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IMPACT OF SOME PLANT OILS ON CONTROLLING SNAP BEAN POD MOLD INFECTIONS AND OXIDATIVE ENZYMES

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ABSTRACT

Botrytris cinerea, *Sclerotinia sclerotiorum* and *Pythium aphendermatum* were highly pathogenic on un-wounded and wounded snap bean pods of cv. Paulista under pathogenicity test. The *in vitro* and *in vivo* results indicated that all tested plant oils reduced the growth and they were effective in reducing the infection and disease severity percentages on detached pods inoculated individually with the three mold pathogens. Eugenol oil was the best one among all tested plant oils where all its tested concentrations inhibited the growth of all tested pathogens and prevented completely the infection on bean pods with any one of the tested pathogens. *S. sclerotiorum* was the most sensitive among all tested pathogens to all tested plant oils with their concentrations, while *B. cinerea* was the least sensitive one followed by *P. aphendermatum*. Also, increasing the concentration of tested plant oils from 0.1-5.0% increased gradually the effect of oils in reducing the growth of tested pathogens. On the other hand, the other tested plant oils differed in their effect in reducing the infection and disease severity % on inoculated pods with the tested pathogens. In this respect, thymol was effective in controlling the infection and disease severity on inoculated pods with *B. cinerea*. Meanwhile, thymol was less effective in controlling the infection with *P. aphendermatum* and *S. sclerotiorum*. Also, anisol, fennchone and camphore oils were moderately effective in controlling the infection of bean pods with *B. cinerea* and *P. aphendermatum*. Also, it was clear that all tested plant oils were effective in controlling bean pod mold infection under greenhouse conditions. Concerning the activities of peroxidase and polyphenoloxidase in inoculated and un-inoculated bean pods of cv. Paulista, the activities of both enzymes were increased in inoculated and un-inoculated bean pods with the tested mold pathogens. Also, the activities of peroxidase and polyphenoloxidase correlated with increasing the incubation periods from 4-13 days. Additionally, treating the inoculated and non-inoculated bean pods with some plant oils encouraged the increase in activities of peroxidase and polyphenoloxidase enzymes. In this respect, the highest activities of

peroxidase were recorded with eugenol oil treatment of inoculated bean pods with the three tested mold pathogens.

INTRODUCTION

Snap bean (*Phaseolus vulgaris L.)* belonging to the family *Fabaceae*, is considered one of the most important leguminous crops cultivated in Egypt where their seeds and pods are rich in calcium, some vitamins, proteins, mineral salts, some amino acids especially lysine and others. Recently, it's highly demanded for exporting to the European market **(HEIA, 2003)**. Snap bean pods could be attacked during storage, transmission, marketing or exporting with many of fungi such as, *Botrytis cinerea* (grey mold), *Pythium aphendermatum* (cottony mycelium) and *Sclerotinia sclerotiorum* (white soft rot), etc...**Snowdon (1992)** and **Suslow** and **Cantwell (1998)** where the previously mentioned fungi could be cause a great losses in quantity or quality of the snap bean pods. In this respect also, **Pearson** and **Hall (1973)** reported that *Pythium ultimum* and *Pythium aphendermatum* caused cottony rot disease of snap bean. **Maiti and Sahambi (1980)** mentioned that infection with *Pythium aphendermatum* on French bean appeared as very rapidly enlarging lesions, eventually girdling and rotting the pods without surface mycelium. **Cheah and Irving (1997)** isolated *Botrytis cinerea* from French beans, where it caused grey mold on pods. **Meinhardt *et al* (2002)** reported that *Sclerotinia sclerotiorum*, the causal agent of white mold of winter bean (*Phaseolus vulgaris*) caused great losses of bean production in Brazil. **Shah and Dillard (2007)** mentioned that *Sclerotinia sclerotiorum* caused the white mold of snap bean is one of the most destructive diseases of snap bean where it caused 2.2% infection to pods out of 40% to the whole bean plants. **Pranab *et al.* (2008)** stated that *Sclerotinia* rot caused by *Sclerotinia sclerotiorum* is the most serious disease on French beans (*Phaseolus vulgaris* L.).**Oliver *et al* (2008)** revealed that *Sclerotinia sclerotiorum* is the causal agent of the white mold of the common bean (*Phaseolus vulgaris* L.). Flowers are generally the first tissue to be colonized by *Sclerotinia sclerotiorum* in bean. **Ahmed (2010 b)** found that the six isolated fungi *i.e., Alternaria* spp., *Botrytis cinerea, Fusarium* spp., *Mucor* sp., *Penicillium* spp. and *Sclerotinia sclerotiorum* from beans cultivated in different locations in Egypt were able to infect pods of snap bean cv. Paulista. **Vieira *et al.*, (2010)** reported that white mold of common bean (*Phaseolus vulgaris*) caused by *Sclerotinia sclerotiorum*, is a major yield-limiting disease during the fall-winter season in Brazil.

