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**Evaluation of some Control Methods of Weeds** and Fungal Soil-Borne Diseases on Cumin and its **Productivity** 

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**C**from different locations of Behira, Ismailia and Minia governorates, Egypt. Many fungal species belonging to the genera Fusarium, Macrophomina, Rhizoctonia and Pythium were isolated from the infected plants. The results indicated that F. oxysporum recorded the highest frequency among the isolated fungi, followed by R. solani, F. solani, M. phaseolina and Pythium sp. The effect of various concentrations of marjoram and peppermint essential oils on the mycelial growth of the isolated fungi was tested in-vitro. Marjoram and peppermint oils completely inhibited the growth of F. oxysporum, F. solani, R. solani and Pythium sp. at the concentration 5ml/L. Field experiments were conducted at the Experi. Farm. of Agric. Res. Stat., Malawy, Minia Governorate, Egypt, during two successive seasons (2017/2018 and 2018/2019) to study the effect of different weed control treatments (hoeing, black polyethylene mulch, clover cover crop, unweeded control) on weed biomass. The results indicated that all weed control treatments significantly decreased the dry weight of grassy weeds, broad-leaved weeds and total weeds 70 days after sowing as compared to the unweeded control. Moreover, the effects of different weed control treatments and seed treatment with the biocides (Plant Guard and Rhizo-N) as well as the essential oils of peppermint and marjoram on the disease incidence, and some yield parameters of cumin plants were evaluated under field conditions. The results indicated that plastic mulch was the most effective in reducing wilt incidence as well as increasing cumin yield, while the unweeded plots had the highest disease incidence and the lowest yield. Furthermore, the application of the biocide Rhizo-N and marjoram essential oil seed dressing (5ml/L) reduced the incidence of wilt and significantly increased seed yield. Thus, it could be suggested any of the biocide Rhizo-N or marjoram essential oil can be formulated to be used for controlling cumin wilt under field

conditions. Moreover, integration of these methods could provide an approach for the management of the disease in infested fields more effectively.

Keywords: Cuminum cyminum, plastic mulch, cover crop, seed treatment, biocides, essential oils

Cumin (*Cuminum cyminum* L.) is an annual plant of the Apiaceae family. Cumin is used in folk medicine as a carminative for stomach disorders, diarrhea, and colic (Parthasarathy *et al.*, 2008).Cumin seeds are used as a spice for flavoring soups, sauces, pickles and for seasoning breads and cakes (Farrell *et al.*, 1985). The seeds contain 2-5 % essential oil, the principal constituent of which is cuminaldehyde (Duke, 2002). The cultivated area of cumin in Egypt reached about 5448 feddan during 2010 growing season and declined to 3489 and 1941 feddan during 2011 and 2012 growing seasons, respectively (Hassan *et al.*, 2014). The decline in the cultivated area with cumin during the last decade may be due to wilting the plants in the latter stages of the plant life, which affects the quantity of the seed yield.

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cumini* is a destructive disease limiting cumin productivity (Dange, 1995; Khare *et al.*, 2014 and Ghoneem *et al.*, 2019). The disease occurs at all stages of crop growth. The leaves of infected plants drop down, the roots exhibit browning of vascular region when split open and fruiting do not occur. The pathogen is internally seed borne as well as soil borne (Khare *et al.*, 2014). Root-rot of cumin is caused by six species of *Fusarium*, *i.e. F. oxysporum*, *F. solani*, *F. monileforme*, *F. dimerum*, *F. equiseti* and *F. lateritium*. In China the root rot has been reported due to *F. oxysporum*, *F. solani*, *Rhizoctonia* sp. and an unidentified Deuteromycetes fungus (Shujuan *et al.*, 2012). These diseases are usually common year after year. This may be due to inappropriate agricultural practices, lack of adequate resistance to these diseases.

Cumin is most vulnerable to weed competition at all stages of its growth due to its slow growth cycle and short stature. Chemical pesticides are the most effective means for controlling weeds and fungal diseases in cumin plants (Bhatnagar *et al.*, 2013 and Birla *et al.*, 2016). However, the excessive use of these chemicals has caused pollution to the environment and hazard effect on human health. Therefore, there is a need to develop alternative strategies for weed and disease management that reduce pesticide use in an integrated approach.

Mulching is one of the effective and safe methods to reduce pests, soil-borne diseases and weed infestation. Mulching involves covering the soil with a layer of material around the crop, which prevents light required for germination of small seeded weeds (Teasdale and Mohler, 2000). Mulches are generally inorganic (black polyethylene and geotextile mulches), organic (wood, bark, and leaves), or living (turf grass, rye, and clover) materials. Polyethylene mulch combined with marigold

intercropping and fungicide application has resulted in an effective control of early blight of tomato caused by *Alternaria solani* (Jambhulkar *et al.*, 2012).

Living mulches are cover crops that are planted between the rows of a main crop and are maintained as a living ground cover during the growing of the main crop. Although living mulches are sometimes referred to as cover crops, they grow at least part of the time simultaneously with the crop. In addition to providing adequate cover to reduce soil erosion and increase soil water infiltration, legume living mulches improve soil nutrient status through addition of organic nitrogen via fixed atmospheric nitrogen which improves soil physical properties (Mohammadi *et al.*, 2012).

Additionally, seed treatment with essential oils and bioagents such as *Trichoderma harzianum* and *Bacillus subtilis* have been realized as an effective alternative for Fusarium wilt management in cumin plants (Tawfik and Allam, 2004). Furthermore, Hashem *et al.* (2010) tested the effectiveness of some essential oils in the control of cumin root rot, of which the essential oils of cumin, basil and geranium showed the highest effectiveness in controlling the disease.

