



Management of coriander stem rot caused by *Sclerotinia sclerotiorum* using certain biocontrol agents and chemical inducers

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Abstract

The aim of the present study was to evaluate certain of bioagents (*Trichoderma harzianum*, *T. hamatum*, *T. viride* and ESRU bioformulation) and chemical inducers (salicylic acid and potassium silicate) on suppression of coriander stem rot disease caused by *Sclerotinia sclerotiorum*. The highest antagonistic bioagent was *T. viride* which reduced the mycelial growth to 3.9 cm in relation to control, being (9.0 cm). Moreover, five concentrations of each of salicylic acid and potassium silicate (0, 100, 250, 500 and 750 ppm) were evaluated against the growth and formation of sclerotia of the causal fungus *in vitro* and in a greenhouse. Salicylic acid completely inhibited both parameters at 250 and 500 ppm., respectively. Meanwhile, potassium silicates gave the same effect at 750 ppm. Under greenhouse conditions, spraying of coriander plants challenged with the causal fungus with any of the tested inducers and the bioagents lead to a critical reduction in the disease severity in relation to the control. Potassium silicate and salicylic acid as well as *T. viride* and ESRU formulation were the most efficient treatments, being 18.52, 22.22, 29.63 and 33.33%, respectively. Moreover, the effect of the tested inducers as a soil drench integrated with the bioagents as foliar spray was performed. Potassium silicate integrated with *T. viride* was the most effective treatment. Two season field experiments (2015-2016 and 2016-2017) showed that potassium silicate as soil drench integrated with *T. viride* as foliar spray was highly effective in reducing the percentage of dead plants and the area under the disease progress curve as well as increasing the weight of 100 seeds during the two seasons. An increase in the activity of polyphenoloxidase, peroxidase and phenylalanine ammonia lyase was recorded in the leaves sprayed with potassium silicate followed by those sprayed with salicylic acid then *T. viride* and the ESRU formulation.

Keywords: Biological control, coriander, integrated control, chemical inducers, oxidative enzymes, stem rot.

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Introduction

Coriander (*Coriandrum sativum* L.) is an annual herbaceous plant belongs to Apiaceae and is the most popular due to their aromatic and medicinal properties. In Egypt, the cultivated area of anise, fennel and coriander is 30418 ha producing 26558 tones with a productivity of 8731 kg ha⁻¹ (FAO, 2014). Many reasons may be led to crop productivity, including diseases, especially those caused by soil borne and seed borne pathogens. Coriander stem rot incited by *Sclerotinia sclerotiorum* (Lib.) de Bary, is the most destructive disease causing economic losses in coriander and different Umbelliferous medicinal plants in Egypt (Hilal et al., 1998) as well as worldwide. This pathogen is able to infect more than 400 plant species around the world (Bolland & Hall, 1994). Moreover, it is geographically international and has globally ecological distribution. So, disease caused by *S. sclerotiorum* is extremely difficult to manage. The disease appears as water soaked spots on the stem then become brown. As the disease progresses the whole stem is girdled and covered with white cottony fungal growth with black sclerotia (Khare et al. 2017). Unfortunately, several disadvantages faced the traditional strategies used in combating the disease such as environmental risks, especially those based on chemical fungicides. So, seeking for safe, effective and alternatives to these traditional methods has become very urgent at the present. Biological control is a standout among the most encouraging other options to control diseases caused by soil borne pathogens. The genus *Trichoderma* has the ability to antagonist the plant pathogens by different mechanisms (Benítez et al. 2004). However, a few of them proved efficiently under field

conditions. So, seeking for strategies to stimulate the antagonistic activity of the beneficial organisms especially under natural conditions has been urgent. Recently, many authors found that chemical inducers such as salicylic acid and potassium silicate improve the antagonistic activity of different bioagents when applied together. For example, Elungi (2009) found that a synergistic effect was observed when Eco-T® the bioproduct entrapping *T. harzianum* was applied to the plant with silicon to control sunflower root rot caused by *Rhizoctonia solani* and *S. sclerotiorum*. The present study was aimed to evaluate certain of bioagents and chemical inducers on suppression of coriander stem rot caused by *S. sclerotiorum* and to estimate the changes in the activity of oxidative-reductive enzyme of the infected coriander plants after treatment.

