

## Effectiveness of Bio-Control Agents and Essential Oils on Controlling Damping-Off Disease in Peanut Plants

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**R**hizoctonia *solani*, *Fusarium solani* and *Macrophomina phaseolina* are the most devastating and economically important in many peanut growing areas in Egypt. The effect of some essential oils, bio-control agents and Vitavax/captan on peanut damping-off was evaluated *in vitro* and *in vivo*. *In vitro*, the obtained results indicated that all concentrations of essential oils of Thyme, Peppermint and Lemongrass significantly reduced the growth of the tested pathogenic fungi. Thyme and Lemongrass oils at 0.50% suppressed completely the mycelia growth of the tested fungi. On the other hand, all bio-control agents decreased mycelia growth of the tested fungi *in vitro* and Plant guard recorded the highest reduction in growth of the pathogenic fungi. Under greenhouse conditions, results indicated that all treatments significantly reduced peanut damping-off (pre- and post-emergence). Vitavax/captan followed by Lemongrass oil and Plant guard recorded the highest reduction of damping-off incidence under greenhouse conditions. Also, the obtained data, under field conditions, indicated that all treatments significantly reduced damping-off in peanut and increased plant growth and yield in both growing seasons. Vitavax/captan followed by Lemongrass and Plant guard recorded the highest reduction of damping-off incidence and the highest increase of plant growth and yield in peanut during both growing seasons.

**Keywords:** Biocides, Essential oils, Peanut, Root-rot diseases

Peanut (*Arachis hypogaea* L.) is one of the most important leguminous and oily crops in Egypt and the world. Damping-off of seedlings caused by *R. solani*, *Sclerotium rolfsii*, *M. phaseolina* and *Fusarium* spp. is among the most destructive diseases, which attack peanut plants causing quantitative and qualitative losses in yield (Mahmoud *et al.*, 2013). A successful disease-control program could involve generally requires the application of several control measures (Dik *et al.*, 2002). Generally, IPM is regarded as the use of environmentally safe practices to reduce the disease incidence and development or use of multiple control tactics integrated into a single pest control strategy (Zinkernagel *et al.*, 2002).

The use of different approach in plant disease control *i.e.*, bio-control agents, plant extracts and natural compounds were used as an IPM program to control damping-off and root rot diseases in many crops (Napier and Oosthusse, 2002; Jirovetz *et al.*, 2005 and Pawar and Thaker, 2006). Among other control measures the use of compounds that induce a systemic plant resistance which were successfully used against several plant diseases incidence affected either plant root

or shoot systems. In this regard, Lemongrass (*Cymbopogon citratus* L.) oil was reported to be antifungal against several plant pathogens. Fungal spore production, spore germination and germ tube length of *Colletotrichum coccodes*, *Botrytis cinerea* and *Rhizopus stolonifer* were inhibited with lemongrass oil treatments (Tzortzakakis and Economakis, 2007; Mironescua and Qeorgescub, 2008; Hadizadeh *et al.*, 2009 and Hussein, 2011).

The use of bio- agents has led in preventing many plant diseases (Sivan and Chet, 1992; Sharon, 2001 and Rose *et al.*, 2003). Recently, it was suggested that *Trichoderma* affects induced systemic resistance ISR mechanism in plants (Shoresh *et al.*, 2005; Hoitink *et al.*, 2006; El-Mougy and Abd El-Kader, 2008 and El-Mougy *et al.*, 2012). Mohamed (2015) found that some antagonists significantly reduced the percentage of disease incidence, disease severity and increased seedlings survival of peanut compared with untreated control.

The aim of this study is an attempt to study the ability of some biocides and essential oils in controlling damping-off incidence compared to a recommended fungicide as well as their impact on plant growth and crop parameters under field conditions.

### Materials and Methods

#### *Sources of pathogens:*

The highly pathogenic fungi, *i.e.* *F. solani*, *R. solani* and *M. phaseolina* were obtained from the Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.

