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CONTROL OF DAMPING-OFF DISEASE IN SOME PLANTS USING ENVIRONMENTALLY SAFE BIOCIDES

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Abstract

Rhizoctonia solani and *Fusarium solani* are causal agents of damping-off disease. Biocides formulations were prepared from the essential oils of fennel, peppermint, oregano and ginger. The potential of these formulations were tested to inhibit the *In vitro* growth of *Rhizoctonia solani* and *Fusarium solani.* The most effective formulations obtained were used against the *In vivo* growth of the studied fungi (pot experiment) on gamma-irradiated seeds of squash and tomato, respectively. The *In vitro* studies showed that the formulated peppermint oil led to complete growth inhibition of *Rhizoctonia solani* and the oregano oil formulation resulted in complete growth inhibition of *Fusarium solani*. The other formulations showed a variably less effect against the investigated fungi. The results of the *In vivo* experiment revealed that the formulated peppermint and oregano oils significantly minimized the pathological symptoms of the inoculated fungi on the studied plants compared to the control. Gamma radiation showed an insignificant result in enhancing the curative effect of the biocides. Chemical fungicide demonstrated fewer effects than the formulated biocides. Physiologically, the formulated biocides add protection to the plant against fungal infection by increasing the levels of the enzymes; Polyphenol oxidase (PPO), Peroxidase (POD) and Phenylalanine ammonia lyase (PAL).The obtained results reveal the potential antifungal effects of biocides against the damping-off disease in squash and tomato and recommend their use as an alternative tool rather than chemical fungicides.

Key words: Damping-off disease, Essential oils, *Rhizoctonia solani*, *Fusarium solani*, Oregano, Peppermint.

Introduction

The soil-borne pathogens are among the most dangerous diseases infecting worldwide crop plants since they attack different plant species. Some species of fungi such as *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc are known for causing damping-off disease for many economically important crops. However, they lead to a decrease in the seed germination and post germination diseases. Some previous reports show that *Rhizoctonia solani* is an active mycelium in the soil and attacks more than 2000 species of plants. Also, considerable losses due to damping-off disease were recorded annually **(**Dawar *et al.*, 2007). Abdel-Monaim *et al.* (2011), revealed that *Rhizoctonia solani* Kühn, *Fusarium solani* (Mart.) Sacc, *F. oxysporum* Shelct and *Macrophomina Phaseolina* (Tassi) infected lupine plants causing damping-off and wilt diseases, which greatly decreased seed yield. Similarly, tomato plants are infected by several soil-borne fungal pathogens like *Fusarium spp.*, *Rhizoctonia solani* and *Sclerotium rolfsii* which cause serious diseases such as wilting and rotting the roots, the very thing that, finally, reduces crop yield and quality (El-Mohamedy *et al.,* 2014). *Fusarium solani* and *Rhizoctonia solani* were reported to cause root-rot in squash *(Cucurbita pepo)*. This leads to great loss in plant yield (Nawar, 2007).

Traditionally, control of such diseases mainly depends on seed treatment, soil application and foliar spray with systemic chemical fungicides (Kazempour, 2004). This unfavorable approach of controlling disease costs much and has a terrible effect on health and environment (Vapnek *et al.,* 2007). In addition, such disease can expectedly develop the microbial resistance to fungicides (Sangeetha *et al.*, 2013). Recently, genetic based methods have been developed to introduce genetically disease-resistant plants. However, these methods are slow and take long time for soil application (Widmer & Laurent, 2006).

The hazardous effects of synthetic chemicals fungicides results in a growing interest in developing alternative and safer treatments. A lot of investigators have developed new biocides-based approaches using formulations of essential oils (Bowers & Locke, 2000; Mario *et al.,* 2002). In the same context, biocide formulations of essential oils have been prepared. These formulations, derived from fennel, peppermint, caraway, oregano, rosemary and ginger, are emulsified with different fixed oils (sesame, olive, cotton and soybean oils) to be used as a carrier. Ginger derived formulation has been used in treating black rot caused by *Alternaria alternata* in tomato fruits (Helal & Abdeldaiem, 2008). Clove and thyme essential oil formulations have been used against blue and green rot caused by *Penicillium italicum* and *P. digitatum,* in orange fruits, respectively (Helal & Abdeldaiem, 2009). Moreover, rosemary formulation has been used against rust disease in irradiated *Vicia faba* plants (Ahmed *et al.,* 2013).

Hence, this research aims to evaluate the effectiveness of different concentrations of our formulated biocides against the *In vitro* growth of *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc and their potential in protecting gamma irradiated and non-irradiated seeds of squash and tomato against *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc, respectively.

Materials and Methods

Seed samples: Seeds of squash and tomato plants were obtained from Vegetable research section, Horticulture Institute, Agriculture Research Center (ARC), Ministry of Agriculture, Egypt.

Isolation and identification of fungi causing damping off: Diseased squash and tomato plants showing root-rot symptoms were collected from various areas in Egypt. The infected roots and the basal stem parts were thoroughly washed with running tap water and were cut into small fragments, superficially sterilized with sodium hypochlorite (1%) for 3 minutes. Then, they were washed several times with sterile distilled water and dried between sterilized filter paper. The sterilized pieces were plated onto potato dextrose agar (PDA) medium and incubated at 25±1ºC. After 3-7 days incubation, the developed fungal colonies were purified by hyphal tip and spore isolation techniques suggested by Dhingra & Sinclair, (1984). The predominant fungal isolates were identified according to their cultural and microscopical characters as: *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc, that cause damping-off in squash and tomato plants, respectively. Isolates were kindly confirmed by Plant Pathology Research Institute, Agriculture Research Center (ARC), Giza, Egypt. Subcultures of the obtained isolates were then kept on PDA slants and stored for further investigations.

