Journal

J. Biol. Chem. Environ. Sci., 2014, Vol. 9(4): 467- 487 www.acepsag. org

POTENTIAL EFFECT OF PLANT ESSENTIAL OILS AS ANTIFUNGAL ACTIVITY AGAINST POSTHARVEST DECAY OF SNAP BEAN PODS.

***Abdel-Mageed, M.H.; *Mohamed, F. G.; **Soltan, H.H.; *Hafez, M.A. and **Abdel-Rahman, F.A.**

*Department of Plant Pathology, Fac. Agric. Moshtohor, Benha University, Egypt. ** Institute of Plant Pathology Res. Agric. Res. Center Giza, Egypt.*

ABSTRACT

The present investigation aims to evaluate the antifungal effects of the plant essential oils against major postharvest pathogens (*i.e*. *Botrytis cinerea* and *Pythium aphanidermatum*) cause decay of Snap bean pods (cv. Xera and Valentino). Three essential oils obtained from Camphor, Cinnamon and Carnation were tested at concentrations 0.25, 0.5 and 1%(v/v). The results of *in vitro* experiment indicated complete inhibition of *B. cinerea* and *P. aphanidermatum* at all concentrations of Cinnamon and Carnation tested. Under field conditions, pre-sprayed Snap bean plants with Cinnamon and Camphor treatments at 1% gave the highest levels of controlling decay of bean pods (cv. Xera) by *B. cinerea*. The same effects were given on pods of Valentino cv. at concentration 0.5% and 1% against the tested pathogen *B. cinerea*. Vaporization of Snap bean pods during storage with Carnation at 100 µL was the best treatment as suppressed completely the disease caused by the two tested mold pathogens (*B. cinerea* and *P. aphanidermatum*), while the same potential effectiveness was obtained on Valentino cv. using Camphor oil at 100 µL with both tested pathogens. Peroxidase and polyphenoloxidase activities were increased at 3 days and 7 days in bean pods taken from plants sprayed with Carnation or Camphor oils comparing with the control. This work might highlights the potential for using essential oils as vaporization for postharvest disease control of Snap bean pods, which was the main objectives of this study.

Keywords: Bean, *B. cinerea, P. aphanidermatum,* polyphenoloxidase, peroxides, plant essential oils.

INTRODUCTION

Bean *(Phaseolus vulgaris* L.) is one of the most important economic vegetable crop in Egypt for both local consumption and exportation for Europe and other countries. Expansion in green bean cultivation has exhibited impressive growth in Egypt during the past several years with a cultivated area of 2.4% of total world cultivated area of bean. Recently, snap bean production of Egypt was increased and reached 251279 ton from 57873 feddan with average production of 4.342 ton/feddan through 2012 season, **Statistics Data, (Min. Agric. ARE, 2012**). The Egyptian annual growth in the production of green beans represents half of the world's total growth **Wijnands (2004).**

Snap bean pods could be attacked during storage, transmission, marketing or exporting with many of fungi such as, *Botrytis cinerea*, *Pythium aphanidermatum* and *Sclerotinia sclerotiorum* which could cause great losses in quantity or quality of the snap bean pods **Snowdon (1992) and Suslow and Cantwell (1998).**

Currently such decay is controlled by pre- and postharvest fungicidal application. Meanwhile, a growing public demand for food without chemical residues. As a result several efforts focus on the reduction of fungicidal application by using new natural antimicrobials including the essential oils. Essential oils of seven Moroccan Labiatae were chemically analyzed and evaluated for their *in vitro* antifungal activity against *Botrytis cinerea* **(Chebli *et al.*, 2003).** Among them, *Origanum compactum* and *Thymus glandulosus* greatly inhibited the mycelial growth of *Botrytis cinerea.* **Soylu *et al.* (2010)** investigated antifungal activities of essential oils obtained from aerial parts of aromatic plants, such as origanum (*Origanum syriacum* L. var. Bevanii), lavender *(Lavandula stoechas* L. var. stoechas) and rosemary (*Rosmarinus officinalis* L.), against *Botrytis cinerea,* which was completely inhibited *in vitro* by essential oil of lavender and rosemary.

The aim of this work was to study the effect of pre and post- harvest treatments of snap bean pods with some plant essential oils *i.e.* Camphor, Cinnamon and Carnation in protecting pods from postharvest rots caused by *B. cinerea* and *Pythium aphanidermatum.*

MATERIALS AND METHODS

Source of plant oils:-

Camphor (*Eucalyptus glabulus*), Cinnamon (*Cinnamon zylanicum*) and Carnation (*Dianthus caryophyllus*) oils were obtained from Cairo Company for oils, Cairo Egypt.

Source of fungal bean pods mold pathogens:

Pathogenic isolates of *Botrytis cinerea* and *Pythium aphanidermatum* which previously isolated from diseased snap bean pods with gray mold and Pythium rot, were taken from Postharvest Diseases Department, Plant Pathology Research Institute-Agriculture Research Center (ARC) Giza, Egypt, where this investigations were carried out.