Therefore, the objective of this study was to assess the effect of black plastic mulch, clover cover crop and hoeing as weed control treatments on biomass of grassy weeds, broad-leaved weeds and total weeds. In addition, to assess the impact of weed control treatments, seed treatments with biocides and essential oils of peppermint and marjoram on the incidence of Fusarium wilt and crop parameters of cumin plants in Middle Egypt region to develop an integrated management strategy for the control of cumin wilt.

#### Materials and Methods

#### Isolation and identification of the associated fungi

Infected cumin plants showing wilt and root rot symptoms were collected from plantations of Beheira, Ismailia and Minia governorates during 2016/2017 growing season. These symptoms were yellowing, stunting, and dried shoots and wilt. Infected roots were thoroughly washed several times with tap water, then cut into small pieces and surface sterilized by immersing in 1% sodium hypochlorite for 2 minutes, washed several times with sterilized stilled water and dried between folds of sterilized filter papers. The surface sterilized pieces were then plated onto potato dextrose agar (PDA) medium in Petri-dishes. The plates were incubated at  $27\pm1^{\circ}$ C for 7 days. The growing fungi were purified using single spore or hyphal tip techniques. Fungi were identified depending on their morphological features according to the description of Booth (1971), Domsch *et al.* (1980) and Niternik and Vandler (1981) and the identification was confirmed by the staff member of Department of Mycology, Plant Disease Survey and Plant Pathology Research Institute, ARC, Giza, Egypt. Pure cultures on PDA slants were kept at low temperature (5°C) for further studies.

#### Pathogenicity test

Pathogenicity test was conducted to all the isolated soil fungi, *i.e. Fusarium* oxysporum, *F. solani, Macrophomina phaseolina, Pythium* sp. and *Rhizoctonia* solani. Sandy clay soil (1 sand: 1 clay) was sterilized by 5 % formalin solution for one week, then left to dry for two weeks before use. Tested fungi were separately cultured on autoclaved sorghum grains medium (100 g sorghum + 100 ml water), at  $27\pm1$ °C for 15 days. Soil infestation with the isolated fungi was applied at the rate of 1 % w/w, which thoroughly mixed with the upper soil surface in plastic pots (20 cm diameter). Pots were watered till saturation one week before sowing to enhance colonization of the fungi. Cumin seeds (cv. Balady) were surface sterilized using 0.1% sodium hypochlorite for one minute, then washed with sterilized distilled water and left to dry. Pots were sown with 20 seeds for each. All treatments were replicated three times. Disease incidence was recorded as percentages of pre-, post-emergence damping-off and wilt 25, 45 and 90 days after sowing, respectively using the following formula (Waller *et al.*, 2002).

Disease incidence % = 
$$\frac{\text{Number of infected plants}}{\text{Total number of planted seeds}} \times 100$$

Fungi were re-isolated from the infected plants and compared with the original isolates.

# Evaluation the effect of the essential oil emulsions on the mycelial growth of the tested fungi in vitro

Essential oils of marjoram and peppermint were obtained from Med. and Arom. Plants Res. Dept., Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. The essential oil emulsions were prepared by adding 10 ml of any of the two essential oils and 5 ml of non-ionic surfactant Tween 80 slowly under gentle stirring until a homogeneous mixture was formed. Then, sterilized water (85 ml) was added to reach the final mixture of each oil to 100 ml, to enhance distribution and complete incorporate the essential oils and then stirred using a magnetic stirrer for 30 min. The efficacy of volatile substances in reducing the fungal growth was tested. Prepared emulsion of the essential oils of marjoram and peppermint were added to sterilized PDA flasks before solidification to obtain the proposed concentrations of 1, 2, 3, 4 and 5 ml/L medium. The bactericide (Chloramphenicol, (0.1mg/L) was added to the medium to avoid bacterial contamination. Three plates (9 cm in diam.) containing potato dextrose agar (PDA) medium were separately inoculated with discs (5-mm-diam.) taken from the edge of 7 days old culture of any of Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium sp. and Rhizoctonia solani, isolated from cumin plants. Petri dishes were incubated at  $25\pm2^{\circ}C$ . Percentages of fungal growth inhibitions were calculated when the fungal growth of the control plates (without treatments) completely covered the plates according to the formula suggested by Topps and Wain (1957) as follows:

% Inhibition = 
$$\frac{A - B}{A} \times 100$$

A= The linear growth (cm) in control treatment.

B= The linear growth (cm) of treated fungus.

*Field experiments* 

Field experiments were carried out at the Experi. Farm of Agric. Res. Stat., Malawy, Minia governorate, Egypt during two successive seasons (2017/2018 and 2018/2019) to evaluate the effect of different weed control treatments (hoeing, black plastic mulch and cover crop), seed treatment with two commercial biocides (Plant Guard and Rhizo-N)and essential oil emulsions (peppermint and marjoram) on the disease incidence, and crop parameters of cumin plants. The layout of the experiment was a split plot design with three replicates for each treatment. The main plots were assigned to the weed control treatments and the sub plots were assigned to the seed treatment. The soil of the experimental site is classified as silty clay. The field was prepared and divided into plots. The plot size was  $10.5 \text{ m}^2$  ( $3.5 \text{ m} \times 3 \text{ m}$ ). Each plot consisted of five rows with 60 cm wide and 3.5 m long. Egyptian cumin seeds (Balady) were planted in hills 20 cm apart at the rate 3 seeds/hill. Seeds were planted on 1<sup>st</sup> November in both seasons in a naturally infested field with the causal of cumin wilt. The recommended dose of nitrogen was applied at a rate of 250 kg/fed using ammonium sulphate (20.5% N). Phosphorus was applied and mixed with the soil before sowing at a rate of 200 kg/fed using calcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub> P). Potassium was applied at a rate of 50 kg/fed using potassium sulphate (48% K<sub>2</sub>O).