Materials and methods

Source of the causal pathogen:

Coriander plants with typical symptoms of stem rot were collected from fields located at Giza governorate, Egypt. Infected stems were washed under tap water, cut into small pieces, surface disinfected by submerging in 2% sodium hypochlorite for 2 min, washed in several changes of sterile water and finally aseptically transferred onto potato dextrose agar medium (PDA) in Petri plates and incubated at 20±1°C for 7 days. The target growing fungus was purified and identified according to their morphological and cultural characteristics according to Nelson et al. (1983) and Domsch et al. (1980) after confirming its pathogenic capability to infect coriander plants (Data not shown).

Isolation and identification of *Trichoderma* isolates: Leave samples of two healthy medicinal plants, *i.e.* Coriander (*Coriandrum sativum* L.) and rosemary (*Rosmarinus officinalis* L.) grown in fields located at Giza governorate, Egypt were collected to isolate the *Trichoderma* from the phyllosphere. The serial dilution technique was followed and three dilutions of each sample were prepared. One milliliter of every sample was pipetted onto a Rose Bengal Agar (RBA) medium into Petri dishes and incubated at $20\pm 1^{\circ}\text{C}$ for 1 week. The confined *Trichoderma* isolates were distinguished utilizing the key of the genus *Trichoderma* (Rifai, 1969).

Effect of the antagonistic activity of the tested bioagents on the mycelial growth of *S. sclerotiorum*: The antagonistic activity of three isolates of *Trichoderma* was estimated against *S. sclerotiorum* by the dual culture technique (Cherif & Benhamou, 1990). One disk (5mm diameter) of the mycelium of the tested pathogen was placed onto one side of the PDA in Petri plates (5 replicates/treatment) and an equal disk of one tested *Trichoderma* isolates was placed onto the other side. The inoculated plates were incubated at $20\pm 1^{\circ}\text{C}$. Plates have two disks of *S. sclerotiorum*, one on each side, were used as control. The effect of *Trichoderma* isolates on plant pathogens was determined by counting the length of mycelial growth in cm when the growth of any of control plates covered the Petri dish.

Effect of the chemical inducers on the mycelial growth and formation of *S.*

***sclerotiorum*:** Four different concentrations (0, 100, 250, 500 and 750 ppm) of each of salicylic acid and potassium silicate were evaluated on the linear growth and formation of sclerotia of *S. sclerotiorum in vitro*. Each treatment was added to individual flask containing sterilized PDA before solidifying and rotated gently to ensure even distribution of the proposed concentration, and then poured into 9 cm Petri dishes. Plates were inoculated with a 5-mm disk of the fungus to be tested at the center of a plate and incubated at 20°C . Five Petri dishes were used for each concentration. PDA-dishes cultures without any inducers were used as control. The fungal growth was measured when the growth of any of control plates covered the Petri dish. The number of sclerotia was counted 10 days later.

Effect of certain of bioagents and chemical inducers on coriander stem rot under greenhouse conditions: Pot experiments were carried out to evaluate the efficacy of the tested inducers and the three isolated *Trichoderma* species as well as a locally produced bacterial formulation innovated by the Environmental Studies and Research Unit (ESRU), Department of Microbiology, Faculty of Agriculture, Cairo University, Egypt labeled as "Biocontrol formulation" against coriander stem rot. ESRU bioformulation is a composite culture of seven potent associative bacteria, *i.e.* *Bacillus polymexa* (3 strains), *Bacillus macerans* (1strain), *Bacillus macerans* (2 strains) and *Enterobacter agglomerans* (1 strain) known with its high efficacy against *S. sclerotiorum* (Fayez et al. 2009). This

experiment was performed at Ornamental, Medical and Aromatic Plant Diseases Department, Orman garden, Giza, Egypt. Completely randomized blocks with nine plants per treatment was used for each tested bioagent. Coriander stems were inoculated using pieces of infested toothpicks by a method described by Pratt et al. (1998). Where, the tips (1.0 to 1.5 cm long) of wooden toothpicks were autoclaved for 20 min in 250 ml distilled water, evacuated, smudged and re-autoclaved in extra water to remove inhibitory substances and autoclaved for a third time in 250 ml distilled water. Sterilized toothpicks were placed on PDA plates inoculated with one disc (5mm) of *S. sclerotiorum* then incubated at $20\pm1^{\circ}$ C for seven days. Plants aged 90 days grown in 35 cm diameter pots were used in this experiment to obtain stems with appropriate diameters to insert the toothpick. A single toothpick piece was inserted into one stem base per plant. Control inoculations were performed with toothpick pieces prepared in the same manner and incubated on non-infested PDA. Stem rot severity was calculating 14 days after inoculation using scale (from 0 to 3) described by Sansford (1995) with some modification, where 0 = healthy plants, 1= less than half stem girdled, 2= more than half stem girdled and 3= whole stem girdled and plant death. A disease severity index (DSI) was calculated for each treatment by a formula suggested by Kim et al. (2000) as follows:

$$DSI = \frac{\sum(\text{rating of each plant})}{3 \times \text{number of plant rated}} \times 100$$

Depending on the results of *in vitro*

studies, the tested *Trichoderma* isolates (3×10^6 spore/ml), the ESRU formulation was used as recommended, where diluted with water to 1:1 (v/v), salicylic acid (100 ml of 250 ppm), and potassium silicate (100 ml of 750 ppm) were sprayed 24 hr. after inoculation by the causal pathogen and 10 days later. Unsprayed plants with any of bioagents and inducers were used as control. A few drops of Tween-20 were added to every 100 ml. of the sprayed materials.

Combined application of the tested bioagents as a foliar spray and chemical inducers as a soil drench on coriander stem rot under greenhouse conditions: The effects of the tested biocontrol agents as foliar spray and the chemical inducers as soil drench individually or in dual application against coriander stem rot were evaluated under greenhouse conditions. Split plot design was completely randomized with soil drench with the chemical inducers each of 100 ml of 250 ppm for salicylic acid and 750 ppm for potassium silicate as the main plot and the foliar spray with the bioagents as subplots. The main plots were divided into three parts. The first part was drenched plants three times with 100 ml salicylic acid once immediately after sowing and two times again, once every 30 days, the second part was drenched plants with 100 ml potassium silicate once immediately after sowing and two times again, once every 30 days and the third part was treated with water. Each main plot was divided into three sub-plot, the first was plants sprayed with *T. viride* at the rate of 3×10^6 spore/ml 24h after inoculating the stem (90 days after sowing) with the causal fungus and repeated again after 10 days,

the second was plants treated with the ESRU formulation (1:1 v/v) 24 h after inoculating the stem with the causal fungus and repeated again after 10 days and the third was non-drenched plants with any of the tested inducers and sprayed with water only. A few drops of Tween-20 were added to each 100 ml. of the sprayed bioagents. The application of water as both soil drench and foliar spray was considered as control. The severity of stem rot was measured as mentioned before. Experimental blocks design; completely randomized with nine plants per treatment were used.

Field experiments: Field experiments were conducted at the Horticultural Research Station, Al-Shaer Island, Al-Qanatir Al-Khairiya, Qalubiya governorate, Egypt. The experiment (Clay soil) was divided into plots of (10.5 m²; 4 rows of 3 m. long x 3.5 m width, 8 hills/row and 5 seeds/row which thinned to two seeds 30 days after sowing). The experimental setup was soil drench with the chemical inducers as the main plots (each inducer was applied four times, once immediately after sowing and three times again every 21 days) and foliar spray with the bioagents as subplots (each bioagent was sprayed five times, once 30 days after sowing and four times again, once every 10 days). Two controls (one with fungicide vitavax-thiram at the rate of 3g/l water as a soil drench and the second control was water only) were used. The experiment was organized as shown before in greenhouse experiments. Two experiments were carried out each was sown on November 1, 2015 and 2016. All agricultural practices, *i.e.* irrigation, weeds and pests control as well as fertilization were applied

according to the standard recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. As the experiment was done under natural field conditions, The efficacy of the tested treatments was evaluated by counting the percentage of dead plants as well as AUDPC (area under disease progress curve) depending on three readings once every 25 days using formula suggested by (Madden et al. 2007) as follows:

$$Ak = \sum_{i=1}^{Ni-1} \frac{(Y_i + Y_{i+1} + 1)}{2} (t_{i+1} - t_i)$$

Where y_i is an assessment of the disease incidence (%) at the i th observation, t_i is time in days at the i th observation, and n is the total number of observations. The weight of 100 seeds was recorded at the time of harvest crop, 120 days after planting.

