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ORIGINAL PAPER

Evaluation the Activities of Wood Vinegar and some Commercial Bio-Products in Management of Soil-Borne Fungi of Two Tagetes Varieties

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ABSTRACT

In Egypt, ornamental and medicinal marigold (*Tagetes minuta*) and (*Tagetes erecta*) plants suffer high yield losses due to damping off and root rot diseases. During surveys conducted in Beni-Suef governorate in 2021 and 2022 seasons, these important diseases were observed. The most frequently occurring fungi in isolation trials from diseased samples were *Alternaria alternata* (Fr.) Keissler., *Fusarium oxysporum* Schlecht., *Fusarium semitectum* Berk. & Rav., *Fusarium solani* (Mart.) Sacc., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary and the fungal-like organism, *Pythium splendens* Braun. *Fusarium oxysporum* was more virulent than the other fungi in pathogenicity tests on the two tested Tagetes varieties, *i.e.*, *Minuta* and *Erecta*. The effect of Bio-Cure B, Bio-Cure F, Rhizo-N, Topsin-M 70, Vitavax 200 and wood vinegar (WV) on the two tested Tagetes varieties and the incidence of damping off and root rot diseases was determined under *in vitro* and *in vivo*. These controlling agents, however, varied in their positive efficiency against diseases. The most effective treatments for controlling these diseases and enhancing plant development parameters were fungicides followed by wood vinegar (WV). All tested treatments significantly reduced the percentages of infection and increased growth parameters in field experiments compared to control treatment.

Keywords: Tagetes, *Tagetes erecta*, *Tagetes minuta*, Wood vinegar, Bio-fungicides, Damping-off, Root-rot

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INTRODUCTION

One of the main ornamental plants in the Asteraceae family is Tagetes (*Tagetes erecta* L.). It is one of the important crops cultivated for commercial purposes as an ornamental crop. The large, beautiful flowers produced by Tagetes range in hue from yellow to orange, and they are scented (Bussmann *et al*. 2020).

The Asteraceae family of herbaceous plants includes the ornamental *Tagetes* spp. A native of Southern South America, *Tagetes minuta* is used as a condiment, a refreshing drink, and for medical purposes (Sadia *et al*. 2013).

In Tengchong City, Yunnan province, China, marigold (*Tagetes erecta* L.) root rot significantly reduced crop yields in 2018. The basement of the marigold stem became water-soaked about a month after seedling emergence, and over time it turned brown and black, occasionally with some burgundy and yellow discoloration. The plant was stunted and wilted. Disease incidence was about 3% in the area investigated (Manlin *et al*., 2020).

Under field conditions, the six-causal pathogenic fungi affecting Tagetes plants were isolated and identified as *Alternaria alternata, A. dianthi, Botrytis cinerea, Fusarium oxysporum, Curvularia lunata, and Sclerotinia sclerotiorum*, respectively, are responsible for leaf spot, Botrytis blight, flower bud rot, stem rot, foliage blight, white mould, foot and root rot, and other diseases. However, the cause of foot and root rot is still under study. The incidence and severity of diseases of Tagetes varied significantly among the locations. Leaf spot, Botrytis blight, foliage blight, and flower bud rot are the main diseases of Tagetes. From the seed of marigold, five fungi were isolated and identified as, *Fusarium oxysporum, Alternaria alternata, Aspergillus flavus, A. niger and Rhizopus stolonifer*. (Anannya**,** 2019).

For organic farming, wood waste recycling can take the place of chemical pesticides which also lowering the environmental risks. The inhibitory effects of wood vinegar's non-volatile and volatile components were assessed in relation to the control of root and crown rot disease of greenhouse cucumber (*Cucumis sativus* L.) brought on by *R. solani* as well as the mycelial growth of *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. Fresh mycelial discs of *R. solani* and *S. sclerotiorum* were placed on artificial media in Petri dishes and various concentrations of wood vinegar to evaluate the effects of the substance (0.75, 0.5, 0.37, 0.25, 0.125, 0.05, 0.025 and 0%). The mycelial growth of both pathogens was considerably

reduced by both the volatile and non-volatile components of wood vinegar (p 0.05). To control the diseases in cucumber plants infected with this pathogen, three concentrations of wood vinegar (0.125, 0.25, and 0.50%) greatly inhibited the mycelial growth of *R. solani*. In comparison to untreated control plants, disease severity was significantly reduced at all concentrations (= 0.05). Comparing the treated control to the wood vinegar-treated control, the pathogen's pathogenicity rate was reduced by up to 87%. (Mahin *et al*., 2015). El-Gindy (2003) found that *Trichoderma harzianum*, *T. lignorum*, and *Bacillus subtilis* considerably decreased the average diameter of *Botrytis fabae* colonies compared to the control. Fluorescent Pseudomonas an antagonistic rhizobacterium, are widely known for suppressing fungal root disease in a variety of crops, including common bean (Ahmad and Tehrani, 2009). *B. subtilis* isolates have been observed to increase plant establishment and seedling vigour in the field, control damping-off, and have inhibitory activity of soil-borne diseases. *Trichoderma* species and *Gliocladium* species, two more fungal bio-control agents, have also inhibited a variety of significant soil-borne plant diseases (Montealegre *et al*., 2005). *Rhizoctonia solani* and *Phytophthora cactorum*, two soil-borne pathogens, were successfully controlled by *T. harzianum*, which also improved plant stand and growth (Yobo *et al*., 2011).

Two years data of field research were used to identify efficient fungicides for groundnut collar rot control. In-field tests conducted on fungicides including Vitavax 200 WP carboxin 37.5 % + thiram 37.5 %), Carboxin, Ipconazole, Kodiak, Thiram, and Mancozeb, the treatment with Vitavax 200 (WP) 4.0g/kg seed reduced the disease incidence (5.16%) and the highest pod yield (1232 kg/ha), followed by ipconazole 3.8 FS (0.1mL) + Thiram 75wp (2.5g/kg seed) and Vitavax 200 wp 3.0g/kg seed (Rakholiya *et al*., 2012). The most effective fungicides for reducing graft failure were Topsin-M70 70% (WP) and Kemazed 50% (WP), followed by Billis 38% (WG), Saprol 19% (DC), Syllit 40% (SC), and Conazol 10% (EC). Bio-Zied (*Trichoderma album*), Rhizo-in (*Bacillus subtilis*), and Bio-Arc (*Bacillus megaterium*) significantly reduced the percentage of disease occurrence when used as alternatives to conventional control methods by 40.06, 26.92, and 25.18%, respectively. The results indicated that phytopathogenic fungi are among the most significant elements impacting the success of grapevine graft unions in Egypt (Abo Rehab *et al*. 2013).

The aim of this study is to assess the effectiveness of wood vinegar and some commercial bio-products in controlling damping off and root rot fungal diseases of two varieties of Tagets *in vitro* and in the greenhouse, as well as *in vivo*.

