Minia Governorate, Egypt

Root rot/ wilt survey of thyme was carried out in two villages, Abbad Sharouna and Elskehk Zyad, belonging to Maghagha district and a village, Al Gindiah which belonging to Beni Mazar district, Minia Governorate during 2016 – 2017 season.

Data in Table 2 show that thyme root rot/wilt is distributed in different fields under experiment. The percentages of diseased plants ranged between 12.1% and 15.2. The highest number of infected plants was showed in Elskehk Zyad village. The disease severity ranged between 5 - 7%. The highest severity of infected plants (7%) was recorded in Elskehk Zyad village, whereas in the other two villages, the percent of disease severity was 5%. Most of the diseased plants were suffered from early wilt symptoms, including weakened growth and yellowing, whereas a few number of them, in of the fields which were visited, were severe from wilt symptoms.

3.2 Isolation and Identification of Fungi Associated with Infected Thyme Root Rotted/Wilted Plants

Twenty six isolates of fungi, belonging to 10 different species, i.e., five isolates of *Fusarium semitectum*, two of *F. solani*, *Fusarium* sp. (three isolates), *Rhizoctonia solani* (five isolates), *Macrophomina phaseolina* (four isolates), two isolates of each of *Mucor* sp. and *Rhizopus stolonifer* and one isolate of either *Alternaria* sp., *Aspergillus flavus*, or *Sclerotium* sp. (Tables 3 and 4). The most dominant genus was *Fusarium* which presented the highest frequency (38.46%). *Fusarium semitectum* and *R. solani* were the dominant isolated fungi (19.23%, each), followed by *M. phaseolina* (15.38%).

Table 2. Root rot/wilt incidence and severity in two years-old thyme plants in Minia Governorate during 2016 -2017

Villages	Infected plants (%)	Disease severity (%)
AbbadSharouna	12.1	5
ElskehkZyad	15.2	7
Al Gindiah	13.3	5
Mean	13.53	5.6

Table 3. Fungi isolated from diseased plants collected from different locations from Minia Governorates

Fungi	No. of isolates	Locality of sample		Frequency (%)
		District	Village	
<i>Fusarium semitectum</i>	5	Maghagha	Abbad Sharona	19.23 ⁽¹⁾
<i>F. solani</i>	2	Maghagha	Abbad Sharona	7.69
<i>Fusarium</i> sp.	2	Maghagha	Elshekh Zyad	7.69
<i>Rhizoctonia solani</i>	5	BeniMazar	Al Gindiah	19.23
<i>Macrophomina phaseolina</i>	3	BeniMazar	Al Gindiah	11.53
<i>Mucor</i> sp.	2	Maghagha	Elshekh Zyad	7.69
<i>M. phaseolina</i>	1	Maghagha	Elshekh Zyad	3.85
<i>Fusarium</i> sp.	1	BeniMazar	Al Gindiah	3.85
<i>Aspergillus flavus</i>	1	BeniMazar	Al Gindiah	3.85
<i>Rhizopus stolonifer</i>	2	BeniMazar	Al Gindiah	7.69
<i>Sclerotium</i> sp.	1	Maghagha	Abbad Sharona	3.85
<i>Alternaria</i> sp.	1	Maghagha	Abbad Sharona	3.85

⁽¹⁾Each figure represents the percentage of isolates in relative to the whole isolated fungi

Table 4. The percent of different isolated fungal genera according to locations

Location (Village)	Percent of different genera of isolated fungi				Total percent of isolated fungi
	<i>Fusarium</i>	<i>Rhizoctonia</i>	<i>Macrophomina</i>	Others	
Abbad Sharouna	26.91	-	-	7.70	34.61
Elskekh Zyad	7.69	-	3.85	7.69	19.23
Al Gindiah	3.85	19.23	11.53	11.45	46.06
Total	38.45	19.23	15.38	26.84	99.91

The highest frequency of fungi was isolated from Al Gindiah village (46.15%), followed by Abbad Sharona village (34.62%) and El Shekh Zyad village (19.23%) (Table 4). *Fusarium semitectum* and *F. solani* were isolated from infected plants collected from Abbad Sharona (Maghagha district), *Rhizoctonia solani* was isolated from Al Gindiah (Bani Mazar), whereas isolates of *Macrophomina phaseolina* was collected from both Al Gindiah (3 isolates) and Elshekh Zyad (one isolate).