Pathogenicity test: The pathogenicity test of the isolated fungi was performed in infested soil in an experimental field as described before (Abdel-Monaim *et al.,* 2011).

Preparation of fungal inoculum and soil infestation: Inoculum of the obtained isolates of *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc, were prepared on autoclaved barley medium (75g washed dried barley grains, 100g washed dried coarses and 5ml tap water) in 500ml glass bottles. Each bottle was inoculated with five discs (0.7cm in diameter) of 4-day-old cultures of each isolate. Bottles were incubated at 25±1 C for 15 days (Abo-Elyousr *et al.,* 2009). For each isolate, the content of 20 bottles was thoroughly mixed in a plastic container and used as a source of inoculum.

Soil and pots were sterilized with a 5% formalin solution for 15 min. Soil was covered with a polyethylene sheet for 7 days to retain the gas and was left to dry for 2 weeks until all traces of formaldehyde disappeared. Pathogen inocula were added to the potted soil at a rate of 3% (w/w) and mixed thoroughly with the soil one week before planting. Five pots were used for controlling (non-infested soil) and as replicates for each isolate. Seeds were sterilized using1% sodium hypochlorite for 2 min, rinsed in distilled water several times and sown in a 10 seed-pot sat rate. These pots were irrigated every three days. All pots were kept under glass-house conditions. Assessing disease percentage of pre- and post-emergence damping off was recorded after 15, 45 and 60 days from planting. Percentages of disease incidence were calculated according to the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Numeber of infected plants}}{\text{Total plant number}} \text{ X } 100$$

Preparation of biocides: Biocides were prepared in liquid formulations (Emulsifiable concentrates) according to previously described method (Abo-El-Seoud *et al.,* 2005).

Irradiation treatment of the seeds: For irradiation treatments, seeds were exposed to different doses of gamma irradiation (0, 10, 20 and 30 Gy) in an experimental ^{60}Co gamma chamber, Nuclear Research Center, Atomic Energy Authority, Egypt.

Evaluation the biocidal effect of formulated oils: Laboratory and pot experiments were performed to study the effect of biocides on fungi isolated from damped and rotten plants as follows:

***In vitro* experiment (Laboratory experiment):** In this experiment, a set of 100ml of PDA preparations was inculcated with one of the tested fungi and then poured in 9cm plates (4 plates for each concentration). Pre-prepared discs impregnated with different biocides concentrations (0, 1000, 2000, 3000, 4000 and 5000 ppm) were applied onto the plates, which were incubated at 25°C. Fungal growth was monitored for 7 days, along with the control plates (0 concentrations). Each experiment was performed in triplicate with the consideration of the average dimensions of the inhibition zone.

***In vivo* experiments (Pot experiments):** The *In vivo* experiments were carried out as described by Somda *et al.* (2007)**.** Based upon the *In vitro* experiment, seeds were soaked in the biocide emulsions (4000ppm) for 8h. According to the preliminary experiment, this concentration had no adverse effects on seeds germination. For comparison, some seeds were soaked in emulsion of Vitavax T fungicide with recommended concentrations. The soaked seeds were air dried for 2h then sown in the potted infested and non-infested soils with the pathogenic fungus at the rate of a 10 seed-pot. Five pots and a set of three replicates were used for each treatment. Cultural practices were performed according to the standard protocols. Percentages of pre- and post-emergence damping-off and plant survival were recorded and calculated.

Biochemical investigations: To investigate the effect of biocides on the plant defense against fungal infection, the activities of three oxidative enzymes (peroxidase (PO), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) were determined in leaves extracts. The extraction was performed according to the method of Biles & Martyn, (1993), where one gram of leaves was grounded in 2ml of sodium phosphate buffer (pH6.5), after using a mortar and pestle. Then, it was transferred to Eppendorf tubes, and centrifuged for 20 min at 12000 rpm at 4ºC. Supernatant was recovered and stored at-8ºC until enzymes were determined. All enzyme activity measurements were performed in triplicates.

Polyphenoloxidase (PPO) activity: It was determined according to the method described by Malik & Singh, (1980) in which 100µl of leaves extract was added to 3ml of freshly prepared reaction mixture containing 0.01M of buffered catechol solution (pH6.5). The absorbance was recorded (at 495nm) every 30 seconds for 3 minutes.

Peroxidase (PO) activity: It was determined according to the description of Hammerschmidt *et al.* (1982), in which 100µl of leaves extract was added to 2.9ml of 100mM sodium phosphate buffer (pH6.5) containing 0.25%(v/v) catechol and 100mM H_2O_2. The absorbance was recorded every 30sec for 3min. Enzyme activity shows an increase in absorbance min^{-1} g^{-1}fresh weight.

