



Essential Oils and Sulfur to Management Potato Late and Early Blight Diseases under Field Conditions

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Received: 05 Dec. 2021

Accepted: 12 Jan. 2022

Published: 20 Jan. 2022

ABSTRACT

Both late and early blights caused by *Phytophthora infestans* and *Aternaria solani* respectively historically an important diseases of potato and tomato plants. Effect of Sulfur and some essential oils applied as alone or in combination to control late and early blight diseases of potato plants during two seasons was studied. The findings of laboratory investigations results revealed that Sulfur at 3.0 g/L and Thyme as well as Citral at 6.0 ml/L resulted in a total reduction in the linear growth of *A. solani* and *P. infestans*. In field trials, the data indicated the all tested treatments altogether decreased the late and early incidence during two seasons. Highest decrease in the incidence of late and early blight diseases was obtained with the combined treatments between Thyme + Sulfur and Citral + Sulfur, which reduced the late blight ranged between 83.3 and 88.2 %. and early blight between 69.2 and 76.9 %, respectively. As for the potato yield during the two winter planting seasons, the results showed that obtaining the highest increase in potato yield was achieved with the combination between Thyme + Sulfur and Citral + Sulfur, which led to an increase in potato yield ranged between 66.7 and 74.3 %. This treatments also showed an increase in potato yield during the two summer planting seasons, which led to an increase in potato yield ranged between 53.8 and 60.3 %. All previous treatments increased the enzyme activities. The best treatments are combined treatments between Thyme + Sulfur and Citral + Sulfur which increase peroxidase, chitinase and β ,1-3- glucanase more than 300, 240 and 203.6 % respectively.

Keywords: Late, early blight, *Phytophthora infestans*, *Aternaria solani*, Sulfur, Thyme, Citral.

1. Introduction

Potato (*Solanum tuberosum* L.) is the most generally developed food crop on the world and has high dietary and monetary worth. Historically, late and early blight caused by *Phytophthora infestans* (Mont.) de Bary and *Aternaria solani* (Ell. and Mart.) Jones and Grout. Which important diseases of potato and tomato plants (Arora *et al.*, 2014; Saied *et al.*, 2016 and Abbas, 2018).

The control of these diseases mainly depends on chemical fungicides that face environmental problems. Therefore, there is an urgent need for safe alternative treatments to control plant diseases (Abd-El-Kareem and Haggag, 2015 Saied *et al.*, 2016 and Abd-El-Kareem *et al.*, 2018).

Sulfur and copper only utilized in organic cultivating to control plant diseases. Sulfur can be utilized as a fungicide and protection against many plant diseases (Abd-El-Kareem *et al.*, 2009).

The effect of sulfur and sulfur compounds on plant diseases directly as biocides and indirectly by enhancing plant resistance in addition to increasing the availability of other elements (Haneklaus *et al.*, 2006 a).

It was found that thyme extract was most effective against the growth of *M. phaseolina* and other pathogenic fungi. The decrease in linear growth is more or less increased by increasing the concentration of plant products (El-Habbaa *et al.*, 2002).

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Furthermore, thyme oils have inhibitory consequences for food waste brought caused by *A. niger* and *A. flavus* (Viuda *et al.*, 2007).

The thyme oil had solid fungicidal impact on *Penicillium* and *Alternaria* (Mironescua and Georgescub, 2008), *A. alternate* (Hadizadeh *et al.*, 2009).

Hussein (2011), it was shown that thyme had the highest effect in controlling peanut damping off diseases and root rot caused by *Rhizoctonia solani*.

Citral oil is one of citrus essential oil fractions and has antifungal activity against *Giotrichum candidum*, *Penicillium digitatum* and *P. italicum* as pathogens of citrus fruit diseases (El-Mohamedy *et al.*, 2002).

Citral essential oil is a business result of citrus essential oil portions and has been accounted for to have antifungal action to *Giotrichum candidum*, *Penicillium digitatum* and *P. italicum* as pathogens of citrus fruit diseases (El-Mohamedy *et al.*, 2002).

The main goals of the current examination are evaluation the impact of Sulfur and some essential oils as single or double treatments to control late and early blight of potato plants.

2. Materials and Methods

2.1. Pathogens

Pathogenic isolate of *Alternaria solani*, and *Phytophthora infestans* the causal agent of early and late blight diseases, was kindly provided by Plant Pathology Department, National Research Centre, Giza, Egypt.