Antioxidant enzymes activity: The leaves of coriander plants (3 month after planting) were collected from the pot experiments 48 hr. after spraying with the tested treatments to determine the enzyme's activity. 0.5 g of coriander leaves was homogenized in a mortar with 30-40 ml phosphate buffer (0.02M) then filtered through cheese cloth and centrifuged at 2000 rpm for 10 min then the extract was made up to 100 ml with the buffer.

Evaluation of polyphenol oxidase activity (PPO): The enzyme activity was assayed spectrophotometrically at 470 nm as described by Palmer (1963). The sample cuvette contained 1 ml 0.033 M potassium phosphate (pH7), 1 ml 0.005

M substrate solution and quantity of enzyme to give a total reaction volume of 3 ml (Palmer, 1963). The blank sample contained the same mixture solution without the enzyme extract.

Evaluation of peroxidase activity (POX): The enzyme activity was assayed spectrophotometrically at 470 nm using guaiacol as a phenolic substrate with hydrogen peroxide (Díaz et al., 2001). The reaction mixture contained 0.15 mL of 4% (v/v) guaiacol, 0.15 mL of 1% (v/v) H₂ O₂, 2.66 mL of 0.1 M phosphate buffer (pH 7) and 40 µL of the enzyme extract. The blank sample contained the same mixture solution without the enzyme extract.

Evaluation of phenylalanine ammonia lyase activity (PAL): 0.5 g of the leaves was homogenized in a mortar with 5 ml cold borate buffer (25 mM) containing 5 mM mercaptoethanol (0.4 ml L⁻¹). The homogenate was centrifuged in a refrigerated centrifuge at 12,000 rpm for 20 min. The supernatant served as enzyme extract. All operations were carried out at 4°C according to the methods of Brueske (1980).

Statistical analysis: Data were analyzed using CoStat software (version 6.4, CoHort Software, USA) according to Gomez and Gomez (1984). The differences among means were compared at 5% level using least significant differences (L.S.D) according to Fisher (1948).

Results

In vitro antagonistic of *Trichoderma*

isolates against *S. sclerotiorum*: The results in Figure (1) indicate that *T. viride* was the most effective isolate where it caused the lowest mycelial growth (3.9 cm) meanwhile, the least effective isolate was *T. hamatum* followed by *T. harzianum* (4.9 and 5.5 cm, respectively) without significant differences at the level of significance ($p < 0.05$).

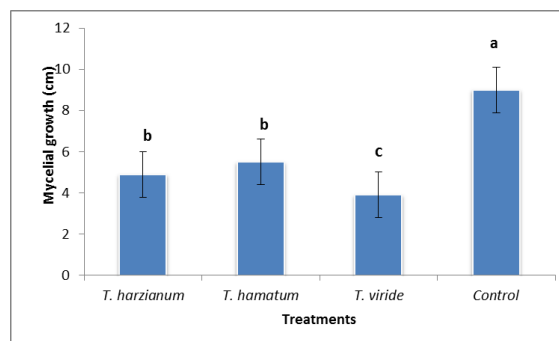


Figure 1: Effect of the tested *Trichoderma* isolates on the mycelial growth of *S. sclerotiorum*. Bars with the same letters are not significantly different ($p < 0.05$)

Effect of salicylic acid and potassium silicate on the mycelial growth and formation of sclerotia of *S. sclerotiorum* in vitro: Five different concentrations (0, 100, 250, 500 and 750 ppm) of each of salicylic acid and potassium silicate were evaluated on the linear growth and formation of sclerotia of *S. sclerotiorum* in vitro (Table 1). The all tested concentrations significantly reduced the linear growth and the number of sclerotia of the tested pathogen in relation to the control. Significant decreases in the fungus growth were observed by increasing the concentrations of the tested elicitors. Salicylic acid completely inhibited the formation of sclerotia and the mycelial growth at 250 and 500 ppm, respectively. Meanwhile, potassium silicates gave the same effect at 750 ppm.

Effect of certain of bioagents and chemical inducers on coriander stem rot under greenhouse conditions:

Effect of the tested bioagents and chemical inducers on coriander stem rot was evaluated under artificial infection by estimating the stem rot severity as shown in figure (2). Potassium silicate

and salicylic acid followed by *T. viride* and ESRU formulation were the most effective in reducing the severity of stem rot to 18.52, 22.22, 29.63 and 33.33%, respectively. Each of *T. harzianum* and *T. hamatum* were the lowest effective treatments (44.44 and 44.44%, respectively).