Phenylalanine ammonia lyase (PAL) activity: It was determined in fresh leaves extract according to the method described by Solecka & Kacperska, (2003) with slight modifications. The extract was prepared by mincing 1g of leaves in 2ml of 50mM borate buffer (pH8.8) using a mortar and pestle at 4ºC. The mixture was centrifuged at 12000 rpm for 10 min at 4˚C and the supernatant was recovered and used for determination of PAL activity. For deproteinization, the reaction mixture (1ml enzyme extract, 2ml sodium borate buffer (pH 8.8) and 1ml of 10^{-2} ML-phenylalanine) was incubated at 30ºC for1h, the reaction was stopped by adding 500µl of 6NaHCl, the mixture was centrifuged for10 min at 12000 rpm then the supernatant was used for determination of the enzyme activity. Enzyme activity appeared in micromoles of trans-cinnamic acid formed in each gram of fresh weight of tissue which was measured at 290nm.

Statistical analysis: All experiments were performed in triplicates. Statistical analysis was carried out using MSTAT-C program version 2.10, (1991). Least significant difference (LSD) was employed to test for significant difference between treatments at $p \leq 0.05$ (Gomez & Gomez, 1984).

Results

Isolation of the tested fungi: In this work isolates of *Rhizoctonia solani* and *Fusarium solani* (Mart.) Sacc were obtained from infected squash and tomato plants, respectively. Initially, isolate of *Rhizoctonia solani* predominated greatly and caused the highest severity of damping-off to squash plants. Also, the isolate of *Fusarium solani* (Mart.) sacc was the major one sticking around the rotten root of tomato and having the highest pathogenic severity to tomato.

Pathogenicity test: The pathogenicity tests were conducted on three cultivars of each investigated plant. The results presented in figure 1 showed that all the tested cultivars were variably infected. The infectivity in all the investigated cultivars (n=9) was 68% up to 90%. Squash cultivars infected with *Rhizoctonia solani* showed a pathogenicity ranged from 73-88%., where the Askandrany cultivar was the most susceptible as it demonstrated the highest percent of diseased plants (88%);(53.7% and 34.3% pre- and post-emergence, respectively). In tomato plant, super strain B (SSB) cultivar was more susceptible to *Fusarium solani* for recording 89.5% disease severity (50.0% and 39.5% pre- and post-emergence, respectively).