3.3 Pathogenicity Test

Data in Table 5 indicated that *Fusarium semitectum* caused the highest percentage (73.3%) of thymes seedling damping off when sowed by seeds in September followed by *R. solani* (50.0%) and *M. phaseolina* (33.3%). The mixture of the three pathogens caused 46.7% damped-off seedlings. Also, the highest seedling damping off severity was occurred by *F. semitectum* (36.0%), followed by *R. solani* (21.7%) then *M. phaseolina* (14.9%), whereas, 22.5% disease severity was occurred by the mixture of the three fungi. When transplants were planted, the percentages of infection caused by *F. semitectum* and *R. solani* were decreased to 51.9 and 22.2%, respectively. No significant

differences were recorded in disease incidence caused by *Macrophomina phaseolina* (33.3% DI) comparing with seed sowing (33.3% DI), while disease severity was reduced (12.7%) when transplanting than seed sowing (14.9%). In general, disease severity was reduced when thyme was cultivated by transplants (12.7 - 21.6%) if compared to seed sowing (14.9 - 36.0%). This indicated that transplants were more tolerant to infection than seedlings initiated from seeds. The infection caused by the mixture of the three tested fungi was decreased from 46.7 to 40.7% and severity from 22.5 to 19.2% when transplants were used for cultivation. The percent of survival plants ranged between 49.0 and 78.0% by using transplants in comparing by seed sowing (26.7 and 66.7%).

Data presented in Table 6 showed that transplanting thyme in April led to reduce root rot/wilt disease caused by all tested fungi in comparing with that transplanted in November. The percent of disease incidence caused by *F. semitectum*, *R. solani* and *M. phaseolina* were 40.7, 18.5 and 25.0%, respectively. Also, disease severity was decreased to 16.4, 8.2 and 11.8% (Table 6) when compared with that cultivated in November (21.6, 11.9 and 12.7%

Table 5. Pathogenicity test, 50 and 90 days after seed sowing and transplanting, respectively

Fungi	Seed sowing in September, 2016; seedling damping-off ⁽¹⁾			Transplants sowing in November, 2016 ⁽²⁾		
	Incidence of seedling damping-off, (%)	Disease severity (%)	Survival plants (%)	Disease incidence (%)	Disease severity (%)	Survival plants (%)
<i>F. semitectum</i>	73.3a ⁽³⁾	36.0a	26.7	51.9a	21.6a	49.0
<i>R. solani</i>	50.0ab	21.6b	50	22.2c	11.9b	78.0
<i>M. phaseolina</i>	33.3b	14.9c	66.7	33.3bc	12.6b	67.8
Mixture of the three fungi	46.7ab	22.5b	53.4	40.7ab	19.2a	60.0
Control	0.0c	0.0d	100.0	0.0d	0.0c	100.0

⁽¹⁾Seedling damping off was recorded 50 days after seed sowing, ⁽²⁾Root rot / Wilt (%) was recorded 90 DAT(days after transplanting), ⁽³⁾ Each figure represents the average of three replicates each contains 10 seeds or 3 transplants

(Table 5) respectively. The percent of survival plants was increased in plants transplanted in April (ranged between 59.3 and 81.5%) if compared with that transplanted in November (49.0 –78.0%).

3.4 Applying Some Antioxidant and Fungicide Compounds to Inhibit the Fungal Linear Growth *in vitro*

3.4.1 The antifungal activity of ascorbic acid and salicylic *in vitro*

Czapek' Dox agar medium supplemented with four different concentrations of Ascorbic acid or Salicylic acid was inoculated separately with the root rot/wilt pathogens of thyme, *F. semitectum*, *R. solani* or *M. phaseolina*. Fungal growth diameters (mm) were measured, the growth inhibition (%) was then estimated for each chemical concentration.

Data presented in Table 7 indicated that both tested compounds reduced the growth of all tested thyme root rot/wilt pathogens. Salicylic acid was the most effective compound in this respect, causing complete inhibition at 200 ppm for *F. semitectum* and *R. solani*, and at 400 ppm

for *M. phaseolina* followed by Ascorbic acid.