#### *Laboratory tests:*

The effects of some essential oils on the linear growth of the pathogenic fungi were evaluated in laboratory. The essential oils, *viz.* Thyme, Peppermint and Lemongrass, at different concentrations were used in this test. Two mL of Tween 80 (0.2% of water volume) were added to the essential oil solutions to obtain an aqueous emulsion (El-Mougy *et al.*, 2012). The essential oils were added individually to conical flasks containing sterilized PDA medium before solidification to obtain the proposed concentrations of 0.25, 0.5 and 1.0% (Mokhtar *et al.*, 2013). A separate PDA flask free of essential oils was used as check control treatment. The supplemented media were poured into Petri dishes 9 cm, nearly 20 ml per each. Mycelial discs 5 mm were taken from the periphery of actively growing PDA cultures of pathogenic fungi, each placed at the center of the Petri dish, then quickly sealed with Para film, and incubated for seven days at 25±1°C. For each treatment, three concentrations were tested, and three replicates were used. The reduction in fungal growth was calculated in relation to its growth in check treatment. The percent growth inhibition (PGI) was calculated using the following formula:

$$PGI = (C - T) / C \times 100.$$

Whereas; PGI= Percentage growth inhibition, C = Radial growth of the pathogen in control plates (cm) and T = Radial growth of the pathogen in dual culture plate (cm). The percent growth inhibition was calculated as mentioned before.

*Effect of some biocides on mycelial growth of pathogenic fungi:*

Biocides presented in Table 1 were mixed with PDA medium. The plate's center was inoculated with pathogenic fungi, i.e. *F. solani*, *R. solani* and *M. phaseolina* discs 5mm-diam. of PDA culture each alone and incubated at 25±1°C for 7 days. Biocides-free plates served as control. Average radial growth was recorded when any plate was covered with the fungal growth.

**Table 1. Biocides used as bio-control agents against pathogenic fungi**

Biocide trade name	Producer name	Bio-agents concentration (cfu/ml)	Dose per kg seed
Rhizo-N	El-Nasr Co.	<i>B. subtilis</i> , 3 × 10 <sup>7</sup>	4.0 g
Bio-zeid	El-Nasr Co.	<i>T. album</i> , 3 × 10 <sup>7</sup>	2.5 g
Plant guard	El-Nasr Co.	<i>T. harzianum</i> , 3 × 10 <sup>7</sup>	4.0 ml

*Effect of different essential oils on damping-off incidence in pots:*

The experiment was carried out under greenhouse conditions in 2015 growing season. Inocula were prepared by growing each of the isolated fungi, *F. solani*, *R. solani* and *M. phaseolina* in 500 ml conical flask containing 200 ml of autoclaved corn meal broth medium and incubated at 25±1°C for 10 – 15 days (Abd El-Moity, 1985). The fungal growth was blended in the blender for two minutes using sterilized water to homogenize the inocula. Plastic pots 30 cm in diameter were sterilized by immersing in formalin 5% solution for 15 minutes, then left to dry for 7 days to ensure getting rid and evaporation the excess poisonous of formalin. Infestation was carried out by adding the blended homogenized fungal inoculum to sterilized soil then watered and left for 7 days to ensure its distribution in the soil. Control pots were watered with the used medium free from the fungus at the same rate. Three replicate pots were used for each particular treatment. Seeds were soaked for 1 h in the essential oils at concentration 0.5 and 1.0%. Five seeds were sown in each pot. All plants were observed daily and watered as needed.

*Disease assessment:*

Disease assessment was recorded as percentages of pre- and post-emergence damping-off after 15 and 30 days from sowing, respectively (Mahmoud et al., 2013). Percentages of pre- and post-emergence damping-off were calculated using the following formula:

$$\text{Pre-emergence (\%)} = \frac{\text{Number of non-germinated seeds}}{\text{Number of planted seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{Number of dead seedlings}}{\text{Number of planted seeds}} \times 100$$

$$\text{Plant survival (\%)} = \frac{\text{Number of survived healthy plants}}{\text{Number of planted seeds}} \times 100$$

*Effect of the tested biocides on damping-off in pots:*

This experiment was conducted in sterilized plastic pots 25 cm diam. containing sterilized loam sandy soil in the greenhouse. Soil was infested with the tested pathogenic fungi as mentioned before. The peanut seeds cv. Giza 3 were treated with the tested biocides (Rhizo-N, Bio-zeid and Plant guard) at the recommended dose as presented in Table 1 after treatment with 4% solution of carboxymethyl cellulose (CMC) as sticker. The same aforementioned methods were used without biocides as control. Five seeds were sown per pot. Three pots as a replicates were used for each treatment. Pre- and post-emergence damping-off and healthy survival were recorded as mentioned before.

