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Evaluation Activities of some Biocontrol Agents, Plant Extracts and Fungicides in Management of Soil-Borne Fungi of Two Basil Varieties.

Abdel-Wahed, G.A.

Ornamental, Medicinal & Aromatic Plants Dis. Res. Dept., Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Under Egyptian conditions Basil (*Ocimum basillicum* L.) plants are subjected to invasion by many soil borne diseases causing considerable losses. Damping off and root rot of basil were found widespread in plantations in Beni-Suef and Fayoum governorates during 2016 and 2017 seasons. The isolated fungi from naturally infected basil plants with these diseases were identified as *Fusarium subglutinans, F. oxysporum, F. moniliforme, F. semitectum, F. nival, F. roseum, Rhizoctonia solani* and *Macrophomina phaseolina. Fusarium moniliforme* and *R. solani* have achieved the highest occurrence percentage in isolation trials from samples of Beni-Suef and Fayoum governorates. *Rhizoctonia solani* was more virulent than the other fungi in pathogenicity tests on the two tested basil varieties, *i.e French and American*. The effects of biocides (Plant guard and Rizo-N), biofertilizers (Biogen and Nitrobien), fungicides (Folio gold, Babon and Amister top) and plant extracts (Basil and Verbascum) on controlling damping off and root rot on two basil varieties were tested under greenhouse and field conditions. The controlling agents, however, were varied in their positive efficiency against diseases. Biocides and fungicides were the superior treatments in controlling diseases and improving plant growth parameters. In the applied field experiments, all treatments tested significantly decreased the percentages of infection and increased the growth parameters compared with control treatment.

Keywords: Basil (*Ocimum basillicum* L.), French basil, American basil, root-rot, biocides, plant extract, fungicides.

Basil (*Ocimum basillicum* L.), Fam. Lamiaceae is an annual crop, commercially cultivated and has been utilized in food, perfumery and medical industries in South Europe, Southeast Asian and American countries. (Chen *et. al*, 2017). The origin of these plants Iran, South Asia, Indonesia, India, Africa, Southern Europe and North America (Hilal and Haready, 1996). It is cultivated in Egypt, especially in Beni-Suef and Fayoum governorates. The cultivated area reached about 2685 fedan in Beni-Suef and 435 fedan in Fayoum (Anon 2015).

Damping-off of seedlings is the most common disease caused by *R. solani* (Moussa, 2002). *Rhizoctonia solani* has a wide host range and causes the disease in a variety of crops, such as lawn grass (Parmeter *et al.*, 1969), tomato and cucumber (Coa *et al*., 2004). One of the most significant diseases of basil is a wilt disease caused by *Fusarium oxysporum* f. sp. *basilica* also *Rhizoctonia solani* is an important soil-borne fungal pathogen causing damping-off and root-rot diseases.

These diseases are a serious constraint to basil production in other parts of the world and are prevalent in the USA (Wick and Haviland, 1992). Many investigators reported that many soil borne diseases can attack basil plants (Davis *et al*., 1993), South Africa (Swart and van Niekerk 2003), Greece (Biris *et al*., 2004), Israel (Rekah *et al*., 2000) and Egypt (Abdel-Wahed, 2018). First report of Fusarium wilt in California was recorded by Davis *et al*. (1993). Elmer *et al*, (1994) studied the vegetative compatibility among *F. oxysporum* f. sp. *basilicum* isolates recovered from basil seed and infected plants.

Regarding the effect of bio-agents against root rot and wilt pathogens, all of, Mathew and Gupta (1998) and Hazarika and Das (1999) emphasized the abilities of *Trichoderma viride*, *T. koningii*, *T. harzianum*, *T. virens* and *Bacillus subtilis* in inhibiting the linear growth of root rot fungi like *R. solani* and *F. solani* of faba bean and other legume crops. Also, El-Gindy (2003) mentioned that *T. harzianum*, *T. lignorum* and *B. subtilis* affected significantly the average diameter of *Botrytis fabae* colonies than the control.

Using biofertilizers and/or organic substances such as humic acid instead of the chemical forms of fungicides or mineral fertilizers could be the way to control soil borne plant pathogens and production of natural clear fruits free from minerals residues (Abdel-Monem *et al*., 2008). In this respect, application of biofertilizers for controlling soil borne pathogenic fungi has been applied to several plant species (ELZeiny *et al*., 2001), Gomaa (1995) found that organic and/or biofertilizers improved vegetative growth and nutritional status.

Ocimum basilicum (basil) has inhibited fungal development on maize kernels. Many studies have shown that basil contains antioxidants such as Beta-carotene and to copherol, which have anti-fungal, antimicrobial and anti-apoptotic properties (Kaya *et al*., 2008 and Isaac and Abu-Tahon 2014).

The aims of this study are; (1) Evaluation the biological control activity of two basil varieties (French and American basil) against diseases by using bio, plant extracts and fungicides treatments, (2) Estimation of the incidence and disease severity of the diseases, (3) Determination the growth parameters of the two basil varieties tested.

Materials and Methods

I- Sampling and Isolation and identification of the associated fungi:

Samples of basil plants exhibited natural symptoms of root rot and wilt were collected from farms located at Beni-Suef and Fayoum governorates during 2016 and 2017 seasons.