The inhibitory effects of antioxidant compounds were ranged from 5.56 - 100%, 3.33 – 100% and 21.11 - 100% for Ascorbic acid and between 2.22 - 100%, 10.0 – 100% and 13.33 to 100%, for Salicylic acid against *F. semitectum*, *R. solani* and *M. phaseolina*, respectively. The percentages of reduction at 100 and 200 ppm of Salicylic acid were higher than Ascorbic acid at the same concentrations. The pathogen growth reduced significantly as the concentrations of the two compounds were increased. The lowest average of linear growth was achieved by Salicylic acid (46.8, 42.6 and 44.8 mm for *F. semitectum*, *R. solani* and *M. phaseolina*, respectively) compared to ascorbic acid (54.4, 57.8 and 47.7 mm, in the same arrange, Table 7).

Significant differences were shown in the average of linear growth values between the evaluated two tested antioxidants (Table 7). The highest averages of linear growth were achieved by Ascorbic acid (54.4, 57.8 and 47.4 mm) followed by Salicylic acid (46.8, 42.6 and 44.8 mm) for *F. semitectum*, *R. solani* and *M. phaseolina*, respectively.

Table 6. Pathogenicity test, 90 days after transplanting in April 2017

Fungi	Disease incidence (%)	Disease severity (%)	Survival plants (%)
<i>F. semitectum</i>	40.7a ⁽¹⁾	16.4a	59.3
<i>R. solani</i>	18.5b	8.2b	81.5
<i>M. phaseolina</i>	25.0b	11.8b	75.0
Mixture of the three fungi	37.0a	9.7b	63.0
Control (free of the fungus)	0.0c	0.0c	100

⁽¹⁾ Each figure represents an average of three replicates each consisting 3 transplants

Table 7. Linear and inhibition growth of thyme root rot/wilt pathogens due to antioxidant compounds application

Compounds	Concen. (ppm)	<i>F. semitectum</i>		<i>R. solani</i>		<i>M. phaseolina</i>	
		Linear growth (mm)	Inhibition (%)	Linear growth (mm)	Inhibition (%)	Linear growth (mm)	Inhibition (%)
Ascorbic acid	0	90 ⁽¹⁾ a	0.0	90a	0	90a	0
	50	86a	5.56	87a	3.33	71b	21.11
	100	53b	31.11	60c	33.33	49c	45.56
	200	43c	52.22	52d	42.22	27f	70.0
	400	0.0d	100	00f	100	00h	100
	mean	54.4A ⁽²⁾		57.8A		47.4A	
Salicylic acid	0	90a	0.0	90a	0	90a	0.0
	50	88a	2.22	81b	10.0	78b	13.33
	100	56b	37.67	42e	53.33	37d	58.89
	200	00d	100	00f	100	17g	78.89
	400	00d	100	00f	100	00h	100
	mean	46.8B		42.6B		44.8B	

¹⁾ Data presented the average of four replicates, ²⁾ Values followed by the same letter(s) within each column don't differ significantly

3.4.2 The effect of some fungicides on the mycelial growth of thyme root rot/wilt pathogens

The effect of three fungicides (namely, Cure –M 72%, Rezolex- Ex-T 50% and Rolex 50%) on the mycelial linear growth of *Fusarium semitectum*,

Rhizoctonia solani and *Macrophomina phaseolina* was tested in laboratory. Data presented in Table 8 showed that all tested fungicide concentrations caused significant reduction on the mycelial growth of the three tested fungi when comparing with the control treatment.

Table 8. Linear and inhibition growth of thyme root rot/wilt pathogens due to fungicides application