# Treatments

#### Weed control treatments

Weed control treatments consisted of

- 1- Hoeing, three times.
- 2- Black polyethylene mulch of plastic films, 25-micron thickness was applied manually. Polyethylene sheets were covered on the rows. Holes were prepared on the plastic sheet in certain space of each plant. Cumin seeds were planted in the previously prepared holes.
- 3- Cover crop plots included clover (*Trifolium alexandrinum*) as the living mulch planted in mid-November at the rate of 30 kg clover seeds/fed. Clover seeds were planted after cumin initial growth on either side of the cumin rows. Clover plants were cut at 45 days after sowing. Clover was sown to compete with total weeds during the early stages of the growth of cumin plants and to improve soil nutrient status and physical properties, reduce soil erosion and increase soil water infiltration.

4- Un-weeded treatment as a control.

#### Seed treatment

Cumin seeds were washed several times with sterilized water and soaked for 30 minutes in the solution or suspension of:

- 1- Biocide Plant Guard (1 ml contains  $30 \times 10^6$  CFU of *Trichoderma harzianum*)
- 2- Biocide Rhizo-N (1 ml contains  $30 \times 10^6$  CFU of *Bacillus subtilis*)
- 3- Marjoram essential oil emulsion
- 4- Peppermint essential oil emulsion
- 5- Water served as a control treatment

The biocides were applied at a concentration of 4ml/L (obtained from the Central Lab. of Organic Agric., ARC), whereas essential oil emulsions were applied at a concentration of 5 ml/L. According to a previous study (Hassanin *et al.*, 2017),these concentrations had no adverse effects on seed germination. The soaked seeds were air dried at room temperature for one hour before sowing.

#### Data collection and analysis

#### a- Weeds measurements

After 70 days from sowing in both seasons, weed samples from one square meter area were randomly collected from each plot. Dry weight of broad-leaved, grassy weeds and total weeds were estimated and determined after drying in an oven at 70°C to constant weight (g).

# b- Frequency percentage of the isolated fungi

% Frequency =  $\frac{\text{No. of fungal colonies for each isolated fungus}}{\text{Total No. of fungal colonies of the isolated fungi}} \times 100$ 

#### c- Disease incidence

The incidence of cumin wilt was determined 90 days after sowing and calculated by the following formula:

Incidence of wilt (%) = 
$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

# d-Vegetative growth and yield traits

Plant samples were randomly collected from all treatments 90 days after sowing. Plant height (cm), number of branches/plant and number of umbels/plant were recorded during both seasons. At harvest, seed yield (kg) of each plot was recorded and then converted to kg/feddan.

The collected data were statistically analyzed according to the method described by Snedecor and Cochran (1980). Least Significant Difference test was used for comparison between means of treatments.

## **Results and Discussion**

Isolation and identification of the associated fungi

Fungi belonging to four fungal genera, i.e. Fusarium, Macrophomina, Rhizoctonia and Pythium were isolated from infected samples of cumin plants. Samples showing root rot and/or wilt symptoms were collected from different locations of South Tahrir district, El-kassasin and Malawy counties, representing Beheira, Ismailia and Minia governorates, respectively during 2016/2017 growing season. Isolated fungi were purified and identified as: Fusarium oxysporum Schlecht, Fusarium solani (Mart.) Appel & Wollenw, Macrophomina phaseolina (Tassi) Goid., Pythium sp. and Rhizoctonia solani Kuhn. Results in Table (1) indicate that F. oxysporum recorded the highest mean frequency of fungi isolated (39.19%), followed by R. solani (24.93%), F. solani (16.29%), M. phaseolina (13.08%) and Pythium sp. (6.51%). Only F. oxysporum was isolated from wilted plants. On the other hand, the other fungi were isolated from cumin plants suffered from root rot. F. oxysporum was the most frequent isolated fungus in the three governorates followed by F. solani for Beheira and Minia governorates and R. solani for the three governorates. Fusarium solani was not isolated from Ismailia governorate and Pythium sp. was not isolated from Beheira and Minia governorates. The widespread of these diseases seem to be function of environmental factors. Minia governorate (Malawy county) is characterized by having a clay soil texture while Beheira governorate (South Tahrir district) and Ismailia governorate (El-Kassasin county) are characterized by having a sandy soil texture. Fusarium spp. recorded the higher frequency as comparing with the other fungi isolated from the three governorates indicating their ability to survive in most soil types. Such finding goes in accordance with the data obtained by Hilal et al. (1993), Hassanin (2013) and Singh et al. (2017).

Incluted funci	%Freq	uency of fu	ngi at gover	norate	Symptoms	
Isolated lungi	Beheira	Ismailia	Minia	Mean	Symptoms	
Fusarium oxysporum	40.73	33.14	43.70	39.19	Wilt	
F. solani	25.00	00.00	23.87	16.29	Root rot	
Macrophomina phaseolina	11.29	17.75	10.19	13.08	Root rot	
Rhizoctonia solani	22.98	29.59	22.24	24.93	Root rot	
Pythium sp.	00.00	19.53	00.00	06.51	Root rot	
Total	100.00	100.00	100.00	100.00	-	

 Table (1): Fungi isolated from infected cumin plants collected from different governorates during 2016/2017 growing season and their frequency.