The infected plants were cut into 1-cm-long pieces and washed with tap water followed by surface sterilization using 2 % sodium hypochlorite for 2 minutes or in 70 % ethanol for 15 sec then washed with sterilized distilled water. The samples were dried between two folds of sterilized filter papers. The surface sterilized pieces were placed on potato dextrose agar (PDA) medium in Petri dishes (9 cm diameter) and incubated at 25°C for 7 days with observation every day as described by Gamliel *et al.* (1996).

The growing fungal colonies were microscopically examined, counted and the frequency of each fungus was calculated. Purification of the isolated fungi was carried out using the single spore or hyphal tip techniques suggested by Dhingra and Sinclair (1995). These fungi were identified according to Leslie and Summerell (2006); Domsch *et al.* (1980) and Plaats-Niterink (1981). The identification was kindly confirmed by Mycology and Plant Diseases Survey Dept., Plant Pathol. Res. Instit., Agric. Res. Center., Giza, Egypt. Pure cultures grown in PDA slants were kept at low temperature (5°C) for further studies. The frequency of isolated fungi was calculated according to the following Formula of Pal *et al* (2007)

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

II-Pathogenicity tests:

Pathogenicity tests were conducted under greenhouse conditions using two varieties of basil seeds and seedlings, *i.e*. French and American at the Agric. Exp. Sta. Sides, Beni-Suef Governorate.

Inocula of the tested fungi, *i.e. F. subglutinans, F. oxysporum, F. moniliforme, F. semitectum, F. nival, F. roseum, R. solani* and *M. phaseolina* were prepared by growing each fungus alone for 20 days at 25°C on maize-meal-sand medium. Formalin-sterilized pots (20-cm-diam) packed with Formalin-sterilized clay and soil (1:1, w/w) were separately infested with each fungus at the rate of 1 % (w/w). Each pot was planted with 25 seeds and / or five seedlings (45 days-old). Four replicates were used per each particular treatment (Land *et al*., 2001).

Disease incidence was recorded as percentages of pre-and post-emergence damping-off and healthy survival seedlings at 30, 60 and 90 days after sowing seeds, respectively. Growth parameters of plants (height, fresh and dry weights) as well as those of roots (length and fresh & dry weights) were also recorded 60 days after sowing. Disease incidence and severity of basil root rot were recorded after 90 days of planting seedlings in infested soil.

The percentages of disease incidence for root rot were calculated according to the following formula of Gamliel *et al.* (1996)

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

Disease severity was also recorded at the same time according to our modification on the disease index of El-sayed, (2017) using the devised scale (0-5), 1 =healthy seedling, 2 = very little root rot, 3 = moderate root rot, 4 = severe root rot, 5 = complete root rot, as follows:

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

Where:

n = Number of roots in each category,
v = Numerical value of each category,
N = Total number of roots in the samples.

II-Disease control

According to the pathogenicity tests, a control; programme was conducted to control aggressive pathogenic isolates of *F. semitectum, R. solani* and *F. moniliforme* and for evaluating their effectiveness on some plant growth parameters. The same procedures of preparing potted infested soil for pathogenicity tests were adopted using 1 % (w/w) inoculum level of fungi. These pots were divided into four groups for four types of control using two types in each method comparing with pots of control. Treatment materials tested and their rates of application are shown in Table (1).

All the aforementioned treatments (Table 1) were used as root dipping treatments and/or seed treatment for 15 min. just before transplanting, except the biofertilizers which were applied as soil treatment. The basil varieties seeds were treated with the aforementioned treatments at the same rates.

Preparation of watery plant extracts:

According to the methods described by Gaafar *et al*. (1989), 100 gm/L Water. of herbs from verbascum (*Verbascum thapsus*) and basil (*Ocimum bacillicum*) were washed under running tap water several times and shacked in double sterile distilled water for four to five times to ensure that no foreign particles on the plant materials .The fresh plant materials were killed immediately in 70% alcohol. Then, each herb was macerated in a blender .The macerated herb was squeezed and the extract was filtered through filter paper Whatman (No.1).The filtrate was centrifuged at 5000 rpm for 30 min .and was sterilized through (0.45µm) porosity Millipore filter. The sterile watery extract was kept in a refrigerator for experimental purpose. They were tested against fungal growth at 50 % concentrations.

Table (1): Fungicides, biocides, plant extracts and biofertilizers, their commercial name, composition and concentrations tested.

Commercial Names	Composition	Concentration used
Fungicides:		
Folio gold	(Mefenoxam 3.75 % + Chlorothalonil 50%)	3 g/ L water or 3 g/ kg seeds
Babon	(Metalaxyl 10.8 + Azoxystrobin 28%)	3 g/ L water or 3 g/kg seeds
Amistar top	(Difenoconazole + Azoxystrobin)	3 g/ L water or 3 g/ kg seeds
Biocides:		
Plant Guard	*Trichoderma harzianum*, $3X10^7$cfu / ml	4 ml/ L water or 4 ml/ kg seeds
Rizo-N	*Bacillus subtilis*, $3X10^7$ cfu / gm	4 g/ L water or 4 g/ kg seeds
Biofertilizers:		
Biogen	*Azotobactervinelauvii&A.chroococcum*,10^6-10^8 cfu/gm	4g / 1.5 kg soil
Nitrobien	A*zotobacter* sp. and *Azospirillum* sp.).	4g / 1.5 kg soil
Plant extracts:		
Basil	(watery Crude extract of *Ocimum bacillicum* herb)	50 %
Verbascum	(watery Crude extract of *Verbascum thapsus* herb)	50 %

