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Effectiveness of Certain Biocides and Essential Oils in Controlling Damping-Off and Root-Rot Diseases of Soybean (*Glycine max* (L.) Merr.)

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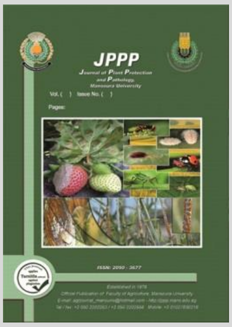

ABSTRACT

Damping-off and root-rot are some of the most important diseases attack soybean (*Glycine max* (L.) Merr.) in Egypt. The effect of biocides, i.e. Bio-Cure-F, Plant guard, and Rhizo-N, and essential oils of eucalyptus, thyme, and lemongrass oils, as well as the fungicide Vitavax-200, were evaluated *in vitro*, greenhouse and under field conditions during the two successive growing seasons, 2018 and 2019 to control damping-off and root-rot diseases of soybean. All the tested biocides and essential oils used significantly inhibited the linear growth of the tested pathogenic fungi, *i.e.*, *Fusarium moniliforme*, *Fusarium solani* and *Rhizoctonia solani* compared with the control. Under greenhouse and field conditions in both growing seasons, obtained results indicated that all the tested biocides and essential oils significantly reduced the percentages of soybean damping-off and root-rot severity furthermore improved photosynthetic pigments content, growth parameters, and seed yield. Vitavax-200 and Plant guard gave the highest values of reduction in this respect, followed by Rhizo-N, lemongrass oil, Bio-Cure-F, thyme oil, and eucalyptus oil respectively. Besides, the quality parameters of soybean seeds showed a favorable elevation in total oil content, on the contrary, there was a general decrease in the seeds protein content. However, the correlations between seeds oil and protein contents were negatively correlated. It could be concluded in biocides and essential oils examined can be recommended for biocontrol of soybean damping-off and root-rot diseases.

Keywords: Soybean, Biocides, Essential oils, Damping-off, Root-rot, Photosynthetic pigments, Oil content, Protein content.

INTRODUCTION

Soybean (*Glycine max* (L). Merr.) is one of the most valuable Fabaceae crops cultivated worldwide (Patkowska and Konopinski, 2013). It represents one of the important sources of oil and protein, soybean seed contains on a dry weight basis about 190 to 230 g kg^{-1} oil and about 380 to 420 g kg^{-1} protein (Bellaloui *et al.*, 2015). Great efforts in Egypt are paid to increase the cultivated area and to increase its productivity *via* following programs concerned with pest control.

Seedling diseases are the most widely distributed diseases of soybean. *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme*, *Fusarium spp*. *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Phytophthora sojae*, *and Pythium spp*. represented the most fungal attack soybean seeds, seedlings and roots causing damping-off, root-rot and wilt diseases resulting in serious economic losses limiting soybean growth and productivity (Hartman *et al.*, 1999.; Abd El-Hai *et al.*, 2016.; El-Gendy *et al.*, 2016 and Yassin *et al.*, 2019).

Plant diseases are conventionally controlled by chemical fungicides. However, due to the potentially harmful effects on the environment because of the use of fungicide, alternative control measures are necessary to replace synthetic organic pesticides to diminish the hazardous side effects of fungicides. (Ozkara *et al.*, 2016).

For disease control several investigator suggested different approach including fungicides, soil fumigation, plant breeding for resistance, biological control, essential oils and natural compounds in integrated pest management (IPM) program to control plant diseases in many crops (Ozcan and Erkmen, 2001; Jirovetz *et al.*, 2005; Hadizadeh *et al.*, 2009 and Abo-Elyousr *et al.*, 2014).

The applications of biotic and abiotic inducers have the potential in controlling plant diseases, however leading to different defense reactions in the host plants, including pathogenesis-related proteins, defense-related enzymes, lignin synthesis, accumulation of phenolic compounds and specific flavonoids in response to microbial infection (Reddy *et al.*, 2014 and Prasannath 2017). Meanwhile, a promising approach to control diseases caused by soil-borne pathogens (Reddy *et al.*, 2014 and Singh *et al.*, 2018).

Essential oils are composed of complex fractions of volatile oils, hydrophobic, lipophilic and liquid that are produced and extracted from plant parts (Velluti *et al.*, 2003; Andrade *et al.*, 2014). Which are known to have various biological properties in plants that may provide potential antibacterial activity of these plant extracts may be due to secondary metabolites and several chemical compounds of relatively complex structures including alkaloids, flavonoids, terpenoids, isoflavonoids, glycosides, tannins, coumarin, phenylpropanoids, terpenes, and organic acids that could adversely influence pathogen growth and disease development (Nychas 1996; Wijesekara *et al.*, 1997 and Andrade *et al.*, 2014). These antimicrobial activities frequently related to the essential oil fraction or to volatile aromatic compounds in the aqueous phase (Gould, 1995; El-Gayyar *et al.*, 2001 and Leal *et al.*, 2003).

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Therefore the present study aimed to study the effectiveness of certain biocides and essential oils for management soybean damping-off and root rots incidence compared to a recommended fungicide also their impact on soybean growth parameters and yield.

MATERIALS AND METHODS

Source of soybean seeds:

Soybean seeds (*Glycine max* (L.) Merr.) cv. Giza 35 was obtained from Legume Crop Res. Dep., Field Crop Res. Inst., Agric. Res. Centre, Giza, Egypt.