*Field experiments:*

Field experiments were conducted in naturally infested soil at Sers El-Lyain Agric. Res. Station in 2015 and 2016 growing seasons. A randomized complete block design with 5 replications was used in each season. A field experiment consisted of plots 7 × 6 m; each comprised of 10 rows 20 cm distance and 30 holes / row was conducted with five plots as replicates for each particular treatment as well as untreated check treatment. The essential oils, *viz.* Thyme, Peppermint and Lemongrass at 0.5% concentration alone were used. Peanut seeds cv. Giza 3 were sown during the two successive seasons 2015 and 2016. Fungicide Vitavax/captan at the rate of 3 g/kg seeds was used as seed dressing. Untreated seeds were planted as a control. Pre- and post-emergence damping-off percentages were determined after 15 and 30 days from sowing, respectively. At harvest, some peanut parameters such as shoot weight g/plant, shoot length cm, root weight g/plant, root length cm and yield kg/plot were recorded.

*Statistical analysis:*

The obtained data were subjected to analysis of variance according Fisher (2002). Means were separated by fisher's protected least significant differences L.S.D at  $p < 0.05$  level (Gomez and Gomez, 1984).

## Results

*Laboratory tests:*

Data presented in Table 2 indicate that all essential oils at different concentrations were significantly inhibited mycelial growth of all the tested fungi. Increasing concentration of essential oils increased mycelial growth inhibition. This inhibition for each of *F. solani*, *R. solani* and *M. phaseolina* was the highest with Lemongrass oil and Thyme oil. Generally, Lemongrass oil was the most effective with all fungi followed by Thyme oil, while Peppermint was the lowest effective with all fungi.



**Table 3. Effect of different biocides on mycelium growth of pathogenic fungi *in vitro***

Biocide name	<i>F. solani</i>		<i>R. solani</i>		<i>M. phaseolina</i>	
	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Rhizo-N	66.00	26.66	53.00	41.11	60.00	33.33
Bio zaid	40.00	55.55	33.23	63.10	37.00	58.88
Plant gaurd	33.00	63.33	28.00	68.88	32.00	64.44
Control	90.00	0.00	90.00	0.00	90.00	0.00
LSD at 5%	2.90	–	0.37	–	4.01	–

*Effect of essential oils on pre- and post-emergence damping-off in pots:*

Data in Table 4 show that there is significant difference among the tested essential oils in decreasing pre- and post-emergence damping-off percentages and increasing healthy survived plant percentage. Lemongrass oil and Thyme oil were the most effective essential oils with all fungi, followed by Peppermint oil. Data showed that the best applied concentration was 0.50%.

**Table 4. Effect of different essential oils on pre- and post-emergence damping-off in pots**

Essential oils	Concentrations %	Damping-off (%)						Plant Survivals (%)		
		Pre-emergence			Post-emergence			<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>
		<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>	<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>			
Thyme oil	0.50	13.31	13.3	20.0	20.0	20.0	20.0	66.6	66.6	60.0
	1.00	3.3	13.3	13.3	13.3	13.3	13.3	73.4	73.4	73.4
	Mean	13.3	13.3	16.7	16.7	16.7	16.7	70.1	70.0	66.6
Peppermint oil	0.50	20.0	20.0	20.0	20.0	20.0	20.0	60.0	60.0	60.0
	1.00	20.0	20.0	20.0	20.0	13.3	13.3	60.0	66.7	66.7
	Mean	20.0	20.0	20.0	20.0	16.7	16.7	60.0	63.3	63.3
Lemon-grass oil	0.50	13.3	13.3	13.3	13.3	13.3	13.3	73.4	73.4	73.4
	1.00	13.3	13.3	6.7	13.3	13.3	13.3	73.4	73.4	80.0
	Mean	13.3	13.3	10.0	13.3	13.3	13.3	73.4	73.4	76.7
Mean	0.50	15.5	15.5	17.8	17.8	17.8	17.8	66.7	66.7	64.5
	1.00	15.5	15.5	13.3	15.5	13.3	13.3	68.9	71.2	73.4
	Mean	15.5	15.5	15.6	16.7	15.5	15.5	67.8	68.9	68.9
Control	–	26.7	26.7	33.3	26.7	26.7	26.7	46.6	46.6	40.0