Concerning the effect of plant oils in controlling bean pod mold infection, **Crisan *et al* (1978)** found that oils of *Anthum graveolens,*

Foeniculum vulgare, Pimpinella anisum and *Majorona hortensis* were particularly active in preventing the spore germination of *Sclerotinia sclerotiorum, Sclerotinia fucheliana* and *Sclerotinia fructigena.* While, **Fahmy (1994)** found that oils of caraway, cumin, and fennel completely inhibited the mycelial growth of *Rhizoctonia solani, Fusarium oxysporum* and *S. rolfsii* while coriander oil had no effect. **Smolinska and Kowalska (2006)** found that extracts of green parts of potato, tomato and rape were toxic especially of *Fusarium* sp., *B. cinerea* and *R. solani*, which infecting the French bean *[Phaseolus vulgaris]*.

As for the oxidative enzymes related to bean pod mould infection, **Reuveni *et al* (1992)** found that peroxidase activity in uninfected muskmelon plants was used to predict the resistance and susceptibility of 527 plants as cultivars or breeding lines and crosses of susceptible and resistant plants. **Ahmed (2001)** recorded that infection of soybean by *Colletoricum dematium* resulted in an increase in peroxidase and polyphenoloxidase activity during the examined periods in all tested cultivars compared with the healthy ones. The highest increase in the enzyme activity was recorded in the resistant and moderately susceptible cultivars compared with the susceptible one. **Ali *et al* (2003)** reported that oxidative enzymes activity provides a positive correlation between the pathogenicity of the tested fungi and inducing the infection of strawberry leaf spots. The higher production of such enzymes was induced in the leaf tissues of the resistant cultivar. **Hassan *et al* (2007)** revealed that, citric and benzoic acids were effective in decreasing the percentages of disease severity of *Botrytis fabae* and/or *B. cinerea* on faba bean plants and they gave the highest levels of peroxidase activities.

This study aimed to throw the light on role of natural plant oils in controlling the bean pod mold fungi and their effect the formatted oxidative enzymes which related to resistance and susceptibility of infection with mold fungi on bean pods.

MATERIALS & METHODS

- Source of isolates

Three different isolates i.e., *Botrytis cinerea* (isolated from Menofia), *Pythium aphendermatum* (isolated from Sharkia) and *Sclerotinia sclerotiorum* (isolated from Behira-) were tested for their pathogenic abilities on healthy snap bean pods of cv. Paulista and used in the further experiments in this study **(Ahmed, 2010)**.

- Pathogenicity tests:

Inoculum preparations:

For preparation of the standard inoculums, the pure fungal isolates of isolated fungi *Botrytis cinerea, Sclerotinia sclerotiorum and*

Pythium aphendermatum were grown separately at optimum temperature of each one of the isolated fungi for seven to ten days on PDA plates. As for isolated fungi forming mycelium without spores *Sclerotinia sclerotiorum*, the inoculum was prepared by brushing the surface of the culture in the presence of 10 ml sterilized water per each dish to become inoculum in form of mycelial suspension. While in case of fungi forming spores *Botrytis cinerea* and *Pythium aphendermatum* , the inoculum was prepared by brushing the surface of the culture in the presence of 10 ml sterilized water per each dish and then the spore suspensions were filtered through muslin. The concentration of spore suspension was adjusted to about $6x10^6$ spores/ml or cfu/ml of both type of tested fungi using a haemocytometer slide.

Healthy snap bean pods of cv., Pulista which obtained from Alayat (Giza) were divided into two groups, one group was scratched while, the other still without scratching then surface sterilized by dipping in 70% ethyl alcohol for one minute and left to dry at room temperature.

Green sterilized snap bean pods were sprayed with the prepared inoculum using an atomizer for each one of the tested fungi. Other sterilized snap bean pods were sprayed with sterilized distilled water as control treatment. All treatments were incubated in foam plates (12×21cm) each containing 9 pods. Three replicates were used for each treatment then all foam plates were kept at suitable temperature for seven to ten days post inoculation. After ten days, all treatments were examined and then the infection percentages as well as the disease severity percentages of inoculated pods were recorded according to **Spalding and Reeder (1974)** as follows:

$$\textbf{Infection percentage} = \frac{\text{Number of diseased pods}}{\text{Total number of the treatment}} \times 100$$

$$\textbf{Disease Severity \%} = \frac{\sum(n \times v)}{4N} \times 100$$

Where:

(n)= Number of infected pods in each category.
(v)= Numerical values of symptoms category.
(N)= Total number of pods.
(4)= Maximum of numerical values of symptoms categories

The four categories are represented as following:

1 = 1- 24% infection/pod
2 = 25- 49% infection/pod
3 = 50- 74% infection/pod
4 = 75- 100% infection/pod

- Effect of plant oils:

a- On the growth of tested mold pathogens under laboratory conditions:

In this experiment, several plant oils i.e., Thymol of thyme (*Thymus vulgaris*), Anisol of anise (*Pimpenella anisum*), Eugenol of clove (*Dianthus curyaphulus*), Camphor oil of camphor (*Eucalyptus citriodora*) and Fennchone of fennel (*Foeniculum vulgaris*) were obtained kindly from the Sector of Perfume and Additives, Hawamdia Sugar Company, Cairo, Egypt, to study their effect on bean pod mold fungi and disease development. Acetone was added to each one of the concentrated plant oils to increase their solubility. Different concentrations i.e., 0.1, 0.2, 0.5, 1.0, 2.5, and 5% were made and each one of the prepared concentrations was added to the melted PDA medium before pouring into Petri plates. Then the poured media were left to solidify. An equal disc (3mmΦ) of each one of the three tested pathogenic fungi *Botrytis cinerea, Sclerotinia sclerotiorum* and *Pythium aphendermatum* was added separately to the center of the Petri plates. Three plates were used for each treatment where, each treatment was replicated 3 times. Control treatment was in form of inoculated plates without adding any one of the tested plant oils. All inoculated plates were incubated at 20-22°C for 7-10 days then the plates were examined. The fungal growth was estimated for each one of the tested fungi and the growth reduction percentages were calculated using the formula suggested by **Fokemma(1973).**

$$\textbf{Reduction percentage} = \frac{de - di}{de} \times 100$$

Where:

de = Mean diameter of growth in control.
di = Mean diameter of growth in treatment.

b-On the bean pods under laboratory conditions:

Healthy snap bean pods (cv.Paulista) obtained from Giza growing's were surface sterilized in 70% ethanol for 1 min., then they were inoculated by each one of the three mentioned fungi. After 24 hours, the inoculated pods were swapped in 5% conc., of each one of the tested oils, i.e., Thymol, Anisol, Eugenol, Camphor, and Fennchone. Control treatment was in form of inoculated pods without oils treating.

All the previous treatments were replicated three times where each treatment contained 27 pods, preserved in foam plates and incubated at 20-22°C for 7-10 days. Disease severity percentage, infection percentage and efficacy percentages were calculated as mentioned before.

- Oxidative enzymes:

a-Determination of oxidative enzymes produced by the three tested pathogens under laboratory conditions:

Czapek's liquid medium containing (20g Sodium nitrite, 1.0 Dipotassium phosphate, 0.5g Magnesium sulphate, 0.01g Ferrous sulphate, 0.5 Potasium chloride, 30g sucrose dissolved in 1000 ml of sterile distilled water) dispensed in 250 cc conical flasks, 100 ml each autoclaved, inoculated and incubated with the three pathogenic fungi *Botrytis cinerea, Sclerotinia sclerotiorum* and *Pythium aphendermatum* at 22°C for 4, 6 and 13 days. Three flasks were used for each particular treatment. After a known incubation period, the cultures were filtrated through filter paper then centrifuged at 3000 rpm for 20 minutes. The clear supernatant culture filtrates (in vitro crude enzymes) were used for determining activities of peroxidase and polyphenoloxidase (PPO).

Peroxidase activity:

Peroxidase assay (based on oxidation of pyrogallol to purpurogallin in the presence of H_2O_2) was determined according to the method described by **Allan and Hollis (1972)**. The reaction mixture contains 0.3 ml of the in vitro crude enzyme + 0.5 ml phosphate buffer solution (pH 7) + 0.3 ml pyrogallol + 1 ml H_2O_2 . The mixture was completed with distilled water up to 3 ml. Peroxidase was expressed as the change in the absorbance of the mixture every 0.5 minute for 5 minutes period at 425 nm by Spectrophotometer (Spectronic 601 MiltonROY).

Polyphenoloxidase activity:

The activity of polyphenoloxidase was measured as mentioned by **Matta and Dimond (1963)**. The reaction mixture contains 1ml of the in vitro crude enzyme + 1ml phosphate buffer solution (7.1 pH) + 1ml catechol and completed with distilled water to 6.0 ml. Polyphnoloxidase was expressed as the change in the absorbance of the mixture every 0.5 minute for 5 minutes period at 495 nm by Spectrophotometer (Spectronic 601 MiltonROY.

b-Determination of oxidative enzymes produced by three tested pathogens on the bean pods under laboratory conditions:

Natural and artificial inoculated snap bean pods with the three pathogenic fungi were used to determine the activities of the oxidative enzymes produced under the in vivo conditions at 4, 6 and 13 days post inoculation. In this respect, 50g of each treatment were blended with 100

ml phosphate buffer solution (7.1 pH) then filtrated, then centrifuged at 3000 rpm for 20 minutes. Clear supernatants were used as crude enzymes to estimate the activities of peroxidase and polyphenoloxidase as mentioned in the previous test.

c- Determination the oxidative enzymes in treated bean pods with plant oils under laboratory conditions:

In this experiment, the tested oils i.e., Thymol, Anisol, Eugenol, Camphor and Fennchone were used at the best effective conc. 5% to evaluate their effect in controlling the snap bean pod rots in vivo. In this respect, healthy snap bean pods (cv. Paulista) were surface sterilized in 70% ethanol for 1 min., then they were swapped in 5% conc., of each one of the tested oils. After 24 hours of treatment, the pods were inoculated by each one of the three mentioned fungi as mentioned before and incubated in foam plates (12x21cm) then incubated at 20-22 °C for seven to ten days. Control treatment was in form of inoculated pods without oils treating and another control treatment was treated with water only. All treatments were replicated 3 times where each replicate contained 9 pods. Disease severity percentages and infection percentages were recorded as mentioned before. After 13 days post inoculation, the oxidative enzymes were determined in infected tissues as mentioned before.