***In vitro* experiments:-**

Effect of natural essential oils on linear growth of *B. cinerea* and *Pythium aphanidermatum*:-

Three natural oils, *i.e.* Camphor, Cinnamon and Carnation were evaluated for their capability to suppress fungal growth of *B. cinerea* and *P. aphanidermatum* in *vitro.* Each oil was embedded in PDA medium at a concentration of 0.25, 0.5 and 1.0% with 0.5% Tween-20. Treated or not-treated medium with oils were poured into 3 Petri dishes per each treatment. After medium solidification, Petri dishes were inoculated with 3-mm discs of 7-day-old culture of *B. cinerea* and *P. aphanidermatum* and incubated at 20+2^{o}C for 5 days. The diameter of developed colonies were measured when fungus mycelium covered one plate in control treatment and the percentage of reduction in the colony diameter was calculated using the formula suggested by **Fokemma (1973)** as follows:

$$\text{Reduction percentage} = \frac{(de - d_i)}{de} \times 100$$

Where:

de = maximum linear growth in control set.

di = maximum linear growth in treatment set.

Efficacy of pre harvest treatments with essential oils under field conditions:-

Under field conditions for the two successive seasons 2011 and 2012, the effect of spraying treatment of snap bean (pre-harvest) with plant oils for controlling rot diseases during harvesting and storage was studied. Three plant oils, Camphor, Cinnamon and Carnation) at rate of 0.5 and 1% were tested. This experiment was carried out at El-Qanater in Qaluobia governorate. Each Plot consisted of three rows, each row 3x0.7 m which is considered as an experiment unit. Snap bean plants (Xera and Valentino cvs.) were sprayed three times, the $_1$st at bloom stage, the 2nd after ten days from the first spray and the 3rd one sprayed before harvest 5 days. As well as plots receiving no plant oils were used as control. After harvest, fresh

Sample of snap bean pods Xera and Valentino cvs. were divided into two parts first sterilized bean pods were inoculated with the prepared spore suspension of *B. cinerea* and *P. aphanidermatum* which prepared by brushing the surface of the culture in the presence of 10 ml of sterilized water per 9-cm petri-plate. The used concentration of spore suspension was adjusted to about $4x10^5$ spores/ml using a haemocytometer. Second, snap bean pods were left without sterilization (as natural infection). Three replicates were used for each treatment (25 pods for each) and packed in polyethylene bags. Natural and artificial inoculated snap bean incubated at 27±1°C and 90 - 95% RH for 15 days the disease severity percentages of infected pods were recorded according to **Spalding and Reeder (1974)** as follows:

$$\% \text{ Infection} = \frac{\text{Number of diseased pods}}{\text{Total number of the treatment}} \text{ X } 100$$

$$\% \text{ Disease Severity} = \frac{\sum (n \text{ x } v)}{4N} \text{ X } 100$$

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, 2 = 25- 49% infection, 3 =50- 74% infection. and 4 =75- 100% infection.

Effects of vaporization of plant oils under modified atmospheric packaging (MAP) on controlling snap bean pods rot during storage:-

Snap bean pods Xera and Valentino cvs. were hand-harvested at mature stage in the last week of October 2012 from El-Qanater, Qaluobia Governorate, and transported within 2 h to the laboratory. The pods were selected for uniform size, color, and absence of defects.

Three natural oils, *i.e.* Camphor, Cinnamon and Carnation each at 50 and 100 µL air vapor, were tested for controlling snap bean pods rot caused by the two tested pathogens. Such treatments subjected for natural and artificial infection. Fresh samples of snap bean pods were washed thoroughly with tap water, sterilized in 70% ethanol for one minute, and a fresh sample of snap bean pods were used without sterilization as natural infection then left to dry at sterilized room temperature condition (25-30°C). Sterilized bean pods were inoculated with spore suspension of *B. cinerea* and *P. aphanidermatum* with $4x10^5$ spores/ml. After 24 hours from incubation **Naffa and Rabie (2006).** Artificial and natural inoculated snap bean pods were vaporized separately with different concentration of plant oils treatment. The artificial and natural inoculated pods were put in Xtend® Easy-Tear bags (StePac Ltd., Israel) (MAP), while control treatment was vapored by air and put in polyethylene bags, and other group from control (inoculated - un-treated) of snap bean pods were put in Xtend® (MAP treatment).

Three replicates were used for each treatment each containing 27 pods. They were placed in 10 L glass jars, plant oils vapor were introduced through pipe lines in and out and each jar along vaporized was sealed with plastic lid **(Fig.1).** But control treatments of snap bean pods were vapor with only air. Plant oils concentrations were vaporized utilizing a breathing pump (Emed) (Model: A1000230 v- 50 Hz 90va Manufacturer: Elettroplastica SpA Via Del Commercio, Travagliato (BS), Italy). Plant oils and control vapor at one time. Severity of infection and disease percentage were recorded as mentioned before.

Enzymatic studies:

Detection of oxidative enzymes after treatments:-

Natural and artificial inoculated snap bean pods with tested fungi were used to determine the activities of the oxidative enzymes, at 3 and 7 days post inoculation. In this respect, 50g of fresh pods of each treatment were blended with 100 ml phosphate buffer solution (7.1 pH) then centrifuged at 3000 rpm for 20 minutes. Clear supernatants were used as crude enzymes to determine the activities of peroxidase and polyphenoloxidase.

Fig. 1: Glass jars of three natural oils, *i.e.* Camphor, Cinnamon and Carnation each at 50 and 100 µL air vapor, and pipe line.

a-Peroxidase activity:

Peroxidase assay 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 Milton ROY)

b-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 Milton ROY).