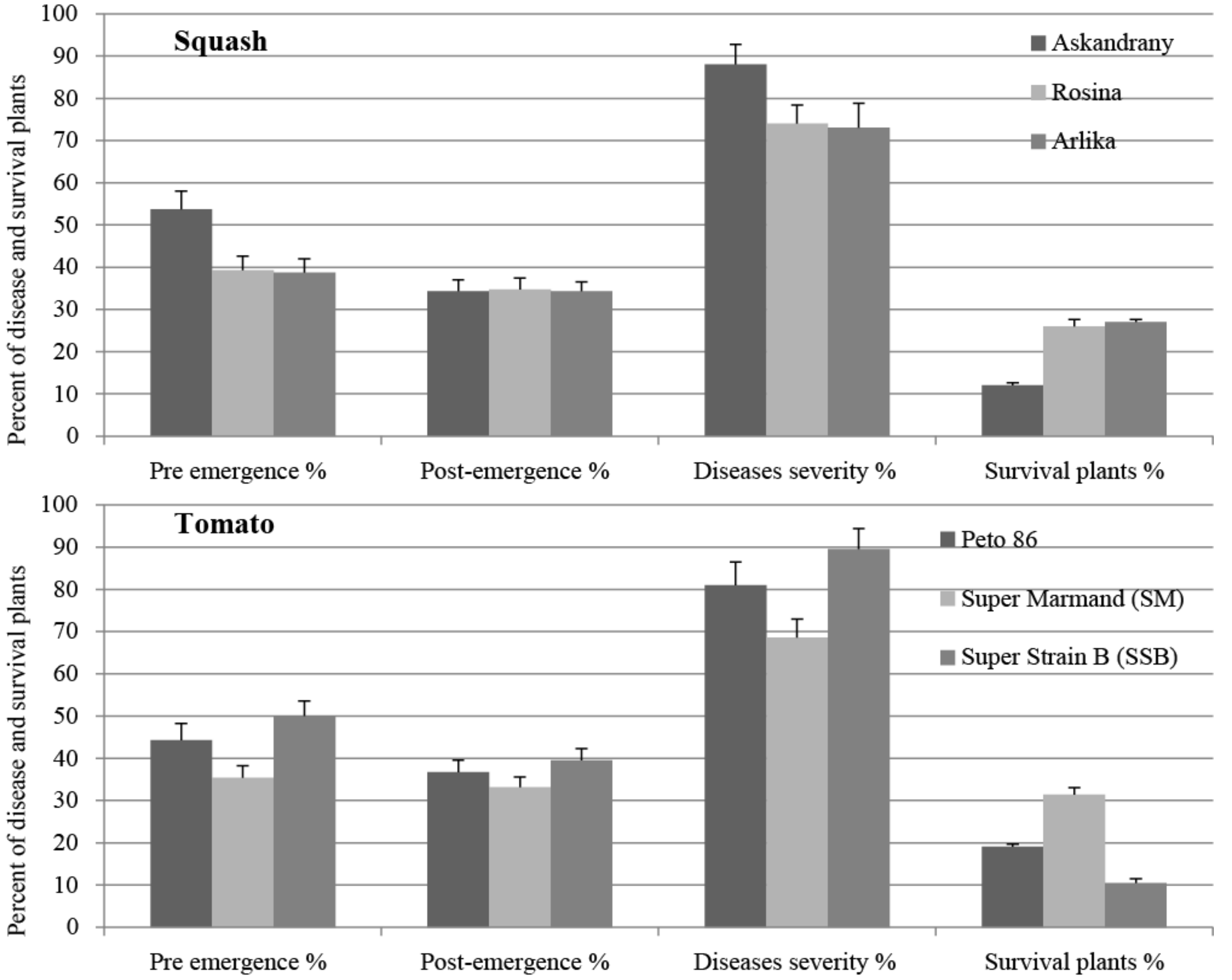

Fig. 1. Susceptibility of squash and tomato cultivar plant to *Rhizoctonia solani* Kühn, *Fusarium solani* (Mart.) Sacc fungi, respectively for causing damping off disease.

Table 1. *In vitro* effect of biocides on linear growth of fungi causing damping-off.

Concentrations (ppm)	Formulated essential oils				
	Fennel	Peppermint	Oregano	Ginger	Mean
	Effect on *Rhizoctonia solani*				
0	0.0	0.0	0.0	0.0	0.0
1000	1.3	1.8	1.0	1.4	1.46
2000	1.7	4.7	2.4	3.1	2.98
3000	2.7	7.6	3.9	4.2	4.60
4000	4.8	N.G	4.6	4.9	5.82
5000	6.2	N.G	6.7	5.3	6.87
Mean	2.78	5.40	3.16	3.14	
	Effect on *Fusarium solani*				
0	0.0	0.0	0.0	0.0	0.0
1000	0.9	1.0	1.8	0.6	1.08
2000	1.3	2.6	4.3	2.8	2.76
3000	2.0	3.9	6.2	3.9	4.00
4000	5.0	5.1	N.G	5.2	6.08
5000	6.7	6.6	N.G	6.1	7.09
Mean	2.66	3.20	5.05	3.11	

Values are diameter of inhibition zones (cm)
N.G = No Growth

L.S.D. at 5%	Biocide	Concentrations	Interaction
Rhizoctonia solani	0.13	0.16	0.32
Fusarium solani	0.09	0.11	0.21

***In vitro* antifungal activity:** Through laboratory investigations, biocides formulated from fennel, peppermint, oregano and ginger were used with 5 different concentrations to examine the potential antifungal activity effect of biocides on isolated fungi and to determine their optimum inhibitory concentrations. The results (shown in table 1) demonstrated that all the prepared biocides were able to suppress the fungi growth, where the degree of inhibition increased with biocides concentrations. The highest concentrations (4000 and 5000ppm) led to either the highest growth inhibition or complete one. The formulated peppermint oil resulted in the highest inhibitory effect on the linear growth of *Rhizoctonia solani* as it inhibited the fungi growth completely with concentrations of 4000 and 5000 ppm. Also, oregano biocide caused complete growth inhibition of *Fusarium solani* at concentrations of 4000 and 5000 ppm.

***In vivo* antifungal activity:** In order to evaluate the *In vivo* efficiency of the formulated essential oils in controlling damping–off disease, a pot experiment was carried out by using irradiated seeds of squash and tomato grown in non-infested or infested soils under greenhouse conditions. The results of *In vitro* experiments showed that peppermint (4000ppm) was used for treating squash seeds challenged by *Rhizoctonia solani*. Moreover, *Fusarium solani* used in oregano formulation (4000ppm) treated tomato and in parallel, the Vitavax T was used in the treatment of two infected plants.

Effect of peppermint biocide on infectivity of *Rhizoctonia solani* on squash plants: Table 2 demonstrates the effect of peppermint biocide on percentages of damping-off in squash seedlings grown in non-infested and infested soils with *Rhizoctonia solani*. The data revealed that the average of the percentages of pre-emergence damping-off in peppermint treated plants were 13.9% and 16.8% in irradiated seeds grown in non-infested and infested soils, respectively. These percentages were fewer than the corresponding values obtained with untreated plants (50.4% & 51.9%) and relatively similar to those of Vitavax-treated plants (14.8% and 17.9%) respectively. The same pattern was observed in post emergence results obtained in seeds grown in non-invested and invested soils. The overall picture depicts that squash irradiated seeds treated with the formulated peppermint oil have significantly higher (73.9% and 68.9%) survival percentages compared to the untreated seeds (9.4%&6.9%) grown in non-infested and infested soils, respectively. No significant difference in the survival rates was observed between peppermint treatments and Vitavax T.

Effect of oregano biocide on infectivity of *Fusarium solani* on tomato plants: Table 3 illustrates the data obtained when infected tomato seeds were treated with oregano biocide. The pre-emergence results showed that oregano decreased about 50% of the damping-off average if compared with that of the untreated plants (from 41.6% or 44.4 to 19.5% or 21.4) in infested ad non-infested soils, respectively. The damping-off percentage shows no significant differences between the biocide and the Vitavax-T. Similar results were seen in plants having a post-emergence damping-off. Also, the average of the survival rate was similarly increased from 26% or 17 % in untreated plants to 64.1% or 59.7 in plants protected with the biocide.

The irradiation of seeds with different doses of gamma radiation did not demonstrate any significant improvement in the survival rates of different plants grown in non-infested or infested soils and treated with peppermint or oregano (Tables 2and 3).