RESULTS

- Pathogenicity test of isolated fungi from naturally infected snap bean pods:

Data in **Table (1)** indicate that *Sclerotinia sclerotiorum* gave the highest infection and disease severity percentages where it recorded 93.0 and 75.0%, respectively on wounded bean pods followed by *Botrytris cinerea* isolate which recorded 85.1% and 72.2% of both infection and disease severity percentages respectively and *Pythium aphendermatum* which gave 78.0% and 67.0% of both infection and disease severity percentages, respectively.

Table (1): Pathogenicity test of the isolated fungi on wounded and un-wounded bean pods (cv. Paulista) incubated at 20°C for 7–10 days.

Tested fungi	Source of isolates	Wounded		Un-wounded	
		In. %	DS %	In. %	DS %
Botrytris cinerea	Menofia	85.1	72.2	52.0	34.3
Pythium aphendermatum	Sharkia	78.0	67.0	48.1	29.0
Sclerotinia sclerotiorum	Behira	93.0	75.0	52.0	33.3

On the other hand, *Botrytris cinerea* gave the highest infection and disease severity percentages where it recorded 52.0% and 34.3% of both, respectively followed by *Sclerotinia sclerotiorum* which recorded 52.0% and 33.3% of both infection and disease severity percentages. While, *Pythium aphendermatum* gave 48.1% and 29.0% of determined infection and disease severity percentages on un-wounded snap bean pods.

- Effect of plant oils

a- Effect of plant oils on the growth of tested pathogens fungi under laboratory conditions:

Data in **Table (2)** indicate that all tested plant oils were able to reduce the growth of selected pod mold pathogens i.e., *B. cinerea*, *P. aphendermatum* and *S. sclerotiorum*. In this respect, eugenol was the best one among all tested plant oils where all its tested concentrations reduced the growth of all tested pathogens to 100%. It was clear from the obtained results that *S. sclerotiorum* was the most sensitive among all tested pathogens to all tested plant oils with their concentrations while *B. cinerea* was the least sensitive one followed by *P. aphendermatum*. Also, it was clear that increasing the concentration of tested plant oils from 0.1-5.0% increased gradually the effect of oils in reducing the growth of tested pathogens except eugenol where it tested concentrations were highly effective. It was pronounced from results that the best reduction% in growth of the tested pathogens was obtained with the concentrations i.e., 2.5 and 5.0%. in case of thymol, anisol, camphor and Fennchone.

b- Effect of some plant oils on bean pod mold infection on detached pods under laboratory conditions:

Data in **Table (3)** illustrate that tested plant oils were effective in reducing the infection and disease severity percentages on detached pods inoculated individually with the three mold pathogens. The results cleared that eugenol treatment was the best where it prevented completely the infection on bean pods with any one of the tested pathogens. In the same time, the efficacy of using eugenol oil in treating bean pods to control the infection with mold pathogens as 100%. On the other hand, the other tested plant oils were differed in their effect in reducing the infection and disease severity % on inoculated pods with the tested pathogens. In this respect, thymol was effective in controlling the infection and disease severity on inoculated pods with *B. cinerea* where the obtained efficacy% were 76.2% and 85.5% respectively. Meanwhile, thymol was less effective in controlling the infection with *P.*

aphendermatum and *S. sclerotiorum* but better than the control treatment. Also, anisol, fennchone and camphore were moderately effective in controlling the infection of bean pods with *B. cinerea* and *P. aphendermatum*. It is clear from the obtained results that fennchone was equal in its effect on *S. sclerotiorum* pod infection with eugenol oil treatment where its efficacy % was 100% followed by camphor which recorded 3.7% of infection and 1.9% of disease severity with efficacy% 94.1 and 96.8% respectively.

Table (2): Effect of plant oils on the growth of tested pathogens fungi under laboratory conditions:

Plant Oils	Conc. (%)	%Reduction in growth of the tested mold fungi		
		B. cinerea	*P. aphendermatum*	*S. sclerotiorum*
Thymol	0.1	0.0	0.0	10.0
	0.2	1.1	2.2	33.3
	0.5	12.2	7.8	44.4
	1.0	16.7	25.6	88.9
	2.5	18.9	66.7	88.9
	5.0	26.7	70.0	88.9
Anisol	0.1	0.0	0.0	16.7
	0.2	0.0	2.2	35.6
	0.5	0.0	16.7	55.6
	1.0	2.2	37.8	76.7
	2.5	30.0	38.9	77.8
	5.0	33.3	43.3	78.9
Eugenol	0.1	100	100	100
	0.2	100	100	100
	0.5	100	100	100
	1.0	100	100	100
	2.5	100	100	100
	5.0	100	100	100
Camphor	0.1	0.0	0.0	0.0
	0.2	0.0	0.0	3.3
	0.5	0.0	0.0	17.8
	1.0	2.2	4.4	44.4
	2.5	32.2	35.6	63.3
	5.0	63.3	61.1	76.7
Fennchone	0.1	0.0	0.0	11.1
	0.2	0.0	0.0	13.3
	0.5	0.0	3.3	44.4
	1.0	0.0	11.1	72.2
	2.5	18.9	32.2	80.0
	5.0	31.1	40.0	80.0
Control		0.0	0.0	0.0
LSD at 5%				
P(plant oils)		0.08	0.08	0.08
C(concenteration)		0.11	0.10	0.10
P x C		0.25	0.22	0.23

Table (3): Effect of some plant oils on bean pod mold infection on detached pods under laboratory conditions.

Plant oils 5% conc.	Efficacy of plant oils on pod mold infection *in vivo*											
	B. cinerea				*P. aphendermatum*				*S. sclerotiorum*			
	In%	Eff.%	DS%	Eff.%	In%	Eff.%	DS%	Eff.%	In%	Eff.%	DS%	Eff.%
Thymol	15.0	76.2	8.3	85.5	37.0	33.5	27.8	45.4	40.7	35.4	31.0	47.7
Anisol	51.9	17.6	28.0	51.2	40.7	26.8	31.0	39.1	37.0	41.3	31.5	46.9
Eugenol	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Camphor	22.2	64.8	12.0	79.1	48.1	13.5	33.0	35.2	3.7	94.1	1.9	96.8
Fennchone	37.0	41.3	20.0	65.2	14.8	73.4	8.3	83.7	0.0	100.0	0.0	100.0
Control	63.0		57.4		55.6		50.9		63.0		59.3	

In% = infection% D.S% = disease severity% Eff% = efficacy%

LSD at 5%	In%: infection%	DS%:disease severity
Plant oils	4.70	3.30
Fungi	3.33	2.33
PxF	8.15	5.71

c- Effect of some plant oils on bean pod mold infection under greenhouse conditions.

Results in **Table (4)** clear that all tested plant oils were effective in controlling bean pod mold infection under greenhouse conditions. Meanwhile, eugenol oil was the best effective plant oil in controlling the bean mold infection under greenhouse conditions where its efficacy percentages were more than 90.0%. Also, all other tested plant oils were effective in controlling bean pod mold infection with the three tested pathogens when compared with the control treatment with significant differences between them.

Table (4): Effect of some plant oils on bean pod mold infection under greenhouse conditions

Plant oils 5% Conc.	Efficacy of plant oils on pod mold infection under greenhouse conditions											
	B. cinerea				*P. aphandermatum*				*S. sclerotiorum*			
	In%	Eff.%	DS%	Eff.%	In%	Eff.%	DS%	Eff.%	In%	Eff.%	DS%	Eff.%
Thymol	15.0	71.2	8.3	76.6	15.0	70.0	8.3	74.3	15.0	72.3	8.3	77.3
Anisol	14.8	71.5	8.3	76.6	22.2	55.6	12.0	62.8	22.2	59.0	12.0	67.1
Eugenol	3.7	92.9	1.9	94.6	3.7	92.6	1.9	94.1	3.7	93.2	1.9	94.8
Camphor	7.4	85.8	4.6	87.0	15.0	70.0	8.3	74.3	15.0	72.3	8.3	77.3
Fennchone	14.8	71.5	8.3	76.6	15.0	70.0	8.3	74.3	14.8	72.7	8.3	77.3
Control	52.0		35.4		50.0		32.3		54.2		36.5	

In% = infection% DS% = disease severity% Eff% = efficacy%

LSD at 5%	In%	DS%
Plant oils	2.80	2.27
Fungi	2.00	1.60
PxF	4.84	3.93

- Oxidative enzymes:

a- Activities of peroxidase and polyphenoloxidase in culture filtrate of the tested mold fungi:

Activities of peroxidase and polyphenoloxidase enzymes were determined in cultures filtrates of the tested mold fungi i.e., *B. cinerea, P. aphendermatum* and *S sclerotiorum* at different incubation period i.e., 4, 6 and 13 days. Data in **Table (5)** indicate that all tested mold fungi were able to produce peroxidase and polyphenoloxidase enzymes in their culture filtrates. It was clear from the obtained results that increasing the incubation periods of the tested fungi from 4-13 days increased gradually the activities of peroxidase and polyphenoloxidase enzymes. The highest activities of peroxidase enzyme were recorded in filtrates of *P. aphendermatum* at all tested incubation periods followed by *B. cinerea* filtrates at 13 days incubation period and *S. sclerotiorum* at the same time of incubation. On the other hand, the determined activities of polyphenoloxidase enzyme were very low in culture filtrates of the tested mold pathogens. The highest activities of polyphenoloxidase were recorded at 13 days of incubation with all tested mold pathogens.