Statistical analysis

All data obtained, were analyzed using Analysis of Variance (ANOVA) for unequal sample sizes and means were separated by Least Significant Differences (LSD) at $p < 0.05$ as described by **Song and Keane (2006).**

RESULTS AND DISCUSSION

Antifungal effects of the plant essential oils on linear growth of *B. cinerea* and *P. aphanidermatum* (*in vitro*):

Data in **Table (1)** indicate that all tested plant oils were able to reduce the mycelial growth of tested pathogens *i.e*., *B. cinerea*, and *P. aphanidermatum*. In this respect, Carnation and Cinnamon were the best as all its tested concentrations reduced the growth of all tested pathogens to 100%. While, Camphor was less effective on the growth of the two pathogenic fungi at all different concentrations, it was clear that increasing the concentration of Camphor oil from 0.25-1% increased gradually the effect of oil in reducing the growth of tested pathogens. The obtained results are in agreement with those obtained by **Rushed (2001)** who found that Camphor, Carnation and Cinnamon oils proved good suppression on *B. cinerea* growth *in vitro*. **Sirirat *et al.* (2009)** reported that vapors of clove oil, Cinnamon oil and lemongrass oil exhibited high inhibitory effects on *B. cinerea*, Cinnamon oil and lemongrass oil all exhibited fungicidal effect on the pathogen, Meanwhile, **Samane and Mohammad (2012)** monition that four essential oils *i.e*., anise, ammi, ziziphora and cinnamon at concentrations (200, 400, 600 and 800 $pL.L^{-1}$) inhibited the growth of *B. cinerea*. On the other hand, **Abdel-Kader *et al* (2012)** found that Cinnamon, clove and thyme essential oils have been found to have inhibitory effects against the mycelia growth of tested fungi *in vitro*. Similar results were obtained by **Riad *et al* (2013)** reported that the inhibitor activity against mycelial growth of *Pythium* sp. was observed at all concentrations of lemongrass, thyme, citral and nerol oils. The Mycelial growth decreased significantly with the increase in concentrations of essential oils and reached minimum mycelia growth at the highest concentration used.

Effect of prehravest treatment with plant oils on rots of snap bean pods caused by *B. cinerea* and *P. aphanidermatum* during cold storage.

Spraying solution of cinnamon, carnation and camphor oils on snap bean plant Xera and Valentino cvs. with three times before harvest at concentrations of 0.5% and 1.0% resulted in a comparable control of *B. cinerea* during cold storage for 15 days. No significant differences were obtained between both tested concentrations of each kind of oils, where oils at 1.0% concentration achieved more control than 0.5%. All tested oils achieved a good control on the two mold pathogens on snap bean pods Xera and Valentino cvs. However, cinnamon, carnation and camphor oils at a concentration of 1.0% highly suppressed *B. cinerea* and *P.*

aphanidermatum development in either naturally infected or artificially inoculated snap bean pods during seasons 2011 and 2012.

Table (1): Antifungal effect of certain plant oils concentrations on the linear growth of pathogenic fungi.

Treatments	Cone. (%)	*Botrytis cinerea*		*Pythium aphanidermatum*	
		Growth (mm)	Efficacy (%)	Growth (mm)	Efficacy (%)
Carnation	0.25	0.00	100	0.00	100
	0,5	0.00	100	0.00	100
	1	0.00	100	0.00	100
Cinnamon	0.25	0.00	100	0.00	100
	0.5	0.00	100	0.00	100
	1	0.00	100	0.00	100
Camphor	0.25	90.00	0,00	80.00	11.11
	0.5	81,66	8,34	71.66	20.38
	1	78.33	12.97	63.33	29.63
Control		90.00		90.00	
LSD at 0,05		0.40		0,85	

Data in **Table 2 and 3.** illustrate that, Carnation oil at both concentration (0.5 and 1.0%) completely inhibited the infection on bean pods with *B. cinerea* with efficacy being 100%, but Carnation oil at 1% completely inhibited the infection on bean pods Xera cv. infected with *P. aphanidermatum* and natural infection with efficacy being 100% in two seasons.

Also, the results cleared that Cinnamon treatment at 1% were the best where they prevented completely the infection on bean pods with *B. cinerea* and consequently, the disease severity was 0.0% during season 2011 and season 2012, at the same time, the efficacy of using Camphor oil at 1% in treating bean pods to control the infection with *B. cinerea* as 100% during season 2011 and 96.27% as disease severity during season 2012, but its effect on infected bean pods with *P. aphanidermatum* were more in reducing the infection and disease severity % where their values were 9.33-12.00 infection and 3.66-3.00% disease severity during season 2011 and season 2012, respectively.

Table (2): Comparative effectiveness of plant oils spray as a pre-harvest bean treatments on *B. cinerea*. *Pythium aphanidermatum* and natural infection 2011.