Table 2. ***In vivo*** **effect of peppermint biocide and vitavax t chemical fungicide on percentage of damping–off and survival of irradiated squash seedlings grown in non-infested and infested soils with** ***Rhizoctonia solani*****.**

Treatments	In non-infested soil					In infested soil				
	Doses of gamma radiation Gy									
	0	10	20	30	Mean	0	10	20	30	Mean
	Damping-off: 1-Pre-emergence %									
Control (Untreated)	51.1	50.9	49.8	49.9	50.43	53.4	52.1	51.6	50.6	51.93
Peppermint Biocide	13.3	13.8	14.9	13.4	13.85	16.4	17.2	16.5	17.1	16.80
Vitavax T fungicide	14.2	13.9	15.8	15.4	14.83	17.8	17.9	18.2	17.9	17.95
Mean	26.20	26.20	26.83	26.23		29.20	29.07	28.87	28.53	
	2- Post-emergence %									
Control (Untreated)	40.3	40.4	40.0	40.2	40.23	42.2	41.3	41.1	40.0	41.15
Peppermint Biocide	11.40	12.60	13.5	11.6	12.28	14.2	13.9	14.9	14.1	14.28
Vitavax T fungicide	11.9	12.7	13.9	12.9	12.85	14.3	15.1	13.7	14.2	14.33
Mean	21.20	21.90	22.47	21.57		23.57	23.43	23.23	22.77	
	Survival plants %									
Control (Untreated)	8.6	8.7	10.2	9.9	9.35	4.4	6.6	7.3	9.4	6.93
Peppermint Biocide	75.3	73.6	71.6	75.0	73.88	69.4	68.9	68.6	67.8	68.68
Vitavax T fungicide	73.9	73.4	70.4	71.7	72.35	67.9	67.0	68.1	67.9	67.73
Mean	52.60	51.90	50.73	52.20		47.23	47.50	48.00	48.37	

L.S.D. at 5%		Treatments	Irradiation	Interaction
Pre-emergence:	1. Non-infested soil	0.29	0.34	0.59
	2. Infested soil	0.29	0.33	0.57
Post-emergence:	1. Non-infested soil	0.29	0.40	0.58
	2. Infested soil	0.29	0.34	0.60
Survival plants:	1. Non-infested soil	0.35	0.41	0 .70
	2. Infested soil	0.53	0.62	1.07

Table 3. *In vivo* effect of Oregano biocide and vitavax t chemical fungicide on percentage of damping –off and survival of irradiated tomato seedlings grown in non-infested and infested soils with *Fusarium solani*.

Treatments	In non-infested soil					In infested soil				
	Doses of gamma radiation Gy									
	0	10	20	30	Mean	0	10	20	30	Mean
	Damping-off: 1-Pre-emergence %									
Control (Untreated)	42.5	41.6	40.3	41.9	41.58	45.1	46.0	41.6	44.7	44.35
Oregano Biocide	18.6	18.9	19.4	20.0	19.23	20.8	21.6	20.5	22.6	21.38
Vitavax T fungicide	19.8	20.1	19.9	20.1	19.98	20.7	20.9	21.2	21.8	21.15
Mean	26.97	26.87	26.53	27.33		28.87	29.50	27.77	29.70	
	2- Post-emergence %									
Control (Untreated)	33.6	31.9	30.8	32.7	32.25	35.3	39.8	38.6	39.2	38.23
Oregano Biocide	15.1	15.8	17.6	17.9	16.60	18.7	18.5	19.8	18.9	18.98
Vitavax T fungicide	15.2	14.6	18.2	18.1	16.53	19.1	19.5	19.5	19.9	19.50
Mean	21.30	20.77	22.20	22.90		24.37	25.93	25.97	26.00	
	Survival plants %									
Control (Untreated)	23.9	26.5	28.9	25.4	26.18	19.6	14.2	19.8	16.1	17.43
Oregano Biocide	65.9	65.3	63.0	62.1	64.08	60.5	59.9	59.7	58.5	59.65
Vitavax T fungicide	65.0	65.3	61.9	61.8	63.50	60.2	59.5	59.3	58.3	59.34
Mean	51.60	52.37	51.27	49.77		46.77	44.56	46.27	44.30	

L.S.D. at 5%		Treatments	Irradiation	Interaction
Pre-emergence:	1. Non-infested soil	0.27	0.31	0.53
	2. Infested soil	0.43	0.49	0.86
Post-emergence:	1. Non-infested soil	0.31	0.32	0.55
	2. Infested soil	0.44	0.52	0.88
Survival plants:	1. Non-infested soil	0.28	0.43	0 .56
	2. Infested soil	0.54	0.62	1.32