Compounds	Conc. (ppm)	<i>F. semitectum</i>		<i>R. solani</i>		<i>M. phaseolina</i>	
		Linear growth (mm)	Inhibition (%)	Linear growth (mm)	Inhibition (%)	Linear growth (mm)	Inhibition (%)
Cure-M 72%,	0	90 ⁽¹⁾ a ⁽²⁾	0	90a	0	90a	0
	50	75c	16.7	80b	11.11	72c	20.0
	100	60d	36.0	66d	26.67	53f	41.11
	200	42e	53.3	32g	64.44	27h	70.0
	400	27f	70.0	00i	100.0	00i	100
	mean	58.8 A		53.6B		48.4B	
Rizolex-Ex-T 50%	0	90a	0	90a	0.0	90a	0.0
	50	82b	8.89	76c	15.56	70c	22.22
	100	40e	55.56	52e	42.22	59e	34.44
	200	20g	77.8	33g	63.33	42g	53.33
	400	00h	100.0	00i	100.0	00i	100.0
	mean	47.8B		61.2A		52.2A	
Rolex 50%	0	90a	0	90a	0.0	90a	0.0
	50	75c	16.7	81b	10.0	79b	12.22
	100	43e	52.2	68d	24.44	64d	28.89
	200	31f	65.6	43f	52.22	31h	65.56
	400	00h	100	24h	73.33	00i	100.0
	mean	46.4B		50.2C		52.8A	

¹⁾ Data presented the average of four replicates, ²⁾ Values followed by the same letter(s) within each column don't differ significantly

Table 9. Average diameter for treatment (cm)

Compounds	Concentration (ppm)	<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>
		Average diameter for treatment (mm)	Average diameter for treatment (mm)	Average diameter for treatment (mm)
Cure-M 72%,	0	90	90	90
	50 - 400	51.0 ⁽¹⁾	44.5	38.0
Rizolex-Ex-T 50%	0	90	90	90
	50 - 400	35.5	40.3	42.8
Rolex 50%	0	90	90	90
	50 - 400	37.3	54	43.5

⁽¹⁾Data presented the averages of four treatments; i.e. 16 replicates

The fungitoxic effect of all tested compounds increased with increasing the fungicide concentration up to the level that induced complete inhibition. Except the fungicides Cure-M and Rolex against *F. semitectum* and *R. solani*, respectively, all tested fungicides caused complete inhibition for fungal growth at 400 ppm. Fungicides Cure-M and Rolex at the maximum concentration tested (400 ppm) caused 70.0 and 73.33% reduction to the growth of *F. semitectum* and *R. solani*, respectively.

Data in Table 9 indicated that *F. semitectum* and *R. solani* were more sensitive to Rizolex T (causing average for colony diameters 35.5 and 40.3 mm, respectively), while *M. phaseolina* was more sensitive to Cure-M, causing 38% average growth diameter, when compared with other compounds. Rolex was the least affected one against all the tested three fungi.

4. DISCUSSION

Seedling damping-off or wilt is one of the first problems of the season in the home gardens or field crops. Also, root rot is a serious disease to agriculture worldwide, continuously decreasing quality and quantity of yields and jeopardizing crop survival. Depending on the causal pathogen, susceptibility of the host and the environmental conditions, entire fields can be lost due to infection with these diseases.

Thyme root rot/wilt was surveyed in three districts belonging to Minia Governorate, Egypt. Data indicated that this disease is distributed in different fields of these districts, ranging between 12.1 and 15.2% and disease severity ranged between 5 -7%. Elshekh Zyad village, belonging to Maghagha district, showed the highest percentage of infection (15.2%) and severity 7%, followed by Abbad Sharouna (Maghagha district) and Al Gindiah (Bani Mazar district). Observation

showed that most of the diseased plants suffer from early wilt symptoms, including weakened growth and yellowing, whereas a few number of diseased plants were severe from wilt.

Twenty six isolates of fungi, belonging to 10 different species, i.e., five isolates of *Fusarium semitectum*, two isolates of *F. solani*, *Fusarium* sp. (three isolates), *Rhizoctonia solani* (five isolates), *Macrophomina phaseolina* (four isolates), two isolates of each of *Mucor* sp. and *Rhizopus stolonifer* and one isolate of either *Alternaria* sp., *Aspergillus flavus*, or *Sclerotium* sp. *Fusarium semitectum* and *Rhizoctonia solani* were the dominant isolated fungi (19.23% each), followed by *Macrophomina phaseolina* (11.53%). The most dominant genus was *Fusarium* which presented the highest frequency (38.45%), followed by *Rhizoctonia solani* (19.23%) and *Macrophomina phaseolina* (15.38%). The highest frequency of fungi was isolated from Al Gindiah village (46.15%), followed by Abbad Sharona village (34.62 %), whereas El Shekh Zyad village (19.23%) was the latest. *Fusarium semitectum* and *F. solani* were isolated from infected plants collected from Abbad Sharona (Maghagha district), *Rhizoctonia solani* was isolated from Al Gindiah (Bani Mazar), whereas isolates of *Macrophomina phaseolina* was collected from both Al Gindiah (3 isolates) and Elshekh Zyad (one isolate).