Treatment (T)	Conc. C%	CV. Xera												CV. Valentino											
		B. cinerea				*P. aphanidermatum*				Natural infection				*B. cinerea*				*P. aphanidermatum*				Natural infection			
		DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF
Carnation	0.5	00	100	00	100	13.33	86.67	3.33	95.27	4.00	90.91	2.00	91.42	22.66	77.34	9.66	86.77	36.00	50.91	15.66	70.45	00	100	00	100
	1	00	100	00	100	00	100	00	100	00	100	00	100	00	100	00	100	5.33	92.73	1.33	97.58	00	100	00	100
Cinnamon	0.5	5.33	94.67	1.33	98.25	3.66	96.34	7.66	89.11	00	100	00	100	00	100	00	100	46.00	37.30	19.66	62.91	6.66	79.18	1.66	90.94
	1	00	100	00	100	1.33	98.67	1.33	98.11	00	100	00	100	00	100	00	100	18.66	74.55	4.66	91.21	00	100	00	100
Camphor	0.5	13.33	86.67	3.66	95.10	26.66	73.34	11.66	83.42	9.33	78.78	3.00	87.14	00	100	00	100	26.66	63.64	8.00	84.91	9.33	70.84	2.33	87.51
	1	00	100	00	100	9.33	90.67	3.66	94.80	2.66	93.95	1.33	94.30	00	100	00	100	20.00	72.73	5.00	90.57	6.66	79.19	1.66	90.94
Untreated		100		76.00		100		70.33		44.00		23.33		100		73.00		73.33		53.00		32.00		18.33	
LSD at 0.05	T	NS		NS		NS		NS		NS		NS		3.14		2.88		NS		NS		NS		NS	
	C	6.20		NS		9.08		6.68		NS		NS		2.56		2.31		9.03		4.58		NS		NS	

Table (3): Comparative effectiveness of plant oils spray as a pre-harvest bean treatments on *B. cinerea*, *Pythium aphanidermatum* and natural infection 2012.

Treatment (T)	Conc. C%	CV. Xera												CV. Valentino											
		B. cinerea				*P. aphanidermatum*				Natural infection				*B. cinerea*				*P. aphanidermatum*				Natural infection			
		DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF
Carnation	0.5	00	100	00	100	12.00	85.48	5.00	91.94	00	100	00	100	20.00	78.26	10.66	83.85	33.33	51.93	18.33	63.34	10.66	68.02	3.66	83.10
	1	00	100	00	100	00	100	00	100	00	100	00	100	4.00	95.65	1.00	98.48	9.33	86.54	2.33	95.34	4.00	88.00	1.00	95.38
Cinnamon	0.5	6.66	92.76	3.66	94.87	24.00	70.97	10.00	83.87	00	100	00	100	00	100	00	100	40.00	42.30	26.00	48.00	00	100	00	100
	1	00	100	00	100	9.33	88.71	2.33	96.24	00	100	00	100	00	100	00	100	20.00	71.15	5.00	90.00	00	100	00	100
Camphor	0.5	9.33	89.86	3.33	95.33	22.67	72.57	11.00	82.26	4.00	88.46	2.66	87.72	2.66	97.11	2.33	96.50	24.00	65.38	10.33	79.24	00	100	00	100
	1	6.00	93.48	2.66	96.27	12.00	85.48	3.00	95.16	00	100	00	100	00	100	00	100	18.66	73.09	6.00	88.00	00	100	00	100
Untreated		92.00		71.33		82.66		62.00		34.66		21.66		92.00		66.00		69.33		50.00		33.33		21.66	
LSD at 0.05	T	NS		NS		8.13		2.92		NS		NS		6.92		4.29		NS		NS		3.75		1.88	
	C	NS		NS		6.64		2.38		NS		NS		5.65		3.50		NS		10.49		3.06		1.53	

On Valentino cv., the results cleared that, Cinnamon and Camphor oils at 1% concentration completely inhibited the infection on bean pods with *B. cinerea* (efficacy 100%) during two seasons, but there effect on infected bean pods with *P. aphanidermatum* were best in reducing the disease severity where their values were 4.66-5.00%, respectively during season 2011 and 5.00- 6.00%, respectively during season 2012. Meanwhile, Carnation at 1% could be able also to prevent the infection on pods infected with *B. cinerea* with efficacy being 100% during season 2011, but its effect on infected bean pods with *P. aphanidermatum* were good in reducing the disease severity % on bean pods with efficacy reached 87.58 - 95.34%, respectively in the two seasons.

On the other hand, all plant oils at high concentration completely inhibited the infection on bean pods Xera and Valentino cvs. under natural infection conditions, except Camphor oils with Valentino cv. These results are in harmony with those obtained by **Rushed (2001)** tested pre-harvest spray of Camphor, Carnation and Cinnamon oils against infection of Flame Seedless and Thompson Seedless grape berries with *B. cinerea* during cold storage. Results showed that all tested natural oils *in vivo* revealed that 1.0 % concentration was very effective. On the other hand, **Fahiem (2010)** who found that plant oils *i.e.* Thymol, Anisol, Eugenol, Camphor oil and Fennchone oils were effective in controlling bean pod mold infection under greenhouse conditions. Regarding greenhouse studies, Eugenol oil was the best effective plant oil in controlling the bean mold infection under greenhouse conditions. Also, all other tested plant oils were effective in controlling bean pod mold infection with *B. cinerea* and *P. aphanidermatum* when compared with the control treatment with significant differences between them. Also, **Samane and Mohammad (2012)** tested four essential oils (anise, ammi, ziziphora and Cinnamon) at five concentrations (0, 200, 400, 600 and 800 $\mu L.L^{-1}$) against fungal pathogen *Botrytis cinerea* the causal agent of gray mould disease of peach *(Prunus persica* L.) under *in vivo* conditions. Results showed that treated fruits with ammi essential oil at concentration 800 $\mu L.L^{-1}$ had the lowest decay and acidity. Thus, these results showed that essential oils have strong impact on post-harvest decay and fruit quality of peach.