Table (5): Peroxidase and polyphenoloxidase activities in culture filtrates of the tested mold fungi after 4, 6 and 13 days of incubation.

Tested Fungi	Incubation period (days)	Activity/minute as optical density	
		Peroxidase	Polyphenol oxidase
Botrytis cinerea	4	0.688	0.001
	6	0.699	0.009
	13	0.832	0.013
Pythium aphendermatum	4	0.877	0.009
	6	0.943	0.012
	13	1.110	0.012
Sclerotinia sclerotiorum	4	0.649	0.000
	6	0.753	0.001
	13	0.765	0.011

b- Activities of peroxidase and polyphenoloxidase in inoculated and un-inoculated bean pods of cv. Paulista under laboratory conditions

As shown in **Table (6)** it was clear from the obtained results that the activities of peroxidase and polyphenoloxidase enzymes were increased in inoculated and un-inoculated bean pods with the tested mold pathogens. It was clear also that activities of peroxidase and

polyphenoloxidase correlated with increasing the incubation periods from 4-13 days. The highest activities of peroxidase and polyphenoloxidase were recorded in extracts of inoculated bean pods with *S. sclerotiorum* at 13 days incubation period. The least activities of peroxidase and polyphenoloxidase were recorded in extracts of un-inoculated (naturally infected) bean pods at 4 days of incubation period. On the other hand, very interest to record that the determined activities of polyphenoloxidase in extracts of inoculated and un-inoculated bean pods at 6 and 13 days of incubation periods were greatly higher than those determined at 4 days.

Table (6): Peroxidase and polyphenoloxidase activities in inoculated and un-inoculated snap bean pods after 4, 6, and 13 days incubation

Treatments	Incubation period (days)	Activity / minute as optical density	
		Peroxidase	Polyphenol oxidase
Botrytis cinerea	4	1.19	0.230
	6	1.35	0.938
	13	1.35	0.957
Pythium aphendermatum	4	1.19	0.306
	6	1.20	1.625
	13	1.43	1.702
Sclerotinia sclerotiorum	4	1.17	0.095
	6	1.20	1.036
	13	1.52	1.811
Un-inoculated (Control)	4	1.17	0.093
	6	1.19	0.630
	13	1.40	0.559

c- Activities of peroxidase and polyphenoloxidase enzymes in inoculated bean pods treated with plant oils under laboratory conditions:

The obtained results in **Table (7)** reveal that treating the inoculated and non-inoculated bean pods with some plant oils encouraged the increase in activities of peroxidase and polyphenoloxidase enzymes. In this respect, the highest activities of peroxidase were recorded with eugenol oil treatment of inoculated bean pods with the three tested mold pathogens. It was clear from the obtained results that the activities of peroxidase in control-1 treatment (inoculated with mold pathogens without treating with any plant oils) were high comparing to the control-2 treatment and the other oil treatments. Also, the activities of peroxidase were high in treated pods with thyme and inoculated with *P. aphendermatum* and *S. sclerotiorum*, followed by pods treated with anisol oil and inoculated with *S. sclerotiorum* and *B.*

cinerea. On the other hand, the highest activities of polyphenoloxidase were recorded in pods treated with camphor, anisol and fennchone and inoculated with *P. aphendermatum* and *S. sclerotiorum* respectively. Also, the treated pods with thyme increased the activity of polyphenoloxidase when inoculated with *P. aphendermatum*. The least activity of polyphenoloxidase was recorded in control-2 treatment (un-treated and un-inoculated).

Table (7): Activities of peroxidase and polyphenoloxidase enzymes in inoculated bean pods treated with plant oils

plant oils 5% Conc.	Activities of oxidative enzymes in infected bean pods treated with plant oils					
	Peroxidase			Polyphenoloxidase		
	B. cinerea	*P. aphendermatum*	*S. sclertiorum*	*B. cinerea*	*P. aphendermatum*	*S. sclertiorum*
Thyme	0.324	2.365	2.001	0.090	0.298	0.059
Anisol	1.081	0.637	2.017	0.099	0.134	0.137
Eugenol	3.868	3.888	2.218	0.044	0.041	0.020
Camphor	0.607	0.648	0.914	0.047	0.285	0.322
Fennchone	0.713	1.194	0.843	0.024	0.130	0.130
Control-1	2.846	2.255	1.984	0.040	0.024	0.011
Control-2	0.979			0.002		

Control 1: without treatment.
Control 2: without pathogen (Natural infection).

DISCUSSION

Snap bean is considered one of the most important leguminous crops cultivated in Egypt where their seeds and pods are rich in calcium, some vitamins, proteins, mineral salts, some amino acids especially lysine and others. It's cultivated for use as green pods or dried seeds. Snap bean pods could be attacked during storage, transmission, marketing or exporting with many of fungi such as, *Botrytis cinerea* (grey mold), *Pythium aphendermatum* (cottony mycelium) and *Sclerotinia sclerotiorum* (white soft rot), etc...**Snowdon (1992)** and **Suslow** and **Cantwell (1998)** where the previously mentioned fungi could be cause a great losses in quantity or quality of the snap bean pods.

