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EFFECT OF PREHARVEST APPLICATION WITH SOME ESSENTIAL OILS AND CHITOSAN AGAINST WHITE AND GRAY MOULDS ON GREEN BEAN PODS

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ABSTRACT

Survey was carried out in different commercial markets *i.e* 6- October, El-Obour, El-moneeb, El-Lewaa, and Bader City at three times during Autumn and Winter growing season. Illustrated results showed that *S. sclerotiorum* and *B. cinerea* are the most important fungi which recorded higher frequency in all different markets. They recorded 28.5 and 24.9%, respectively . The highest infections were recorded in Autumn growing season, as compared to Winter growing season. Pathogenicity tests indicated that all isolates of *S. sclerotiorum* and *B. cinerea* caused the highest infection in green bean pods under wounded or unwounded technique. Complete inhibition of linear growth of *S. sclerotiorum* and *B. cinerea* was obtained with thyme, marjoram, eucalyptus and soft guard at concentration of 3.0%, halfa-gar and artemisia at 1.0%, chitosan at 0.06% and lemongrass at 0.5%. Results indicated that the highest reduction in disease incidence was obtained with thyme at concentration of 3.0%, chitosan at 0.06% and lemongrass at 0.5%. All treatments significantly reduced white and gray mould diseases of green bean pods under different storage conditions when applied either pre or postharvest treatments. The most effective treatments are lemongrass, chitosan, thyme and soft guard, which reduced the white and gray mould diseases during the Autumn and Winter growing seasons.

Keywords: White mould - gray mould - green beans - postharvest diseases - essential oils - chitosan.

INTRODUCTION

White and gray mould, caused by the plant pathogenic fungi *Sclerotinia sclerotiorum* (Lib.) de Bary and *Botrytis cinerea* Pers.Fr., are the most important diseases attack green beans under pre- or postharvest and storage conditions (Shah *et al.,* 2002 ; Huang *et al.,* 2006 and Ocamb, 2008). There is a growing need to developing alternative approaches for controlling postharvest diseases of green beans pods.

Essential oils are composed of many different volatile compounds and the make up of the oil quite often varies between species. It seems that the anti-fungal and anti-microbial effects are the result of many compounds acting synergistically (Ocamb and McReynolds, 2008).

Using some essential oils by spraying or dipping fruits for controlling postharvest diseases of several fruits has been reported by several investigators (Nguefack *et al.,* 2007; Somda *et al.,* 2007 and Segvie *et al.,* 2007). The following essential oils resulted in controlling postharvest diseases of several fruits included artemisia (*Artemisia herba- alba*) (Hoballah, 2006 and Saleh *et al.,* 2006); lemongrass (*Cymbopogon citratus*) (Adegoke and Odesola, 1996; Nguefack *et al.,* 2007; Somda *et al.,* 2007 and Tzortzakis and

Economakis, 2007) ; halfa-Gar (*Cymbopogon proximus*) (Hoballah , 2006) ; eucalyptus (*Eucalyptus globulus*) (Feng and Zheng, 2007; Kanherkar *et al.,* 2007 and Shahnaz *et al.,* 2007) ; marjoram (*Majorana hortensis*) (El-Sherbieny *et al.,* 2002 and Dimitra *et al.,*2003) and thyme (*Thymus vulgaris*) (Angelini *et al.*, 2006; Nguefack *et al.,* 2007 and Segvie *et al.,* 2007).

On the other hand, chitosan is a by-product in seafood industry was reported by many investigators as a protective safe material against many pathogens (Bautista *et al*., 2004; El Hassni *et al*., 2004 and Banos *et al*., 2006).

The aim of this work is to study the effect of some essential oils, *i.e* thyme, marjoram, eucalyptus, halfa-gar, artemisia and lemongrass in addition to chitosan as its or commercial product (soft guard) against *S. sclerotiorum* and *B. cinerea* fungi. Moreover, applying them as non-conventional safe and cheap materials for controlling pre and postharvest diseases of green bean pods.