2.2. Plant material

Potato tubers (cv. Nicola) obtained from Dept., of Vegetables Crop Research, Agricultural Research Centre, Giza, Egypt were used in this study.

2.3. Essential oils and Sulfur

Thyme and Citral were obtained from the Delta Aromatic Company, Giza, Egypt. Wile, sulfur were obtained from the Ephco, Misr, Egypt.

2.4. Laboratory experiments

2.4.1. Inhibitor effect of Sulfur and some essential oils on the linear growth of *A. solani* and *P. infestans* in vitro

Three concentrations of Sulfur *i.e.* 1.0, 2.0 and 3 g/ L while, Thyme and Citral at concentration of 2.0, 4.0 and 6.0 mL/L were tested against linear growth of *A. solani* and *P. infestans* *in vitro*. Each of tested concentrations were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (9- cm – diameter). Plates were individually inoculated at the center with equal disks (6-mm- diameter) of 10-days old culture of *Alternaria solani* or *P. infestans*. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at 25 ± 2 °C. The average linear growth of fungus was calculated after 10 days.

2.5. Field experiments

Potato tubers cv. Diamont were planted under field conditions in Bahada village, Qalubeia Governorate, during four successive cultivation seasons.

2.6. Experimental design

Experiments were planted in complete randomized design, five replicates were used for each treatment. Each replicate was represented by 50 plants. Irrigation and fertilization were carried out as recommended.

2.6.1. Treatments

Tree chemicals *i.e.* Sulfur at concentration of 3.0 g/ L, Thyme and Citral at 6.0 mL/ L. were tested as single treatments. Meanwhile combined treatment between Thyme + Sulfur or Citral + Sulfur were applied to study their effect on early and late blight diseases. Fungicide, Redomil-plus (2g/l) was also used as comparison treatment.

2.6.2. Application

All tested treatments were applied on potato plants which had 4-5 compound leaves and every 15 days intervals up to 90 days of planting. Meanwhile combined treatment were applied at 7 days between first application and combined treatment. Fungicide was sprayed every 15 days.

2.6.3. Disease assessment

Early and late blight scale from 0 to 4 according to Cohen *et al.*, (1991) was used, as follows:

- (0) No leaf lesions (1) 25% or less (2) 26-50% (3) 51-75% (4) 76-100% infected area of plant leaf

Disease was recorded after up to 90 days of planting.

2.7. Testing of sulfur and some essential oils on enzyme activities

2.7.1. Extraction of enzymes

Plant leaves (g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.1) (Goldschmidt *et al.*, 1968) at the rate of 1/3 w/v. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was used to determine enzyme activities.

2.7.2. Peroxidase assay

Peroxidase activity was measured by incubation 0.1 mL of enzyme extract with 4 mL of guaiacol solution for one minute at 25°C and absorbance at 470 nm was determined. The guaiacol solution consisted of 3 ml of 0.05 M K. phosphate, pH 7, 0.5 mL of 2% guaiacol and 0.5 mL of 0.3% H₂O₂ (Abeles *et al.*, 1971). Peroxidase activity was expressed as the increase in absorbance at 470 nm / gram fresh weight / one minute using Spectrophotometer (Spectronic 20-D).

2.7.3. Chitinase assay

The substrate colloidal chitin was prepared from chitin powder according to the method described by Ried and Ogryd-Ziak (1981) as mentioned before. Determination of chitinase activity was carried out according to the method of Monreal and Reese, (1969). One mL of 1% colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) in test tubes, one mL of enzyme extract was added and mixed by shaking. Tubes were kept in a water bath at 37°C for 60 minutes, then cooled and centrifuged before assaying. Reducing sugar was determined in 1 mL of the supernatant by dinitrosalicylic acid (DNS). The reaction was stopped by heating the tubes for 5 minutes at 100°C. The tubes were cooled and 3 mL of distilled water were added before assay. Optical density was determined at 540 nm using Spectrophotometer (Spectronic 20-D). Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

2.7.4. β -1, 3 – glucanase assay

The method of Abeles and Forrence (1970) was used to determine β -1, 3 – glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. The method was carried out as 0.5 mL of enzyme extract was added to 0.5 mL of 0.05 M of potassium acetate buffer (pH 5) containing 2% laminarin. The mixture was incubated at 40°C for 60 minutes. The reaction was stopped by adding 1 mL of dinitrosalicylic acid reagent and heating the tubes for 5 minutes at 100°C. The tubes were cooled and 3 mL of distilled water were added before assay. The optical density was determined at 500 nm using Spectrophotometer (Spectronic 20-D). β - 1, 3– glucanase activity was expressed as mM glucose equivalent released / gram fresh weight tissues / 60 minutes.