IV-Field experiment:

Field experiments were carried out in the new lands at village of Sayyidna Al-Khidir, Yusef Al-Siddiq County, Fayoum governorate during two successive seasons 2016 and 2017. Basil seedlings (45 days-old) however, were planted in the chosen field which was planted with basil several times before it was used for our experiments. The field experiments were carried out using the complete randomized block design trial. The plot was divided into three sections, each of a single row 50 cm wide and the sections were separated by a 1.5 m free area. The basil seedlings of each treatment were treated individually with the desired materials. Seedling treatment and soil drench were used as two different treatments. In the first trail, basil seedlings were immersed in the desired solution (treatments). In control, sterile distilled water was used for seedling immersing. In the soil treatments the controlling agent was added to the soil in the hole of planting (25 mL hole-1), then the seedlings were transplanted. During the flowering period (at 45 days old), plants were uprooted, and different morphological parameters were assessed including root and shoot weight, plant height, branching, number of flowers and production of leaves (Dorrance and McClure, 2001.)

Statistical analysis

The obtained data were statistically analyzed for computing LSD according to the procedure outlined by Snedecor and Cochran (1989).

Results

1-Isolation, identification of the associated fungi and their frequencies:

Isolation trails carried out from natural infected basil plants showing typical symptoms of root rot or wilt collected from two governorates, Beni-Suef and Fayoum yielded many fungal isolates (Table, 2). The isolated and identified fungi are presented in Table (2). *Fusarium moniliforme* (20 %) and *R. solani* (20 %) were the most frequently isolated fungi from Beni-Suef governorate. Whereas, *F. oxysporum* (21.92) and *R. solani* (17.54) were the most frequently isolated fungi from Fayoum governorate. On the other hand, *F. oxysporum*(5.33), *F. semitectum* (8), *F. subglutinans* (6.14) and *F. nival* (6.57) were isolated at low frequencies from the two surveyed governorates. Concerning the occurrence of the isolated fungi, samples of Fayoum governorate recorded the highest number of isolated fungi, being 228, followed by samples of Beni-Suef (150 isolates).

Table (2): Frequency% of the isolated fungi from infected basil plants collected from governorates.

Fungi	Beni-Suef		Fayoum		Mean of Frequency (%)
	No. of isolates	Frequency (%)	No. of isolates	Frequency (%)	
F. subglutinans	15	10	14	6.14	7.7
F. oxysporum	8	5.33	50	21.92	15.3
F. noniliforme	30	20	30	13.15	15.9
F. semitectum	12	8	22	9.64	9.0
F. nival	15	10	15	6.57	7.9
F. roseum	20	13.33	24	10.52	11.6
R. solani	30	20	40	17.54	18.5
M. phaseolina	20	13.33	33	14.47	14.10
Total	150	100.00	228	100.00	100.00

2- Pathogenicity tests:

All fungi tested (Table, 3) were able to infect the two tested basil varieties, *i.e*. French and American when sown as seeds in soil. Since they significantly increased pre- and post-emergence damping-off compared with the control treatment (seeds grown in uninfested soil). The reverse was true with the results of healthy survived seedlings. The highest percentages of pre-and post emergence of two varieties were due to infection by *F. moniliforme* and *R. solani*. *F. moniliforme* recorded 25 and 28

% and 24 and 27 % on French and American varieties respectively, while *R. solani* recorded 25 and 45% and 23 and 44% on French and American varieties respectively. In contrast, *F. oxysporum* and *Macrophomina phaseolina* were the least fungi in this respect. On the other hand, *R. solani* resulted in significant percentage of pre- and post emergence compared with the other fungi, whereas *M. phaseolina* recorded the lowest values amongst the most pathogenic fungi in this respect. Also, all fungi significantly reduced plant height (cm), fresh weight (g.) and dry weight (g.) of the tested basil plants compared with those of control treatment. In general, *Fusarium moniliforme* and *R. solani* had the highest negative effects on growth parameters of the two tested varieties. In contrast, *F. oxysporum* and *M. phaseolina* were the least aggressive fungi in this respect.

Table (3): Pathogenicity tests of the isolated fungi and their effects on some basil growth parameters.

Varieties	Fungi	Damping-of		Survivals plants (%)	Reduction (%)	Seedling Height (cm)	Fresh Weight/g	Dry Weight/g
		Pre-emergence (%)	Post-emergence (%)					
French basil	*F. oxysporum*	7.0	20.0	73.0	24.1	35.2	43.5	10.9
	F. moniliforme	25.0	28.0	47.0	51.1	25.4	25.4	6.8
	M. phaseolina	10.0	15.0	75.0	22.0	45.4	50.3	13.9
	F. semitectum	16.0	30.0	54.0	43.8	36.0	33.3	12.3
	R. solani	25.0	45.0	30.0	68.8	25.5	25.6	7.0
	Control (uninfested soil)	0.0	4.0	96.0		50.4	56.0	15.9
	Mean	13.8	23.6	62.5	41.6	36.3	39.0	11.1
American basil	*F. oxysporum*	8.0	28.0	64.0	34.0	32.5	20.4	9.5
	F. moniliforme	20.0	30.0	44.0	54.7	23.2	23.4	5.5
	M. phaseolina	11.0	16.0	73.0	24.8	43.3	33.6	12.8
	F. semitectum	18.0	31.0	51.0	47.8	32.0	30.4	10.4
	R. solani	27.0	46.0	27.0	72.2	23.5	24.5	6.0
	Control (uninfested soil)	0.0	3.0	97.0		55.0	55.0	16.0
	Mean	15.0	25.7	59.3	46.7	34.9	31.2	10.0
L.S.D.at 5%:								
	Fungi (F) =	2.30	5.20	7.40		3.55	4.0	2.5
	Varieties (V) =	n.s	n.s	n.s		n.s	n.s	n.s
	F×V =	5.45	10.65	15.75		8.44	11.20	1.50

*Reduction according to the control treatment.