Table 1: Effect of salicylic acid and potassium silicate on mycelial growth and formation of sclerotia of *S. sclerotiorum*.

Treatments	Concentrations(ppm)	Mycelial growth (cm)	Number of sclerotia
Salicylic acid	0	9.0	12.4
	100	9.0	3.8
	250	3.4	0.0
	500	0.0	0.0
	750	0.0	0.0
Potassium silicate	0	9.0	12.4
	100	9.0	10.0
	250	9.0	10.0
	500	5.2	0.0
	750	0.0	0.0
LSD at 5% for:			
Treatments (T) =		0.1	0.5
Concentrations (C) =		0.2	0.7
T × C =		0.3	0.8

Combined application of the tested bioagents as foliar spray and chemical inducers as soil drench on coriander stem rot under greenhouse conditions:

Effect of the tested bioagents as a foliar spray combined with the chemical inducers as a soil drench on coriander stem rot was evaluated under artificial infection by estimating the stem rot severity as shown in Table (2). Potassium

silicate as soil drench combined with *T. viride* as foliar spray was the most effective in reducing the severity of stem rot to 14.81% Combined application of potassium silicate and ESRU formulation followed by combining application of salicylic acid and *T. viride* were occupied the second rank which significantly reducing the stem rot severity to 22.22 and 25.92%, respectively.

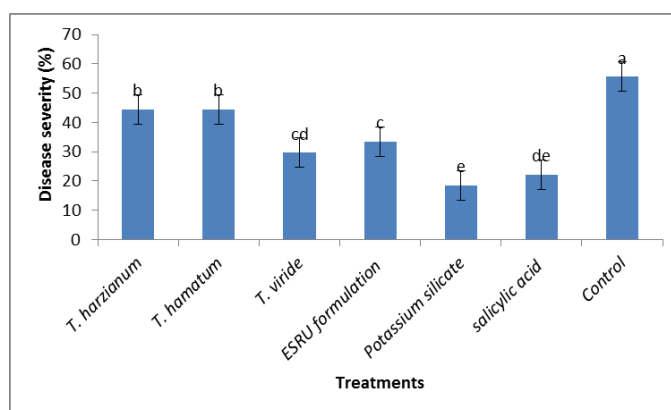


Figure 2: Effect of foliar spray of the tested bioagents and chemical inducers on the severity of coriander stem rot caused by *S. sclerotiorum*. Bars with the same letters are not significantly different ($p < 0.05$).

Table 2: Effect of combined application of the tested bioagents as foliar spray and chemical inducers as soil drench on stem rot severity caused by coriander stem rot under greenhouse conditions.

Treatments		Disease severity (%)
Soil drench	Foliar spray	
Salicylic acid	<i>T. viride</i>	25.92
	ESRU formulation	33.33
	Water	40.74
Potassium silicate	<i>T. viride</i>	14.81
	ESRU formulation	22.22
	Water	37.04
Water	<i>T. viride</i>	37.04
	ESRU formulation	48.15
	Water	66.67
LSD at 5% for:		
Soil drench (S) =		2.80
Foliar spray (F) =		5.60
SXF =		7.40

Field experiments: Table (3) shows that the tested chemical inducers and the bioagents as well as the fungicide vitavax thiram resulted in significant reduction in the percentage of dead coriander plants as well as the AUDPC values under field conditions. Application of potassium silicate as soil drench combined with *T. viride* as foliar spray was the most effective treatment in reducing the percentage of dead plant in both seasons experiment to 5.21 and 9.90%, respectively and the AUDPC to 36.47 and 104.51, respectively. The fungicide vitavax thiram was the most superior treatment which gave the lowest percentage of dead plants. Combined

application of salicylic acid as soil drench and ESRU formulation as foliar spray was the lowest effective treatment. Results shown in Table (3) show that the reduction in the percentage of dead plants was significantly reflected on the weight of the 100 seeds. In general, the fungicide vitavax thiram was the most efficient treatment in increasing the weight of 100 seeds in both seasons, being 0.43 and 0.48 g, respectively followed by combining application of potassium silicate as soil drench and *T. viride* or ESRU formulation as foliar spray, being 0.41, 0.41 and 0.29 g, in the first season, respectively and 0.45, 0.45 and 0.39 g in the second season.