#### Pathogenicity test

All tested fungi (Table 2) were found to be pathogenic to cumin plants with different percentages of pre- and post-emergence damping-off. The highest percentage (40.0%) of pre-emergence damping-off was caused by *M. phaseolina* isolate No. (1). Meanwhile, *R. solani* isolate No. (1), *F. oxysporum* isolate No. (1) and *F. solani* isolate No. (1) recorded the highest percentages of post-emergence damping- off, being40.00, 33.33 and 26.67%, respectively. On the other hand, symptoms of wilt caused by *F. oxysporum* appeared at 90 days after planting. *F. oxysporum* (1) isolated from Behiera governorate recorded the highest percentage of cumin wilt in the growing cumin plants (26.67%). These results are similar to those reported by Hilal *et al.* (1993) and Nada *et al.* (2014).

	% Dam	ping-off		%
Tested fungi	Pre-	Post-	% Wilted	Survived
C	emergence	emergence	plants	plants
Fusarium oxysporum (1)	5.00	33.33	26.67	35.00
Fusarium oxysporum (2)	3.33	11.67	25.00	60.00
Fusarium oxysporum (3)	5.00	18.33	23.33	53.34
F. solani (1)	23.33	26.67	0.00	50.00
F. solani (3)	13.33	11.67	0.00	75.00
Macrophomina phaseolina (1)	40.00	26.67	0.00	33.33
Macrophomina phaseolina (2)	33.33	23.33	0.00	43.34
Macrophomina phaseolina (3)	31.67	11.67	0.00	56.66
Rhizoctonia solani (1)	26.67	40.00	0.00	33.33
Rhizoctonia solani (2)	18.33	33.33	0.00	48.34
Rhizoctonia solani (3)	23.33	33.33	0.00	43.34
Pythium sp. (2)	33.33	5.00	0.00	61.67
Control (without fungus)	0.00	0.00	0.00	100.00
L.S.D. at 5%	1.47	3.43	-	-

 Table (2): Percentages of pre- and post- emergence damping- off as well as wilt infection used by the tested fungi, 25, 45 and 90 days after sowing, respectively, greenhouse experiment.

(1) Beheira governorate isolate.

(2) Ismailia governorate isolate.

(3) Minia governorate isolate.

Evaluation of the essential oil emulsions effect on the mycelial growth of the tested fungi in vitro

Data shown in Table (3) indicate that the different concentrations of marjoram and peppermint oil emulsions significantly inhibited the growth of the tested fungi in comparison with the control. Marjoram and peppermint emulsion oils completely *Egypt. J. Phytopathol.*, Vol. **47**, No. 2 (2019)

inhibited the growth of *F. oxysporum, F. solani, R. solani* and *Pythium* sp. at 5 ml/L. On the other hand, *M. phaseolina* was the lowest affected fungus with the tested essential oils. Positive effectiveness of the present essential oils on mycelial growth of the fungi tested might be due to the increase in the permeability of the fungal cell walls as well as inhibition in the fungal detoxificating enzymes of the antifungal oil substances. The essential oils are also capable to affect respiration of the fungal cell (oxygen uptake) and having toxic substances acting as antisporulation compounds (Inouye *et al.*, 1988).

Essential					с	once	ntrati	on (n	nl/L)	(C)				M
oil	Fungi (B)	(	)		1		2		3		4	4	5	Mean
emulsions (A)	rungi (D)	*	**	*	**	*	**	*	**	*	**	*	**	
	F. oxysporum	9.0	0.0	5.8	35.5	3.2	64.4	1.7	81.1	0.8	91.1	0.0	100	3.41
am	F. solani	9.0	0.0	5.7	36.6	2.8	68.8	1.1	87.7	0.9	90.0	0.0	100	3.25
rjor	M. phaseolina	9.0	0.0	7.6	15.5	4.9	45.5	2.7	70.0	1.7	81.1	0.5	94.4	4.40
Maı	R. solani	9.0	0.0	7.1	21.1	4.6	48.8	2.3	74.4	1.5	83.3	0.0	100	4.08
~	Pythium sp.	9.0	0.0	6.0	33.3	4.3	52.2	2.0	77.7	0.7	92.2	0.0	100	3.66
]	Mean	9.0	-	6.44	-	4.96	-	1.96	-	1.12	-	0.10	-	3.93
nt	F. oxysporum	9.0	0.0	6.0	33.3	4.6	48.8	3.0	66.6	1.3	85.5	0.0	100	3.98
nir	F. solani	9.0	0.0	7.1	21.1	6.3	30.0	3.6	60.0	1.6	82.2	0.0	100	4.60
Den	M. phaseolina	9.0	0.0	7.0	22.2	4.6	48.8	3.0	66.6	1.9	78.8	0.7	92.2	4.36
idə	R. solani	9.0	0.0	6.2	31.1	4.1	54.4	2.1	76.6	1.0	88.8	0.0	100	3.73
Ъ.	Pythium sp.	9.0	0.0	5.6	37.7	3.9	56.6	2.8	68.8	0.5	94.4	0.0	100	3.63
Mean		9.0	-	6.38	-	4.70	-	2.90	-	1.26	-	0.14	-	4.06
L.S.D. at 5	5%		A =	0.15,	B = 0 B	).08, XC	C = 0	).12, 7 A	AXB	B = 0 X C=	.40, A	XC	= 0.1	0,

 Table (3). Effect of different concentrations of marjoram and peppermint essential oil emulsions on mycelial growth of the isolated fungi.

\* Linear growth (cm); \*\* inhibition (%).

Weeds biomass  $(g/m^2)$ 

The most dominant weed species in both growing seasons included two species of annual grassy weeds, i.e. Avena sp. and Phalaris minor L. and fourteen species of annual broad-leaved weeds, i.e. Anagallis arevensis L., Medicago polymorpha L., Ammi majus L., Brassica nigra L., Coronopus squamatus L., Capsella bursa-pastoris L., Sonchus oleraceus L., Chenopodum albam L., Rumex dentatus L., Malva parviflora L., Melilotus indica L., Euphorbia helioscopia L., Cichorium endivia L. and Beta vulgaris L.