2.8. Determination of tuber yield

Total tuber yield per replicate (Kg) or ton per Hectar, was weighted and the average of yield per treatment was determined.

2.9. Statistical analysis

Data were entered into CoStat6303Win.exe software and analyzed statistically by the analysis of variance (ANOVA) and the means were compared by Duncan's multiple range test at $P < 0.05$. Data for percentage of disease incidence were statistically analyzed after arcsine square-root transformation; however, untransformed data are presented as mean values \pm standard errors (SE).

3. Results

3.1. In laboratory trials

3.1.1. Effect of Sulfur and some essential oils on mycelial growth of *A. solani* and *P. infestans*

Three concentrations of Sulfur *i.e.* 1.0, 2.0, 3 g/L; Thyme and Citral at 2.0, 4.0, 6.0 mL/L were tested against linear growth of *A. solani* and *P. infestans in vitro*.

The results presented in Fig (1) show that all tried treatments reduced the growth of *A. solani* and *P. infestans*. Complete reduction was obtained for both tested fungi with sulfur at 3.0 g/L and thyme plus citral at 6.0 ml/L. Moderate effect was achieved with sulfur at 2.0 g/L and thyme plus citral at 4.0 ml/L. Reducing the growth of both tested fungi more than 76.2 and 73.3% for *P. infestans* and *Alternaria solani* respectively.