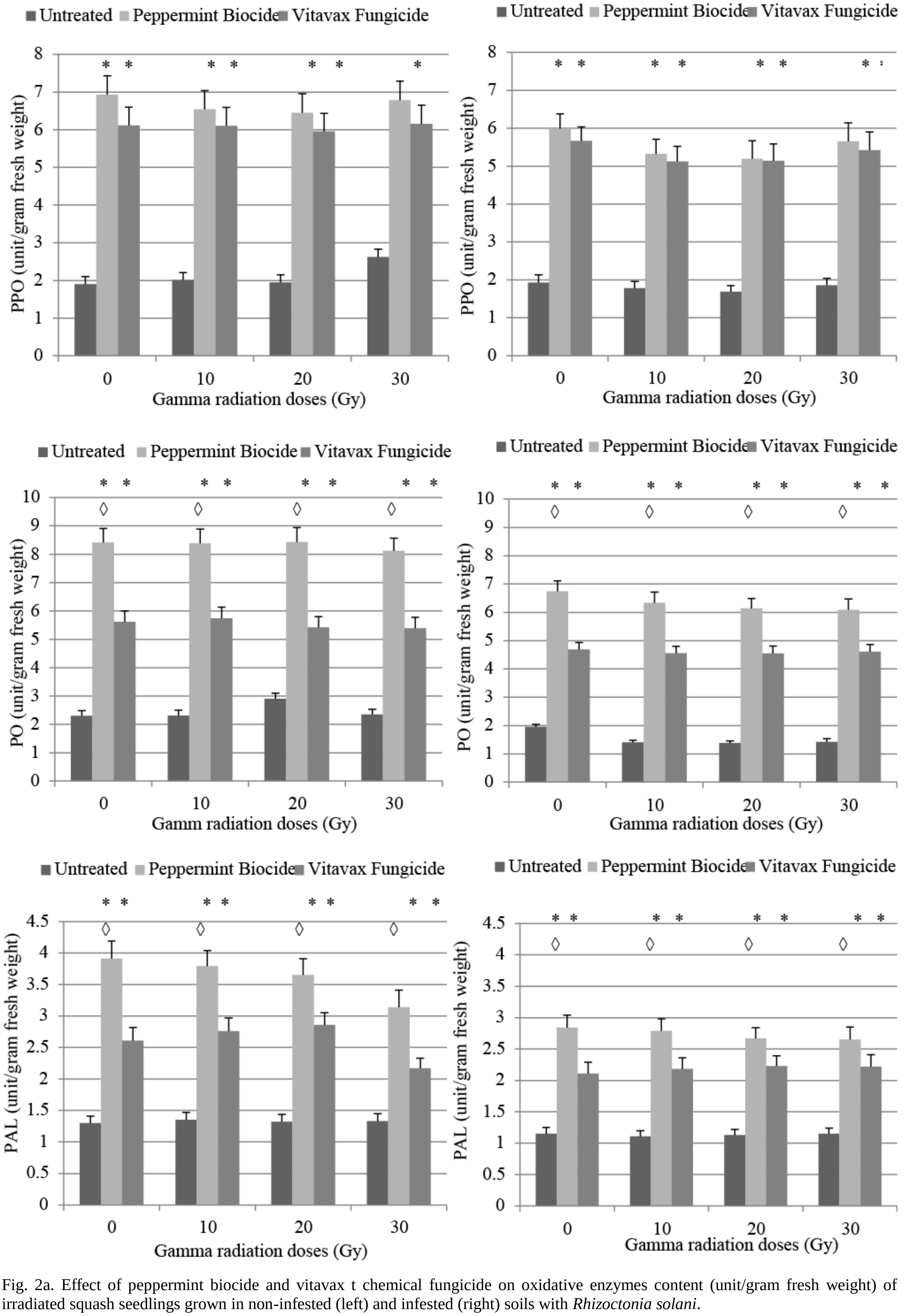

Fig. 2a. Effect of peppermint biocide and vitavax t chemical fungicide on oxidative enzymes content (unit/gram fresh weight) of irradiated squash seedlings grown in non-infested (left) and infested (right) soils with *Rhizoctonia solani*.