Data presented in Table (4) indicate that all the tested fungi were pathogenic to basil plants when grown as seedlings, since they significantly increased infection by root rot and disease severity than the control. *Fusarium moniliforme* recorded the highest percentages of infection and disease severity on French variety, being 62.0

and 53.8 %, whereas in case of American variety, the percentages of infection and disease severity were 64 and 55.5 %, respectively. In contrast, *M. phaseolina* was the least effective one in this respect in case of French variety (12% root rot and 8.2 % disease severity), meanwhile, the previously mentioned fungus caused 14.0 root rot and 10.3% disease severity in American variety.

Table (4): Root-rot incidence (%) and disease severity of basil plants planted as seedlings in infested soil after 60 days, under greenhouse conditions.

Varieties	Soil infested with	Root-rot Infection (%)	Disease severity (%)
French basil	*F. oxysporum*	20.0	6.5
	F. moniliforme	65.0	53.8
	M. phaseolina	15.0	8.2
	F. semitectum	45.0	33.8
	R. solani	50.0	43.9
	Control (uninfested soil)	0.0	0.0
	Mean	39.0	24.4
American basil	*F. oxysporum*	20.0	8.5
	F. moniliforme	65.0	55.5
	M. phaseolina	15.0	10.3
	F. semitectum	45.0	35.5
	R. solani	55.0	45.6
	Control (uninfested soil)	0.0	0.0
	Mean	40.0	25.9
L.S.D.at 5% :			
	Fungi (F) =	6.50	7.40
	Varieties (V) =	n.s	n.s
	F×V=	12.33	13.50

As for fresh weight, plant height and dry weight /plant, it was obvious that their values were significantly reduced due to infection by each of the tested fungi compared to the control treatments (Table, 5). The lowest growth parameter values including fresh weight (50.7 g.), dry weight (27.7g.) and plant height (14 cm) were recorded from French basil plants grown in infested with *F. moniliforme*. The same manner was also achieved when American basil plants grown in the soil artificially infested with the same fungus mentioned before. The least aggressive fungus in reducing plant growth parameters studied was *M. phaseolina*.

Table (5): Effect of the tested pathogenic fungi on some growth parameters of basil, 60 days after transplanting in infested soil, under greenhouse conditions.

Varieties	Soil infested with	Fresh weight / plant (g)	Dry weight / plant /g	Plant height / plant (cm)
French basil	*F. oxysporum*	151.2	77.5	33.0
	F. moniliforme	50.7	27.7	14.0
	M. phaseolina	157.5	82.2	35.0
	F. semitectum	147.5	79.2	25.0
	R. solani	66.2	36.2	17.0
	Control (uninfested soil)	249.5	132.2	61.0
	Mean	137.1	72.5	30.8
American basil	*F. oxysporum*	140.2	70.6	30.0
	F. moniliforme	48.0	25.5	12.0
	M. phaseolina	135.6	80.0	32.0
	F. semitectum	120.6	70.0	23.0
	R. solani	60.5	30.5	15.0
	Control (uninfested soil)	250.8	130.0	60.0
	Mean	125.8	67.8	28.7
L.S.D.at 5% :	Fungi (F) =	7.50	6.40	5.70
	Varieties (V) =	10.0	n.s	n.s
	F×V=	18.60	13.20	11.0

3. Controlling Experiments

3.1. Greenhouse experiments:

The effect of different control treatments with fungicides, biocides**,** plant extracts and biofertilizers on managing pre- and post- emergence damping-off caused by the most aggressive fungi (*F. semitectum*, *F. moniliforme* and *R. solani*) of both basil verities was evaluated under greenhouse conditions. Results shown in Table (6) indicate that in general, all the tested treatments, biocides, biofertilizers, fungicides and plant extracts when used as seed dressing treatment significantly decreased pre- and post- emergence damping-off caused by the three tested fungi and increased values of healthy survived plants compared with the control (untreated seeds). The biocides were the superior treatment in this regard.

Data presented in Table (7) show that basil plants can be efficiently protected against root rot using the tested control treatments (biocides, biofertilizers, plant extracts and fungicides) as seed treatment, just before sowing. Biocides were the most effective treatments in decreasing root rot infection. All treatments used as seedlings dressers significantly decreased the percentages of root rot of the two basil varieties caused by each of *F. semitectum*, *R. solani* and *F. moniliforme* compared with the control Check (untreated seedlings).

Data in Table (8) reveal that all experimented treatments resulted in significant increases in the growth parameters plant of the two basil varieties. Biocides were the most active treatments in increasing growth parameters compared to the other tested treatments. Plant extracts were the least effective treatment in this respect.