Sources of the pathogens:

The pathogenic fungi *i.e., Fusarium moniliforme, Fusarium solani* and *Rhizoctonia solani*, isolated from naturally infected soybean plants showing symptoms of damping-off and root-rot, were kindly provided by Legume and Forage Dis. Res. Dept., Plant Pathol. Res. Inst., Agric. Res. Cent., Giza, Egypt. The cultures of these fungi were maintained on malt extract agar (Malt extract 20g, Peptone 3g and Agar 18g) slants under a phosphate buffer (pH 6.5) at 4±0.5°C (Boeswinkel 1976).

The tested Biocides:

Bio-Cure-F, Contain *Trichoderma viride,* 2×10^6 CFU/g, 4.0 g per kg seed; Plant guard Contain *Trichoderma harzianum,* 3×10^7 CFU/g, 4.0 g per kg seed and Rhizo-N Contain *Bacillus subtilis*, 3×10^7 CFU/mL 4.0 mL per kg seed.

Source of the essential oils:

Pure-grade essential oils of eucalyptus [*Eucalyptus globules* (*a.i.* cineol 80%)], thyme [*Thymus vulgaris* (*a.i.* thymol, 60%)] and lemongrass [*Cymbopogon citratus* (*a.i.* citral 85%)] were obtained from Chemical Industrial Development Company (CID), Egypt. and stored in dark glass bottles at 4±1°C until use.

The tested fungicide Vitavax-200 (WP 75%):

Common name: Carboxin and Thiram, Active Ingredients: Carboxin (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide) & Thiram (tetramethyl thiuram disulfide). Dose per kg seed: 3g/Kg Seed

Effect of some biocides on the fungal linear growth of the tested pathogens:

The biocides, *i.e.* Bio-Cure-F, Plant Guard and Rhizo-N, were added individually to sterilized PDA medium and poured into Petri-dishes 9 cm, before solidification to obtain the concentration 1000 ppm. PDA medium free from biocides was used as a control. Then used after 24 h. Mycelial discs (5 mm) taken from the previously prepared cultures of the fungal isolates, each was placed at the center of the Petri-dishes and incubated at 28±1°C. The averages of the linear growth were recorded when the check treatment plates covered with the fungal growth. Three replicates were used for each treatment and the control. The percentage of growth inhibition (GI %) for each treatment was calculated concerning its growth in check treatment using the following formula:

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

Where; GI % = Percentage of growth inhibition,

C = Linear growth of the pathogen in control plates (cm) and

T = Linear growth of the pathogen in dual culture plate (cm).

The effect of some essential oils on the fungal linear growth of the tested pathogens:

The essential oils, *i.e.* eucalyptus, thyme, and lemongrass, were added individually to sterilized PDA medium and poured into Petri-dishes 9 cm, before solidification to obtain the concentrations of 0.5, 1.0 and 2.0% (Mokhtar *et al.*, 2013). PDA medium free from essential oils was used as a control. Mycelial discs (5 mm) taken from the previously prepared cultures of the fungal isolates, each was placed at the center of the Petri-dishes and incubated at 28±1°C. The averages of the linear growth were recorded when the check treatment plates covered with the fungal growth. Three replicates were used for each treatment and the control. The percentage of growth inhibition (GI %) for each essential oil was calculated as previously mentioned.

Preparation of pathogens inocula

Inocula of *F. moniliforme, F. solani,* and *R. solani* were prepared by growing in glass bottles (500 ccs) containing 150 g sterilized sorghum grains medium. The bottles were inoculated with five actively growing disks (5 mm) for each bottle taken from seven days old cultures. The bottles were incubated at 28±1°C for 15 days; during this period the bottles were vigorously shaken every three days to ensure uniform distribution of the fungal growth. After the incubation period, the glass bottles were then evacuated, air-dried at room temperature and crushed in a mill to pass through a 3-mm sieve. The dried crushed inocula stored in paper bags at 4±1°C until added to the soil within one week (Gaskill, 1968 and Leslie and Summerell, 2006).

Greenhouse experiments

The trials were carried out in the greenhouse of the Plant Pathol. Res. Inst., Agric. Res. Cent., Giza, Egypt. Pots (30 cm in diameter) with a bottom drainage hole were sterilized by dipping in a 5% formalin solution for 15 minutes and left for one week till complete formalin evaporation. Pots were filled with steam disinfested sandy clay soil 1:2 (V/V). Soil infestation was achieved by mixing the inoculum of the tested pathogenic fungi, *i.e. F. moniliforme, F. solani* and *R. solani*, with the soil at the rate of 2% of soil weight (Abdel-Monaim 2011). Sterilized un-inoculated grounded sorghum grains were added to the disinfested soil at the same rate for used as healthy control. The infested soil was mixed thoroughly and watered every 2 days for a week before planting to stimulate the fungal growth and ensure its distribution in the soil. Apparently healthy uniformity seeds of soybean (cv. Giza 35) were surface disinfested by immersing in sodium hypochlorite (1%) for 2 min and washed several times with sterilized water, then left for air dry on screen cloth with paper towel underneath to absorb the excess water at room temperature for approximately two hours.

The biocides, Bio-Cure-F, Plant Guard, and Rhizo-N were used at the rate of 4g, 4g, and 4 ml/kg seeds, respectively. Disinfested soybean seeds (cv. Giza 35) were immersed in the tested biocides for 20 min before sowing. Arabic gum solution (5%) was used as a sticker. Soybean seeds were soaked in the essential oils of eucalyptus, thyme, and lemongrass at the concentration of 2.0% for 30 min. just before sowing. The fungicide Vitavax-200 WP 75% was used for dressing the seeds at the rate of 3g/kg seeds. Seeds soaked in water only were served as untreated control for both infested and non-infested soil.