MATERIALS AND METHODS

I- Sampling and isolation from infected seeds and plant roots:

During 2021 growing season, naturally infected *T. minuta* and *T. erecta* plants with damping off and root rot diseases were collected from fields in Beni-Suef governorate. Infected parts were cut into small pieces and thoroughly washed under running water to remove any soil particles. These items were surface sterilized by soaking in sodium hypochlorite solution at a concentration of 2.5% for two minutes and washed three times with sterilized water and dried between sterilized channel sheets. Four pieces were aseptically transferred onto a potato dextrose agar (PDA) in Petri-dishes (9cm in diam.), plates were kept under observation after incubation for three to seven days at 27°C (Christensen, 1957). Using the single-spore technique and/or the hyphal tips of grown fungi were individually cut and transferred on new PDA plates, and then the fungi were identified according to their morphological and microscopic characteristics (Gilman, 1957; Domsch *et al.*, 1980; Singh, 1991 and Dhingra and Sinclair, 1995). Mycology Research and Plant Disease Survey Department, Plant Pathology Research Institute, ARC, Giza, Egypt, confirmed the identification. For thorough studies, pure cultures were placed on PDA slants and kept at low temperature (5°C). The frequency of isolated fungi was calculated according to the following formula of Abdel-Wahed (2007).

$$\textbf{Frequency \% of fungus} = \frac{\textbf{No of each isolated fungus colonies}}{\textbf{Total Number of all isolates}} \times 100$$

II- Pathogenicity studies:

All the isolated and identified fungi were used in a pathogenicity test using seeds and seedlings at Agric. Exp. Sta. Sides, Beni-Suef governorate. Inocula of the tested fungi *i.e., Alternaria alternata, F. oxysporum, F. semitectum, F. solani, Rhizoctonia solani, Sclerotinia sclerotiorum* and the fungal-like-organism, *Pythium splendens* were prepared and

incubated at 27°C except for *S. sclerotiorum*, which was incubated at 18±2°C, the examined fungi and, *P. splendens* were cultured on autoclaved sorghum grains medium (100 g. sorghum grains + 50 g. washed sand + 100 mL. distilled water) and incubated at the aforementioned temperature degree, 1% w/w of the desired inoculum was placed on the soil and thoroughly mixed with the soil of pots 30 cm except for the control treatment which was left without inoculation. The pots (30 cm) were watered one week before planting to encourage fungi colonization. Each pot was planted with 25 seeds. Four replicates were used per each particular treatment (Land *et al*., 2001).

The prepared soil (1 sand, 1 peat moss, and 1 clay, w, w, w), Clay and sand were autoclaved for 30 minutes at 121°C over the course of three days in order to sterilize them which was then left to aeration for week before being used.

At 15, 30, and 90 days after sowing, disease incidence was recorded as pre-and post-emergence damping-off, root rot and healthy survival plants, respectively.

At 90 days after planting, disease incidence and severity of Tagetes root rot were recorded. Formula of Gamliel *et al*. (1996) was used to calculate the percentages of disease incidence for root rot

Ninety days after sowing, growth parameters of plants (height, fresh and dry weights) were recorded.

$$\textbf{Disease Incidence (\%)} = \frac{\textbf{Number of infected plants}}{\textbf{Total No. of Planted seeds}} \times 100$$

The percentages of pre-, post-emergence damping-off were calculated at 15 and 30 days, respectively, as well as percentage of survived plants was calculated at the end of the experiment using the following formula (Sarhan, 2020):

$$\textbf{Pre-emergence (\%)} = \frac{\textbf{No. of non-germinated seeds}}{\textbf{Total No. of sown seeds}} \times 100$$

$$\textbf{Post-emergence (\%)} = \frac{\textbf{No. of post-emerged dead seedlings}}{\textbf{Total No. of planted seeds}} \times 100$$

$$\textbf{Survived plants (\%)} = \frac{\textbf{No. of survived plants}}{\textbf{Total No. of sown seeds}} \times 100$$

Root rot disease severity was also recorded at 90 days after planting according to our modification on the disease index of El-Sayed (2017) using the devised scale (1-5), 1 =healthy seedling, 2 = very little root rot, 3 = moderate root rot, 4 = severe root rot, 5 = complete root rot, as follows:

$$\textbf{Disease severity \%} = \frac{\textbf{Sum of (n} \times \textbf{v)}}{\textbf{5N}} \times 100$$

Where:

n = Number of infected roots in each category.

v = Numerical value of each category.

N = Total number of roots in the samples.

III- Diseases control:

1- ***In vitro:***

Effect of the tested materials on fungal growth:

The inhibitory effect of Topsin-M 70% WP, Vitavax 200, Wood vinegar, Bio-Cure B, Bio-Cure F and Rhizo-N (Table, 1) against the fungal linear growth of the three highly pathogenic fungi to Tagetes plants was carried out using the poisoned food technique. Before solidification (at 45°C), each of the tested materials was added to PDA medium at different concentrations *i.e*., 0.0, 50, 100, 150 and 200 ppm for Topsin-M 70% WP and Vitavax 200 and 0.0, 100, 200, 300, 400 and 500 ppm for Wood vinegar, Bio-Cure B, Bio-Cure F and Rhizo-N, the medium was placed into Petri dishes (9 cm in diameter). Disks of 5 mm in diameter bearing the growth of the tested fungus, each was placed in the center of PDA plate, and all were incubated at 27°C except of *S. sclerotiorum* was at 18±2°C. The plates were observed until completing the growth of control plates and recorded. Each treatment consisted of five replicate plates. Unpoisoned inoculated PDA medium was used as control. The linear growth of each fungus was recorded after 7 days - incubation. Percentage of Reduction % for each tested material was calculated according to the formula suggested by (Atwa, 2018) as follows:

$$\textbf{Reduction (\%)} = \frac{\textbf{Linear growth of control - Linear growth of treatment}}{\textbf{Linear growth of control}} \times 100$$

Effect of Wood vinegar (WV) on mycelial growth of the tested fungi:

Wood vinegar (WV), also known as pyroligneous acid, is a translucent brown liquid that is created when the smoke from the process of making charcoal condenses. The names "pyrolysis oil," "pyrolysis liquid," "wood liquid," "liquid smoke," "liquid wood," "bio-oil," "bio-crude oil," and "wood distillate" are all synonyms for "WV" (Zulkarami *et al*., 2011).

Major groups of compounds in WV include hydroxy aldehydes, hydroxy ketones, sugars, carboxylic acid and phenolic acid (Guillen and Manzanos, 2002).

Table (1): Commercial names, composition, concentrations, and the producers of the tested materials used through lab. experiments.

Commercial names	Composition	Concentration used in lab.	The Producers
Bio-Cure B	*Pseudomonas fluorescence* 1×10^{9}cells mL^{-1}	0.0, 100, 200, 300, 400, 500 ppm	Produced by El-Nasr Co. for Fertilizers & Bioproducts, Egypt.
Bio-Cure F	*Trichoderma viride* 2×10^{6}cells ml^{-1}	0.0, 100, 200, 300, 400, 500 ppm	Produced by El-Nasr Co. for Fertilizers & Bioproducts, Egypt.
Rhizo-N	*Bacillus subtilis* 3×10^{7}cells g^{-1}	0.0, 100, 200, 300, 400, 500 ppm	Produced by El-Nasr Co. for Fertilizers & Bioproducts, Egypt.
Topsin-M 70% WP	70 % thiophanate-methyl (1,2–bis (3-methoxy carbonyl-2 thiouredio) benzene)	0.0, 50, 100, 150, 200 ppm	Cerexagri-Nisso LLC, Japan
Vitavax 200 75 % WP	37.5% carboxin (5,6 –dihydro-2 methyl – 1,4 –oxathin -3-carboxanilide) +37.5% Thiram (tetramethyl thiuram disulfide)	0.0, 50, 100, 150, 200 ppm	Cerexagri-Nisso LLC, Japan
Wood vinegar (WV)	Both volatile and non-volatile components	0.0, 100, 200, 300, 400, 500 ppm	Japanese Chinese Medicine Research institute

Greenhouse trials:

At Sids Research Station, Beni-Suef governorate, Egypt, greenhouse studies were conducted to throw light on the effect of Topsin-M 70 % WP, Vitavax 200, Wood vinegar, Bio-Cure B, Bio-Cure F, and Rhizo-N on growth and production of two Tagetes varieties (Minuta and Erecta). Fungal inocula were processed as stated before under the pathogenicity experiment. Seven days prior to sowing, 4 pots filled with sterilized sandy clay soil were infested with the desired fungal inoculum. For each treatment, four pots (30 cm) with approximately 25 seeds per each were sown. The following products were used at the rate of 1 g./ kg seeds for Topsin-M 70 % WP, 3 g./ kg seeds for Vitavax 200, 4 g./ kg seeds for Rhizo-N and 4 mL/ kg seeds from each of Wood vinegar, Bio-Cure B and Bio-Cure F. Similar to the procedures outlined under the Pathogenicity Test, soil infestation was performed. The three pathogenic fungi, *F. oxysporum*, *F. solani* and *R. solani* were however, used independently. In order to achieve equal distribution, the fungicides used as seed dressing were thoroughly mixed with the seeds in plastic bags with a little amount of Arabic gum solution (5%).