The isolated fungi from naturally infected snap bean pods were able to infect the wounded and un-wounded bean pods of cv. Paulista. *Sclerotinia sclerotiorum* gave the highest infection and disease severity percentages on wounded bean pods followed by *Botrytris cinerea* isolate and *Pythium aphendermatum*. On the other hand, *Botrytris cinerea* gave the highest infection and disease severity percentages on un-wounded snap bean pods followed by *Sclerotinia sclerotiorum* and *Pythium*

aphendermatum. These results are in harmony with those obtained by **Abd El- Moity, (1976)** who stated that *Botrytis cinerea* was obviously pathogenic causing fruit rots on beans and **Stojanovski (1986)** who, reported that infection of bean with *Sclerotinia sclerotiorum* occurs only through direct hyphal growth from infected tissues. Epidemics are initiated by mycelium and ascospores produced by *Sclerotinia*. Also, **Shah and Dillard (2007)** mentioned that *Sclerotinia sclerotiorum* caused the white mold of snap bean is one of the most destructive diseases of snap bean where it caused 2.2% infection to pods out of 40% to the whole bean plants. Similar results were obtained also by **Ahmed (2010** a) who, found that the six isolated fungi *i.e., Alternaria* spp., *Botrytis cinerea, Fusarium* spp., *Mucor* sp., *Penicillium* spp. and *Sclerotinia sclerotiorum* from beans cultivated in different locations in Egypt were able to infect pods of snap bean cv. Paulista. Also, **Vieira *et al* (2010)** reported that white mold of common bean (*Phaseolus vulgaris*) caused by *Sclerotinia sclerotiorum*, is a major yield-limiting disease during the fall-winter season in Brazil.

As for the effect of plant oils, the *in vitro* and *in vivo* results indicated that all tested plant oils reduced the growth of selected pod mold pathogens i.e., *B. cinerea*, *P. aphendermatum* and *S. sclerotiorum* as well as, they were effective in reducing the infection and disease severity percentages on detached pods inoculated individually with the three mold pathogens. Eugenol oil was the best one among all tested plant oils where all its tested concentrations inhibited the growth of all tested pathogens and prevented completely the infection on bean pods with any one of the tested pathogens and consequently, the disease severity. It was clear from the obtained results that *S. sclerotiorum* was the most sensitive among all tested pathogens to all tested plant oils with their concentrations while *B. cinerea* was the least sensitive one followed by *P. aphendermatum*. Also, it was clear that increasing the concentration of tested plant oils from 0.1-5.0% increased gradually the effect of oils in reducing the growth of tested pathogens except eugenol where its tested concentrations were highly effective. On the other hand, the other tested plant oils differed in their effect in reducing the infection and disease severity % on inoculated pods with the tested pathogens. In this respect, thymol was effective in controlling the infection and disease severity on inoculated pods with *B. cinerea*. Meanwhile, thymol was less effective in controlling the infection with *P. aphendermatum* and *S.*

sclerotiorum but better than the control treatment. Also, anisol, fennchone and camphore oils were moderately effective in controlling the infection of bean pods with *B. cinerea* and *P. aphendermatum*. Also, it was clear that all tested plant oils were effective in controlling bean pod mold infection under greenhouse conditions. However, eugenol oil was the best effective plant oil in controlling the bean mold infection under greenhouse conditions. The obtained results are in agreement to somewhat with those obtained by **Crisan *et al* (1978)** who mentioned that oils from *Anthum graveolens, Foeniculum vulgare, Pimpinella anisum* and *Majorona hortensis* were particularly active against *S. sclerotiorum, S. fucheliana* and *S. fructigena*. Similar results were obtained by **Fahmy (1994)** who found that oils of caraway, cumin and fennel completely inhibited the mycelial growth of *Rhizoctonia solani, Fusarium oxysporum* and *S. rolfsii*. Also, **Smolinska and Kowalska (2006)** found that the most toxic extracts in controlling *Botrytis cinerea, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Fusarium* sp., which infecting the French bean [*Phaseolus vulgaris*] were obtained from green parts of potato, tomato and rape.

As for the oxidative enzymes, all tested mold fungi were able to produce peroxidase and polyphenoloxidase enzymes in their culture filtrates. Also, increasing the incubation periods of the tested fungi from 4-13 days increased gradually the activities of peroxidase and polyphenoloxidase enzymes. Concerning the activities of peroxidase and polyphenoloxidase in inoculated and un-inoculated bean pods of cv. Paulista, the activities of both enzymes were increased in inoculated and un-inoculated bean pods with the tested mold pathogens. Also, the activities of peroxidase and polyphenoloxidase correlated with increasing the incubation periods from 4-13 days. Additionally, treating the inoculated and non-inoculated bean pods with some plant oils encouraged the increase in activities of peroxidase and polyphenoloxidase enzymes. In this respect, the highest activities of peroxidase were recorded with eugenol oil treatment of inoculated bean pods with the three tested mold pathogens. It was clear from the obtained results that the activities of peroxidase in control-1 treatment (inoculated with mold pathogens without treating with any plant oils) were high comparing to the control-2 treatment and the other oil treatments. Also, the activities of peroxidase were high in treated pods with thyme and inoculated with *P. aphendermatum* and *S. sclerotiorum*, followed by