3.2. Field experiment:

The effect of the nine control treatments using biocides, biofertilizers, plant extracts and fungicides on the incidence of root rot of two basil vars. under field conditions during 2016 and 2017 growing seasons is presented in Table (9) and Figs. (1, 2, 3 and 4). The obtained results reveal that these treatments significantly reduced the infection percentages and increased fresh weight and dry weight and essential oil compared to the untreated plants. Plant guard as biocide resulted in the highest level of disease reduction compared to the check treatment. Moreover, biocides gave the best results in decreasing the root rot during the two growing seasons. Plant extracts were the lowest effective treatments in this regard. Regarding to infection, there was significant reduction with the most treatments in case of French variety, being 25.7and 28.4 % achieved in seasons 2016 and 2017. While, for the American variety, the significant reduction recorded 27 and 29.7% in seasons 2016 and 2017, respectively. Increases in fresh weight (gm) to 1012 (gm) and 964 (gm) in seasons 2016 and 2017 in case of French variety, while, for American variety the increases recorded were 987 (gm) and 874 (gm) in seasons 2016 and 2017. With respect to dry weight values it was noticed they were significantly increased with the most control treatments tested, in case of French variety, if compared with the control treatment either in season 2016 or 2017. The same was also true in case of American variety during the two growing seasons 2016 and 2017.Control treatments significantly caused increases in the determined essential oils than the check treatment Mean values of essential oil (ml)/100g. fresh herb were 0.30 and 0.33 (ml)/100g in seasons 2016 and 2017 in case of French variety, while for American variety the mean values were 0.28 and 0.34 (ml)/100gin seasons 2016 and 2017, respectively.

Table (6): Influence of different control treatments on the incidence of basil damping-off caused by *F. semitectum, F, moniliforme* and *R. solani* under greenhouse conditions.

Variety	Treatments	Effect of treatments on:								
		Fusarium semitectum			*Rhizoctonia solani*			*Fusarium moniliforme*		
		Pre- Emergence (%)	Post- Emergence (%)	Survival (%)	Pre- Emergence (%)	Post- Emergence (%)	Survival (%)	Pre- Emergence (%)	Post- Emergence (%)	Survival (%)
French basil	Biocides									
	Plant Guard	4.0	13	83	7	15	78	9	24	67
	Rhizo-N	7.0	10	83	6	11	83	12	15	73
	Biofertilizers									
	Biogen	12	17	29	13	17	70	17	25	58
	Nitrobien	14	19	67	12	21	67	22	27	51
	Plant extract									
	Basil	10	13	77	11	18	71	25	29	46
	Verbascum	12	20	68	14	23	63	27	33	40
	Fungicides									
	Folio gold	8	22	70	9	24	67	13	17	70
	Babon	9	25	66	10	20	70	15	19	66
	Amistar top	7	27	66	8	22	70	17	23	60
	Check	25	65	10	25	60	15	30	43	27
	Mean	10.8	23.1	61.9	11.3	23.1	65.4	18.7	25.5	60.4
American basil	Biocides									
	Plant Guard	6	14	80	9	17	74	10	25	65
	Rhizo-N	9	11	80	8	13	79	13	17	60
	Biofertilizers									
	Biogen	14	18	68	14	18	68	19	27	54
	Nitrobien	15	20	65	13	22	65	24	28	48
	Plant extract									
	Basil	11	15	74	15	20	65	20	30	44
	Verbascum	13	22	65	16	24	60	28	34	38
	Fungicides									
	Folio gold	9	23	68	10	19	71	14	18	68
	Babon	10	27	63	11	21	68	17	20	63
	Amistar top	8	22	70	9	20	71	18	24	58
	Check	27	68	5	28	61	11	33	44	23
	Mean	12.2	24	63.8	13.3	23.5	63.2	20.2	26.7	52.1
L.S.D. at 5%		Treatments (T) = 3.50, Varieties (V) = 2.30, Fungi (F) = 4.1 T × V = 4.20, T × F = 4.50, V × F = 5.0, T ×V × F =5.30								

Table (7): Effect of different control treatments on percentages of infection by root rot on basil plants, grown in the infested soil, 90 days after transplanting, under greenhouse conditions