Effect of some plant oils vapor under modified atmosphere packaging (MAP) and cold storage on disease incidence of snap bean pod rots under cold storage:-

Exposing snap bean pods Xera and Valentino cvs. to vapors of plant oils *i.e.,* cinnamon, carnation and camphor oils at the rate of 50 and 100 µL then put in MAP were used for study their effect on the disease incidence on snap bean pods Xera and Valentino cvs. after inoculation with *B. cinerea* and *P. aphanidermatum* fungi as well as natural infection, and storage at 8±1°C and 90 - 95%RH for 18 days.

Data in **Table (4)** cleared that, both concentrations of oils used as vaporization were obviously effective against *B. cinerea* and *P. aphanidermatum* infection and development compared with control and used MAP only without any treatment by oils. All plant oil treatments significantly inhibited infection of snap bean pods with *B. cinerea* and *P. aphanidermatum.* While no significant differences were found among plant oils treatments. Vaporization of snap bean pods cv. (Xera) with Carnation at 100 µL after harvest was the best effective treatment among the tested plant oils where it is prevented completely the infection and consequently the disease severity percentages of all the two tested mold pathogens on snap bean pods with efficacy 100%. Camphor oil at 50 and 100 µL was the most suppressive treatment against grey mold and Pythium rot on snap bean pods (Xera, cv.), where it reduced decay percentage to 8.64-3.70% and 6.17-123% in snap bean pods infected with *B. cinerea* and *P. aphanidermatum,* respectively. Cinnamon caused great reduction in the severity of grey mold and Pythium rot when it used at 100 µL with bean pods.

Moreover, vaporization of snap bean pods Valentino cv. with Camphor oil at 100 µL was more effective for reducing the infection and disease severity of *B. cinerea* and *P. aphanidermatum* on snap bean pods Valentino cv. than other oil treatments by results 4.94 -3.70% infection and 1.24- 0.93% disease severity, respectively. Cinnamon and carnation at both concentrations were highly effective for reduction the infection and disease severity of *B. cinerea* and *P. aphanidermatum* on snap bean pods Valentino cv. compared with control treatments.

Meanwhile, all oil treatments at higher concentration completely inhibited the infection of snap bean pods Xera and Valentino cvs. with others from natural infection with efficacy 100%. While, MAP was the least effective on reducing disease severity of snap bean pod rots caused by the two pathogenic fungi and natural infection compared with all plant oil treatments.

On the other hand, all plant oil treatments at 1% concentration completely inhibited the infection of bean pods Xera and Valentino cvs. from natural infection with efficacy 100%. The obtained results are in agreement to somewhat with those obtained by **Rushed (2008)** found that Cinnamon and Carnation oils at 0.25% and 0.5% caused almost no dry rot on potato tubers kept at 10°C under 85-90% RH for 90 days. Similar results were obtained by **Sirirat *et al.* (2009)** who, found that vapors of Clove oil, Cinnamon oil and lemongrass oil exhibited strong inhibitory effects on *B. cinerea.* These results have shown that the essential oils derived from clove, Cinnamon and Lemongrass might be used as alternative options for the control of gray mould on postharvest organic fruits. Also **Nemat *et al.* (2012)** tested three essential oils of *Satureja hortensis, Zataria multiflora* and *Carum copticom* at three concentrations 100, 200 and 300 ppm. The results showed that a 200 ppm of all three essential oils was able to effectively control the growth of the Botrytis mycelium on both PDA and the actual fruit of strawberries.

Table (4): Effect of vaporization by some plant oils on bean pod mold infection on detached pods.

Treatment	Conc. %	CV. Xera												CV. Valentino											
		B. cinerea				*P. aphanidermatum*				Natural infection				*B. cinerea*				*P. aphanidermatum*				Natural infection			
		DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF	DI	EF	DS	EF
Carnation+MAP	50	8.69	90.40	3.71	93.32	6.17	90.20	2.47	94.36	00	100	00	100	11.11	88.89	4.32	93.26	12.34	84.68	4.94	91.11	6.17	84.38	1.54	93.07
	100	00	100	00	100	00	100	00	100	00	100	00	100	6.17	93.83	2.16	96.63	9.87	87.75	4.63	91.67	00	100	00	100
Cinnamon+MAP	50	7.46	91.72	3.10	94.42	8.64	86.28	4.94	88.93	00	100	00	100	12.34	87.66	5.25	91.81	12.34	84.68	7.71	86.12	6.17	84.38	1.85	91.67
	100	5.33	94.09	1.26	97.73	7.40	88.25	2.78	93.66	00	100	00	100	6.17	93.83	2.47	96.15	8.64	89.28	3.40	93.88	00	100	00	100
Camphor+MAP	50	8.64	90.41	2.47	95.55	6.17	90.20	1.85	95.78	00	100	00	100	8.64	91.36	3.70	94.23	13.58	83.14	5.87	89.43	3.70	90.63	.93	95.81
	100	3.70	95.89	.93	98.33	1.23	98.05	.62	98.59	00	100	00	100	4.94	95.06	1.24	98.07	3.70	95.41	.93	98.33	00	100	00	100
MAP		25.93	71.23	18.52	66.67	18.52	70.58	9.26	78.87	4.93	87.52	2.78	85.92	43.31	56.69	29.63	53.78	45.68	43.30	31.79	42.78	16.05	59.37	7.10	68.05
Untreated		90.12		55.56		62.96		43.83		39.50		19.75		100		64.10		80.56		55.56		39.50		22.22	
LSD at 0.05		7.55		5.41		6.76		3.68		3.10		0.66		10.79		5.49		8.92		8.09		4.66		4.44	

Polyphenoloxidase Activity.