Table 3: Effect of combined application of the tested bioagents as foliar spray and chemical inducers as soil drench on dead plants percentages and area under disease progressive curve (AUDPC) under field conditions.

Treatments		Dead plant (%)		AUDPC		Weight of 100 seeds	
Soil drench	Foliar spray	2015-2016	2016-2017	2015-2016	2016-2017	2015-2016	2016-2017
Salicylic acid	<i>T. viride</i>	7.29	14.06	49.85	117.92	0.39	0.42
	ESRU formulation	8.85	18.75	55.92	136.13	0.36	0.40
Potassium silicate	<i>T. viride</i>	5.21	9.90	36.47	104.51	0.41	0.45
	ESRU formulation	5.73	13.54	36.47	73.41	0.41	0.45
Vitavax Thiram WP		4.17	6.77	31.62	57.11	0.43	0.48
Control		25.52	34.38	176.20	250.35	0.29	0.39
L.S.D 0.05		3.21	5.07	17.02	36.29	0.02	0.01

Estimation the activity of oxidative-reductive enzymes: Data presented in

figures (3, 4 and 5) show the changes in the activity of some enzymes, *i.e.*

polyphenol oxidase (PPO), peroxidase (POX) and phenylalanine ammonia lyase (PAL) due to spraying of coriander plants with the tested inducers and the bioagents compared with the control. In general, results indicate that there was an increase in the activity of the three enzymes in the leaves of all sprayed plants compared with control treatment. Plants sprayed

with potassium silicate showed the highest activity followed by those sprayed with salicylic acid, *T. viride* and the ESRU formulation, being 0.434, 0.412, 0.306 and 0.261, respectively for polyphenoloxidase, 0.305, 0.292, 0.278, respectively for peroxidase and 0.361, 0.349, 0.322 and 0.285, respectively for phenylalanine ammonia lyase.

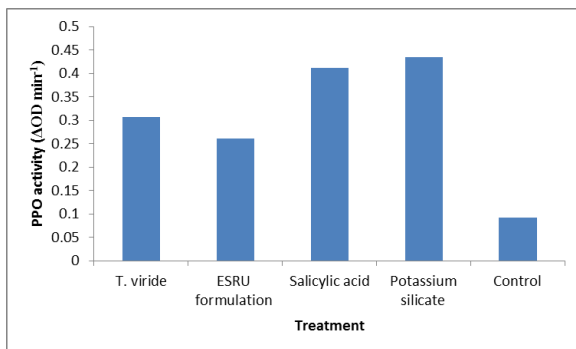


Figure 3: The activity of polyphenoloxidase (PPO) due to spraying of infected coriander plants by *S. sclerotiorum* with the tested chemical inducers and the bioagents.

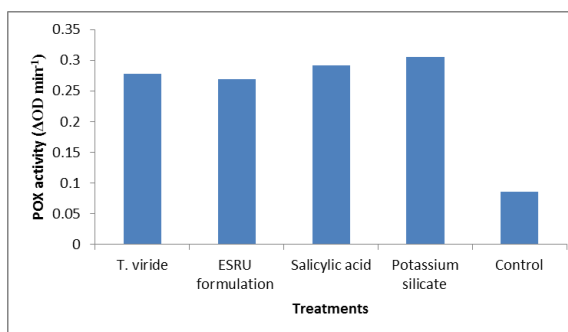


Figure 4: The activity of peroxidase (POX) due to spraying of infected coriander plants by *S. sclerotiorum* with the tested chemical inducers and the bioagents.

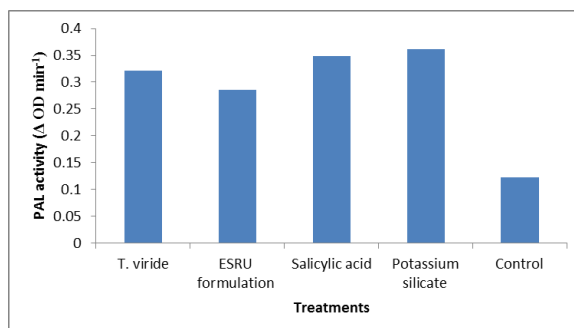


Figure 5: The activity of phenylalanine ammonia lyase (PAL) due to spraying of infected coriander plants by *S. sclerotiorum* with the tested chemical inducers and the bioagents.

Meanwhile, untreated leaves (control) showed the lowest values, being 0.092, 0.086 and 0.123, respectively.