MATERIALS AND METHODS

I. Survey of postharvest diseases of green bean pods

Commercial samples of green bean pods of Poulista cv. were collected three times during marketing season from trade markets located at 6-October, El- Obour, El-Moneeb, El-Lewaa and Badr City during Autumn and Winter growing seasons 2005 / 2006. Green bean pod samples were classified in two groups, apparently healthy and decayed pods. Decayed pods were stored at 23- 25˚C for 4 days with daily examinations. The frequency of isolated fungi were recorded.

a. Isolation and purification of pathogenic fungi

Samples of diseased green bean pods were carefully washed with tap water, cut into small pieces, disinfested in 70% ethyl alcohol for one minute then dried between folds of sterilized filter paper. Disinfested small pieces were transferred into Petri dishes containing Water Agar medium and incubated at 23- 25˚C for 4 days. The developed fungi from diseased specimens were transferred into PDA medium plates. The isolated fungi were purified using single spore method for spore forming fungi according to the technique described by Ezekiel (1930) or by hyphal tip transfers method mentioned by Brown (1924).

b. Identification of pathogenic fungi

Identification of isolated fungi was carried out in Plant Pathology Laboratory, Plant Pathology Department, National Research Center. Isolated fungi from decayed pods were identified according to Gilman (1957), Ellis (1971) and Barnett and Hunter (1972). While *Sclerotinia sclerotiorum* identification was achieved according to its morphological characteristics of mycelium and sclerotia (Abuel-Ela, 1993). Purified fungi were maintained on PDA slant under refrigerator conditions at 5˚C as stock cultures for further studies.

2. Pathogenicity tests

Pure fungal isolates were tested to study their pathogenic ability for inducing postharvest diseases on green bean pods cvs. Poulista and Bronco.

a. Inoculum preparation

Spore suspension (10^6 spores/ml) of pure isolates of *Alternaria alternata, Botrytis cinerea*, *Fusarium semitectum* and *Pythium debaryanum* (sporangia) were prepared by culturing each fungus on PDA slant in 500 ml bottles for 10 days at 23 - 25°C. Spores were released in sterilized water using a brush and filtered through muslin cloth to exclude mycelial growth. Spores were counted using haemocytometer slide.

Inoculum of *S. sclerotiorum* was prepared as a mycelial suspension. The fungus was grown on PDB (Potato Dextrose Broth) medium at 20˚C for 10 days, and mass of growth was blended. Inoculum of *S. sclerotiorum* was prepared as mycelial fragments (Soltan, 1993).

b. Inoculation of green bean pods

Apparently healthy green bean pods cvs. Poulista and Bronco obtained from EL-Behera Governorate, were surface sterilized by dipping in 70% ethyl alcohol for one minute and washed several times with sterilized water then dried at room temperatures. Sterilized bean pods were artificially wounded (by tooth burst). Inoculation of green bean pods was carried out by spraying wounded or unwounded green bean pods with prepared inoculum using an atomizer for each fungus at the rate of 100 ml spore suspension (10^6 spores / ml) / 100 bean pods. While, inoculum of *S. sclerotiorum* were used a mycelial fragments at the same rate. Sprayed green bean pods with the same amount of sterilized water served as control. The inoculated and un- inoculated green bean pods were packed in carton poxes (23 × 12 × 4 cm), each pox contained a uniform 50 pods and covered with polyethylene bags to increase the relative humidity. Three replicates were used for each treatment. Inoculated pods were stored at 23-25˚C, and pods inoculated with *S. sclerotiorum* were stored at 20˚C .

c. Disease assessment

The percentages of infected pods were recorded 7 days after inoculation according to Spalding and Reeder (1974) as follows:

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

3. Source of plant essential oils and chitosan

Several essential oils *i.e* Artemisia (*Artemisia herba-alba*), Halfa-gar (*Cymbopgon proximus*), Eucalyptus (*Eucalyptus globulus*), Lemongrass (*Cymbopogon citratus*), Marjoram (*Majorana hortensis*) and Thyme (*Thymus vulgaris*) were obtained from Cultivation and Production of Medicinal and Aromatic Plants Dept., National Research Center, Dokki, Cairo. Chitosan powder was obtained from SIGMA, and soft guard (commercial product from chitosan) was obtained from TECHNOGREEN Company.