Five seeds of the previous treatments were sown in each pot and pots were irrigated directly. Three replicated pots were used for each particular treatment. All pots were irrigated when it was necessary and were fertilized with 0.1% 15:15:15 (N:P:K) fertilizer solution after the first month and kept under greenhouse conditions.

Disease assessment

Disease assessment was recorded as percentages of pre-and post-emergence damping-off after 15 and 30 days after sowing, respectively (Mahmoud *et al*., 2013). Also, the percentages of the survived plants were counted and recorded 60 days after sowing .Percentages of pre- and post-emergence damping-off as well as the survived plants were calculated using the following formulas:

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

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

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

Field experiments

Field experiments were carried out during the successive growing seasons of 2018 and 2019 at Giza Agric. Res. Stat., Agric. Res. Cent., Giza Governorate, Egypt, in a field has a back history of natural infestation with fungi causing damping-off and root-rot diseases, to investigate the effect of some biocides and essential oils for managing damping-off and root-rot diseases. Soybean seeds (cv. Giza 35) were treated in the same manner of the greenhouse experiment. The treated soybean seeds were sown in the field on 6th May 2018 and 5th May 2019. The field trial (24 plots) was designed in complete randomized block with three replicates. The area of each plot was 10.5 m^2 consisted of five rows; each row was 3.5 m length and 0.6 m width. The seeds were sown in hills, 20 cm apart on both sides of the row ridge, with one seed per hill**.** Rhizobium (*B. japonicum*) formula (400g/feddan) (obtained from Biofertilizers Production Unit, Soils Water and Environment Res. Inst., Agric. Res. Centre (ARC), Giza, Egypt) was mixed with approximately 100 kg of moistened fine sandy soil then added to seed furrow during sowing. All other recommended agricultural practices were followed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

Random samples of ten soybean plants were collected at the harvest stage from each plot to determined growth parameters, *i.e.* plant height (cm), number of pods per plant, the weight of one hundred seed (g) and weight of seeds per plant were recorded. Also, the grown plants in each plot were harvested and seed yield as kg/plot was weighed then the yield per feddan was calculated.

Disease assessment

Disease assessment was recorded as percentages of pre-and post-emergence damping-off as well as root-rot after 15, 30 and 60 days after sowing, respectively (Mahmoud *et al*., 2013). Percentages of pre-, post-emergence damping-off and root-rot were calculated using the following formula:

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

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

$$\text{Root-rot (\%)} = \frac{\text{Number of dead plants}}{\text{Number of sown seeds}} \times 100$$

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

Determination of photosynthetic pigments:

Photosynthetic pigments (chlorophyll a, b, and carotenoids) were extracted (Robinson and Britz, 2000), from the blade of 3rd leaf from the tip (terminal leaflet) were determined (Spomer *et al.*, 1988) using the following equation. Three replicated for each particular treatment were taken randomly from each treatment from the two growing seasons.

Chlorophyll A (mg/g) = (16.5×E665 - 8.3×E650)/5.
Chlorophyll B (mg/g) = (33.8×E650 - 12.5×E665)/5.
Total Chlorophyll (mg/g) = (25.5×E650 - 4×E665)/5.
Carotenoids (mg/g) = (4.2×E452.5 - 0.0264×Chl.A - 0.496×Chl.B)/5.

Determination of oil and protein content:

Mature seeds from each treatment were taken for this purpose. Three replicates for each particular treatment were taken randomly from each treatment of both the two growing seasons (2018 and 2019) for oil and protein content analyses.

Oil content (OC): Oil content was determined gravimetrically after extraction using petroleum ether, in a Soxhlet instrument, technique 920.85 (AOAC, 1990), expressed in dry matter %.

Protein content (PC): Protein content was obtained by total nitrogen determination, according to the micro Kjeldahl method, technique 920.87 (AOAC, 1990), using a 6.25 conversion factor and expressed in dry matter % as well.

Statistical analysis

The collected data were subjected to one way ANOVA as outlined by Gomez and Gomez (1984). Least significant difference (LSD) and Duncan multiple range test were automated to compare treatment means at 5% probability level.

RESULTS AND DISCUSSION

1. Effect of the tested biocides on linear growth of the tested fungi *in vitro*:

Data in Table (1) show that all the tested biocides, *i.e.* Bio-Cure-F, Plant Guard and Rhizo-N significantly inhibited the linear growth of the tested pathogenic fungi, *i.e. F. moniliforme*, *F. solani* and *R. solani* compared with the control. Plant Guard was the most effective one in this regard, being 72.22, 65.92 and 70.74, % growth reduction followed by Bio-Cure-F, being 66.67, 57.41 and 65.19 % then Rhizo-N, being 39.26, 41.48 and 46.67%, respectively. In addition, *R. solani* was the most sensitive fungus to all the tested biocides by average growth inhibition 60.87% followed by *F. moniliforme* (59.38%). Meanwhile, *F. solani* was the lowest sensitive fungus (54.94%).

The current results showed that the use of the tested biocides including fungal and bacterial bioagents was able to inhibit the mycelial growth of tested three pathogenic fungi. These results were supported by other findings that approved the efficiency of many bioagents to inhibit pathogenic fungi by producing one or more of inhibitory substances such as antibiotic(s), hydrolytic enzymes, indole acetic acid, siderophore or hydrogen cyanide (Soria *et al.*, 2012; Abo-Elyousr *et al.*, 2014; Sarhan and Shehata, 2014 and Prasad *et al.*, 2017). *Trichoderma* spp. and *Bacillus* spp. are the most promising groups of rhizospheric able to inhabit pathogenic soil-borne microorganisms, where they showed antagonistic activity against *Rhizoctonia* spp., *Fusarium* spp., *Sclerotium rolfii*, *Macrophomina phaseolina*, *Phytophthora* spp. and *Pythium* spp. caused legume diseases (Kazmar *et al.*, 2000; Spadaro and Gullino, 2005 and Abo-Elyousr *et al.*, 2014).