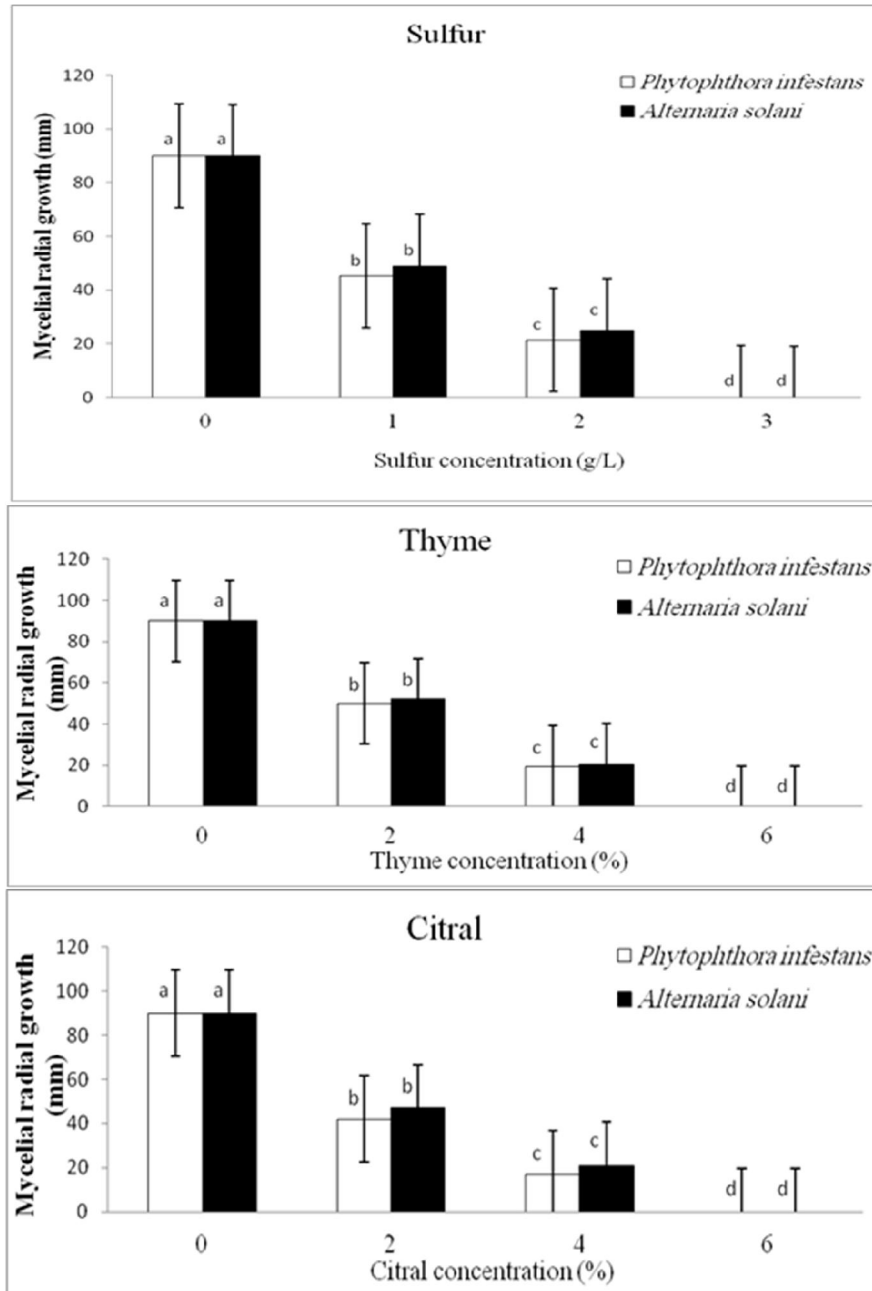


Fig. 1: *In vitro* antifungal activity of sulfur, thyme and citral against *Phytophthora infestans* and *Alternaria solani*.

3.2. Field experiments

Three chemicals *i.e.* Sulfur at concentration of 3.0 g/ L, Thyme and Citral at 6.0 mL/ L. were tested as single or combined treatments against early and late blight diseases. Fungicide, Redomil-plus (2g/L) was also used as comparison treatment. Moreover, their effects on potato yield and enzyme activities.

3.2.1. Effect on late blight incidence

Data in Table (1) reveal that tested treatments significantly suppressed late blight incidence during two growing seasons. The highest inhibition of disease incidence was obtained Thyme + Sulfur and Citral + Sulfur which reduced late blight incidence ranged between 83.3 and 88.2 %. Meanwhile, single treatments showed satisfactory impact.

Table 1: Late blight of potato plants in response to different concentrations of sulfur and some essential oils alone or in combination during two winter cultivation seasons

Treatment	Late blight incidence			
	First season	Efficacy %	Second season	Efficacy %
Sulfur	1.8±0.11 c	47.1	2.0±0.23 c	44.4
Thyme	2.6±0.11 b	26.7	2.8±0.11 b	22.2
Citral	2.5±0.05 b	26.5	2.5±0.10 b	30.6
Thyme + Sulfur	0.5±0.05 d	85.3	0.5±0.10 d	86.1
Citral + Sulfur	0.4±0.10 d	88.2	0.6±0.05 d	83.3
Redomil-plus	0.5±0.10 d	85.3	0.7±0.10 d	80.6
Control	3.4±0.11 a	0.0	3.6±0.17 a	0.0

Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

3.2.2. Effect on potato yield

Data in Table (2) reveal that tested treatments increase the potato yield. The best treatments were obtained with combined treatments between Thyme + Sulfur and Citral + Sulfur which increase potato yield ranged between 66.7 and 74.3 %. Meanwhile, single treatments showed satisfactory impact.

Table 2: Potato yield in response to sulfur and some essential oils alone or in combination during two winter cultivation seasons

Treatment	Potato yield (Ton / ha)			
	First season		Second season	
	Yield	Increase (%)	Yield	Increase (%)
Sulfur	11.2±0.20 b	60.0	11.5±0.11 b	53.3
Thyme	9.4±0.23 c	34.3	10.0±0.11 c	33.3
Citral	9.6.4±0.11 c	37.1	10.5±0.20 c	33.3
Thyme + Sulfur	12.0±0.23 a	71.4	12.5±0.05 a	66.7
Citral + Sulfur	12.2±0.05 a	74.3	12.8±0.46 a	70.7
Redomil-plus	11.2±0.20 b	60.0	12.2±0.11 a	62.7
Control	07.0±0.23 d	0.0	07.5±0.17 d	0.0

Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

3.3.3. Effect on early blight incidence

Results in Table (3) reveal that all treatments suppressed the early blight incidence. The highest suppression of disease incidence was obtained with Thyme + Sulfur and Citral + Sulfur which reduced early blight incidence ranged between 69.2 and 76.9 %. Meanwhile, single treatments showed satisfactory impact.

3.3.4. Effect on potato yield

Results in Table (4) reveal that tested treatments increase the potato yield. The most elevated increment was acquired with combined treatments between Thyme + Sulfur and Citral + Sulfur which

increase potato yield ranged between 53.8 and 60.3 % respectively. Meanwhile, single treatments showed satisfactory impact.