Discussion

Stem rot incited by *Sclerotinia sclerotiorum* is one of the most important

diseases that cause economic losses in coriander and different Umbelliferous medicinal plants (Hilal et al., 1998). With increasing awareness of the serious environmental implications of the chemical fungicides in agricultural ecosystems, different strategies have been emerging as alternative means to these compounds (Elungi, 2009).

Biological control using beneficial organisms alone, or as mixtures to reduce the excessive use of chemical fungicides in integrated plant disease management, has become urgent need in these days. Isolation of fungal bioagents from the healthy coriander (*Coriandrum sativum* L.) and rosemary (*Rosmarinus officinalis* L.) plants yielded three isolates of *Trichoderma*, i.e. *T. harzianum*, *T. hamatum* and *T. viride*. All the tested chemical inducers, i.e. salicylic acid and potassium silicate as well as the tested bioagents resulted in significant reduction in the mycelial growth of the causal fungus compared with the control. In the trials of the chemical inducers the reduction in the mycelial growth of the causal pathogen as well as the formation of sclerotia was gradually increased by increasing the concentration of the inducers. Under greenhouse conditions, spraying of coriander plants artificially inoculated with the causal fungus with any of the tested chemical inducers and the bioagents brought about a critical decrease in the severity of the disease compared with control. Potassium silicate and salicylic acid as well as *T. viride* and biocontrol formulation were the most efficient treatments in this respect. Moreover, the treatment of soil drench with potassium silicate combined with *T. viride* as foliar spray was more efficient in reducing the severity of the disease compared with spraying the bioagent on plants of undrenching soil. In this concern, Hilal et al. (2006) reported that spraying of caraway, coriander and fennel plants three or seven days with each of bion, chitosan, oxalic acid, salicylic acid and switch before artificially inoculated of any of these plants with *S. sclerotiorum*

lead to a critical decrease in the disease severity compared to the control. Moreover, Fagodiya (2016) found that treated seed and soil as dual treatment with *T. viride* resulted in significant reduction in coriander stem rot disease in comparison to treat seeds or soil alone. It has been found from the two season field experiments that the treatment of soil drench with potassium silicate combined with foliar spray with *T. viride* or ESRU formulation very effective in reducing the percentage of dead plants and AUDPC compared with the control. Furthermore, the fungicide vitavax thiram was the superior treatment in both experiments. Many authors confirmed the exciting potential for increased disease resistance through silicon supplements (Sait, 2010). The way of supplying silicon to the plants are greatly manages the action of the plants to protect themselves from infection by the pathogens (Rodrigues et al., 2015). Where, it may be acting as a physical barrier or by boosting the defence weapons of the plants when supplied to the plant through the roots. Meanwhile, it acts by changing the pH or the osmotic potential on the leaf surface when supplied as foliar spray (Rodrigues et al., 2015). Liang et al. (2005) observed that the root silicon amendment significantly reduced the cucumber powdery mildew disease severity compared to foliar spray. The present study clearly showed that as the percentage of coriander plant death under field conditions was reduced by the tested materials the weight of 100 seeds was increased. In general, treatment of soil drench with potassium silicate combined with foliar spray with *T. viride* or ESRU formulation was more efficient in increasing the weight of 100

seeds compared with the control treated with water only. However, the fungicide vitavax thiram was the most effective treatment in both seasons. The mechanism of plant defence is complex and involved different weapons such as phenols and oxidative enzymes such as polyphenoloxidase (PPO), peroxidase (POX) and phenylalanine ammonia lyase (PAL) (Arun et al., 2010). PPO and POX enzymes oxidize the phenols to quinones. The quinones increased the rigidity of the plant cell wall by increasing its lignifications. Meanwhile, PAL enzyme catalyzes the trans-cinnamic acid via the L-deamination of phenylalanine. It is commonly considered the principal enzyme in the biosynthesis of phenolic compounds (Aldesuquy et al., 2015). In this study the activity of PPO, POX and PAL enzymes was increased in the treated leaves compared to the control. Potassium silicate recorded the highest activity of the three enzymes followed by those sprayed with salicylic acid then *T. viride* and the ESRU formulation. Meanwhile, untreated leaves which conserved as control recorded the lowest activity. Increasing of the three enzymes in coriander leaves treated with the tested material may be pointing to that these materials induced the weapons of the plants against the tested pathogen.

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