Table 1. Effect of the biocides Bio-Cure-F, Plant guard and Rhizo-N on linear growth of the three tested pathogenic fungi *in vitro*.

Biocides	*F. moniliforme*		*F. solani*		*R. solani*	
	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Bio-Cure-F	30.00	66.67	38.33	57.41	31.33	65.19
Plant guard	25.00	72.22	30.67	65.92	26.33	70.74
Rhizo-N	54.67	39.26	52.67	41.48	48.00	46.67
Control	90.00	0.00	90.00	0.00	90.00	0.00
Mean	36.56	59.38	40.56	54.94	35.22	60.87
LSD at 5%	3.85	–	2.92	–	2.37	–

2. Effect of the tested essential oils on the linear growth of the tested pathogenic fungi *in vitro*:

Data presented in Table (2) indicate clearly that the three tested essential oils of eucalyptus, thyme and lemongrass oils significantly inhibited the linear growth of three tested fungi at all tested concentrations. Increasing concentration of the essential oils increased mycelial growth inhibition. Lemongrass oil was the most effective one on all fungi followed by thyme oils then eucalyptus oil. *F. solani* was the most sensitive fungus to the tested essential oils followed by *R. solani* then *F. moniliforme*.

Obtained results are in agreement with the previous observations who found that essential oils have biological activities such as antibacterial activity against plant fungal pathogens (Hadizadeh *et al.*, 2009 and Andrade *et al.*, 2014). These antimicrobial activities frequently related to the essential oil fraction of volatile aromatic compounds in the aqueous phase (Gould, 1995; Leal *et al.*, 2003; Hadizadeh *et al.*, 2009 and Andrade *et al.*, 2014). Similar result was found by Velluti *et al.* (2003) who found that the five essential oils clove, palmarosa, cinnamon, lemongrass and oregano, inhibited the growth of *Fusarium proliferatum*. Mahmoud *et al.* (2013) who found that the plant oils of cumin, thyme, garlic, and cardamom reduced the linear growth of *S. rolfsii*, *F. solani*, *M. phaseolina* and *R. solani*. They added that this reduction was increased by increasing concentration of the tested plant oils. Recently, El-Wakil and Ahmed (2019) reported that the tested oils of eucalyptus (*Eucalyptus globulus*), lemon tea tree (*Melaleuca alternifolia*), peppermint (*Mentha piperita*), lemon (*Citrus limonum*) and angelica (*Angelica archangelica*) inhibited the mycelial growth of *R. solani* at concentrations of 0.5, 1.0, 1.5 and 2.0%. The different fungal species tested gave various degrees of inhibition depends on the essential oils (Pattnaik *et al.*, 1996).

Table 2. Effect of the essential oils of eucalyptus, thyme and lemongrass oils at different concentrations on the linear growth of the three tested pathogenic fungi *in vitro*.

Essential oils	Concentrations (%)	*F. moniliforme*		*F. solani*		*R. solani*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Eucalyptus oil	0.5	50.00	44.44	36.00	60.00	45.00	50.00
	1.0	35.00	61.11	15.00	83.33	28.00	68.89
	2.0	15.00	83.33	8.00	91.11	10.00	88.89
	Mean	33.33	62.96	19.67	78.15	27.67	69.26
Lemongrass oil	0.5	30.00	66.67	20.00	77.78	25.00	72.22
	1.0	10.00	88.89	0.00	100.00	8.00	91.11
	2.0	0.00	100.00	0.00	100.00	0.00	100.00
	Mean	13.33	85.19	6.67	92.59	11.00	87.78
Thyme oil	0.5	35.00	61.11	25.00	72.22	30.00	66.67
	1.0	12.00	86.67	7.00	92.22	7.00	92.22
	2.0	0.00	100.00	0.00	100.00	0.00	100.00
	Mean	15.67	82.59	10.67	88.15	12.33	86.30
Mean	0.5	38.33	57.41	27.00	70.00	33.33	62.96
	1.0	19.00	78.89	7.33	91.85	14.33	84.07
	2.0	5.00	94.44	2.67	97.04	3.33	96.30
	Mean	20.78	76.91	12.33	86.30	17.00	81.11
Control	–	90.00	-	90.00	-	90.00	-
LSD at 0.05 for:							
Essential oils (A) =		2.12		2.65		2.71	
Concentrations (B) =		2.88		2.95		3.02	
Interaction (A×B) =		5.91		5.79		5.85	

3. Effect of some biocides and essential oils on the incidence of damping-off under greenhouse conditions:

Results in Table (3) reveal that all the tested biocides and essential oils as well the fungicide Vitavax-200 resulted in significant reduction to the percentages of pre- and post-emergence damping-off caused by the tested pathogenic fungi with significant increase to the survived plants compared with untreated infected control. As well, there is a significant difference among the tested treatments. The fungicide Vitavax-200 and Plant Guard treatments gave the highest effect with all the tested pathogenic fungi , being 89.78 and 86.11% of survived plants followed by Rhizo-N, lemongrass oil, Bio-Cure-F , being 77.11, 72.56 and

70.22%, respectively. On the other hand the lowest percentages of survived plants, being 65.04 and 60.78% were attributed to thyme oil and eucalyptus oil, respectively compared to 42.77% for untreated infested control treatment.