In terms of disease assessments, pre- and post-emergence damping off percentages, as well as root rot diseases and plant survival were assessed accordingly after 15, 30, and 90 days from planting. At 90 days after planting, measurements of growth parameters of the tagetes plants' growth including plant height (cm) and fresh and dry weights, were made for each treatment (Sarhan, 2020).

***In vivo* experiments:**

Field experiments were carried out in two successive growing seasons, 2021 and 2022, in the fields at Sayyidna Al-Khidir village in Yusef Al-Siddiq County, Fayoum governorate, using the complete randomized complete block design trial to examine the effects of Topsin-M 70 % WP, Vitavax 200, Wood vinegar, Bio-Cure B, Bio-Cure F and Rhizo-N on soil borne diseases suppression and enhancement of growth and productivity of two Tagetes varieties (Minuta and Erecta) grown under natural infection. Tagetes seedlings 45 days old and 15 cm long were transplanted in the selected field. Seeds were obtained from the Medicinal & Aromatic Plants Res. Sta., Sides, Beni-Suef governorate, Hort. Res. Inst., Agric. Res. Center.

Tagetes transplants were dipped for 15 minutes just before transplanting in 1 g. /L water containing each of Topsin-M 70 % WP, 3 g./l water for Vitavax 200, 4 g./l water, Rhizo-N and 4 mL/l water from for each of Wood vinegar, Bio-Cure B and Bio-Cure F. Seedlings were transplanted in three replicate plots for each treatment. Each plot (4×5 m; 20 m^2) consisted of 7 rows, and 112 seedlings were transplanted in each plot, 16 in each row, at a distance of 25 cm. The soil was mechanically ploughed and

planked twice throughout each season. Calcium super-phosphate (15.5% P_2O_5) was provided as a source of phosphorus at a rate of 200 kg/fed during soil preparation for transplanting.

Seedlings 15 cm length, both treated and untreated, were placed 20 cm in between. To maintain steady development, weeds were manually removed during the growing season. Nitrogen was applied as ammonium sulphate (20.6 % N) at the recommended rate of 400 kg/fed as follows: At a rate of 75 kg/fed, potassium sulphate (48% K_2SO_4) was added as a source of potassium. The seedlings were directly transplanted after irrigation in the presence of irrigation water. Biweekly, the plants were irrigated, and all other agricultural procedures were carried out as needed.

At 90 days after transplanting, the percentage of infected plants exhibiting root rot disease symptoms was recorded. At the time of harvest, fresh weight per plant (g) and dry weight and essential oil production were also measured.

Essential oils extraction:

According to Guenther (1961), 100 g. fresh *T. minuta* leaves were hydro distilled in a Clevenger apparatus in triplicate. The proportion of essential oils was calculated. Prior to GC analysis, the essential oils were collected, dried over anhydrous sodium sulphate, and stored in a refrigerator.

Gas chromatographic analysis (GC) of the essential oils:

Using a gas chromatography device, the volatile oil sample GC analysis was completed. a gas chromatograph called the DsChrom 6200 that has a flame ionization detector, Column: BPX-5, 30m of 0.25mm ID by 0.25mm film of 5% phenyl (equiv.) polysillphenylenesiloxane. Size of sample: 1 l, Temperature programme ramps up from 70° to 200°C at a rate of 10°C per minute, Temperature of the detector (FID) 280°C, Gas for carriers nitrogen, N_2, H_2, and air flow at 30 mL per minute each. By comparing their retention periods to those of the real samples injected under the identical circumstances, the main constituents of the volatile oils were identified. The area of the peak corresponding to each compound was used to compute the relative percentage of each component (Adams, 2007).

Isolation of essential oils and gas chromatographic analysis were carried out at the Medicinal and Aromatic Plants Research Department Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Statistical analyses:

Using a computer programme, the collected data were statistically analyzed in accordance with the guidelines set forth by Gomez and Gomez (1984) (costate). L.S.D. was used to compare means at a level of probability of 5%.

RESULTS

I- Isolation, purification and identification of the isolated fungi and their frequencies:

Seven different fungal species were isolated from naturally infected *T. minuta* and *T. erecta* showing the typical symptoms of damping-off and root rot diseases collected from different locations in Beni-Suef governorate. These fungi species were identified according to their morphological characteristics in the naturally infested soil Fig. (1). Data in Table (2) reveal that seven fungal species had been isolated and recognized from the samples. The highest means of frequency, though, (%) isolated fungi from *T. minuta* and *T. erecta* were *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc. followed by *Fusarium oxysporum* Schlecht., *Sclerotinia sclerotiorum* (Lib.) de Bary and *Pythium splendens* Braun isolates and constituted 18.52, 16.77, 16.49, 14.70 and 13.17 % on the average, respectively of the total isolates recovered (203) from both *T. minuta* and *T. erecta* collected samples, Whereas *Alternaria alternata* **(**Fr.) Keissler. (7.87 %) and *Fusarium semitectum* Berk. & Rav. (12.43 %) were isolated at low frequencies.

II - Pathogenicity tests:

When the seeds were sown in the infested soil, all of the tested fungi (Table, 3) and Fig. (2) were able to infect the two tested Tagetes varieties, *Tagetes minuta* and *Tagetes erecta*, as compared to the control treatment (seeds sown) in uninfested soil), planting in the infested soil significantly increased pre- and post-emergence damping-off. With the results of healthy seedlings that survived, the opposite was true. The two Tagetes varieties showed the highest pre- and post-emergence and root rot percentages due to infection by *F. solani* and *R. solani* on *Tagetes minuta* and *Tagetes erecta* varieties, *R. solani* recorded 52, 33, 11 and 56, 27, 10 % on *Tagetes minuta* and *Tagetes erecta* varieties, respectively.

On *Tagetes minuta* and *Tagetes erecta* varieties, *F. oxysporum* recorded 12, 59, 20 and 12, 59, 18 %, respectively. The least infection in this respect was caused by *A. alternata*. *A. alternata* recorded the lowest percentages among the most pathogenic fungi in this respect.