The activities of polyphenoloxidase enzymes were estimated in pods inoculation with *B. cinerea* and *P. aphanidermatum* as well as natural infection as a reflection to spray of different plant oils *i.e.* Carnation, Cinnamon and Camphor in concentration (at 1%) on snap bean plants (Xera and Valentino cvs.) and storage at 8±1°C and 90 - 95% RH.

Data in **Table (5)** showed that the activities of polyphenoloxidase enzymes were reduced in pods inculcated with *B. cinerea* at 3 days after spraying of snap bean plants Xera and Valentino cvs. with all tested plant oils compared with control. Meanwhile, the activities of polyphenoloxidase enzymes were high at 7 days in pods infected with *B. cinerea* as a reflection to spray of all tested plant oils on snap bean plants cv. Xera.

On the other hand, Camphor increased the activities of polyphenoloxidase at 7 days in pods cv. Valentino inoculated with *B. cinerea* followed by pods after spraying cinnamon oil on bean plants when compared with carnation oil treatment and control check. On the other hand, the highest activities of polyphenoloxidase were recorded during three days in snap bean pods cv. Xera infected with *P. aphanidermatum* after spraying of bean plants with carnation oil treatment compared with other treatments and control. Data cleared that, the increase of polyphenoloxidase activity was associated with increasing resistance against infection by the two tested pathogen.

Table (5): Effect of spraying some plant oils under field conditions on polyphenoloxidase activity in naturally infected and artificially inoculated bean pods with the two tested fungi.

Treatments	Polyphenoloxidase enzymes activity after incubation days																	
	cv. Xera									cv. Valentino								
	B. cinerea			*Pythium aphandermatum*			Natural infection			*B. cinerea*			*Pythium aphandermatum*			Natural infection		
	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days
Carnation	100	0.133	0.186	100	0.799	0.256	100	0.223	0.162	100	0.081	0.072	92.73	0.112	0.133	100	0.231	0.090
Cinnamon	100	0.061	0.249	98.07	0.473	0.206	100	0.118	0.092	100	0.051	0.096	74.56	0.621	0.055	100	0.117	0.098
Camphor	100	0.117	0.156	90.07	0.558	0.093	93.95	0.152	0.101	100	0.089	0.141	72.73	0.802	0.057	79.19	0.178	0.084
Untreated		0.203	0.142		0.087	0.076		0.062	0.084		0.187	0.090		0.527	0.122		0.088	0.087
Healthy	0.011									0.008								

Ef= Efficacy **DI**= Disease infection

In this respect, spraying of bean plant cv Xera with all tested oils treatment increased the activities of polyphenoloxidase at 3 and 7 days in pods infected with *P. aphanidermatum* and naturally infected compared with control. Meanwhile, the highest activities of polyphenoloxidase were obtained at 3 days in snap bean pods cv Valentino infected with *P. aphanidermatum* after spraying of bean plants with Camphor oil treatment compared with other treatments. Spraying of bean plants (Valentino, cv.) with all plant oils treatment increased the activity of polyphenoloxidase at 3 days in naturally infected pods compared with control. Also, data showed that, the least activity of polyphenoloxidase was recorded in healthy pods compared with other treatments and control check. These results are in agreement with those obtained by **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. Also, susceptible strawberry cultivars produced such oxidative enzymes. However, higher production of such enzymes was induced in the leaf tissues of the resistant cultivar. Also, **Naffa and Rabie (2006)** found that polyphenol oxidase activity increased in cucumber fruits inoculated with *Botrytis cinerea* and *Sclerotinia sclerotiorum.* Similar results were obtained by **Fahiem (2010)** who found that treating the inoculated and non-inoculated snap bean pods with some plant oils encouraged the increase in activities of polyphenoloxidase enzymes. The highest increase in the enzyme activity was recorded in pods treated with Camphor, anisol and fennel and inoculated with *P. aphanidermatum* and *S. sclerotiorum* respectively.

Peroxidase activity:

The activities of peroxidase enzymes were estimated in pods inoculated with *B. cinerea* and *P. aphanidermatum* as well as naturally infected as a reflection to spray of different plant oils i.e. Carnation, Cinnamon and Camphor in concentration (at 1%) on snap bean plants (Xera and Valentino cvs.) and storage at 8±1°C and 90 - 95% RH.

Data in **Table (6)** showed that, the activities of peroxides were increased at 3 days in bean pods cv Xera infected with *B. cinerea* also it increased during 7 days in bean pods inoculated with *B. cinerea* and *P. aphanidermatum* and in bean pods (Valentino, cv.) inoculated with *P. aphanidermatum* as well as natural infection after spraying Carnation on snap bean plants. The highest activity of peroxides were recorded in bean pod cv. Xera infected with *B. cinerea* at 7 days when snap bean plant pre-sprayed with carnation compared with control and other treatments. Data showed that, the increase of peroxides activity was associated with increasing resistance against infection by the two tested pathogens.

Table (6): Effect of spraying of some plant oils under field conditions on peroxides enzymes activity on naturally infected and artificially inoculated bean pods with the two pathogenic fungi.