Similar results have been reported the use of biological control and/or essential oils in managing soybean and others legume crops damping-off, root-rot and wilt diseases under greenhouse and field conditions (Mokhtar *et al.*, 2013; Abd EL-Hai *et al.*, 2016; El-Gendy *et al.*, 2016; ELmorsy *et al.*, 2016 and Yassin *et al.*, 2019).

Table 3. Effect of some biocides and essential oils as well as the fungicide Vitavax-200 on the percentages of the infection by damping-off as well as the survived plant of soybean grown in artificially infested soil with *F. moniliforme*, *F. solani* and *R. solani* under greenhouse conditions.

Treatment	Damping-off (%)						Plant Survivals (%)			
	Pre-emergence			Post-emergence						
	F. moniliforme	*F. solani*	*R. solani*	*F. moniliforme*	*F. solani*	*R. solani*	*F. moniliforme*	*F. solani*	*R. solani*	*Mean*
Bio-Cure-F	15.67	14.67	14.67	15.00	16.67	16.00	69.66	70.66	70.33	70.22
Plant guard	9.33	1.67	10.67	7.67	8.33	8.33	89.00	87.66	81.66	86.11
Rhizo-N	11.00	11.67	11.33	11.67	13.00	13.33	77.33	77.00	77.00	77.11
Eucalyptus oil	20.33	20.33	20.33	16.00	18.33	17.33	59.34	59.34	63.67	60.78
Lemongrass oil	13.33	14.33	13.67	13.00	15.00	14.67	72.34	72.00	73.33	72.56
Thyme oil	18.88	17.67	17.67	15.33	17.33	16.67	63.45	64.66	67.00	65.04
Vitavax-200	5.33	5.67	5.33	3.33	3.67	4.33	89.00	89.00	91.34	89.78
Mean	13.33	13.67	13.33	11.67	13.33	12.67	73.00	73.00	75.00	73.67
Control (infested soil)	28.67	28.33	29.67	27.03	27.67	29.33	43.00	42.00	43.30	42.77
Control (non-infested soil)		2.00			0.00			98.00		
LSD at 5%	4.82	5.75	4.12	3.67	4.45	3.25	8.78	11.24	9.31	-

4. Effect of biocides and essential oils on the infection by damping-off and root-rot under field conditions:

Results in Table (4) show that all the tested biocides and essential oils significantly reduced the percentages of damping-off and root-rot of soybean plants as compared with untreated control in the two successive growing seasons (2018 and 2019) at Giza governorate, Egypt. Such results were concomitant with a significant increase in the survived soybean plants treated with biocides and essential oils over the untreated control. As for the treatments, Vitavax-200 and Plant Guard gave the highest values in reduction of both diseases as well as increasing the survived plants, being 77.83 and 77.33% compared to the untreated control, 38.17%, followed by Rhizo-N, lemongrass oil, Bio-Cure-F, and thyme oil as they recorded 74.33, 66.33, 61.17 and 61.0% respectively. Meanwhile, the lowest values even in decreasing diseases or increasing survival soybean plants 55.83% was attributed to eucalyptus oil treatment.

Table 4. Effect of some biocides and essential oils and the fungicide Vitavax-200 on the percentages of the infection by damping-off and root-rot as well as the survived plants of soybean grown under field conditions during the growing seasons of 2018 and 2019.

Treatments	Damping-off %		Root-rot	Plant
	Pre-emergence %	post-emergence %	severity %	survivals %
		2018		
Bio-Cure-F	15.67c	17.33b	6.67ab	60.33c
Plant guard	8.33a	10.00a	6.33ab	75.33d
Rhizo-N	10.33ab	11.33a	5.67a	72.67d
Eucalyptus oil	17.33c	19.67b	8.67b	54.33b
Lemongrass oil	14.67bc	16.67b	7.33ab	61.33c
Thyme oil	16.33c	17.33b	7.67ab	58.67bc
Vitavax-200	7.00a	10.67a	5.33a	77.00d
Control	24.33d	24.67c	15.33c	35.67a
		2019		
Bio-Cure-F	14.67c	16.67b	6.67ab	62.00b
Plant guard	7.33a	8.67a	4.67a	79.33d
Rhizo-N	8.33ab	10.33a	5.33a	76.00cd
Eucalyptus oil	16.67c	17.67b	8.33b	57.33b
Lemongrass oil	10.67b	11.33a	6.67ab	71.33c
Thyme oil	14.33c	15.33b	7.00ab	63.33b
Vitavax-200	6.67a	10.00a	4.67a	78.67d
Control	21.67d	24.33c	13.33c	40.67a
		Mean		
Bio-Cure-F	15.17bc	17.00bc	6.67bc	61.17c
Plant guard	7.83e	9.33d	5.50c	77.33e
Rhizo-N	9.33de	10.83d	5.50c	74.33e
Eucalyptus oil	17.00b	18.67b	8.50b	55.83b
Lemongrass oil	12.67cd	14.00c	7.00bc	66.33d
Thyme oil	15.33bc	16.33bc	7.33bc	61.00c
Vitavax-200	6.83e	10.33d	5.00c	77.83e
Control	23.00a	24.50a	14.33a	38.17a
L.S.D at 0.05				
Seasons (S)	N.S	N.S	N.S	N.S
Treatments (T)	2.55	2.23	1.73	3.63
S × T	N.S	N.S	N.S	N.S

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.; N.S indicated P<0.05% not significant.