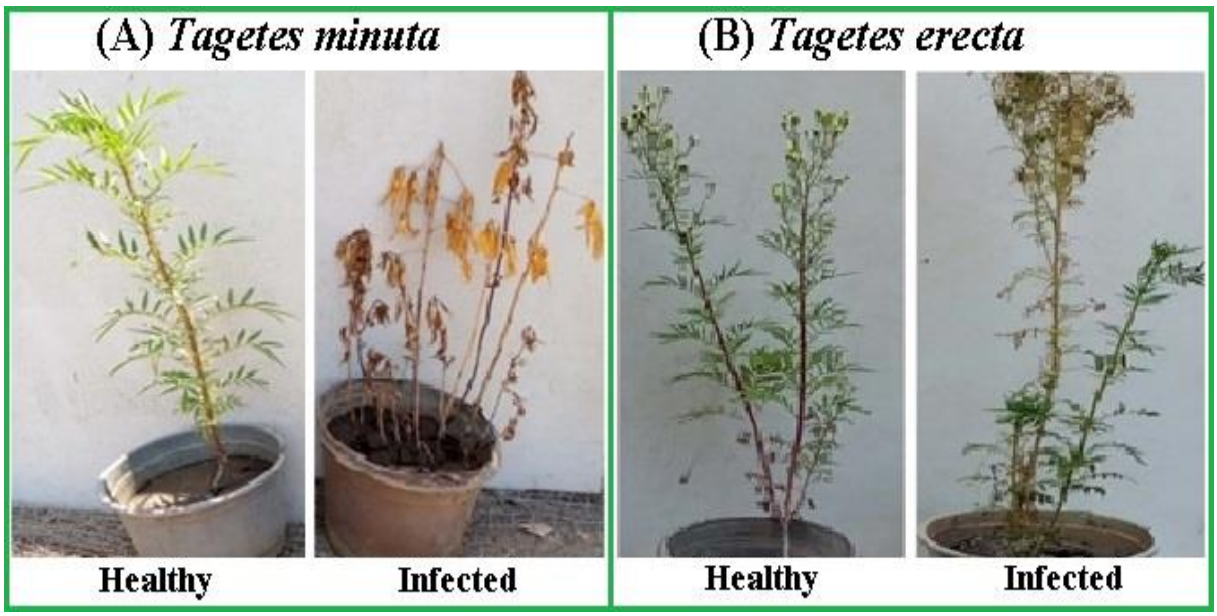

Fig. (1): Natural infection in a nursery with root rot on *T. erecta* (A) and *T. minuta* (B) showing infection by root rot on right and healthy plant on the left. The seeds were planted in pots filled with natural infested soil collected directly from fields.

Table (2): Frequency percentages of the isolated fungi from the rotted plants roots of two tagetes varieties collected from Beni-Suef governorate.

Fungi and fungal-like – organism	*Tagetes minuta*		*Tagetes erecta*		Mean of Frequency (%)
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)	
Alternaria alternata	15	7.38	20	8.36	7.87
Fusarium oxysporum	33	16.25	40	16.73	16.49
Fusarium semitectum	25	12.31	30	12.55	12.43
Fusarium solani	35	17.24	39	16.31	16.77
Pythium splendens	28	13.79	30	12.55	13.17
Rhizoctonia solani	37	18.22	45	18.82	18.52
Sclerotinia sclerotiorum	30	14.77	35	14.64	14.70
Total	203	100.00	239	100.00	100.00

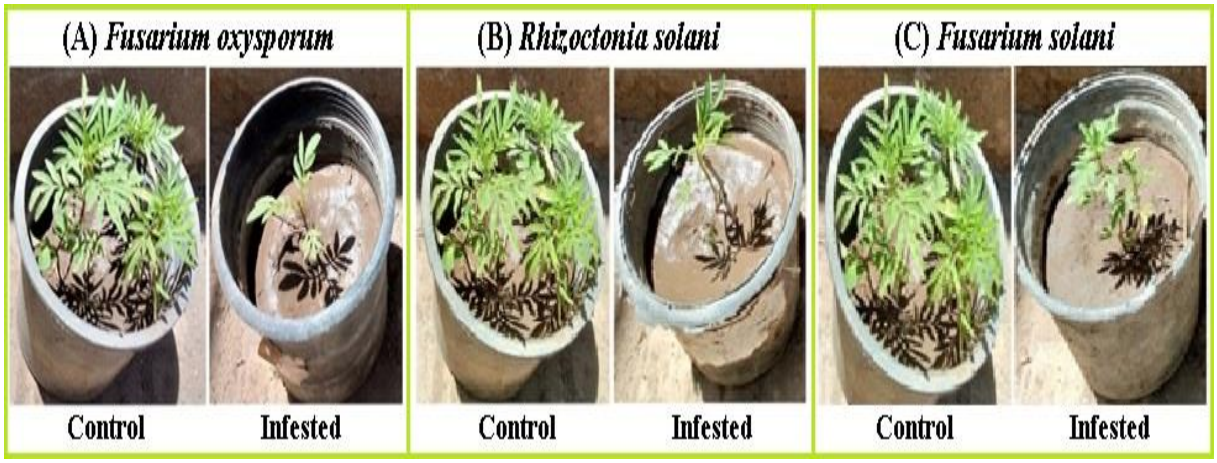

Fig. (2): *Tagetes minuta* plants (sown by seeds) were grown in soil artificially infested with the most aggressive fungi, *F. oxysporum* (A), *R. solani* (B), *F. solani* (C).

Table (3): Percentages of pre-and post-emergence damping-off, Root-rot (%) and plant survivals of two Tagetes varieties after 15, 30 and 90 days of planting, in infested soil under greenhouse conditions.

Tagetes spp.	Fungi	Pre-emergence (%)	Post-emergence (%)	Root-rot (%)	Plant Survivals (%)
Tagetes minuta	*A. alternata*	8	12	15	65
	F. oxysporum	12	59	20	9
	F. semitectum	24	42	11	23
	F. solani	28	50	15	7
	P. splendens	24	31	13	32
	R. solani	52	33	11	4
	S. sclerotiorum	24	31	33	12
	Control (uninfested soil)	0.0	0.0	0.0	100.0
	Mean	20.5	30.4	14.7	31.5
Tagetes erecta	*A. alternata*	12	18	13	57
	F. oxysporum	12	59	18	11
	F. semitectum	20	30	10	40
	F. solani	20	50	20	10
	P. splendens	24	31	15	30
	R. solani	56	27	10	7
	S. sclerotiorum	28	27	29	16
	Control (uninfested soil)	0.0	0.0	0.0	100
	Mean	21.5	25.0	14.3	33.8
LSD at 0.05 %:	Fungi (F) =	0.34	4.10	4.30	6.43
	Varieties (V) =	0.23	0.54	0.450	0.78
	F×V =	3.21	8.67	8.20	9.98

All of the tested fungi were pathogenic to Tagetes plants when the seedlings were transplanted in the infested soil. Data tabulated in Table (4) show significant increase by root rot and disease severity caused by the tested fungi to the control. The highest percentages of infection and disease severity were obtained by *R. solani* in *Tagetes minuta* variety, as they were 80.0 and 57.0 %, respectively. In contrast, the percentages of root rot and disease severity in case of *Tagetes erecta* variety were 80.0 and 59.0 %, by the same fungus. *A. alternata*, on the other hand, was the least aggressive in this respect for *Tagetes minuta* variety (20% root rot and 15% disease severity), whereas the same fungus caused 20 % root rot incidence and 12 % disease severity in case of *Tagetes erecta* variety.

Presented data in Table (5) show that all tested fungi significantly decreased plant height (cm), fresh weight (g), and dry weight (g) of the tested Tagetes plants compared to those of the control treatment. The effect of the tested fungi on the growth parameters of Tagetes plants was determined 90 days after transplanting the two tested varieties. *F. oxysporum*, *F. solani* and *R. solani* generally had the highest detrimental effects on growth parameters respectively. The least aggressive fungus in this respect was *A. alternata*.

III-Disease control

1. *In vitro*:

1.1. Effect of fungicides on fungal growth:

According to data shown in Table (6), both fungicides tested reduced the mycelial growth of the three tested fungi, namely *F. oxysporum*, *F. solani* and *R. solani* the causals of damping-off and root rot on *Tagetes minuta* and *Tagetes erecta*, in comparison to the control treatment. With increasing concentration, the fungicides' inhibitory activity was increased significantly at 200 ppm concentration, Vitavax/Thiram was significantly the most effective fungicide against mycelial growth; it completely inhibited the growth of the three tested fungi and completely prevented their growth at the concentration of 200 ppm.

1.2. Effect of Bio-Cure B and Bio-Cure F on the linear growth (cm) of three pathogenic fungi to Tagetes plants:

According to data shown in Table (7), Bio-Cure B and Bio-Cure F significantly reduced the mycelial growth of the three tested fungi. The concentrations of Bio-Cure B and Bio-Cure F significantly increased the inhibitory activity of such treatments. 500 ppm concentration, Bio-Cure F completely inhibited the mycelial growth of the three tested fungi, making it significantly the most effective.