Variety	Treatments	Percentages of infection with:											
		Fusarium semitectum				*Rhizoctonia solani*				*Fusarium moniliforme*			
		30D	60D	90D	M	30D	60D	90D	M	30D	60D	90D	M
French basil	Biocides												
	Plant Guard	15.0	20.0	20.0	18.3	15.0	25.0	30.0	23.3	20.0	30.0	35.0	28.3
	Rhizo-N	15.0	20.0	30.0	21.7	20.0	30.0	35.0	28.3	30.0	40.0	45.0	38.3
	Biofertilizers												
	Biogen	20.0	30.0	35.0	28.3	30.0	35.0	35.0	33.3	35.0	40.0	50.0	41.7
	Nitrobien	25.0	35.0	35.0	31.7	35.0	35.0	40.0	36.7	20.0	35.0	40.0	31.7
	Plant extract												
	Basil	35.0	40.0	40.0	38.3	30.0	35.0	40.0	35.0	25.0	35.0	45.0	35.0
	Verbascum	25.0	30.0	35.0	30.0	25.0	30.0	35.0	30.0	25.0	30.0	35.0	30.0
	Fungicides												
	Folio gold	30.0	35.0	40.0	35.0	15.0	25.0	30.0	23.3	25.0	30.0	35.0	30.0
	Babon	20.0	30.0	35.0	28.3	25.0	30.0	40.0	31.7	25.0	35.0	40.0	33.3
	Amistar top	20	25.0	40.0	28.3	25.0	35.0	45.0	35.0	30.0	35.0	40.0	35.0
	Check	75	80.0	85.0	80.0	70.0	75.0	80.0	75.0	75.0	75.0	85.0	78.3
	Mean	28.0	34.5	39.5	33.9	29.0	35.5	41.0	35.1	31.0	38.5	45.0	38.1
American basil	Biocides												
	Plant Guard	15.0	15.0	25.0	18.3	20.0	25.0	30.0	25.0	25.0	30.0	35.0	30.0
	Rhizo-N	20.0	25.0	30.0	25.0	25.0	30.0	35.0	30.0	35.0	40.0	45.0	40.0
	Biofertilizers												
	Biogen	25.0	30.0	40.0	31.7	30.0	35.0	40.0	35.0	35.0	40.0	50.0	41.7
	Nitrobien	25.0	30.0	45.0	33.3	35.0	40.0	45.0	40.0	25.0	40.0	45.0	36.7
	Plant extract												
	Basil	35.0	40.0	45.0	40.0	30.0	35.0	45.0	36.7	25.0	35.0	35.0	31.7
	Verbascum	30.0	35.0	40.0	35.0	25.0	35.0	40.0	33.3	25.0	35.0	25.0	28.3
	Fungicides												
	Folio gold	30.0	35.0	45.0	36.7	20.0	25.0	35.0	26.7	25.0	35.0	40.0	33.3
	Babon	20	25.0	45.0	30.0	25.0	25.0	30.0	26.7	25.0	35.0	45.0	35.0
	Amistar top	25.0	30.0	35.0	30.0	30.0	30.0	35.0	31.7	30.0	40.0	50.0	40.0
	Check	75.0	80.0	85.0	80.0	75.0	75.0	85.0	78.3	75.0	75.0	85.0	78.3
	Mean	30.0	34.5	43.5	36.0	31.5	32.5	42.0	36.3	32.5	40.5	45.5	36.7
L.S.D. at 5%		Treatments (T) = 4.30, Varieties (V) = 1.50, Fungi (F) = 3.50 T × V = 7.60, T × F = 8.50, V × F = 8.70, T ×V × F = 9.40											

Table (8): The effect of different control treatments on some of growth parameters of two basil varieties in infested soil 60 days after transplanting, under greenhouse conditions.

Variety	Treatments	*F, semitictume*			*Rhizoctonia solani*			*F. moniliforme*		
		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)
French basil	Biocides									
	Plant Guard	100	750	90	100	760	95	105	750	96
	Rhizo-N	120	800	100	125	810	110	123	805	105
	Biofertilizers									
	Biogen	70	600	80	75	610	85	80	605	80
	Nitrobien	80	650	85	85	660	90	82	650	95
	Plant extract									
	Basil	75	680	70	80	690	75	85	695	80
	Verbascum	85	700	75	90	710	95	93	705	92
	Fungicides									
	Folio gold	90	700	85	94	703	92	95	706	93
	Babon	95	720	80	93	725	86	95	730	85
	Amistar top	98	730	90	100	735	96	95	740	95
	Check	55	400	50	55	400	50	60	390	50
	Mean	86.8	67.3	80.5	89.7	680.3	87.4	91.3	677.6	87.1
American basil	Biocides									
	Plant Guard	95	730	85	96	735	89	93	738	90
	Rhizo-N	110	780	95	100	785	90	105	780	94
	Biofertilizers									
	Biogen	65	590	75	70	595	80	80	600	80
	Nitrobien	75	630	80	80	640	85	85	650	84
	Plant extract									
	Basil	70	640	65	75	645	70	80	650	75
	Verbascum	80	650	70	83	660	75	75	665	80
	Fungicides									
	Folio gold	85	680	80	86	684	82	87	689	85
	Babon	90	700	75	95	710	80	94	705	75
	Amistar top	95	710	85	90	705	86	85	700	86
	Check	60	410	55	62	63	55	60	400	53
	Mean	82.5	652	76.5	83.7	622.2	79.2	84.4	658.3	80.2
L.S.D. at 5%	Treatments (T) = 4.70, Varieties (V) = 2.50, Fungi (F) = 5.20 T×V = 7.50, T×F = 9.50, V×F = 10.30, T×V×F = 12.40									

Table (9): Effect of different control treatments on controlling basil root rot infection (%) and on some growth parameters of basil plants during 2016 and 2017 growing seasons under field conditions.