LSD at 0.05 for:

Essential oils (A) = 6.6    7.9    6.3    9.3    5.8    8.6    12.7    9.9    5.8  
 Concentrations(B) = n.s.    n.s.    2.3    2.0    3.1    3.1    n.s.    n.s.    n.s.  
 Interaction (A×B) = 7.7    11.7    12.6    11.8    7.7    10.8    14.8    11.2    20.4

*Effect of the tested biocides on damping-off incidence in pots:*

Data in Table 5 show that there is a significant difference among the tested biocides in decreasing percentage of pre- and post-emergence damping-off and increasing percentage of healthy survived plants and control treatment. Plant guard was the most effective biocide with each of *F. solani*, *R. solani* and *M. phaseolina* followed by Rhizo-N and Bio-zeid. Generally, Plant guard was the most effective in pots, followed by Rhizo-N and Bio-zeid. However, the differences among the biocides, in most cases, were not significant.

**Table 5. Effect of three biocides on pre- and post-emergence damping-off and healthy survived plants in pots**

Biocide	Damping-off (%)						Plant survivals (%)		
	Pre-emergence			Post-emergence			<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>
	<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>	<i>F. solani</i>	<i>R. solani</i>	<i>M. phaseolina</i>			
Rhizo-N	11.1	12.2	10.0	10.0	10.0	10.0	78.9	77.8	80.0
Bio-zeid	13.3	14.4	12.2	11.1	12.2	10.0	75.6	73.5	77.8
Plant guard	10.0	9.2	8.7	6.7	6.7	5.3	83.3	84.1	86.0
Control	33.3	33.3	28.6	28.9	30.0	26.7	37.8	36.7	44.7
LSD at 5%	5.6	5.3	5.7	7.2	6.0	5.2	10.7	11.0	9.8

*Effect of different essential oils compared with Vitavax/captan on damping-off on peanut plants under field conditions during 2015 and 2016 growing seasons:*

The obtained data in Table 6 show that all treatments significantly reduced pre- and post-emergence damping-off and increased the survived seedlings of peanut compared with untreated control. Data also showed that the fungicide treatment as seed dressing gave significant disease reduction in pre- and post-emergence damping-off values compared with untreated control.

**Table 6. Effect of essential oils on damping-off disease of peanut plants under field conditions**

Treatment	Seasons 2015			Seasons 2016		
	Damping-off %		Plant survivals %	Damping-off %		Plant survivals %
	Pre-emergence	post-emergence		Pre-emergence	post-emergence	
Thyme oil	16.67	21.33	62.00	17.33	22.00	60.67
Peppermint oil	18.00	22.00	60.00	19.33	25.33	55.34
Lemongrass oil	14.67	18.00	67.33	16.00	21.33	62.67
Vitavax/captan	0.00	10.00	90.00	10.00	10.00	80.00
Control	33.33	40.00	26.67	36.00	48.00	16.00
LSD at 5%	5.12	6.31	9.34	5.89	7.54	9.24

*Effect of different biocides compared with Vitavax/captan on pre and post-emergence damping-off on peanut plants under field conditions during 2015 and 2016 seasons:*

Data presented in Table 7 show that all tested biocides significantly reduced damping-off (pre- and post- emergence damping-off) and increased survived plants compared with check treatment (control) during both growing seasons (2015 and 2016). Rhizo-N and Plant guard were the best biocides treatments for controlling damping-off diseases in both growing seasons while; Bio-zeid was the lowest one in this respect. On the other hand, peanut seeds treated with the fungicide Vitavax/captan recorded the lowest damping-off (pre- and post- emergence) incidence and the highest plant survivals compared with biocide treatments.