Obtained results are in agreement with many researchers who found that the bio-agents and essential oils were effective in controlling damping-off in soybean and other legumes crops. Abo-Elyousr *et al.*, (2014) found that the bioagent *Trichoderma harzianum* and two types of compost control the root rot disease caused by *Rhizoctonia solani* in soybean under greenhouse, and field conditions. Abd El-Hai *et al.*, (2016) The bioagents *B. subtilis* and *Saccharomyces cerevisiae* were significantly decreasing soybean damping-off. Yassin *et al.*, (2019) indicate that soybean seeds treated with bioagents, Mycorrhizae and fungicide under field conditions significantly reduced damping-off disease, as well the fungicide Rizolex-T and *Trichoderma harzianum* were the best treatments. Similar achievements were obtained for the use of essential oils. Mohamed *et al.*, (2006) found that lemongrass and clove oils have significantly minimized the guar damping-off incidence and increased percentages of healthy survival under greenhouse and field conditions. Abdel-Monaim *et al.*, (2011) reported that water leaf extracts of *Calotropis procera, Nerium oleander, Eugenia jambolana, Citrullus colocynthis, Ambrosia maritime, Acacia nilotica* and *Ocimum basilicum* and fruit extracts of *C. colocynthis, C. procera* and *E. jambolana* reduced damping-off and wilt diseases caused by *F. oxysporum* f. sp. *lupini*. Mahmoud *et al.*, (2013) stated the effect of four plant oils, cumin, thyme, garlic, and cardamom on peanut damping-off and root rots incidence. Similar results Mokhtar *et al.*, (2013) indicated that *T. harzianum* and some essential oils alone or in combination with compost significantly reduced the peanut crown rot disease. El-Gendy *et al.*, (2016) found that neem and eucalyptus significantly reduced the percentages of soybean damping-off under greenhouse and field conditions. Abdel-Monaim *et al.*, (2017) reported that aqueous extracts and organic solvent extracts of three wild medicinal plants (*Lawsonia inermis, Eugenia jambolana*, and *Moringa oleifera*) under greenhouse and field conditions significantly reduced damping-off and root-rot in faba bean plants.

5. Effect of biocides and essential oils on growth parameters and yield of soybean plants under field conditions:

Data shown Table (5) clearly show that under field conditions, the tested biocides and essential oils treatments significantly improved crop growth parameters, *i.e.* plant height, the pods number per plant, one hundred seed weight and the seed yield/feddan compared with the untreated control in both seasons. The highest significant increase in the plant height was recorded with the treatments Plant Guard, Vitavax-200 and Rhizo-N compared with the untreated control. On the other hand, no significant differences were recorded between the treatments of Bio-Cure-F, eucalyptus oil, thyme oil, and lemongrass oil and the untreated control. In both seasons, all treatments significantly increased the pods number per plant, the weight of one hundred seed and seed yield/feddan as compared with untreated control. The maximum number of pods per plant was recorded with Vitavax-200 and Plant Guard treatments, followed by Bio-Cure-F, thyme oil lemongrass oil, and Rhizo-N, respectively. The maximum one hundred seed weight was recorded with Vitavax-200 treatment followed by Plant Guard, Bio-Cure-F, Rhizo-N, lemongrass and thyme oils, respectively. Also, the maximum seed yield/feddan was recorded with Vitavax-200 treatment followed by Plant Guard, Rhizo-N, Bio-Cure-F, lemongrass and thyme oils, respectively. Generally, in both seasons, Vitavax-200 and Plant Guard gave the highest values of all crop parameters. Whereas, the lowest values in the pods number per plant, weight of one hundred seed and seed yield/feddan were attributed to eucalyptus oil treatment.

Table 5. Effect of some biocides and essential oils on plant height, number on pods, 100-seed weight and seed yield of soybean under field conditions during the growing seasons of 201٨ and 2019.

Treatments	Plant height (cm)	No. of pods/plant	100-seed weight (g)	Seed yield (kg/fed.)
		2018		
Bio-Cure-F	70.26a	98.91b	17.36bc	1556.2b
Plant guard	98.58c	114.02c	18.8cd	1871.3cd
Rhizo-N	92.12bc	92.99b	17.04ab	1672.1bc
Eucalyptus oil	76.44a	90.36b	16.67ab	1440.5b
Lemongrass oil	82.92ab	92.58b	16.70ab	1609.5b
Thyme oil	73.34a	96.66b	16.14ab	1511.8b
Vitavax-200	93.23bc	136.06d	19.44d	2025.8d
Control	84.79abc	73.44a	15.42a	1118.0a
		2019		
Bio-Cure-F	78.31a	105.94c	17.66b	1744.3c
Plant guard	102.88d	119.20d	19.09c	1997.3d
Rhizo-N	92.40bcd	93.35b	17.32b	1720.4bc
Eucalyptus oil	79.36ab	93.26b	16.85b	1513.9b
Lemongrass oil	83.24abc	94.56b	17.11b	1673.6bc
Thyme oil	81.09ab	102.13bc	16.67b	1667.6bc
Vitavax-200	94.57cd	136.41e	19.87c	2194.2d
Control	80.05ab	77.31a	14.82a	1143.8a
		Mean		
Bio-Cure-F	74.29a	102.43d	17.51c	1650.2c
Plant guard	100.73b	116.61e	18.95d	1934.3d
Rhizo-N	92.26b	93.17bc	17.18bc	1696.3c
Eucalyptus oil	77.90a	91.81b	16.76bc	1477.2b
Lemongrass oil	83.08a	93.57bc	16.90bc	1641.5c
Thyme oil	77.21a	99.39cd	16.41b	1589.7bc
Vitavax-200	93.90b	136.24f	19.65d	2110.0e
Control	82.42a	75.37a	15.12a	1130.9a
L.S.D at 0.05				
Seasons (S)	N.S	N.S	N.S	N.S
Treatments (T)	8.72	6.70	0.98	152.05
S × T	N.S	N.S	N.S	N.S

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.; N.S indicated P<0.05% not significant.