Pathogenicity test proved that *F. semitectum* caused the highest percentage (73.3% disease incidence and 32.5% disease severity) of thymes seedling damping off when sowed by seeds in September, followed by *R. solani* (50.0% DI and 21.7% DS) and *M. phaseolina* (33.3% DI and 14.1% DS). The mixture of the three pathogens caused 46.6% damped-off seedlings and disease severity was 22.5%. The decrease on the percent of infected plants with *M. phaseolina* in comparing with mixture of fungi may be due to

competition for nutrients or due to secretion of one of them some antimicrobial growth toxic substances. When transplants were planted, the percentage of infection caused by *F. semitectum* and *R. solani* was decreased to 51.0 and 22.0%, and disease severity ranged between 12.0 – 21.6%. Also, seed sowing in November caused high percent of disease incidence and severity than transplanting in April, in addition to the percent of survival plants was the lowest. So, this data indicate that thyme cultivation using transplants at April is the best comparing with sowing seeds at November, that may be due to provide suitable environmental conditions for thyme planting. These data are in agree with that obtained by several authors [3,12-16], who reported that *R. solani* and *M. phaseolina* caused damping-off, root rot and charcoal rot on thyme seedlings. The physiological conditions of the stock plants [39], time and place of cutting collection [40] and environmental conditions [41, 42] significantly affect the efficiency of transplanting propagation of many woody and ornamental plants. Koné et al. [43] reported that the planting date is an important factor which should be taken into account when developing an integrated strategy to fight infectious diseases.

Management of the fungal pathogens has been studied applying different strategies under the laboratory, field and greenhouse conditions [44]. The most affected method to avoid soil-borne pathogens is to using fungicides which could be harmful to soil, water, living organisms, environment and reduce useful soil microorganisms [45]. Therefore, management strategies is pointed to alternative applications to fungal diseases control, which can play an important role in integrated pest control systems to reduce the depending on fungicides [46]. Antioxidant compounds are frequently mentioned to use as resistance inducers due to it is safety applications, to reduce incidence and severity of many disease plants, including soil borne pathogens [47,48].

Salicylic acid, Ascorbic acid, Citric acid and other organic acids are known for many years ago for their antimicrobial properties which have been widely used in foodstuff industries and agricultural practices [49,50]. Seed-borne and soil-borne fungal diseases may be controlled using the antioxidant compounds [51,52].

In the present investigation, both Salicylic and Ascorbic acids (as free radical scavenging

compounds) reduced the growth of *F. semitectum*, *R. solani* and *M. phaseolina*, thyme root rot/wilt pathogen agents, *in vitro*. The pathogen growth reduced significantly as the concentration of the two compounds was increased. Salicylic acid was the utmost effective compound in this respect, causing complete inhibition at 200 ppm for *F. semitectum* and *R. solani* and at 400 ppm for *M. phaseolina*, followed by Ascorbic acid. The percentages of reduction at 100 and 200 ppm of Salicylic acid showed antifungal activity higher than Ascorbic acid at the same concentrations. The lowest average of linear growth for different pathogens tested was achieved by Salicylic acid when comparing with Ascorbic acid application.

The efficacy of Salicylic acid (SA) as antifungal agent against various plant pathogenic fungi has been explained by different mechanisms in many studies. Ismail et al. [53] indicated that among some phenolic compounds, salicylic acid had an inhibitory effect on germ tube length and number of spore germination of *F. oxysporum* f. sp. *lycopersici* and *Aspergillus fumigatus*. In addition, Dwivedi [54] found that the growth of *Pythium aphanidermatum* was completely inhibited at 200 μgml^{-1} of SA. The growth of *Fusarium solani* was completely inhibited at 200 ppm [28], they added that the toxicity of SA increased as the concentration of the compound increased in the medium. Salicylic acid has been identified as a natural signal that triggers resistance responses in certain plants [55] and it has been implicated as an endogenous signal substance in systemic acquired resistance.