1.3. Effect of Rhizo-N and vinegar (WV) on the linear growth (cm) of the three pathogenic fungi to Tagetes plants:

Data shown in Table (8) indicate that, in comparison to the control treatment, both Rhizo-N and vinegar (WV) significantly inhibited the mycelial growth of the three tested pathogenic fungi to the tested Tagetes varieties, The inhibitory effect of both Rhizo-N and vinegar (WV) was significantly increased as their concentrations increased. The concentration of 500 ppm vinegar (WV) was significantly the most effective in inhibiting the mycelial growth where it completely inhibited the growth of all the three tested fungi.

Table (4): Root-rot incidence and disease severity (%) on plants of two Tagetes varieties raised from seedlings transplanted in infested soil after 90 days, under greenhouse conditions.

Tagetes spp.	Fungi and fungal-like – organism	Root-rot (%)	Disease severity (%)
Tagetes minuta	*A. alternate*	20	15
	F. oxysporum	60	55
	F. semitectum	20	17
	F. solani	40	33
	P. splendens	20	12
	R. solani	80	57
	S. sclerotiorum	20	18
	Control (uninfested soil)	0.0	0.0
	Mean	32.5	25.8
Tagetes erecta	*A. alternate*	20	12
	F. oxysporum	60	52
	F. semitectum	40	28
	F. solani	40	35
	P. splendens	20	13
	R. solani	80	59
	S. sclerotiorum	20	11
	Control (uninfested soil)	0.0	0.0
	Mean	35.0	26.2
L.S.D.at 5%	Fungi (F) =	5.40	6.30
	Varieties (V) =	0.60	0.40
	F×V=	10.30	12.40

Table (5): Effect of the tested pathogenic fungi on some growth parameters of two Tagetes varieties, 90 days after transplanting in infested soil, under greenhouse conditions.

Tagetes spp.	Fungi and fungal-like – organism	Plant height (cm)	Fresh weight/ plant (g)	Dry weight/plant (g)
Tagetes minuta	*A. alternata*	40.0	100.0	32.3
	F. oxysporum	15.2	44.6	13.6
	F. semitectum	37.6	60.6	19.2
	F. solani	23.8	55.4	17.4
	P. splendens	33.6	57.3	18.4
	R. solani	20.7	45.9	16.5
	S. sclerotiorum	25.7	62.3	20.2
	Control (uninfested soil)	50.0	120	43.0
	Mean	30.4	68.2	22.5
Tagetes erecta	*A. alternate*	35.0	95.0	29,7
	F. oxysporum	13.4	40.4	10.6
	F. semitectum	30.8	54.8	15.5
	F. solani	20.9	50.7	12.5
	P. splendens	30.8	50.9	11.6
	R. solani	16.3	42.6	9.4
	S. sclerotiorum	22.3	55.8	22.6
	Control (uninfested soil)	52.0	125	45.9
	Mean	27.6	64.4	19.7
LSD at 0.05 %:	Fungi (F) =	5.40	4.70	4.80
	Varieties (V) =	8.60	0.30	0.60
	F×V=	15.40	11.80	10.0

2. Greenhouse experiments:

Under greenhouse conditions, controlling of pre- and post-emergence damping-off caused by the most aggressive fungi (*F. oxysporum*, *F. solani*, and *R. solani*) on both tagetes varieties was assessed using various control treatments with Bio-Cure B, Bio-Cure F, Rhizo-N, Topsin-M 70, Vitavax 200, and vinegar (WV). Results in Table (9) show that, in general, all tested treatments, significantly reduced pre- and post-emergence damping-off caused by any of the three tested fungi and increased values of healthy survived plants in comparison to control (untreated seeds). Vitavax 200 was much better course of action in this case.

Data in Table (10) and Figs (3 and 4) show that all treatments increased the growth parameters of the two tagetes varieties plants in a significant way when compared to the other tested treatments, Vitavax 200 was the most effective on increasing growth parameters. The least successful treatment in this respect was Bio-Cure B.

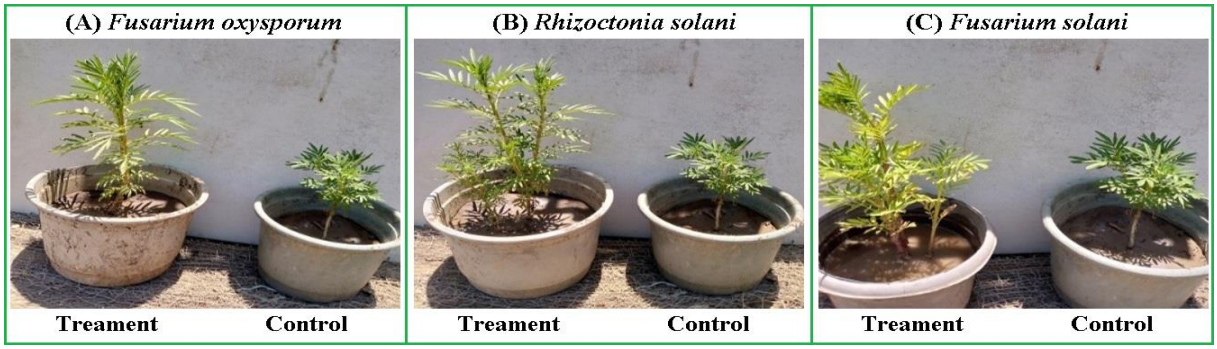

Fig. (3): Effect of Wood vinegar (WV) treatment on infection by *F. oxysporum* (A), *R. solani* (B), *F. solani* (C) on *T. erecta* plants under greenhouse conditions. Control plants on the right.

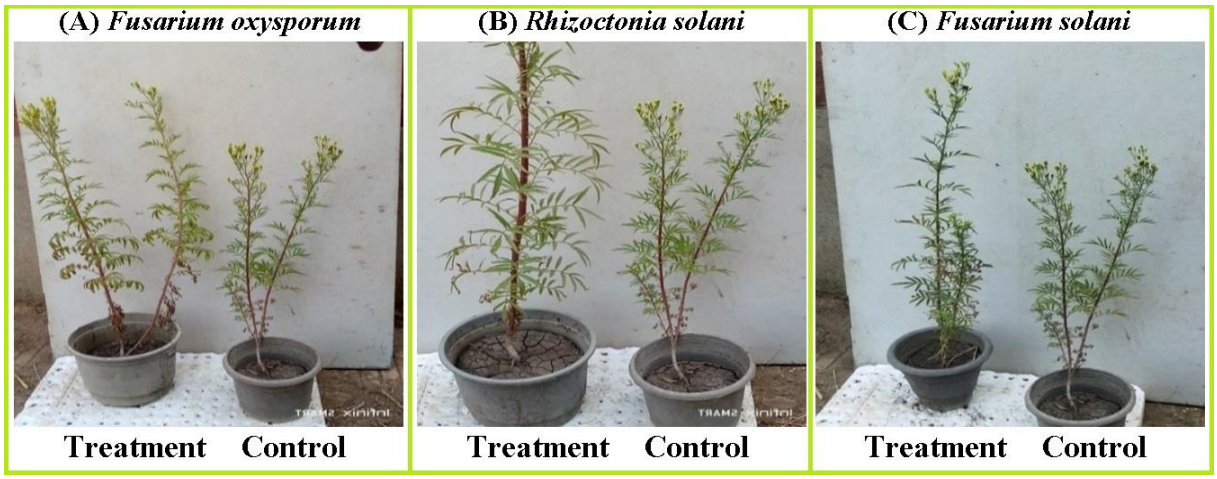

Fig. (4): Effect of Wood vinegar (WV) treatment on infection by *F. oxysporum* (A), *R. solani* (B), *F. solani* (C) on *T. minuta* plants under greenhouse conditions. Control plants on the right.