These results are in harmony with those obtained by many investigators who found an antifungal activity of some bioagents and natural plant products, which can play an important role in the resistance to infection by soil pathogens and consequently, improved crop parameters (Mahmoud *et al*., 2013; Abd El-Hai *et al*., 2016; El-Gendy *et al*., 2016; Abdel-Monaim *et al*., 2017 and Yassin *et al*., 2019)

Mahmoud *et al.* (2013) studied the effect of four plant oils of cumin, thyme, garlic, and cardamom and found significant reduction to peanut damping-off and root rot incidence and improved crop parameters. Abd El-Hai *et al*., (2016) found that *Bacillus subtilis* and *Saccharomyces cerevisiae* significantly decreased soybean damping-off and root-rot diseases field conditions and improved crop parameters. El-Gendy *et al.* (2016) found that neem and eucalyptus significantly reduced the percentages of soybean damping-off under greenhouse and field conditions and improved crop parameters. Abdel-Monaim *et al.* (2017) reported that aqueous and organic solvent extracts of three wild medicinal plants, *i.e. Lawsonia inermis*, *Eugenia jambolana*, and *Moringa oleifera* significantly reduced faba bean damping-off and root rot and improved crop parameters under greenhouse and field conditions. Yassin *et al.* (2019) indicated that soybean seeds treated with bioagents, mycorrhizae and the fungicide Rizolex-T under field conditions significantly reduced damping-off and dead plants. They added that *Trichoderma harzianum* and the fungicide Rizolex-T, were the best treatments in this regard. In addition, all bioagent treatments significantly improved crop parameters.

6. Effect of biocides and essential oils on Photosynthetic pigments of soybean plants under field conditions:

Data presented in Table (6) show that biocides and essential oils treatments significantly increased the amounts of photosynthetic pigments, *i.e.* chlorophyll a, b, total chlorophyll and carotenoids in the treated soybean leaves compared with the untreated control in the two successive growing seasons. Vitavax-200 and Plant Guard gave the highest values followed by Rhizo-N, lemongrass oil, Bio-Cure-F, thyme oil, and eucalyptus oil respectively. Reveal good parameters reflecting the plant healthy due to the treatment by biocides and essential oils.

The positive effect of the biocides and essential oils on increasing chlorophylls may be due to the decrease of infection by damping-off and root-rot diseases. Many investigators supported this idea since they stated that increasing the amount of photosynthetic pigments is a good parameter indicates the plant health condition (Abd El-Hai *et al*., 2010 and Abd El-Hai *et al*., 2016). Tohamey and El-Sharkawy (2014) reported that the essential oils, *i.e.* chamomile, thyme, cumin, basil, eucalyptus, and garlic oils decreased wheat leaf rust severity as well amount of photosynthetic pigments.

7. Effect of biocides and essential oils on oil and protein contents of soybean seed:

Data presented in Table (7) show that biocides and essential oils treatments significantly increased seed oil content, whereas decreased in seed protein content compared with the untreated control in both seasons. The correlations between oil and protein were negatively correlated. Vitavax-200 and Plant guard gave the highest values in oil content (22.80 and 21.70%) followed by Rhizo-N, lemongrass oil, Bio-Cure-F, thyme oil and eucalyptus oil (20.98, 20.73, 20.54, 20.35 and 20.02%) compared with the untreated control (19.11%) respectively. Thyme oil the only treatment gave significantly highest value in protein content (38.28%) followed by eucalyptus oil, Bio-Cure-F, lemongrass oil, Rhizo-N, Vitavax-200, and Plant guard (37.03, 36.12, 35.72, 35.61, 35.14 and 34.67%) non-significant and lower than with the untreated control (36.74%) in both seasons respectively. Seed composition is known to be influenced by biotic and abiotic factors (Wilcox and Cavins, 1995). This explanation is supported by other workers like Hoffman *et al*., (1998) who found correlations between *Sclerotinia sclerotiorum* (SSR) incidence, seed protein, and oil content, seed protein content significantly increased from 41 to 44% when SSR incidence increased, but Seed oil content was negatively ($P < 0.05$) correlated to SSR incidence. Ziems *et al*., (2007) found that soybean infection with bean pod mottle virus had decreased seed oil and increased protein. Bellaloui *et al*., (2008) indicated that charcoal rot infection in soybean may alter seed composition and nitrogen fixation as protein concentration was significantly ($P<0.05$) higher for the susceptible cultivars. It has also been reported a reduction in oil concentration correlated with an increase in protein concentration and not always, lower yield (Brim and Burton, 1979 and Wilson, 2004). This result could be explained by the theory of Burton (1985) who indicate the negatively correlated between the oil and protein contents of soybean. The present studies are in agreement with the above researches for the inverse relationship between oil and protein concentrations (when protein and oil concentration in the healthy treated and naturally infested control are compared).

Table 6. Effect of biocides and essential oils on photosynthetic pigments of soybean plants under field conditions, during the growing seasons 2018 and 2019.