Variety	Treatments	2016				2017			
		Infection (%)	Fresh weight / (g)	Dry weight / (g)	Essential oil (ml) /100g. fresh herb	Infection (%)	Fresh weight / (g)	Dry weight / (g)	Essential oil (ml) /100g. fresh herb
French basil	Biocides								
	Plant Guard	15	1200	80	0.4	15	1100	80	0.5
	Rhizo-N	20	1100	70	0.5	20	1000	70	0.6
	Biofertilizers								
	Biogen	20	1000	60	0.2	25	950	55	0.3
	Nitrobien	25	1050	55	0.1	30	1000	50	0.2
	Plant extract								
	Basil	30	950	40	0.3	35	900	35	0.3
	Verbascum	25	940	45	0.2	30	860	40	0.3
	Fungicides								
	Folio gold	20	1040	70	0.7	25	1000	66	0.7
	Babon	25	1060	75	0.6	25	1050	70	0.7
	Amistar top	25	1080	86	0.7	30	1060	80	0.8
	Check	65	700	20	0.01	65	720	15	0.01
	Mean	27	1012	60.1	0.3	30.3	964	56.1	0.33
American basil	Biocides								
	Plant Guard	15	1050	65	0.3	15	1000	60	0.3
	Rhizo-N	20	1100	75	0.4	20	900	65	0.5
	Biofertilizers								
	Biogen	25	960	50	0.2	40	850	44	0.2
	Nitrobien	30	1040	40	0.1	45	800	33	0.2
	Plant extract								
	Basil	35	940	30	0.2	50	750	28	0.3
	Verbascum	25	930	35	0.1	45	700	32	0.2
	Fungicides								
	Folio gold	25	1030	60	0.5	25	980	55	0.6
	Babon	25	1040	70	0.4	30	1020	65	0.4
	Amistar top	20	1050	80	0.6	20	1040	70	0.7
	Check	65	730	25	0.01	65	700	20	0.01
	Mean	28.5	987	53	0.28	35.5	874	47.2	0.34
L.S.D. at 5%									
Treatments (T) =		4.20	20.50	5.10	0.013	3.90	25.60	3.80	0.014
Varieties (V) =		1.30	15.0	n.s	0.010	n.s	n.s	n.s	0.015
T × V =		7.40	30.60	10.40	0.012	9.50	35.60	6.50	0.016

Rhizo-N Treatment | Plant Guard Treatment | Folio gold Treatment

Fig (1): Basil plants treated with Rhizo-N, Plant Guard and Folio gold

Babon Treatment | Amistar top Treatment | Biogen Treatment

Fig (2): Basil plants treated with Babon, Amistar top and Biogen

Nitrobien Treatment | Basil extract Treatment | Verbascum extract Treatment

Fig (3): Basil plants treated with Nitrobien, Basil extract and Verbascum extract

Fig (4): Basil plants untreated.

Discussion

Damping-off and root rot diseases are distributed in Beni-Suef and Fayoum Governorates. It seems that warm weather might be favorable for infection and development of these diseases. These results are in agreement with those obtained by Moussa (2002) and Huang *et al*. (2011).

Eight fungal isolates were recovered from rotted root basil samples collected from basil plantations in Beni-Suef and Fayoum governorates. Frequency of the isolated fungi was variable. *Rhizoctonia solani* and *F. moniliforme* were the most frequently isolated fungi followed by *F. roseum* and *M. phaseolina* from damped-seedlings. *F. oxysporum* was the most frequently isolated followed by *R. solani* from root rotted samples. According to the available literatures, *R. solani* and *F. oxysporum* were recorded for the first time on basil plants by Coa *et al.* (2004). In this study, *F. oxysporum* from diseased basil showed specific pathogen to *Ocimum basillicum* L. (Armstrong and Armstrong 1981).

Pathogenicity tests proved that *F. oxysporum*, *F. moniliforme, M. phaseolina*, *F. semitectum* and *R. solani* were pathogenic on var. French basil and American basil and showed typical symptoms of root rot. *Rhizoctonia solani* and *F. moniliform*e caused the highest pre- and post- emergence damping-off, whereas *M. phaseolina* and *F. semitectum* caused the lowest pre- and post- emergence damping-off. These results are in agreement with those obtained by Moussa (2002); Huang *et al*. (2011) and Abdel-Wahed (2018).

Different *Fusarium* spp. are known as a causal pathogens of root rot diseases of many plants (Persson *et al.*, 1997; Hashem *et al.*, 2010). *Fusarium oxysporum* f. sp. *basilica* is known as the main pathogen of sweet basil wilt, and crown and root rot. (Gamliel *et al.*, 1996; Lori *et al.*, 2014). Our results are in accordance with those of Elmer *et al.* (1994), Gamliel *et al.* (1996), Lori *et al.* (2014), who found that all isolates of *F. oxysporum* were virulent on sweet basil seedlings in the greenhouse. In accordance with our findings, Persson *et al.* (1997) noticed that *F. solani* could not be a causal agent of the vascular wilt, however most *F. oxysporum* isolates caused a significant root rot. The difference in disease incidence of many isolates, as well as the increase of disease severity for different crops were mentioned (Hashem *et al.*, 2010; Okoh *et al.*, 2010).

Due to its medicinal and aromatic properties, basil plants must grow under clean weather and free from chemical residues particularly fungicides. Thus using biofertilizers, plant extracts and biocides has become a good target as fungicides alternatives (El-Deeb *et al*., 2002.)

Traditionally, plant diseases are controlled by the application of synthetic fungicides (Eckert and Ogawa, 1988). However, the potential impact on environment as well as on human health largely limits their application (Eckert *et* *al*., 1994). It is reported that some microbes become fungicide-resistant (Holmes and Eckert, 1999), and thus a fungicide effect on controlling fungal infection may be greatly reduced so, a trail to induce systemic resistance against root rot/wilt in basil was done.

Results of the present study revealed that basil plants infected with root-rot showed a reduction in all growth parameters. This reduction may be due to the toxins produced by the fungus, which affect the stomatal function leading to uncontrolled transpiration and excessive loss of water, thus leading to dead plants. In addition, the decrease in shoot dry weight might be related to increased respiration rate, decompartmentalization due to membrane degradation (Ahmed *et al*., 2009).