**Table 7. Effect of biocides on damping-off of peanut plants under field conditions**

Treatment	Seasons 2015			Seasons 2016		
	Damping-off (%)		Plant Survivals (%)	Damping-off (%)		Plant Survivals (%)
	Pre-emergence	post-emergence		Pre-emergence	post-emergence	
Rhizo-N	14.00	10.00	76.00	16.00	12.00	72.00
Bio-zeid	18.00	16.00	66.00	20.00	18.00	62.00
Plant guard	12.00	10.00	78.00	14.00	12.00	74.00
Vitavax/captan	0.00	10.00	90.00	10.00	10.00	80.00
Control	33.33	40.00	26.67	36.00	48.00	16.00
LSD at 5%	6.20	6.80	11.11	5.80	6.10	8.32

*Effect of different essential oils on peanut growth and yield under field conditions:*

Data in Table 8 show significant differences and positive correlation among the tested essential oils as seed treatment and improving peanut plant growth compared with control treatment. Lemongrass oil and Thyme oil were the best essential oils compared with the control in the two growing seasons. Data also showed that the fungicide treatment as seed dressing gave significant disease reduction in pre- and post-emergence damping-off values and yield increase as well.

*Effect of different biocides on peanut growth and yield under field conditions:*

Data presented in Table 9 show that all biocides and the fungicide *Vitavax/captan* significantly increased plant growth (shoot length, shoot weight, root length and root weight) and pod yield weight per plot compared with control during both growing seasons (2015 and 2016). The fungicide *Vitavax/captan* recorded the highest plant growth and pod yield /plot followed by Plant guard treatment. Meanwhile, Bio-zeid treatment recorded the lowest increase in plant growth in this respect in both growing seasons.

**Table 8. Effect of essential oils on plant growth parameters and yield of peanut plants under field conditions during growing seasons 2015 and 2016**

Treatment	Shoot weight (gm)		Shoot length (cm)		Roots weight (gm)		Root length (cm)		Yield (Kg / plot)	
	Season 2015	Season 2016	Season 2015	Season 2016	Season 2015	Season 2016	Season 2015	Season 2016	Season 2015	Season 2016
Thyme oil	75.0	101.3	28.0	36.0	25.3	32.0	28.0	30.0	31.0	34.0
Peppermint oil	70.0	98.4	26.0	33.0	21.6	29.0	26.0	28.0	29.6	31.1
Lemongrass oil	81.0	103.2	30.0	40.0	27.3	37.2	30.3	33.0	33.9	35.0
Vitavax/captan	125.0	144.0	32.1	44.2	30.2	39.0	35.0	38.3	45.1	53.2
Control	38.1	60.3	25.0	28.0	21.7	23.6	21.0	25.0	15.1	17.3
L.S.D. at 5%	2.1	2.3	1.1	1.3	2.1	2.2	1.9	1.7	2.8	2.0

**Table 9. Effect of biocides on plant growth and yield of peanut plants under field conditions during growing seasons 2015 and 2016**

Biocides	Shoot weight (gm/plant)		Shoot length (cm)		Root weight (gm/plant)		Root length (cm)		Yield (kg / plot)	
	Season 2015	Season 2016	Season 2015	Season 2016	Season 2015	Season 2016	Season 2015	Season 2016	Season 2015	Season 2016
Rhizo-N	83.4	107.0	28.0	39.0	25.0	35.7	31.2	33.2	35.2	37.1
Bio-zeid	81.0	45.7	27.2	36.0	23.0	31.9	28.2	30.2	33.5	35.3
Plant guard	95.0	139.2	30.0	41.3	28.7	36.8	33.1	36.0	39.2	42.3
Vitavax/captan	125.0	144.0	32.1	44.2	30.2	39.0	35.0	38.3	45.1	53.2
Control	38.1	60.3	25.0	28.0	21.7	23.6	21.0	25.0	15.1	17.3
LSD at 5%	8.2	7.4	1.7	2.0	1.08	1.3	1.2	1.3	2.9	2.9

### Discussion

Several fungi were found to be responsible for the damping-off of peanut seedlings growing in many locations in Egypt. The role of three essential oils, i.e. Thyme, Peppermint and Lemongrass and three biocides *i.e.* Rhizo-N, Bio-zeid and Plant guard as well as the fungicide Vitavax/captan in controlling damping-off of peanut caused by *R. solani*, *F. solani* and *M. phaseolina* was evaluated under greenhouse and field conditions.