All weed control treatments significantly decreased the dry weight of grassy weeds, broad-leaved weeds and total weeds70 days after sowing as compared to the unweeded control (Table 4). The highest reductions in total dry weight of weeds were obtained with black plastic mulch, being 46.06 g/m<sup>2</sup> and 43.80 g/m<sup>2</sup>, followed by *Egypt. J. Phytopathol.*, Vol. **47**, No. 2 (2019)

hoeing, being 51.50 g/m<sup>2</sup>, 46.20 g/m<sup>2</sup> then cover crop (73.63 g/m<sup>2</sup>, 64.63g/m<sup>2</sup>) in the first and second growing seasons, respectively compared to unweeded treatment as shown in Table (4).

		Weed biomass $(g/m^2)$									
Weed control	Grassy	weeds	Broad-lea	ved weeds	Total weeds						
treatments	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season					
Unweeded control	329.76	316.03	117.86	96.43	447.63	412.46					
Hoeing	39.56	34.40	11.93	11.80	51.50	46.20					
Plastic mulch	35.46	29.70	10.60	14.10	46.06	43.80					
Cover crop	53.86	42.30	19.76	22.33	73.63	64.63					
L.S.D. at 5%	13.54	11.41	6.33	6.26	12.11	11.10					

Table (4). Effect of weed control treatments on biomass of grassy weeds, broadleaved weeds and total weeds during 2017/2018 and 2018/2019 growing seasons.

The effectiveness of plastic mulch in controlling weeds has also been reported by Ricotta and Masiunas (1991) who evaluated black polyethylene mulch and herbicide application for weed control in basil and rosemary plants and mentioned that plastic mulch provided the best weed control within the rows, although some weeds emerged through the holes in the plastic.

Reductions in weed biomass have been reported with subterranean clover living mulch and increased soybean yield by 91 % relative to weedy control plots (Ilnicki and Enache, 1992). Moreover, Chase and Mbuya (2008) reported that weed biomass was lowest with rye and black oat living mulches when used in organic broccoli production. Mulching and cover crops suppress weeds through their physical presence with soil surface by shading, allelopathic activity and blocking the light required for germination of many small-seeded weed species or by competing for water and nutrients (Teasdale and Mohler, 1993).

#### Field experiment

#### Disease incidence

The most commonly observed wilt disease symptoms in cumin plants were yellowing, browning, and wilting of lower leaves, possibly with some internal vascular discoloration in the stem. Data in Tables (5) and (6) reveal that wilt incidence was significantly affected by different weed control treatments, seed treatment and their interactions. Wilt incidence ranged from 1.3-8.7% in different weed control treatments during the second growing season.

The highest disease incidence was recorded in the unweeded plots (8.7%) on the average), while the lowest disease incidence (1.3%) and the maximum percentage of survived plants (98.7%) on the average) were recorded in plastic mulched plots (Table, 6). This treatment was closely followed by plots with cover crop which 2.7\% disease incidence on the average in the second growing season.

				Fir	st seasc	on (201	on (2017/2018)				
		Wilt i	nciden	ce (%)	)		Survi	ved plan	ts (%)		
0 14 4 4				Weed	d contro	ol treat	ments (A	A)			
(B)	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	
Control	16.7	13.3	10.0	10.0	12.5	83.3	86.7	90.0	90.0	87.5	
Peppermint oil	6.7	3.3	0.0	3.3	3.3	93.3	96.7	100.0	96.7	96.7	
Marjoram oil	3.3	0.0	0.0	0.0	0.8	96.7	100.0	100.0	100.0	99.2	
Plant Guard	6.7	3.3	3.3	3.3	4.2	93.3	100.0	96.7	96.7	95.8	
Rhizo-N	6.7	0.0	3.3	3.3	3.3	93.3	96.7	96.7	96.7	96.7	
Mean	8.0	4.0	3.3	4.0	-	92.0	96.0	96.7	96.0	-	
L.S.D at 5%			A= 0.7	7		A= 1.3					
			B = 0.3	3		B= 1.0					
		Α	$\times$ B=	0.9			A	$\times B = 1$	.5		

Table (5): Effect of weed control treatments and seed treatment on wilt incidence (%) and plant survival (%) during 2017/2018 growing season.

Table (6): Effect of weed control treatments and seed treatment on wilt incidence (%) and plant survival (%) during 2018/2019 growing season.

	_			Secon	d seaso	n (2018/2019)				
		Wilt in	ncidenc	æ (%)		Survived plants (%)				
			1	Weed	control	treatm	ents (A)	)		
Seed treatment (B)	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean
Control	16.7	10.0	6.7	6.7	10.0	83.3	90.0	93.3	93.3	90.0
Peppermint oil	10.0	3.3	0.0	0.0	3.3	90.0	96.7	100.0	100.0	96.7
Marjoram oil	6.7	0.0	0.0	0.0	1.7	93.3	100.0	100.0	100.0	98.3
Plant Guard	6.7	0.0	0.0	3.3	2.5	93.3	100.0	100.0	96.7	97.5
Rhizo-N	3.3	3.3	0.0	3.3	2.5	96.7	96.7	100.0	96.7	97.5
Mean	8.7	3.3	1.3	2.7	-	91.3	96.7	98.7	97.3	-
L.S.D at 5%			A= 0.6					A= 1.1		
			B = 0.3			B= 1.5				
		А	$\times \mathbf{B} = 0.$	7			А	$\times B = 2.$	0	

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Weed control can play a role in reducing disease incidence which can ultimately reduce fungicide use. Jambhulkar *et al.* (2012) found that plastic mulching reduced early blight of tomato by hindering the conidial movement. Reducing disease incidence by cover crops also has been reported by several authors. Ristaino *et al.* (1997) reported that a wheat cover crop reduced Phytophthora blight of peppers due to reduced dispersal of propagules of the causal agent. Moreover, ahairy vetch and Austrian winter pea cover crop reduced fruit rot incidence in tomato plants compared to plants in fallow plots (Nyochembeng *et al.*, 2014).