Treatments	peroxides enzymes activity after incubation days																	
	cv. Xera									cv. Valentino								
	B. cinerea			*Pythium aphandermatum*			Natural infection			*B. cinerea*			*Pythium aphandermatum*			Natural infection		
	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days	Ef of DI	After 3 days	After 7 days
Carnation	100	1.87	2.05	100	1.36	1.82	100	1.66	1.76	100	1.70	1.65	92.73	1.74	1.83	100	1.48	1.68
Cinnamon	100	1.86	1.54	98.07	1.80	1.66	100	1.61	1.65	100	1.50	1.72	74.56	1.85	1.68	100	1.70	1.79
Camphor	100	1.64	1.88	90.07	1.67	1.70	93.95	1.59	1.77	100	1.57	1.93	72.73	1.82	1.51	79.19	1.53	1.61
Control		1.86	1.87		1.70	1.74		1.73	1.85		1.83	1.69		1.77	1.81		1.62	1.66
Healthy	0.87									0.79								

Ef= Efficacy DI= Disease incidence

In this respect, snap bean plants sprayed with Cinnamon caused increase in peroxides activity at 3 days in bean pods cv Xera inoculated with *B. cinerea* and *P. aphandermatum* and in bean pods cv Valentino inoculated with *P. aphandermatum* as well as naturally infected after harvest and storage. Also the activities of peroxides enzymes were increased during 7 days in pods (Valentino cv.) infected with *B. cinerea* and naturally infected pods when spraying bean plants with Cinnamon compared with control and healthy (untreated-un-inoculated). While, Camphor was more effective to increase the activities of peroxides after 3 and 7 days in bean pods (Valentino, cv.) infected with *B. cinerea.* Meanwhile, the least activity

of peroxidase was recorded in healthy treatment (un-treated un-inoculated). Compared with control. The obtained results could be interpreting in light the findings of **Reuveni *et al.* (1992)** who 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. Peroxidase activity was increase with time in both susceptible and resistant plants. Determination changes in peroxidase and polyphenoloxidase activity and newly developed peroxidase isozymes and polyphenoloxidase in pre-sprayed plants with some pre-harvest treatments. Increasing of peroxidase activity was associated with increasing resistance against infection by many diseases, through the accumulation of phenolic compounds playing a role in disease resistance **Mitlirass *et al.* (2006**). Similar results were obtained by **Hassan *et al.* (2007)** revealed that, citric and benzoic acids were the most effective one, since they recorded the lowest percentages of disease severity of *Botrytis fabae* and *B. cinerea* on faba bean plants and the highest levels of peroxidase activities. Moreover, pretreated faba bean plants showed some new isozymes and increment in the density of original isozymes, especially in infected plants. In this respect, **Hegazi (2010)** evaluated some essential oils as biocontrol agents against powdery mildew on *Zinnia elegans,* L. A field experiment was carried out during the two successive seasons of 2006 and 2007 using marjoram, clove, Cinnamon, garlic, ginger and fennel oils, added as foliar spray at 2 levels of 0.05 and 0.1 ppm. peroxidase and polyphenol oxidase activities were determined after 24 hour from the last spray in leaves samples. It was found that the activities of peroxidase (POX) and polyphenoloxidase (PPO) enzymes were increased as a result of oils sprayed on plants.

REFERENCES

Abdel-Kader, M.M., N.S. El-Mougy, N.G. El-Gammal, F. Abd- El-Kareem and M.A. Abd-Alla (2012): Laboratory evaluation of some chemicals affecting pathogenic fungal growth. J. Appl. Sci. Res, 8(1): 523-530.

Ali, I.N.; A.O. El-Shimy; N.M. Eisa; and A.Z. Tadrous, (2003): Enzymes activities in relation to strawberry leaf spot pathogens. Minufiya J. Agric. Res., 28(3): 721-735.

Allan, A. I. and J. P. Hollis. (1972): Sulfide inhibition of Oxidases In rice roots. Phytopathology 62: 634-636.

Chebli, B.; L.M. Achouri; H. Idrissi and H. Mohamed (2003): Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr. Journal of Ethnopharmacology, 89: 165-169.

Fahiem, M.A. (2010): Studies on bean fruit in Egypt. M. Sc. Thesis, Fac.

Agric., Benha Univ. 121pp.

Fokemma, N.J. (1973): The role of saprophytic fungi in antagonism against *Drechslera sorokiniana (Helminthosporium sativum)* on agar plates and on rye leaves with pollen. Physiol. Plant Pathol, 3:195-205.

Hassan, Maggie E.M.; S. S. Abd El-Rahman; I. H. El-Abbasi and M.S. Mikhail (2007): Changes in peroxidase activity due to resistance induced against faba bean chocolate spot disease. Egypt. J. Phytopathol., 35(1): 35-48.

Hegazi M.A. (2010): Efficacy of Some Essential Oils on Controlling Powdery Mildew on Zinnia *(Zinnia Elegans*, L.). J. Agric. Sci., 2(4):.

Matta, A.I. and A.F. Dimond (1963): Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathol. 53: 574-578.

Min. Agric. ARE, (2012): Statistics data of Ministry of Agric. ARE

Mitlerass, K.; D.M. Treutter; W. Plessl; E.F. Elstner and I. Heiser. (2006): Modification of primary and secondary metabolism of potato plants by nitrogen application differentially affects resistance to *Phytophthora infestans* and *Alternaria solani*. Plant Biol., 8:653-661.

Naffa, Azza M.A. and S.M.H. Rabie (2006): Role of Dipping cucumber fruits in salt solutions and packaging to control pathogenic fungi and preserving quality during cold storage. Minufiya J. Agric. Res., 20(12): 1403-1417.

Nemat, A.E.; M. Behdad and H. Zeinali (2012): Antifungal effects of three plant essential oils against *Botrytis cinerea* : the cause of gray mold on strawberry. J. Res. Agric. Sci., 8(2): 165-170.