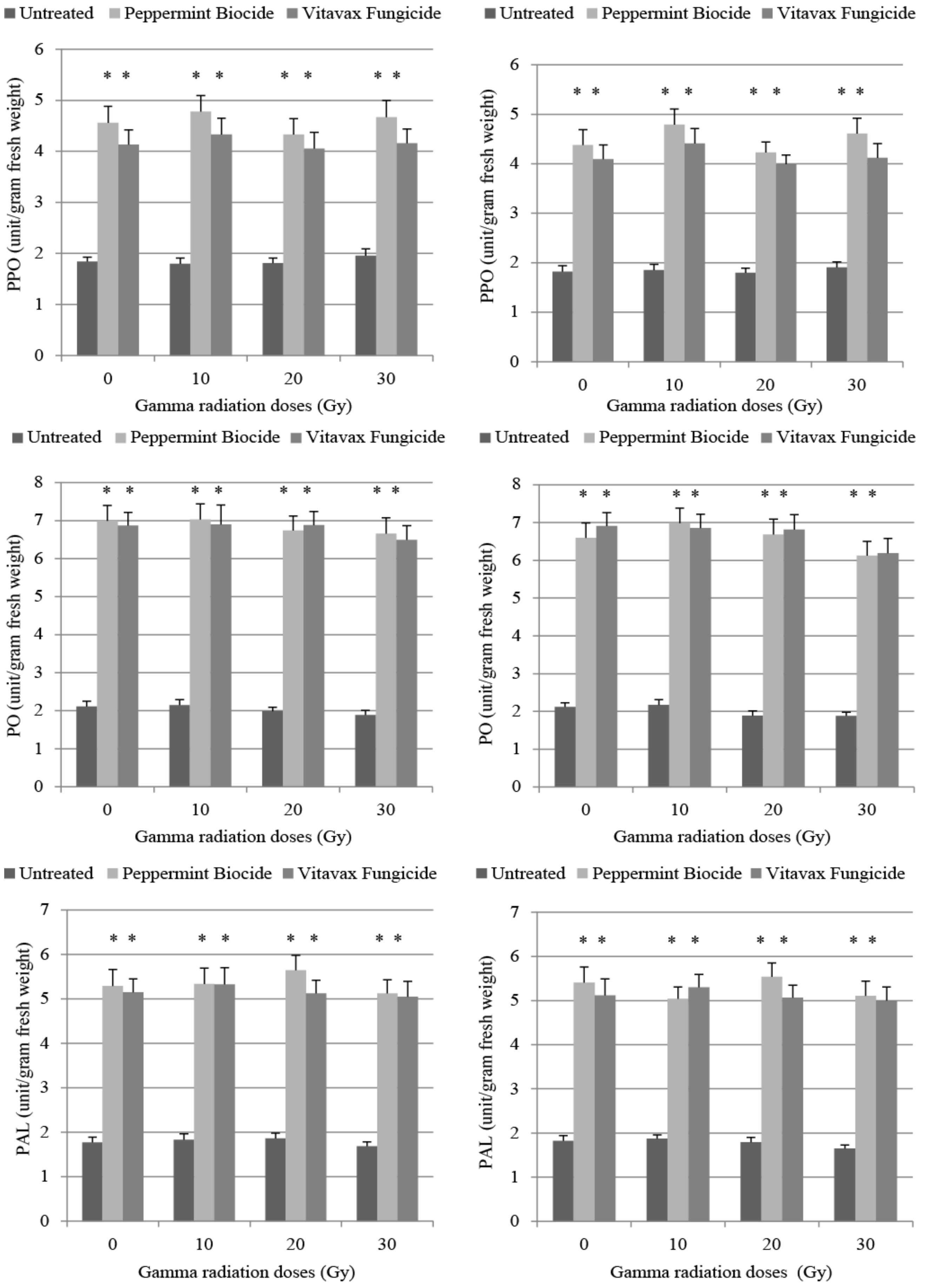

Fig. 2b. Effect of oregano biocide and vitavax t chemical fungicide on oxidative enzymes content (unit/gram fresh weight) of irradiated tomato seedlings grown in non-infested (left) and infested (right) soils with *Fusarium solani*.

Biochemical investigations: Fig. 2 demonstrates the changes in the activities of the oxidative enzymes: polyphenoloxidase (PPO), peroxidase (PO) and phenylalanine ammonia lyase (PAL) in the infected plants treated with biocides or Vitavx-T. In squash (Fig. 2a), there is a variable increase in the activity of the 3 enzymes of plants treated with peppermint, compared with the untreated plants ($p<0.001$, with all doses of gamma radiation) in both infested and non-infested soils. Also, peppermint shows a significant increase in both PO and PAL (but not in PPO) activities, compared with plants treated with Vitavax-T ($p<0.001$, with all doses of gamma radiation. In tomato plant (Fig. 2b), both oregano biocide and Vitavax-T significantly and similarly increase the activities of PPO, PO and PAL, compared with the untreated plants. However, no significant differences are noticed among the plants grown in non-infested, infested soils and treated with oregano or Vitavax ($p>0.05$, with all doses of gamma radiation).

Discussion

A lot of soil-borne fungi attack squash and tomato plants during their various growth stages from seedling till maturity causing damping-off and wilt diseases. The present study investigated the antifungal potential of biocides derived from peppermint and oregano, compared to Vitavax-T. This approach constantly used essential oil-based biocides in the treatment of damping-off in squash (Yang *et al.,* 2012**),** and tomato plants (Gwinn *et al.,* 2010; El-Mougy *et al.,* 2012). However, these studies did not formulate the essential oils used. In our previous work, we formulated the extracted essential oil with fixed oils, as a carrier, and emulsifiers to control the post harvest pathogens infecting tomato plant (Helal & Abdeldaiem, 2008) and orange (Helal & Abdeldaiem, 2009). Our previous work demonstrated that the formulation of biocides had overcome the degradation of the essential oils and prolonged its protective effect (Abo-El Seoud *et al.,* 2005).

Thus, the formulated biocides are used in treating damping-off in squash and tomato. The pathogens causing seeds damping-off and plant wilt diseases were isolated and identified. On the other side, *Rhizoctonia solani* Kühn and *Fusarium solani* (Mart.) Sacc were found to be the predominant species infecting squash and tomato plants, respectively. Out of 4 biocides (prepared from fennel, peppermint, oregano and ginger**),** only 2 (peppermint and oregano) demonstrated the most effective antifungal activity. Peppermint appears to be the most effective biocide (100%) against *Rhizoctonia solani* kühn, whereas oregano is the most effective against *Fusarium solani* (Mart.) Sacc.

Similar studies (Khaledi *et al.,* 2015) have demonstrated that the essential oils derived from peppermint, caraway and thyme showed the highest antifungal activity against the same species investigated (*Rhizoctonia solani*). The essential oil of peppermint had the lowest minimum inhibitory concentration (MIC) for *R. solani* that indicated the sensitivity of *R. solani* to the biocide used. Also, McMaster & co-workers, (2013), demonstrated that both crude and fractionated components of the essential oil derived from oregano showed broad-spectrum and dose-dependent inhibitory against mycelia of *Rhizoctonia solani* and *Fusarium oxysporum*. In addition, Ibrahim & Al-Ebady, (2014), reported that the essential oil of oregano showed a very strong antifungal activity against *Fusarium* spp., and some other species (*Aspergillus niger* and *Penicillium* spp.). Consequently, the formulated peppermint and oregano oils were selected to be used in the *In vivo* applications. It seems that the mode of application of biocides does not play an important role. However, other investigators (Plodpai *et al.,* 2013) reported a significant reduction in the severity of plant diseases caused by *R. solan*i using foliar application rather than soaking one used.