Similarly, soaking cumin seeds in essential oils of marjoram and peppermint at a concentration of (5 ml/L) significantly decreased the percentages of wilted plants, as compared to the control. Marjoram essential oil was more effective than peppermint oil in reducing the percentages of disease incidence (Tables 5 and 6). Furthermore, the application of marjoram oil resulted in the highest percentages of survived plant when compared with other treatments during both seasons. These findings are in agreement with an earlier report on similar effects of essential oils on the reduction of wilt and root rot incidence in green beans (El-Mougy et al., 2007). The efficacy of essential oils as antifungal agents is well documented. The major constituents of marjoram essential oil were determined to be cis-sabinene hydrate and terpinene-4ol that comprise more than 60% of the oil constituents (Gharieb, 2006). Several of these components have been previously tested for their biological activity against a number of fungi. In this respect, Terzi et al.(2007) found that terpinen-4-ol, gammaterpinene and 1,8-cineole, the principal components of Melaleuca alternifolia essential oil had highly inhibitory effect against the mycelial growth of some pathogenic fungi and among the tested compounds, terpinen-4-ol was the most effective one. Hence, it could be suggested that the antifungal activity of marjoram oil is due to the presence of the main component terpinene-4-ol in the oil. Moreover, Edris and Farrag (2003) confirmed the inhibitory effect of menthol (one of the major constituents in peppermint essential oil) on the growth of the fungi Sclerotinia sclerotiorum and Rhizopus stolonifer, and they stated that menthol was the only constituent responsible for the antifungal properties of peppermint essential oil. The antifungal activity might be due to the highly lipophilic nature of terpenes and low molecular weight that leads to disrupting of the cell membrane, causing cell death or inhibiting the sporulation and germination of the fungi (da Silva Bomfim et al., 2015).

The application of Plant Guard and Rhizo-N significantly reduced the incidence of wilt disease (Tables 5 and 6). Rhizo-N was more effective than Plant Guard in reducing the disease incidence resulting in a higher percentage of survived plants during the first season (Table 5). Whereas, the effects of these treatments were similar during the second season. Rhizo-N has proved to be an effective biocontrol agent against a broad range of plant pathogens. Podile and Prakash (1996) showed that groundnut seeds bacterized with *B. subtilis* caused a reduction in the disease incidence of crown rot in infested soil with *Aspergillus niger*, suggesting a possible *Egypt. J. Phytopathol.*, Vol. **47**, No. 2 (2019)

role of *B. subtilis* in biological control of *A. niger. B. subtilis* antagonizes fungal pathogens by producing antifungal peptides that cause lysis of the phytopathogenic fungal cell wall (Gong *et al.*, 2014). *Bacillus* species are also capable of producing enzymes like chitinase having a very strong lytic activity (Zhao *et al.*, 2014). Disease suppression induced by *Trichoderma* spp. has been reported by Aghnoom *et al.* (1999) who mentioned that cumin seeds treated with *Trichoderma harzianum* T2 isolate, lowered disease incidence caused by *F. oxysporum* f.sp. *cumini* by 65.4% and this treatment was found to be more effective than seed treatment with the fungicides. Moreover, Tawfik and Allam (2004) mentioned that the lowest percentage of cumin infection was found in pre-sowing seed treatment with *T. harzianum*. Disease suppression could be due to competition for nutrients. *Trichoderma* has a superior capacity to mobilize and take up soil nutrients compared to other organisms (Mohiidin *et al.*, 2010).

Additionally, the interactions between weed control treatments and seed treatment were also significant (Tables 5 and 6). Plants were completely free from wilt incidence in all weed control treatments with cumin seeds soaked in marjoram essential oil (5 ml/L) in both seasons. Also, there was no occurrence of the disease in hoeing and plastic mulch plots with seeds soaked in *B. subtilis* and peppermint oil in the  $1^{st}$  season.

Furthermore, it has been observed from the results of this study, that the efficacy of marjoram essential oil was similar to the commercial biocides Plant Guard and Rhizo-Nin controlling cumin wilt. Thus, it could be suggested that marjoram essential oil can be formulated to be used for controlling cumin wilt under field conditions.

#### Vegetative growth parameters and yield of cumin plants

The effect of weed control treatments, seed treatment and their interaction on cumin growth and yield are presented in Tables (7-10). All treatments had positively affected the growth and yield parameters of cumin plants in both seasons. In 2018, the lowest plant height, number of branches/plant, number of umbels/plant and seed yield (kg/fed.) were recorded under unweeded control, while the highest plant height and number of branches were obtained by plants grown in plastic mulched plots. However, there was no significant difference between plastic mulch and hoeing treatments (Tables7 and 8). In 2019, hoeing significantly improved seed yield (kg/fed.) and was closely followed by plastic mulch plots. Enhancement of cumin growth and yield under black plastic mulch might be due to the effective control of weeds, which reduced weed competition for light, water and essential nutrients. Similar findings were also reported by some investigators (Ashrafuzzaman et al., 2011 and Pradeep et al., 2017). Ricotta and Masiunas (1991) also reported an increase in fresh and dry weights of basil yield and rosemary in polythene mulched plots than unmulched plots, unlike parsley yield that did not respond to the mulch. Black plastic mulching has been found to have several advantages resulting in

enhancing performance and growth of plants. These advantages include maintaining high soil water contents thus improving water use efficiency, optimizing soil temperature and suppression of weeds (Bond and Grundy, 2001 and Tarara, 2002).