pods treated with anisol oil and inoculated with *S. sclerotiorum* and *B. cinerea*. On the other hand, the highest activities of polyphenoloxidase were recorded in pods treated with camphor, anisol and fennel and inoculated with *P. aphendermatum* and *S. sclerotiorum* respectively. The obtained results could be interpreting in light the findings of **Ahmed (2001)** who recorded that infection of soybean by *Colletoricum dematium* resulted in an increase in peroxidase and polyphenoloxidase activity during the examined periods in all tested cultivars compared with the healthy ones. The highest increase in the enzyme activity was recorded in the resistant and moderately susceptible cultivars compared with the susceptible one. However, **Ali *et al* (2003)** showed that determination of oxidative enzymes activity provides a positive correlation between the pathogenicity of the tested fungi and inducing the infection of strawberry leaf spots. The higher production of such enzymes was induced in the leaf tissues of the resistant cultivar. Also, similar results were obtained by **Hassan, *et al* (2007)** who revealed that, citric and benzoic acids were effective in decreasing the percentages of disease severity of *Botrytis fabae* and/or *B. cinerea* on faba bean plants and they gave the highest levels of peroxidase activities.

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تأثير بعض الزيوت النباتية علي مقاومة أصابات أعفان قرون الفاصوليا والانزيمات المؤكسدة

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أظهر إختبار القدرة المرضية لفطريات عفن قرون الفاصوليا أن الفطريات بوتريتس سينريا و سكليروتينيا سكليروشيوم و بيثيم أفندرماتم كانت عالية المرضية علي قرون الفاصوليا صنف بوليستا المجروحة والغير مجروحة. وقد أشارت نتائج الإختبارات المعملية والحيوية أن كل الزيوت النباتية المختبرة خفضت من نمو المسببات المرضية المختبرة لعفن القرون كما كانت فعالة أيضا في تخفيض النسب المئوية للعدوي والشدة المرضية علي القرون المعداة فرديا بفطريات العفن الثلاثة. وكان زيت (القرنفل)هو الأفضل من بين كل الزيوت المختبرة حيث ثبطت كل تركيزاته المختبرة نمو كل المسببات المرضية المختبرة ومنعت تماما الإصابة علي قرون الفاصوليا. وقد كان الفطر سكليروتينيا سكليروشيوم هو الأكثر حساسية من بين كل الفطريات المختبرة لكل الزيوت النباتية المختبرة بكل تركيزاتها بينما كان الفطر بوتريتس سينريا هو الأقل حساسية متبوعا بالفطر بيثيم أفندرماتم. كما أدت زيادة تركيز الزيوت النباتية من ٠ر١ – ٥ر٠ % إلي زيادة تأثيرها تدريجيا علي تخفيض النمو والإصابة بالمسببات المرضية. وعلي الجانب الآخر فقد تباينت بعض الزيوت النباتية المختبرة في تأثيراتها علي تخفيض العدوي والشدة المرضية حيث كان زيت (الزعتر)هو الأكثر فعالية في التحكم بالإصابة والشدة المرضية علي القرون المعداة بفطر بوتريتس سينريا وفي نفس الوقت كان الزعتر هو الأقل فعالية في مقاومة الإصابة بفطريات سكليروتينيا سكليروشيوم و بيثيم أفندرماتم. أيضا كانت زيوت اليانسون والشمر والكافور متوسطة التأثير في مقاومة الإصابة بعفن قرون الفاصوليا الناتج عن الإصابة بفطريات بوتريتس سينريا و بيثيم أفندرماتم. وقد كان واضحا أيضا أن كل الزيوت النباتية المختبرة كانت فعالة في مقاومة عفن قرون الفاصوليا تحت ظروف الصوبة. وفيما يتعلق بنشاطات إنزيمي البيروكسيديز والبولي فينول أوكسيديز في قرون الفاصوليا (صنف بوليستا) الملقحة وغير الملقحة بفطريات عفن القرون المختبرة ، فقد تزايدت نشاطات كلا الإنزيمين في قرون الفاصوليا الملقحة وغير الملقحة. وقد أدت زيادة فترة التحضين من ٤–١٣ يوم إلي زيادة نشاط إنزيمي البيروكسيديز والبولي فينول أوكسيديز، بالإضافة إلي ذلك ، فقد أدت معاملة قرون الفاصوليا الملقحة وغير الملقحة بفطريات العفن المختبرة ببعض الزيوت النباتية إلي تشجيع زيادة نشاط إنزيمي البيروكسيديز والبولي فينول أوكسيديز حيث سجل أعلي نشاط لإنزيم البيروكسيديز مع المعاملة بزيت القرنفل لقرون الفاصوليا الملقحة بفطريات العفن الثلاثة المختبرة.