Table 3: Early blight incidence of potato plants in response to sulfur and some essential oils alone or in combination during two summer cultivation seasons

Treatment	Early blight incidence			
	First season		Second season	
	Disease incidence	Efficacy %	Disease incidence	Efficacy %
Sulfur	1.0±0.11 bc	58.3	1.2±0.11 bc	53.8
Thyme	1.2±0.11 b	50.0	1.4±0.10 b	46.2
Citral	1.2±0.10 b	50.0	1.2±0.11 bc	53.8
Thyme + Sulfur	0.7±0.10 c	70.8	0.6±0.10 d	76.9
Citral + Sulfur	0.6±0.10 c	75.0	0.8±0.05 cd	69.2
Redomil-plus	0.7±0.10 c	70.8	1.0±0.11 c	61.5
Control	2.4±0.11 a	0.0	2.6±0.10 a	0.0

Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

Table 4: Potato yield in response to sulfur and some essential oils alone or in combination during two summer cultivation seasons

Treatment	Potato yield (Ton / ha)			
	First season		Second season	
	Yield	Increase (%)	Yield	Increase (%)
Sulfur	11.8±0.11 b	31.1	10.7±0.11 c	37.2
Thyme	11.2±0.11 b	24.4	10.0±0.20 c	28.2
Citral	11.2±0.11 b	24.4	11.0±0.11 bc	41.0
Thyme + Sulfur	14.0±0.57 a	55.6	12.0±0.10 ab	53.8
Citral + Sulfur	14.0±0.57 a	55.6	12.5±0.11 a	60.3
Redomil-plus	11.5±0.17 b	27.8	10.0±0.30 c	28.2
Control	09.0±0.30 d	0.0	07.8±0.08 d	0.0

Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

3.3.5. Effect on enzyme activities

Results in Table (5) reveal that tested treatments increased enzyme activities. The most elevated increment was acquired with combined treatments between Thyme + Sulfur and Citral + Sulfur which increase peroxidase, chitinase and β , 1-3- glucanase more than 300, 240 and 203.6 % respectively. Meanwhile, single treatments showed satisfactory impact.

Table 5: Enzyme activities of potato plants in response to sulfur and some essential oils alone or in combination

Treatment	Enzymatic activity					
	Peroxidase	Increase %	Chitinase	Increase %	β ,1-3 glucanase	Increase %
	Sulfur	1.0±0.10 b	100.0	2.3±0.10 b	130.0	6.3±0.10 b
Thyme	1.2±0.10 b	140.0	2.3±0.10 b	130.0	6.4±0.05 b	128.6
Citral	1.0±0.10 b	100.0	2.0±0.10 b	100.0	6.3±0.30 b	125.0
Thyme + Sulfur	2.0±0.20 a	300.0	3.4±0.10 a	240.0	8.5±0.10 a	203.6
Citral + Sulfur	2.1±0.05 a	320.0	3.6±0.10 a	260.0	8.7±0.10 a	210.7
Redomil-plus	1.1±0.05 b	120.0	2.5±0.30 b	140.0	5.5±0.00 c	96.4
Control	0.5±0.10 c	0.0	1.0±0.10 c	0.0	2.8±0.10 d	0.0

Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$.

4. Discussion

Potato is the most generally developed food crop on the world and has high dietary and monetary worth. Late and early blight has historically been an important disease of potato and tomato plants (Arora *et al.*, 2014; Saied *et al.*, 2016 and Abbas, 2018). The control of these diseases mainly depends

on chemical fungicides that face environmental problems. Therefore, there is an urgent need for safe alternative treatments to control plant diseases (Abd-El-Kareem and Haggag, 2015 Saied *et al.*, 2016 and Abd-El-Kareem, *et al.*, 2018).

Sulfur and sulfur compounds affect plant diseases directly as biocides and indirectly by enhancing plant resistance (Datnoff *et al.*, 2006). In the current research, data showed that under laboratory experiment, complete reduction of *A. solani* and *P. infestans* was obtained with Sulfur at 3.0 g/ L and Thyme as well as Citral at 6.0 mL/ L. While, in field trails all tested treatments suppressed the late and early blight incidence during two seasons. As for potato yield, results revealed that the high potato yield was obtained with combination between Thyme + Sulfur and Citral + Sulfur. In this respect, Ellis and Fern (1992) mentioned that sulfur inhibited fungal spore germinating and should be used before disease progression to get an effective result.