Treatments	chlorophyll A (mg/g)	chlorophyll B (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
		2018		
Bio-Cure-F	1.266d	0.751c	2.017d	0.232b
Plant guard	1.411g	0.827d	2.238g	0.319d
Rhizo-N	1.365f	0.800d	2.165f	0.296d
Eucalyptus oil	1.218b	0.705b	1.923b	0.208ab
Lemongrass oil	1.313e	0.767c	2.079e	0.263c
Thyme oil	1.240c	0.734bc	1.974c	0.229b
Vitavax-200	1.459h	0.881e	2.341h	0.356e
Control	1.162a	0.646a	1.808a	0.189a
		2019		
Bio-Cure-F	1.350c	0.801c	2.151c	0.250b
Plant guard	1.504f	0.883d	2.387e	0.339d
Rhizo-N	1.456e	0.853d	2.309d	0.316d
Eucalyptus oil	1.299b	0.752b	2.050b	0.219ab
Lemongrass oil	1.402d	0.793bc	2.195c	0.283c
Thyme oil	1.345c	0.783bc	2.128c	0.243b
Vitavax-200	1.557g	0.942e	2.499f	0.377e
Control	1.236a	0.687a	1.923a	0.199a
		Mean		
Bio-Cure-F	1.308c	0.776c	2.084cd	0.241b
Plant guard	1.458f	0.855d	2.313f	0.329d
Rhizo-N	1.411e	0.827d	2.237e	0.306d
Eucalyptus oil	1.259b	0.729b	1.987b	0.214ab
Lemongrass oil	1.358d	0.780c	2.137d	0.273c
Thyme oil	1.293c	0.759bc	2.051c	0.236b
Vitavax-200	1.508g	0.912e	2.420g	0.367e
Control	1.199a	0.667a	1.866a	0.194a
L.S.D at 0.05				
Seasons (S)	N.S	N.S	N.S	N.S
Treatments (T)	0.018	0.026	0.038	0.020
S × T	N.S	N.S	N.S	N.S

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.; N.S indicated P<0.05% not significant.

Table 7. Effect of some biocides and essential oils on oil and protein contents of soybean seeds under field conditions during the growing seasons of 2018 and 2019.

Treatments	Oil (%)			Protein (%)		
	2018	2019	Mean	2018	2019	Mean
Bio-Cure-F	20.54cd	20.54bc	20.54cd	35.51ab	36.72a	36.12ab
Plant guard	21.75f	21.64d	21.70f	34.85a	34.49a	34.67a
Rhizo-N	21.00e	20.95c	20.98e	35.73ab	35.48a	35.61ab
Eucalyptus oil	20.03b	20.01b	20.02b	37.16ab	36.89a	37.03ab
Lemongrass oil	20.74de	20.72c	20.73cd	35.41ab	36.03a	35.72ab
Thyme oil	20.39c	20.30bc	20.35bc	38.69b	37.88a	38.28b
Vitavax-200	22.91g	22.69e	22.80g	34.59a	35.68a	35.14ab
Control	19.29a	18.93a	19.11a	37.06ab	36.42a	36.74ab
L.S.D at 0.05						
Seasons (S)			N.S			N.S
Treatments (T)			0.331			2.146
S × T			N.S			N.S

Values in the column followed by different letters indicate significant differences among treatments according to LSD at 0.05.; N.S indicated P<0.05% not significant.

CONCLUSION

The biocides and essential oils used proved significant reductions of soybean damping-off and root-rot diseases and improved the crop parameters, photosynthetic pigments amounts, seed yield, and quality. Thus, these biocides and essential oils can be recommended to use for managing damping-off and root-rot diseases of soybean to minimizing the fungicides hazard on the human health and environment.

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فاعلية بعض المركبات الحيوية والزيوت الأساسية في مكافحة مرض سقوط البادرات واعفان الجذور في فول الصويا (*Glycine max* (L.) Merr.)

إيهاب علي ضياء سرحان
معهد بحوث أمراض النباتات – مركز البحوث الزراعيه – ١٢٦١٩ – الجيزه – مصر.

موت لبادرات واعفن الجذور من اهم الأمراض لتي تصيب فول الصويا (*Glycine max* (L.) Merr.) في مصر. تم تقييم تأثير المبيدات الحيوية بيوكيور-إف ، بلانت جارد ،و ريزو-إن وكذلك لزيوت الاسلسيه ، زيت لكافور ،زيت لزعتر ،و زيت حشيشة الليمون ،وذلك في المعمل والصوبة وتحت ظروف الحقل في الموسمين لمتتاليين 2019-2018 لمكافحة أمراض موت البادرات واعفن الجذور في فول الصويا. كل المبيدات الحيوية والزيوت الاسلسية المختبرة ثبطت معنويا النمو الخطي للفطريات الممرضه المختبره *Fusarium moniliforme*, *Fusarium solani* and *Rhizoctonia solani*. تبين من النتائج المتحصل عليها تحت ظروف الصوبة والحقل أن كل لمعاملات من لمبيدات الحيوية ولزيوت الاسلسيه لمختبره أدت إلى خفض معنوي لنسبة حدوث مرض سقوط البادرات واعفان الجذور كما تحسنت كمية صبغات لتمثيل الضوئي و صفات النمو ونيادة انتاج البذور. حقق لمبيد فيتافاكس-٢٠٠ والمبيد الحيوي بلانت جارد اعلي القيم في تقليل الشدة المرضية وزياده النباتات السليمه المتبقية ،وكذلك تحسين صفات النمو وزيادة انتاج لبذور متبوعة ب ريزو-إن ،زيت حشيشة الليمون ،بيوكيور-إف ،زيت الزعتر ،و زيت الكافور علي لتوالي. إلى جانب نلك ، أظهرت معايير جودة بذور فول الصويا ارتفاعًا إيجابيًا في إجمالي محتوى الزيت ،وعلى العكس من ذلك كان هناك انخفاض عام في محتوى البذور من البروتين. حيث كانت الارتباطات بين محتوى البذور من الزيت والبروتين مرتبطة بشكل سلبي. ونتيجة لذلك أظهرت هذه الدراسة أن مثل هذه المبيدات الحيوية والزيوت الأساسية يمكن استخدامها في المكافحة الحيوية لأمراض سقوط البادرات واعفان الجذور في فول الصويا.