The fungicide seed, transplant and soil treatments are simple and cheap applications for reducing root rot/wilt diseases and are widely used in different crops. Several fungicides have been recommended for these applications. Our investigation revealed that the fungitoxic effect of fungicides Cure –M 72%, Rizolex- Ex-T 50% and Rolex 50% increased with increasing the fungicide concentration up to the level that induced complete fungal growth inhibition. Except the fungicides Cure-M and Rolex against *F. semitectum* and *R. solani*, respectively, all tested fungicides caused complete inhibition for fungal growth at 400 ppm. While, Cure–M and Rolex, at 400 ppm, the maximum concentration tested, caused 70 and 73% reduction of the linear growth of *F. semitectum* and *R. solani*, respectively. *Fusarium semitectum* and *R. solani* showed more sensitive to Rizolex T (causing average for colony diameters 35.5 and 40.3 mm,

respectively, while *M. phaseolina* was higher sensitive to Cure-M, causing 38% average growth diameter. Rolex was the least affective against all tested three fungi. Similar results were obtained by several researchers. Metalaxyl (one of the active ingredient compounds of Cure; 8%, and Rolex; 30%), has been on the market for over 30 years to control *Oomycetes*, especially *Pythium* spp. and *Phytophthora sojae* on different vegetables and ornamental plants [56]. The authoress also reported that seed treatments with metalaxyl and ethaboxam could help protect against stand loss associated with *Oomycetes* seedling diseases. In USA, Metalaxyl and azoxystrobin with other 12 active ingredient substances were recommended to control ornamental and cut-flower plants [57]. The fungicidal action of Cure-M 72% (Ridomil), Rizolex- Ex-T 50% and Rolex 50% was evaluated by several investigators [57-59] who reported that these compounds have wide range of activities against fungal and bacterial pathogens belonging to different microorganism classes. Also, among the tested fungicides, metalaxyl-M + mancozeb (Cure M), mancozeb alone, and copper oxychloride inhibited all tested strains of pathogenic bacteria at 1000 ppm *in vitro* [59], while *in vivo* experiment they found that only copper oxychloride show protective activity against the studied diseases, this may mean that the antibacterial properties of the other fungicides, i.e., metalaxyl-M + mancozeb (Cure M), mancozeb alone, did not correspond with their activity on the plant organs used in the *in vivo* experiment. Cure-M 72% contains a systemic, benzenoid fungicide (metalaxyl) and a contact fungicide (mancozeb) and ensures double protection effects. Metalaxyl belongs to Group D Phenylamide – Acylamine fungicide, which disrupts nucleic acids synthesis. Mancozeb, a dithiocarbamate fungicide, is a multisite protective fungicide, inhibits spore germination and remains on the leaf surface and interferes with six different biochemical processes within fungal pathogen cell.

Rizolex Ex-T 50WP is a protective contact action, wettable powder fungicide, was recommended for the control of *Rhizoctonia* seedling damping off on ornamental, flowering, vegetable (bean and potatoes) and field (cotton) crops as well as for controlling *Corticium rolfsii* on chinchinchee bulbs. Thiram is used as a seed protectant, to protect fruit, vegetable, ornamental and turf crops from a variety of fungal diseases. Thiram has insecticidal and herbicidal effects. The mechanism of action associated with the

pesticidal activity of the dithiocarbamates is the inhibition of metal-dependent and sulfhydryl enzyme systems in fungi, bacteria, plants, insects and mammals [60].

5. CONCLUSION

Thyme root rot/wilt had been shown in different districts in Minia governorate, Egypt. Recently, the distribution of this disease increased annually, that may be due to thyme re-cultivation in the same fields for long time. Ascorbic and Salicylic acids and fungicides under investigation proved to be inhibit the growth of thyme root rot/wilt causing pathogens under laboratory condition.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Eman W.R. Ghebrael (Researcher in Pl. Pathol. Res. Institute, ARC, Giza, Egypt) for her helpful advice on pathogenicity test and identification of the isolated fungi. The authors also would like to thank the anonymous reviewers for their insightful suggestions and careful reading of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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