The direct toxicity of sulfur (S) and the circuitous debilitation of metals silicon should be recognized from supplement interceded opposition systems, which have been noticed for all essential miniature and macronutrients, Si and Al. Datnoff *et al.*, (2006) uncovered that the fungicidal impact of the components S applied to the leaves has been taken advantage of since the finish of the nineteenth century. Essential S has been utilized productively against diseases of grapes by fine buildup (*Uncinula necator*) ever since. The experience of Bourbos *et al.*, (2000) demonstrated that notwithstanding the immediate fungicide impact of S applied on the leaves, there was a supplement based impact of S applied in the dirt which brought about a lower contamination pace of grape leaves and delivered a lot better return contrasted with the controls. Schnug (1997) predicted that the decrease in atmospheric S deposition and thus the nutritional status of agricultural crops since the mid-1980s might have serious consequences for the stability of current agro-ecosystems, in particular, highlighted the increased susceptibility of plants to fungal pathogens such as light leaf spot (*Pyrenopezza brassicae*). Sulfur fertilization inhibited disease severity of several host/pathogen interaction by 5–50% and 17–35% in the plastic house or field trials, respectively. Sulfur can induce resistance (SIR) through enhancement of plants resistance against fungal infections which can triggered sulfur-involving metabolic processes through sulfate-based fertilizer strategies applied to the soil (Haneklaus *et al.*, 2006).

Thyme extract was most effective against the growth of *M. phaseolina* and other pathogenic fungi. The decrease in linear growth is more or less increased by increasing the concentration of plant products (El-Habbaa *et al.*, 2002). In the current study, the results indicated that the complete reduction of *A. solani* and *P. infestans* was obtained using thyme and citral at 6.0 ml/L while the results in field trails indicated that tested treatments suppressed the incidence of late and early blight and were obtaining an increase in potato yield with co-treatments of thyme + sulfur and citral + sulfur. In this regard, thyme oils showed suppression impact on food diseases caused by *A. niger* and *A. flavus* (Viuda *et al.*, 2007).

Thyme oil has antifungal impact on *Penicillium* and *alternaia* (Mironescua and Georgescub, 2008), *A. alternata* (Hadizadeh *et al.*, 2009). Hussein (2011) , showed that thyme had the highest effect in controlling peanut coagulation diseases and root rot caused by *Rhizoctonia solani*. Common thyme essential oil has shown complete inhibition of fungal growth for many plant pathogens (Soliman and Badea, 2002).

Furthermore, Bartyńska and Budzikur-Ramza (2001) described the high harmfulness of eucalyptus, lavender and rosemary oils against *Fusarium* spp. In actuality, in our examination, the contagious development of the inspected *Fusarium* species. Just thyme oil showed a measurably huge hindrance of parasitic development for all *Fusarium* species explored.

Sulfur (S) metabolites have been found to be resistant against fungal pathogens, total S, cysteine, glutathione, PR proteins, phytoaloxins and glucosinolates (Haneklaus *et al.*, 2006 b). The results in this study showed that all the tested treatments significantly increase enzyme activities, the most effective treatments are the combined treatments of thyme + sulfur and citral + sulfur that increase peroxidase, chitinase and β ,1-3-glucanase more than 300, 240 and 203.6% respectively. In this regard, (Blossom *et al.*, 2007) detailed that the impact of S treatment on chose S-containing metabolites that are related with the rise of opposition comparable to parasitic disease with *Pyrenopezza brassicae* was tried in long haul field explores different avenues regarding oilseed assault in northern Germany and Scotland S preparation essentially expanded the all out S, cysteine content, glutathione (GSH) and glucosinolates (GSL) in rapeseed sleek leaf tissues upon stem extension. Visible contaminations with *P. brassicae* brought about a huge expansion in cysteine content, with S preparation prompting an extra increment.

Since cysteine is a forerunner to any remaining S metabolites, this might be a significant key to SIR. Simultaneously, the substance of glutathione was fundamentally diminished in the influenced leaf tissues, since S-treatment debilitated this impact. Obviously disease related changes in GSH content are related with various stages in the host/microorganism relationship. Disease with *P. brassicae* expanded the movement of L-cysteine desulfhydrase (Bloem *et al.*, 2004).

Bloem *et al.*, (2007) detailed that it very well may be shown that S treatment brought about expanded outflow of hydrogen sulfide from rapeseed oilseeds in the field. In investigates grape plants during the underlying phase of disease by *Uncinula necator* when indications were not yet noticeable, the plants delivered raised degrees of H₂S; This impact has all the earmarks of being free of show S.

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