The obtained results revealed that cumin growth and yield traits in case of cover crop plots was significantly lower than plastic mulch treatment but higher than the unweeded control treatment. This decrease in growth parameters in cumin plants may be due to competition with clover as the living mulch. Previous studies showed that the species, when selected as living mulches do not suppress weeds selectively, but suppress the crop as well; therefore, living mulches must be managed carefully to reduce their competition with the crop. In this respect, Chase and Mbuya (2008) mentioned that black oat, rye, and annual ryegrass as living mulches were effective in inhibiting weed biomass, but interfered with broccoli growth, which resulted in reduction in the yield. Marks (1993) suggested that reduced growth of the main crop might be due to competition for water or some other limited resource, or the mulch may be having an allelopathic effect.

				F	lant he	ight (c	m)			
	Fi	rst seas	son (20	17/201	8)	See	cond se	eason (2	2018/20	)19)
<b>a</b> 1				Weed	control	treatm	ents (A	<b>(</b> )		
(B)	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean
Control	12.11	23.22	22.72	22.11	20.04	13.05	23.61	23.61	24.44	21.17
Peppermint oil	12.22	24.16	23.88	24.88	21.28	13.44	26.05	24.66	25.22	22.34
Marjoram oil	13.22	25.50	25.61	25.00	22.33	14.33	26.66	26.88	26.00	23.46
Plant Guard	12.88	24.00	24.94	25.44	21.81	14.55	24.55	25.94	25.44	22.62
Rhizo-N	13.11	24.61	24.66	24.06	21.61	15.27	25.94	25.11	24.77	22.77
Mean	12.70	24.32	24.36	24.29	-	14.12	25.36	25.14	25.17	-
L.S.D at 5%		А	. = 0.7	71		A = 0.73				
		E	B = 0.6	8		B = 0.52				
		A>	< B = 1	.82		$A \times B = 1.75$				

 Table (7): Effect of weed control treatments and seed treatment on plant height (cm) of cumin plants during 2017/2018 and 2018/2019 growing seasons.

				Numb	per of b	ranche	es/plan	t		
	Fir	st seas	on (20	017/20	18)	Second season (2018/2019)				
	Weed control treatments (A)									
Seed treatment (B)	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean
Control	3.11	5.16	7.05	4.10	4.85	3.05	5.94	6.61	5.27	5.21
Peppermint oil	3.55	6.66	6.21	5.99	5.60	3.77	6.72	6.94	6.27	5.92
Marjoram oil	3.22	7.83	7.94	6.27	6.31	3.66	6.99	7.27	6.94	6.21
Plant Guard	3.55	6.66	6.27	5.77	5.56	3.94	5.88	6.49	6.21	5.63
Rhizo-N	3.60	7.27	7.27	6.66	6.20	4.27	6.94	7.38	6.88	6.36
Mean	3.40	6.71	6.94	5.75	-	3.73	6.49	6.93	6.31	-
L.S.D at 5%		Α	= 0.2	34		A = 0.48				
		В	= 0.3	37		B = 0.47				
		A >	$\mathbf{B} = 0$	).75			A	$\times \mathbf{B} = 0$	0.93	

Table (8): Effect of weed control treatments and seed treatment on number of branches/plant of cumin plants during 2017/2018 and 2018/2019 growing seasons.

Similarly, seed treatment influenced cumin growth and yield parameters, significant differences in plant height and number of branches/plant were observed among various essential oils or biocontrol agent treatments (Tables7-10). In both years, the highest mean values were obtained with marjoram oil application (Tables 7 and 8), whereas the highest number of umbels/plant and seed yield (kg/fed.) were obtained with Rhizo-N application (Tables 9 and 10). The stimulatory effect of essential oils and bioagents on plant growth has been confirmed by some investigators (Bardia and Rai, 2007; El-Mohamedy and Abd El-Baky, 2008). The beneficial effects of Trichoderma spp. on plant growth has been reported by several authors. Cucumber plants inoculated with T. harzianum significantly increased dry weight, shoot length and leaf area and enhanced the availability of P and Fe to plants (Yedidia et al., 2001). Bacillus subtilis has been widely used for plant growth promotion in a number of ways including its ability to produce phytohormones such as IAA, gibberellins, cytokinins and increase root and shoot cell division and elongation (Idris et al., 2004) and by increasing the availability of N, P, and Fe in the soil (Kim et al., 2011 and Ortíz-Castro et al., 2014), as well as its ability to produce volatile organic compounds which play a significant role in promoting plant growth by regulating the synthesis or metabolism of phytohormones (Tahir et al., 2017). The interactions between weed control treatments and seed treatment on vegetative growth and seed yield were also significant (Tables 7-10). Maximum values were obtained from hoeing and plastic mulch plots with seeds soaked in Rhizo-N or marjoram essential oil in the first and second growing seasons.