Efficiency of biocides, biofertilizers, plant extracts and fungicides tested in controlling the diseases was confirmed. All these treatments gave significant reduction in infection percentages than the check (without treatment). The same reaction was also recorded in increasing plant parameters. In general, biocides and fungicides were the superior treatments in the previous tests.

Root rot and wilt fungal diseases effectively checked with various control measures such as biofertilizers (Abd El-Megid, 2003 and Abo-El-Ela, 2003), biological control agents (Fahim *et al*., 2000 and Abo-El-Ela, 2003), fungicides (Hilal *et al*., 2000 and Abo-El-Ela, 2003). Most of these control means positively improved growth parameters of many different plants as mentioned with biofertilizers (Abd El-Megid, 2003 and Abo-El-Ela, 2003), biological control agent (Fahim *et al*., 2000 and Abo-El-Ela, 2003). In most cases, these useful responses might be attributed to an indirect effect associated with control of the plant fungal pathogens similar to those reported on the efficiency of fungicides (Salt, 1978). On the other hand, precious values of the superior treatments (biocides and fungicides) in controlling the studied disease and in improving plant growth parameters in comparison with those of the fungicide tested were somewhat similar to those mentioned by Abd El-Megid (2003) and Abo-El-Ela (2003)

Biological control had attained importance in modern agriculture in order to curtail the hazards of the intensive use of chemicals for pest and disease control (Tuber and Baker 1988).

Our results are in agreement with those recorded by Hassanein *et al.* (2008), who found that the reduction in growth may also be related to the accumulation and action of phenolics produced from the degradation of cell wall lignin mainly via depolymerization resulting from fungal elicitors. On the other hand, treatment of plants with *O. basilicum* extract was highly effective on growth promotion and protection of basil against root rot and this might be carried out in a number of ways including antibiotic production, induced resistance and reducing the production of the mycotoxins fumonis secreted by the pathogen (El-Khallal 2007). Similar results were obtained by Hassanein *et al*. (2008), who found that the fresh weight of tomato plant was increased when plants were treated with leaf extracts of neem and chinaberry, and these treatments have the potential for use in the control of root rot disease. Also, basil extract, have potential substances such as tocopherol, phenols, flavonoids and high levels of important carotenoids, which are responsible for its antioxidant properties (Dean *et al*.2005).Treatment of some plants with plant extracts provided control of many fungal diseases through metabolic changes in plants, including induction of phenol biosynthesis enzymes, antioxidant defensive enzymes and phenol accumulation (Kamalakannan *et al*., 2004).

Conclusion

Despite the efficiency of pesticides in reducing the percentage of infection and increasing plant measurements, we recommend the use of biocides (Rizo-N and Plant Guard) for the following: (1) effective means, (2) safe for humans and the environment, (3) easy to use by farmers, (4) Pretty cheap price. We also recommend the use of the French basil variety in agriculture, as it gave the lowest percentage of infection beside it has good plant growth parameters.

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Corresponding author: Abdel-Wahed, G.A.
E-mail: gomaaarafat847@yahoo.com

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تقييم كفاءة بعض المعاملات الحيوية والمستخلصات النباتية والمبيدات الفطرية في مقاومة الفطريات المحمولة بالتربة التي تصيب نوعين من الريحان

جمعه عرفات عبد الواحد

قسم بحوث أمراض نباتات الزينة والطبية والعطرية ، معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، الجيزة ، مصر

تتعرض نباتات الريحان (*Ocimum basillicum* L.) تحت الظروف المصرية للاصابة من قبل العديد من الأمراض التي تنقلها التربة مما يتسبب ذلك في خسائر كبيرة. تم رصد انتشار واسع لموت البادرات واعفان الجذور في زراعات الريحان في محافظتي بني سويف والفيوم في خلال موسمي ٢٠١٦ و ٢٠١٧. تم تعريف الفطريات المعزولة من نباتات الريحان المصابة طبيعياً بهذه الأمراض على أنها *Fusarium subglutinans* ، *F. oxysporum* ، *F. moniliforme* ، *F. semitectum*، *F. nival* ، *F. roseum*، *Rhizoctonia solani*، و *Macrophomina phaseolina*. حقق *Fusarium moniliforme* و *R. solani* أعلى نسبة حدوث في تجارب العزل من عينات محافظتي بني سويف والفيوم. كان *Rhizoctonia solani* ألاكثر ضراوة من الفطريات الأخرى في اختبارات القدرة المرضية على صنفعي الريحان المختبرين الفرنسي والامريكي. تم اختبار تأثير المبيدات الحيوية (Plant guard and Rizo-N)، و الأسمدة الحيوية (Biogen and Nitrobien)، والمبيدات الفطرية (Folio gold, Babon and Amister top)، والمستخلصات النباتية (Basil and Verbascum) على مكافحة موت البادرات و اعفان الجذور علي صنفين من الريحان تحت ظروف الصوبة والحقل. تباينت عوامل المكافحة المختبره في كفائتها الإيجابية ضد الأمراض. كانت المبيدات الحيوية والمبيدات الفطرية افضل المعاملات في مكافحة الأمراض وتحسين قياسات النبات الخضرية. في تجارب الحقل التطبيقية ، أدت جميع المعاملات المختبرة إلى انخفاض معنوي في نسب الإصابة وزيادة قياسات النبات الخضرية مقارنة بالكنترول الغير معامل.