3. *In vivo* studies:

3.1. Effect of various control measures on the infection by root rot diseases of two tagetes varieties grown under naturally infested field conditions in 2021 and 2022 growing seasons:

This experiment was carried out under naturally infested field conditions using transplanting of two Tagetes varieties. Data, Table (11) and Fig (5), show the effect of using each of Bio-Cure B, Bio-Cure F, Rhizo-N, Topsin-M 70, Vitavax 200, and vinegar (WV) on the incidence of root rot of two tagetes vars. in the field during 2021 and 2022 growing seasons. The obtained data show that these treatments significantly decreased the infection percentages and increased fresh weight, dry weight, and essential oil. The highest level of disease decrease was noticed with Topsin-M 70 and Vitavax 200 when compared to the control treatment. Moreover, Vitavax 200 had the highest effect in reducing root rot over the course of the two growing seasons. All treatments tested reduced the infection percentages on *Tagetes minuta* and *Tagetes erecta*, in comparison to the control treatment. The least effective treatment in this regard was Bio-Cure B.

Fig. (5): Effect of Wood vinegar (WV) (B), Vitavax 200 (C) and Topsin-M (D), treatments on root rot infection on *T. minuta* plants grown under naturally infested field conditions, Control plants (A), 2022 growing season.

3.2. Effect of various control measures on fresh weight /plant (g) of two tagetes varieties grown in naturally infested field conditions in 2021 and 2022 growing seasons:

Data presented in table (11) show increases in fresh weight (g) to mean 1028.6 (g) and 981.4 (g), respectively for *Tagetes minuta*, in seasons 2021 and 2022. The increases for *Tagetes erecta* variety were 1090 (g) and 1018.6 (g) in seasons 2021 and 2022, respectively.

3.3. Effect of various control measures on dry weight /plant (g) of two tagetes varieties grown in naturally infested field conditions in 2021 and 2022 growing seasons:

In regard to dry weight parameters (Table 11) it was found that most of control treatments tested, in the case of *Tagetes minuta* variety, significantly increased dry weight as compared to the control treatment either in season 2021 or 2022. The *Tagetes erecta* variety showed something similar during the two growing seasons of 2021 and 2022.

3.4. Effect of various control measures on essential oil (mL) /100g fresh herb of two tagetes varieties plants grown in naturally infested field conditions in 2021 and 2022 growing seasons:

Most of the measured essential oils were significantly generated during control treatments than during check treatments. In seasons 2021 and 2022, *Tagetes minuta* variety's mean essential oil (mL)/100g fresh herb mean values were 0.14 and 0.13 (mL), respectively during the two growing seasons of 2021 and 2022, while means of the corresponding figures for *Tagetes erecta* variety were 0.15 and 0.14 (mL) Table (11).

Essential oils constituents:

The relative proportion of the various components in an essential oil determines its quality. Data in Table (12) show that different treatments had an impact on the key constituents' content and enhanced the essential oil's primary chemical compositions in comparison to the untreated control. Twenty components were found through gas chromatographic examination of the volatile oil constituents from the essential oil of *T. minuta* plants. A comparison between composition of *T. minuta* oil shows that plants which raised from seeds treated with wood vinegar and Vitavax 200 gave the highest content of Tagetone (16.23 & 16.02%) and β -ocimene (34.78 & 34.99 %), respectively, followed by the essential oil Dihydrotagetone, being 10.88 & 10.99 %, respectively.

Table (6): *In vitro* effect of two fungicides and their four concentrations on the linear growth of three pathogenic fungi isolated from two Tagetes varieties on PDA medium, incubated at 27°C for 7 days.

Varieties	Con. ppm	Linear growth, in cm													
		Topsin-M							Vitavax / Thiram						
		F. oxysporum	Reduction %	*F. solani*	Reduction %	*R. solani*	Reduction %	*Mean*	*F. oxysporum*	Reduction %	*F. solani*	Reduction %	*R. solani*	Reduction %	Mean
Tagetes minuta	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0
	50	4.0	55.5	5.0	44.4	4.2	53.3	4.4	3.5	61.1	3.0	66.6	3.6	60	3.4
	100	2.2	75.5	3.4	62.2	3.0	66.6	2.6	2.5	72.2	2.1	76.6	2.5	72.2	2.4
	150	0.5	94.4	2.5	72.2	2.1	76.6	1.7	0.4	95.5	0.2	97.7	0.5	94.4	0.4
	200	0.0	100	1.3	85.5	0.3	96.6	0.5	0.0	100	0.0	100	0.0	100	0.0
	Mean	3.1	65.0	4.2	52.9	3.7	58.6	3.6	3.0	65.8	2.9	68.2	3.1	65.3	3.0
Tagetes erecta	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0
	50	3.0	66.6	4.0	55.5	4.0	55.5	3.6	3.6	60	3.5	61.1	3.5	61.1	3.5
	100	2.0	77.7	3.0	65.8	2.5	72.2	2.5	2.7	70	2.5	72.2	2.2	75.5	2.5
	150	0.2	97.7	2.0	77.7	2.0	77.7	1.4	0.5	94.4	0,5	94.4	0.4	95.5	0.5
	200	0.0	100	1.1	87.7	0.1	98.8	.4	0.0	100	0.0	100	0.0	100	0.03
	Mean	2.8	68.8	3.8	57.7	3.5	61.1	3.4	3.2	64.4	3.1	65.0	3.0	65.8	3.1
L.S.D.at 5 %		Fungi (F) = 0.31, Concentrations (C) = 0.40, Treatments (T) =0.30, F×C = 0.52, F×T = 0.60, C×T = 0.43, F×C×T= 1.20													

Table (7): *In vitro* effect of five concentrations of Bio-Cure B and Bio-Cure F on the linear growth of three pathogenic fungi isolated from two Tagetes varieties on PDA medium, incubated at 27°C for 7 days.

Varieties	Con. ppm	Linear growth, in cm													
		Bio-Cure B							Bio-Cure F						
		F. oxysporum	Reduction %	*F. solani*	Reduction %	*R. solani*	Reduction %	*Mean*	*F. oxysporum*	Reduction %	*F. solani*	Reduction %	*R. solani*	Reduction %	Mean
Tagetes minuta	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	9.0	0.0	9,0	0.0	9,0	0.0	9.0
	100	6.3	30	7.5	16.6	6.5	27.7	6.8	7.7	14.4	6.6	26.6	7.3	18.8	7.2
	200	5.6	37.7	6.0	33.3	5.5	38.8	5.7	6.3	30	5.4	40	6.0	33.3	5.9
	300	3.9	56.6	4.4	51.1	4.8	46.6	4.4	4.2	53.3	4.6	48.8	5.0	44.4	4.6
	400	2.2	75.5	3.4	62.2	3.1	65.5	2.9	3.6	60	3.9	56.6	4.2	53.3	3.9
	500	1.4	84.4	2.0	77.7	2.4	73.3	1.9	0.0	100	0.0	100	0.0	100	0.0
	Mean	4.7	47.7	5.4	40	5.2	42.2	5.1	5.5	38.8	5.4	40	5.0	44.4	5.3
Tagetes erecta	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0
	100	3.2	64.4	4.2	53.3	4.3	52.2	3.9	3.5	61.1	3.4	62.2	3.6	60	3.5
	200	2.5	72.2	3.2	64.4	2.4	73.3	2.7	2.5	72.2	2.4	73.3	2.3	74.4	2.4
	300	0.3	96.6	2.4	73.3	2.2	75.5	1.6	0.4	95.5	0,4	95.5	0.3	96.6	0.4
	400	0.1	98.8	1.3	85.5	0.2	97.7	0.5	0.2	97.7	0.0	100	0.0	100	0.0
	500	0.0	100	0.3	96.6	0.0	100	0.1	0.0	100	0.0	100	0.0	100	0.0
	Mean	2.5	72.2	3.4	62.2	3.0	66.6	2.9	2.6	71.1	2.5	72.2	2.5	72.2	2.5
L.S.D.at 5 %		Fungi (F) = 0.11, Concentrations (C) = 0.20, Treatments (T) =0.10, F×C = 0.22, F×T = 0.40, C×T = 0.23, F×C×T= 1.30													

Table (8): *In vitro* effect of five concentrations of Rhizo-N and wood vinegar (WV) on the linear growth of three pathogenic fungi isolated from two Tagetes varieties on PDA medium, incubated at 27°C for 7 days.