				Num	ber of	umbel	s/plant	;		
	Fi	rst seas	son (20	017/20	18)	Sec	cond se	eason (	2018/2	019)
			ſ	Weed	control	treatn	nents (A	A)		
Seed treatment (B)	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean
Control	4.11	17.66	16.27	16.27	13.57	4.94	18.38	15.88	12.88	13.02
Peppermint oil	4.88	19.94	20.10	15.83	15.18	4.72	18.88	20.11	14.88	14.64
Marjoram oil	5.44	20.22	21.49	16.61	15.94	5.83	19.44	22.27	15.88	15.85
Plant Guard	4.55	18.33	17.66	19.27	14.95	5.72	17.66	17.88	15.55	14.20
Rhizo-N	6.83	22.00	21.38	18.83	17.26	6.11	22.22	22.44	16.11	16.72
Mean	5.16	19.63	19.38	17.36	-	5.46	19.31	19.71	15.06	-
L.S.D at 5%		А	L = 1.7	0'0		A = 1.19				
		E	<b>B</b> = 1.4	9		B = 1.56				
		A>	$\langle \mathbf{B} = 2 \rangle$	.74			А	$\times \mathbf{B} = 2$	2.80	

Table (9): Effect of weed control treatments and seed treatment on number of umbels/plant of cumin during 2017/2018 and 2018/2019 growing seasons.

Table	(10): Effect of	of weed contro	ol treatments	and seed tro	eatment on	seed yield
	(kg/fed.) of	f cumin during	g 2017/2018 an	nd 2018/2019	9 growing se	easons.

				Numb	er of u	mbels/	plant			
	F	irst seas	son (201	7/2018	3)	Sec	cond se	eason (	2018/2	.019)
			W	eed co	ontrol t	reatme	nts (A	)		
Seed treatment (B)	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean	Unweeded control	Hoeing	Plastic mulch	Cover crop	Mean
Control	61.77	417.40	52.19	432.2	427.1	337.1	312.1			
Peppermint oil	64.76	426.30	421.30	340.2	313.2	73.59	442	439.8	332.6	321.9
Marjoram oil	66.46	478.80	468.30	338.2	337.9	85.8	469.6	481.5	365.2	350.5
Plant Guard	67.73	459.80	464.20	346.5	334.6	71.05	464.6	452.9	334.2	330.6
Rhizo-N	64.64	481.90	490.80	339.3	344.2	81.02	474.5	454.9	375.0	346.4
Mean	65.07	452.80	449.9	343.8	-	72.73	456.6	451.2	348.8	-
L.S.D at 5%		1	A = 7.80		A = 6.44					
		]		B = 7.59						
		Ax	B = 12.7	6			А	$\mathbf{xB} = 1$	1.88	

## Conclusion

In conclusion, plastic mulching had a positive effect on suppression of weeds, reduced disease incidence of Fusarial wilt and enhanced vegetative growth and yield of cumin plants. In addition, seed treatment with any of the Rhizo-N (4ml/L) or marjoram essential oil at a concentration of 5 ml/L controlled wilt disease and was comparable to, or more effective than that provided by the commercial chemicides, showing potential for being used in controlling the disease. Thus, integration of these methods could provide an approach for the management of the disease in infested cumin fields more effectively.

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٣- المعمل المركزي لبحوث الحشائش ، مركز البحوث الزراعية ، الجيزة ،

مصر

تم جمع نباتات كمون مصابة بمرض عفن الجذور والذبول من عدة مواقع تتبع محافظات البحيرة والإسماعيلية والمنيا - مصر. تم عزل عدة فطريات تابعة لأجناس Macrophomina ، Fusarium ، مصر. تم عزل عدة فطريات Pythium من النباتات المصابة واختبرت قدرتها المرضية في إصابة الكمون. أظهرت النتائج أن الفطر F. oxysporum سجل أعلى معدل تكرار من العزل Pythium ، M. phaseolina ، F. solani ، R. solani ، وتلاه الفطريات المعلي لدراسة تأثير تركيزات عديدة من زيوت البردقوش و النعناع الفلفلي على نمو الفطريات المعزولة. أدت المعاملة بزيوت البردقوش والنعناع الفلفلي إلى تثبيطت لنمو الفطريات عند تركيز 5 مل/لتر.

أجريت تجارب حقلية في محطة بحوث ملوي محافظة المنيا خلال موسمي أمريت تجارب حقلية في محطة بحوث ملوي محافظة المنيا خلال موسمي لمقاومة الحشائش النجيلية وعريضة الأوراق (العزيق اليدوي ، التغطية بالبلاستيك الأسود ، محصول مغطى ، كنترول) على وزن الحشائش. أظهرت النتائج أن جميع معاملات مقاومة الحشائش أدت إلى انخفاض معنوي في الوزن مقارنة بالكنترول. أيضا ، تم دراسة تأثير معاملات مقاومة الحشائش ومعاملات نقع بذور الكمون في مبيدات حيوية مثل Plant Guard معنوي في الوزن النعناع الفلفلي والبردقوش على الإصابة المرضية بالذبول وكذلك صفات النمو الخصري ومحصول الكمون. أظهرت النتائج أن التغطية بالبلاستيك أدت إلى أفضل القيم من حيث خفض معدل الإصابة المرضية بالذبول وكذلك صفات النمو محصول الكمون ، بينما سجلت معاملة الكنترول أعلى معدل إصابة وأقل محصول. أيضا ، أدى نقع البذور في المييد الحيوي معارب وألف على البكتيريا Rhizo-N المون أو زيات الميد الحيوي معارب وأنه محصول. أيضا ، أدى نقع البذور في المييد الحيوي محسول الذي يحتوي على المتيريا معاملة الكنترول أعلى معدل إلى إلى خفض معدل الإصابة بالذبول ، كما أدى إلى زيادة معنوية في محصول البذر إلى خفض

بالتالي ، يمكن التوصية باستخدام أي من المبيد الحيوي Rhizo-N أو زيت البردقوش في مكافحة مرض الذبول تحت ظروف الحقل ، وأيضا يمكن الجمع بين هذه الطرق لمكافحة المرض في الحقول الملوثة بالفطريات المسببة للمرض.