The results presented in this study indicated that all treatments, in most cases gave significant reduction in damping-off (pre- and post- emergence) of seedlings grown under greenhouse and field conditions compared with control. Thyme and Lemongrass oils at 0.50% gave the highest effect in controlling damping-off. On the other hand, Plant guard also reduced pre-and post-emergence damping-off. Data also, showed that there is a relation between the essential oils concentration and their effect on the incidence of damping-off. Data clearly indicated that increasing the concentration of essential oils led to increase their effect in reducing incidence of the disease (Jirovetz *et al.*, 2005; Pawar and Thaker, 2006; Mironescua and Georgescub, 2008; Hadizadeh *et al.*, 2009 and Hussein, 2011). Therefore, biological control of plant pathogens has become an important component of plant disease management practices. Plant guard is effective bio-control agent for controlling a number of soil-borne plant pathogens (Sharon *et al.*, 2001 and Rose, 2003). In the present study, results indicated that biocides significantly reduced the growth area of the pathogenic fungi in vitro conditions. Such results concerning the inhibitory effect of various fungal antagonists on soil-borne plant pathogens were reported previously by many investigators (El-Mougy and Abd El-Kader, 2008 and El-Mougy *et al.*, 2012).

Also, all treatments significantly increased peanut yield throughout the two growing seasons compared with untreated control. The highest increase was recorded due to using Plant guard and Lemongrass oils. Data also showed that the fungicide treatment as seed dressing gave significant disease reduction in pre- and post-emergence damping-off compared with untreated control. Similar results were also reported by several investigators (Nofal *et al.*, 1990; Siddaramaiah, 1979 and El-Nagar *et al.*, 1990).

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فاعلية المركبات الحيوية والزيوت الأساسية في مكافحة  
مرض سقوط البادرات في الفول السوداني

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تعتبر الفطريات *Rhizoctonia solani* و *Fusarium solani* و *Macrophomina phaseolina* من أهم وأخطر المسببات المرضية في الفول السوداني في مصر. تم دراسة تأثير بعض الزيوت الأساسية (زيت الزعتر والفلفل وحشيشه الليمون) والمركبات الحيوية (ريزو ان- بيوزيد- بلانت جارد) مقارنة بمبيد الفيتافاكس كابتن على مرض سقوط البادرات تحت ظروف الصوبة والحقل . حيث وجد أن كل التركيزات المستخدمة من زيت الزعتر والفلفل وحشيشه الليمون تخفض معنويا النمو الميسليومي للفطريات الممرضة المستخدمة في الدراسة . فقد وجد أن استخدام زيت حشيشه الليمون أحدث تثبيطا كاملا لنمو لنمو الفطريات الممرضة عند تركيز 0.5 % . من ناحية أخرى تثبتت المركبات الحيوية من النمو الميسليومي للفطريات الممرضة وكان مركب بلانت جارد أكثر تلك المركبات تثبيطا لها. تحت ظروف الصوبة أدت كل المعاملات المستخدمة في الدراسة إلى خفض معنوي لمرض سقوط البادرات في الفول السوداني . أيضا تبين من النتائج المتحصل عليها تحت ظروف الحقل أن كل المعاملات أدت إلى خفض معنوي لنسبة حدوث مرض سقوط البادرات وزيادة صفات النمو والمحصول في كلا موسمي الدراسة. وكان مبيد فيتفاكس /كابتن يليه زيت حشيشه الليمون والمركب الحيوي بلانت جارد أفضل تلك المعاملات خلال موسمي الدراسة.