The present study showed that *R. solani* and *F. solani* were highly pathogenic agents causing high rates of pre- and post-emergence damping-off in plants seedling. In general, the *In vivo* infectivity percent of the untreated cultivars that were investigated ranged from 68% up to 90%. Squash cultivar (Askandrany) was the most susceptible. It demonstrated the highest percent of diseased plants (88%). A similar result was previously reported by Hassan & El-Kot, (2008). In tomato, super strain B (SSB) cultivar was more susceptible to *F. solani.* Some investigators obtained a moderate response on the same cultivar infected with *F. oxysporum* (Zaghloul *et al.,* 2008), whereas others found that super strain B is more resistant to *F. oxysporum* (Khorsandi *et al.,* 2009).

The obtained data indicated that soaking seeds of squash in biocides formulating peppermint oil leads to a significant reduction of damping off and wilt diseases and induced 10-fold increase in the survival of the plants grown in non-infested soil, compared with the non-treated plants. Although the survival of the plants in non-sterilized was lower if compared to the survival rate in sterilized soil, the biocides performance was better in non-sterilized soil. This was indicated by the unexpectedly higher increase in the survival rates (15-fold) of squash plants grown in infested soil. However, no significant differences were observed in the survival rates of tomato plants grown in infected and non-infested soils.

Also, not only did soaking tomato seeds in the emulsion of formulated oregano oil reduce the damping off and wilts diseases to a great extent, but showed an increase in the percentage of survival plants, compared to the non-treated control as well. Several studies have tested the same or other different plants in controlling the same pathogen or others and found similar effects (Abdel-Monaim *et al.,* 2011; El-Mougy *et al.,* 2012).

The protective effect of biocides may be explained on the basis that biocides may induce a direct inhibitory effect on the pathogen survival and reduce its infectivity. Another suggestion comes through the possible effect of biocides on the host cultivars, where they may increase their resistance to infection. The bioactive compounds included in the formulated essential oil may affect the pathogens through attacking its cell walls and cell membranes. This results in affecting the permeability and release of intra-cellular constituents, and in interfering with membrane function (Tian *et al.*, 2012). Also, the lipophilic properties of essential oil components may help the oil to penetrate the plasma membrane, and affect the cellular physiology. One important approach was repeatedly raised by several investigator represented by the ameliorative effect of biocides on enzymes involved in plant's defense system (Mathre *et al.*, 1995). In the present work, the activities of the oxidative enzymes: polyphenoloxidase (PPO), peroxidase (PO) and phenylalanine ammonia lyase (PAL) were investigated after treating the infected plants with biocides. The data obtained revealed a significant increase in the activity of the 3 enzymes in plants grown in infected or non-infected soils, compared to those treated with the chemical fungicide. The increased activity of oxidative enzymes plays an important role in enhancing the plant systemic resistance against different pathogens and subsequently suppresses the pathogenicity (Kagale *et al.*, 2004). The mechanism of action of the oxidative enzymes was attributed to the role of Peroxidase (PO) in controlling the availability of hydrogen peroxide (H2O2) in the cell wall, which is a prerequisite for the cross-linking of phenolic groups in response to pathogen interactions with the host (Ballester *et al.*, 2010). PPO is involved in the oxidation of polyphenols into quinines by using molecular oxygen as an electron acceptor and lignification of plant cells during microbial infection (Chittoor *et al.*, 1999). Thus, it was suggested that PPO may participate in defense reactions and confer hypersensitivity to plants resistance (Li & Steffens, 2002)

PAL is involved in the metabolic pathways of phenylpropanoids and the biosynthesis of p-coumaric acid derivatives, phytoalexin, and lignins that contribute to plant defense systems. Also, PAL participates in the biosynthesis of the defense hormone salicylic acid, which is required for both local and systemic acquired resistance in plants (Dixon & Paiva, 1995).

Although the formulated biocides used enhanced the plant resistance, seeds irradiation with low doses of gamma radiation but did not give the promising effect. The effect of gamma radiation was investigated by several investigators. The outcome of many studies demonstrated that gamma radiation induces an inconsistent effect. Some reports showed that the radiation leads to reduction in germination, seedling size and challenging pathogens in some plants such as maize (Marcu *et al.,* 2013). In contrast, other studies demonstrated that low doses led to increase the yields of some crops such as squash (Ebrahimzadeh *et al.,* 2013) and tomato (Wiendl *et al.,* 2013)

Conclusion

In conclusion, this study demonstrates that the formulated essential oils (biocides) derived from peppermint and oregano oils reveal antifungal properties against *Rhizoctonia solani* Kühn, and *Fusarium solani* (Mart.) Sacc in squash and tomato, respectively. In spite of the possible cost and low yield limitations of the formulated extracts, this approach provides a promising natural antifungal agent which can be environmentally safer, enhance the plant resistance and confer an effective control of phytopathogens in agriculture.

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