Reuveni, R.; M. Shimoni; Z. Karchi and J. Kuc (1992): Peroxidase activity as a biochemical marker of resistance muskmelon (*Cucumis melo*) *Pseudoperonospora cubersis.* Phytopath., 82: 749753.

Riad, S.R.; M.M. El-Mohamedy; A.F. Abd-El-Kareem and N.S. El-Mougy (2013): Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens *in vitro.* Journal of Agricultural Technology, 9(6):1507- 1520.

Rushed, D.I.S. (2001): Pathological studies on grape prepared for exportation. M. Sc. Thesis, Fac. Agric., AL-Azhar Univ. 176pp.

Rushed, D.I.S. (2008): Studies on fungal post-harvest diseases of potatoes prepared for exportation and processing. PhD. Thesis, Fac. Agric., AL-Azhar Univ. 244pp.

Samane, M. and M.H. Aminifard (2012): Effect of Essential Oils on Postharvest Decay and Some Quality Factors of Peach (*Prunus persica* var. Redhaven). J. Biol. Environ. Sci, 6(17): 147-153.

Sirirat, S.; W. Rungprom and S. Sawatdikarn (2009): Antifungal activity

of essential oils derived from some medicinal plants against gray mould (*Botrytis cinerea*) As. J. Food Ag-Ind. Special Issue, S229-S233.

Snowdon, A.L. (1992): reported that Pythium, Fusarium caused white cottony rot, *Botrytis cinerea* caused gray mould, *Sclerotinia sclerotiorum* caused white soft rot to bean pods. Color atlas of postharvest diseases and disorders of fruits and vegetables. Vol. 2, CRC press, Boca Raton FL, pp. 236-262.

Song, W. and A.J. Keane (2006): "Parameter Screening Using Impact Factors and Surrogate- Based ANOVA Techniques", AIAA-2006-7088, 11th AIAA/ISSMO Multidisciplinary Analysis and Optimization Conference, Renaissance Portsmouth, Portsmouth, Virginia, 6 - 8 Sep 2006.

Soylu, M.; S. Kurt and S. Soylu (2010): *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea.* International Journal of Food Microbiology 143: 183-189.

Spalding, D.H. and W.F. Reeder (1974): Post-harvest control of *Sclerotinia* rot of snap bean pods with heated and unheated chemical dips. Plant Dis. Report., 58: 59-62.

Suslow, T.V. and M. Cantwell (1998): Peas - Snow and Snap Pod Peas.
Perishables Handling Quarterly, Univ. of Calif., Davis CA, 93:15-16.

Wijnands, J. (2004): The impact on the Netherlands of the Egyptian greenhouse vegetable chain. Report 5.04.10, Agricultural Economics Research Institute (LEI), The Hague, Netherlands.

إمكانية تأثير الزيوت العطرية كمضادات فعالة ضد أعفان قرون الفاصوليا ما بعد الحصاد

محمد هارون عبد المجيد* - فتحي جاد محمد* - حمادة حماد سلطان - محمد السيد حافظ* - فايز عبد الرحمن****

*قسم أمراض النبات – كلية الزراعة بمشتهر – جامعة بنها – مصر.

** معهد بحوث أمراض النباتات – مركز البحوث الزراعية – مصر.

تتعرض قرون الفاصوليا بعد الحصاد إلى العديد من المسببات المرضية أكثرها خطورة الفطرين بوتريتس سيناريا وبيثيم أفنيدرماتم. وتهدف هذه الدراسة إلى الحد من خطورة هذه المسببات بإستخدام بعض الزيوت العطرية كمضادات آمنة لأعفان ما بعد الحصاد في قرون الفاصوليا صنفي أكزيرا وفلانتينو. استخدم ثلاث زيوت نباتية وهم زيت الكافور والقرفة و القرنفل بتركيزات ٠,٢٥ ، ٠,٥ ، ١%. وقد أظهرت النتائج المعملية قدرة كلاً من زيت القرفة والقرنفل على تثبيط النمو الميسليومي لكلا الفطرين بشكل كامل مع كل التركيزات المستخدمة. وتحت الظروف الحقلية وجد أن رش نباتات الفاصوليا صنفي أكزيرا وفلانتينو والمتسببة عن فطر بوترايتس سيناريا، كذلك أظهر صنف فلانتينو مقاومة عند تركيز ٠,٥. كما أوضحت النتائج أن تبخير قرون الفاصوليا أثناء التخزين بواسطة زيت القرنفل بتركيز ١٠٠ميكروليتر كان الأفضل في التثبيط التام لأعفان القرون المتسببة عن كلا الفطرين البوترايتس سيناريا وبيثيم أفنيدرماتم. كما أن تبخير قرون الفاصوليا صنف فلانتينو بواسطة زيت الكافور بتركيز ١٠٠ميكروليتر ثبطا تماماً إصابة قرون الفاصوليا بواسطة البوترايتس سيناريا وبيثيم أفنيدرماتم. وأوضحت النتائج أيضاً أن رش نباتات الفاصوليا قبل الحصاد بكل من زيت القرنفل والكافور أدت إلى زيادة نشاط إنزيمات البولي فينول أوكسيداز والبيروأكسيداز بعد ٣ و ٧ أيام من التخزين. حيث انعكس ذلك على زيادة قدرة القرون على مقاومة الإصابة بكلا الفطرين.