Varieties	Con. ppm	Linear growth, in cm													
		Rhizo-N							vinegar (WV)						
		F. oxysporum	Reduction %	*F. solani*	Reduction %	*R. solani*	Reduction %	*Mean*	*F. oxysporum*	Reduction %	*F. solani*	Reduction %	*R. solani*	Reduction %	Mean
Tagetes minuta	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	9.0	0.0	90	0.0	9.0	0.0	9.0
	100	5.4	40	4.4	51.1	5.0	44.4	4.9	4.3	52.2	5.6	37.7	4.3	52.2	4.7
	200	2.4	73.3	3.6	60	2.0	77.7	2.6	1.6	82.2	2.4	73.3	3.5	61.1	2.5
	300	1.9	78.8	2.5	72.2	1.6	82.2	2.0	0.0	100	1.2	86.6	2.6	71.1	1.2
	400	0.7	92.2	1.5	83.3	1.0	88.8	1.0	0.0	100	1.0	88.8	0.5	94.4	0.5
	500	0.0	100	0.3	96.6	0.2	97.7	0.1	0.0	100	0.0	100	0.0	100	0.0
	Mean	3.2	64.4	3.5	61.1	3.1	65.5	3.2	2.5	72.2	3.2	64.4	3.3	63.3	3.0
Tagetes erecta	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0
	100	3.5	61.1	4.2	5.3	4.5	50	4.0	3.2	64.4	3.3	63.3	3.6	60	3.4
	200	2.1	76.6	3.3	63.3	2.2	75.5	2.5	1.7	81.1	2.0	77.7	2.3	74.4	2.0
	300	0.2	97.7	2.2	75.5	1.0	88.8	1.1	0.5	94.5	0,9	90	0.9	90	0.8
	400	0.1	98.8	1.0	88.8	0.5	94.5	0.6	0.1	98.8	0.5	94.5	0.3	96.6	0.6
	500	0.0	100	0.1	98.8	0.3	96.6	0.1	0.0	100	0.0	100	0.0	100	0.0
	Mean	2.5	72.2	3.3	63.3	2.9	67.7	2.8	2.4	73.3	2.6	71.1	2.7	70	2.6
L.S.D.at 5 %		Fungi (F) = 0.20, Concentrations (C) = 0.30, Treatments (T) =0.20, F×C = 0.32, F×T = 0.50, C×T = 0.32, F×C×T= 1.00													

Table (9): Influence of different control treatments on the incidence of damping-off on two Tagetes vars. caused by, *F. oxysporum, F. solani and R. solani,* 90 days after transplanting under greenhouse conditions.

Tagetes spp.	Treatments	Percentages of infection by								
		F. oxysporum			*F. solani*			*R. solani*		
		Pre-emergence (%)	Post-emergence (%)	Plant survival (%)	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)
Tagetes minuta	Bio-Cure B	16	23	61	16	26	58	12	20	68
	Bio-Cure F	20	25	55	16	19	65	16	20	64
	Rhizo-N	12	18	70	20	23	57	12	25	63
	Topsin-M 70	8	17	74	8	18	74	8	15	77
	Vitavax 200	4	12	84	8	13	79	4	20	76
	Wood vinegar	12	13	75	12	20	68	12	17	71
	Control	20	70	10	28	54	18	24	56	20
	Mean	13.1	25.4	61.2	15.4	24.7	56.9	12.5	24.7	62.8
Tagetes erecta	Bio-Cure B	20	24	56	16	22	62	16	22	62
	Bio-Cure F	16	11	73	20	15	65	12	18	70
	Rhizo-N	12	17	71	12	19	69	12	23	65
	Topsin-M 70	8	15	77	4	16	80	8	15	77
	Vitavax 200	4	10	86	4	10	86	4	15	81
	Wood vinegar	12	17	71	12	17	71	12	12	76
	Control	28	54	18	28	55	17	24	55	21
	Mean	14.2	21.1	64.7	13.7	22	64.3	12.5	22.8	64.7
L.S.D. at 5%		Treatments (T) = 2.60, Varieties (V) = 1.40, Fungi (F) = 1.0, T × V = 3.10, T × F = 2.90, V × F = 4.0, T ×V × F =4.40								

Table (10): The effect of different control treatments on some growth parameters of two Tagetes varieties grown in infested soil 90 days after transplanting, under greenhouse conditions.

Tagetes spp.	Treatments	*F. oxysporum*			*F. solani*			*R. solani*		
		Plant height (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Fresh weight (g)	Dry weight (g)
Tagetes minuta	Bio-Cure B	60	400	130.3	55	380	125.6	50	360	115.8
	Bio-Cure F	70	460	151.2	66	450	149.4	60	455	153.6
	Rhizo-N	76	500	163.4	73	470	155.5	70	460	156.6
	Topsin-M 70	82	550	182.7	80	540	179.9	77	530	175.7
	Vitavax 200	90	600	199.5	86	590	190.5	80	580	190.8
	Wood vinegar	80	520	172.9	75	511	171.5	70	500	167.8
	Control	30	200	65.8	33	210	70.6	35	220	70.6
	Mean	69.7	461.4	152.2	66.5	450.1	149	63.1	443.6	147.3
Tagetes erecta	Bio-Cure B	65	430	135.7	59	395	130.7	54	366	122
	Bio-Cure F	73	470	155.8	71	460	155.5	63	462	164.7
	Rhizo-N	80	520	166.7	75	485	166.4	75	473	173
	Topsin-M 70	85	565	185.5	83	455	185.4	81	540	183
	Vitavax 200	94	610	210	91	610	196.2	86	595	196.6
	Wood vinegar	83	532	178.6	82	520	175.6	75	509	177
	Control	31	205	65.0	32	200	71	34	210	71.4
	Mean	73	476	156.7	70.4	446.4	154.4	66.8	450.7	155.4
L.S.D. at 5%		Treatments (T) = 2.30, Varieties (V) = 0.50, Fungi (F) = 3.30, T×V = 4.40, T×F = 5.60, V×F = 5.20, T×V×F = 6.40								

Table (11): Effect of various control measures on percentages of infection by root rot diseases and some growth parameters including fresh weight /plant (g), essential oil (mL)/100g fresh herb and dry weight /plant (g) of two tagetes varieties grown under naturally infested field condition in 2021 and 2022 growing seasons.

Tagetes spp.	Treatments	2021 growing season				2022 growing season			
		Root rot infection %	Fresh weight /plant (g)	Dry weight / plant(g)	Essential oil (mL) /100g. fresh herb	Root rot infection %	Fresh weight /plant (g)	Dry weight / plant (g)	Essential oil (mL) /100g. fresh herb
Tagetes minuta	Bio-Cure B	25	1000	320.7	0.11	26	990	320	0.10
	Bio-Cure F	27	950	315.6	0.10	29	940	312.6	0.90
	Rhizo-N	20	1100	360.6	0.15	22	1000	315.8	0.11
	Topsin-M 70	12	1200	399.5	0.20	14	1100	355.9	0.18
	Vitavax 200	8.0	1300	430.8	0.25	10	1250	400.8	0.27
	Wood vinegar	17	1150	380.8	0.18	19	1100	360.7	0.16
	Control	60	500	165.7	0.01	63	490	160.5	0.01
	Mean	24.1	1028.6	339.1	0.14	26.1	981.4	318.0	0.13
Tagetes erecta	Bio-Cure B	23	1050	350.8	0.13	24	1000	340.8	0.11
	Bio-Cure F	24	1040	330.7	0.11	25	960	320.7	0.10
	Rhizo-N	17	1200	370.9	0.17	19	1050	330.6	0.12
	Topsin-M 70	10	1250	410	0.21	11	1140	380.6	0.20
	Vitavax 200	6	1360	450.5	0.24	7	1380	420.7	0.25
	Wood vinegar	15	1220	390.6	0.19	15	1130	375.4	0.17
	Control	62	510	162.7	0.01	60	470	162.6	0.01
	Mean	22.4	1090	352.3	0.15	23	1018.6	333.0	0.14
L.S.D. at 5%	Treatments (T)=	3.10	10.50	4.20	0.012	2.80	20.60	2.60	0.013
	Varieties (V) =	0.60	10.0	3.40	0.011	1.50	13.00	6.50	0.014
	T × V =	6.30	20.50	9.50	0.013	8.60	30.70	5.60	0.015

Table (12): Effect of different tested treatments on the main components of *T. minuta* essential oil.

Compounds	Treatments						
	Wood vinegar	Bio-Cure B	Bio-Cure F	Rhizo-N	Topsin-M 70	Vitavax 200	Control
β -Pinene	4.37	3.43	2.99	3.67	3.99	4.65	2.76
β -Myrcene	3.79	2.89	2.56	3.21	3.56	3.89	1.89
Palmitic	3.77	2.98	2.67	3.34	3.76	3.78	2.23
α-Phellandrene	2.99	1.99	1.78	2.12	2.45	2.76	1.54
β-caryophyllene	3.22	2.21	1.89	2.78	2.98	3.02	0.50
Dihydrotagetone	10.88	9.99	8.45	9.23	9.76	10.99	8.0
Myrcene	3.88	2.87	2.67	3.23	3.67	3.89	2.25
Tagetone	16.23	15.21	15.98	16.78	16.99	16.02	15.8
β -ocimene	34.78	33.98	33.67	34.34	34.76	34.99	33.o
Linalool	2.72	1.67	1.23	1.87	2.32	2.87	0.78
Decanal	2.88	1.87	1.45	2.12	2.56	2.99	0.98
Nonanal	3.90	2.99	2.87	3.32	3.78	3.89	2.54
Carvone	4.22	3.32	2.98	3.76	3.88	4.21	2.65
Citral	4.86	3.87	3.21	4.01	4.56	4.87	2.99
Limonine	6.98	6.12	5.87	6.67	6.87	6.99	5.6
Hexahydrofarnesyl acetone	0.84	0.65	0.27	0.87	0.99	0.88	0.03
Germacrene B	1.88	1,99	1.87	1.87	1.99	1.76	1.2
Camphene	2.96	3.76	3.43	3.89	4.02	4.65	2.98
Estragole	2.88	1.99	1.89	2.01	2.32	2.56	1.56
Alpha-gurjunene	2.67	1.78	1.56	1.87	1.99	2.12	1.02

DISCUSSION

Several soilborne fungi can infect the studied two tagetes varieties, resulting in damping-off and root rot diseases that have been a significant problem in Egypt for the decade. However, significant and harmful diseases were earlier noted on Tagetes by Ananya (2019). Isolation trails from naturally root-rotted Tagetes plants, yielded seven fungal species, *Alternaria alternata, F. oxysporum, F. semitectum, F. solani, Rhizoctonia solani, Pythium splendens*, and *Sclerotinia sclerotiorum.* In this regard, Abdel-Wahed (2007) in Egypt isolated some of these fungi from chamomile plants showing the same disease symptoms.

Results of the pathogenicity tests on the two tagetes varieties showed that *F. oxysporum, F. solani* and *R. solani* were pathogenic and showed the typical symptoms of damping-off and root rot diseases. The highest pre- and post-emergence damping off was caused by *R. solani* and *F. oxysporum*, whereas the lowest pre- and post-emergence damping off was caused by *A. alternata*. These results are in agreement with those of Abdel-Wahed (2007).

Continuous monocropping may have a negative influence on soil fertility, microbial diversity, and crop yield (Monneveux *et al*., 2006). Management root rots of the two tagetes varieties were studied *in vitro* with the use of Bio-Cure B, Bio-Cure F, Rhizo-N, Topsin-M 70, Vitavax 200, and vinegar (WV). Emara (2005) found that the application of *Bacillus* spp. and *Rhizobium* spp. increased the plants uptake of P and the water status within its tissues, which in turn increased the plants amino acids and activated its rates and improved the action of succinic and lactic acids, which stimulated root growth. When compared to the other bio-products, Rhizo-N was the most effective agent against damping-off. A nitrogen-fixing bacterium named *Gluconacetobacte diazotrophicus* can also create phytohormones like indole acetic acid (IAA) and gibberellic acid (GA), solubilize plant macro- and micronutrients like P and Z, and acts as a bio control agent against phytopathogenic organisms (Saravanan *et al*., 2007). *Trichoderma* spp. are well known for their ability to inhibit the growth of pathogens either directly through mycoparasitism and enzyme synthesis, or indirectly through competition for resources necessary for life (Ragab *et al*., 2015). Fluorescent Pseudomonads produce a wide range of antifungal metabolites, including hydrogen cyanide pyroluteorin (Wang *et al*. 2021).

WV's anti-fungal properties have been demonstrated in a variety of plant diseases (Kadota and Niimi, 2004). While Yatagai (2004) proposesd that the antifungal effects of WV may be caused by phenolic and creosol compounds, Yodthong *et al*. (2008) suggested that the antifungal characteristics of WV may be caused by an interaction between acetic acid and phenolic compounds present in the substance. WV includes 15 macro and micro elements, the majority of which are essential to plant functions like photosynthesis, including calcium, cadmium, chromium, copper, iron, potassium, manganese, sodium, zinc, arsenic, molybdenum, phosphorus, lead, and bromine (Zulkarami *et al*. 2011). By reacting with these substances, acetic acid simultaneously exists with calcium and iron cations, forming a complex in which ionic bonds replace covalent bonds. As a result, the soil is protected against both the leaching of other useful elements and the precipitation of iron (Taiz and Zieger, 2006). For the control of *Macrophomina phaseolina* and *R. solani*, traditional fungicide, chemical treatments with integrated disease management are now believed to be only partially effective. As a result, farmers continue to be concerned about diseases (Ketta *et al*., 2021). The control techniques were tested during the growing seasons of 2021 and 2022 in naturally contaminated field soil. During the two experimental seasons, there was a considerable reduction in the incidence of root rot diseases and an increase in fresh weight/plant in all disease control experiments. However, fungicides were far superior to the competition, followed by wood vinegar and Rhizo-N. Additionally, a sizable rise in dry weight was observed as a result of all treatments.

CONCLUSIONS

The present investigation evaluated the efficacy of Wood vinegar and some commercial Bio- products agents compared to some chemical fungicide in management some soil-borne fungi of two Tagetes varieties. Results of the present study recommend the possibility of using Wood vinegar and some commercial Bio-products agents in management soil-borne fungi of two Tagetes varieties as safe biocontrol agents for controlling soil-borne fungi of two Tagetes varieties and increasing their productivity. Under nursery and/or field conditions, it can be recommended that treating Tagetes seeds and transplants with wood vinegar

and biocides are alternatives to chemical fungicides can be used commercially to control damping off and root rot diseases.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest

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