4. Testing of some treatments on linear growth of *S. sclerotiorum* and *B.cinerea*.

Thyme , marjoram , eucalyptus , halfa-gar , artemisia , lemongrass, and soft guard at 4 concentrations e.g. 0.5 , 1.0 , 2.0 and 3% except that lemongrass at 0.25% in addition to the previous concentrations and chitosan at 0.02, 0.04, and 0.06 were added to conical flasks containing sterilized PDA medium before its solidification to obtain final concentrations and mixed

gently with 0.3% Tween-80, then disbanded in sterilized Petri-dishes (9 cm-diameter). The petri-dishes were individually inoculated with equal disks (6-mm- diameter) taken from 10 days old cultures of *S. sclertiorum* (Bader City isolate) and *B.cinerea* (El-Obour isolate) ,then incubated at 20°C and 23-25˚C respectively.. Linear growth of fungi was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 petri-dishes as replicates and 5 replicates for each treatment were used (Mishra and Dubey 1993).

5. Experiment trials

Thyme, marjoram, eucalyptus, halfa-gar, artemisia, lemongrass and soft guard were prepared at 4 concentrations *e.g.* 0.5 , 1.0 , 2.0 and 3.0% except that lemongrass at 0.25% in addition to the previous concentrations and chitosan at 0.02, 0.04 and 0.06% were added to conical flasks containing sterilized PDA medium before its solidification to obtain final concentrations and mixed gently with 0.3% Tween-80 (Sigma) to enhance oil solubility, then disbanded in sterilized Petri- plates (9 cm-diameter). Plates were individually inoculated with equal disks (5 mm-diameter) taken from 5 days old cultures of *S. sclerotiorum* (Badr City isolate) and *B. cinerea* (El-Obour isolate), then incubated at 20°C. Linear growth of fungi was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates and 5 plates for each fungus were used as control (Mishra and Dubey 1993).

6. Field experiments

Experiments were carried out, in Omar Makram Village (Badr City, El-Behera Governorate). The promising materials in laboratory experiments were applied as foliar spraying under field conditions.

a. Plant materials

Green bean seeds c.v. Poulista were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt.

b. Growing seasons

Four experiments were carried out during 2006 / 2007 and 2007 / 2008 growing seasons as follow :

1. First Autumn growing season (November 2006 – January 2007) 2. Second Autumn growing season (November 2007 – January 2008)
3. First Winter growing season (February- April) 2007
4. Second Winter growing season (February - April) 2008

c. Experiments design

Field experiments were conducted under natural infection in plots (4×8 m), each comprised of 8 rows and 32 holes / row . Thinning was practiced before the first irrigation (Mohaya) to secure two plants /hill. Field experiments were conducted in a complete randomized block design with three replicates (plots) for each particular treatment. as well as untreated plants. Green bean seeds cv. Poulista was sown at rate of 26 kg / feddan.

d. Treatments

Different doses of different materials in laboratory experiments were applied as foliar spraying as follow :-

Thyme, marjoram, eucalyptus and soft guard at concentration of 3.0% lemongrass at 0.5%, chitosan at 0.06%, halfa-gar and artemisia at 1.0% in addition to treated plants with the same amount of water (control) and [Fungicide Sumisclex 50% WP (procymidone) at 0.1% during Winter 2008 growing season] were applied under field conditions to study their effect on pre and or posharvest diseases of green bean pods .

e. Applications

1. First growing seasons (Autumn 2006 / 2007 and Winter 2007)

Experiment was divided into three trials as follow :-

- Firs trial was sprayed once.
- Second trial was sprayed twice
- Third trial was sprayed three times

2. Second growing seasons (Autumn 2007 / 2008 and Winter 2008)

The same applications in first growing seasons were applied in second growing season, except that trial which sprayed once time was excluded during postharvest trials.

3. Spraying time

First spray was carried out at 70% of formed flowers.
Second spray was applied after 7days of the first spray.
Third spray was applied at 48 h before harvest.

4. Disease assessment

Green bean pod diseases were recorded as percentage of infected pods.

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

5. Determination of green bean pods yield

Total yield of green bean pods was determined as kg / m^2.

6.Testing of preharvest application

Thyme , marjoram , eucalyptus and soft guard at concentration of 3.0% lemongrass at 0.5%, chitosan at 0.06%, halfa-gar and artemisia at 1.0% were tested under field conditions to study their effect against preharvest diseases of green bean pods under natural infection . Preharvest diseases were recorded after 70 days of sowing.

Healthy green bean pods from each treatment were harvested and transferred to Plant Pathology Department, National Research Center. Each treatment was divided into two groups. The first group was stored under natural infection conditions and the second was stored under artificial inoculation conditions to study their efficacy against postharvest diseases of green bean pods under different storage conditions as follow:-

a.Green bean pods stored under natural infection

1. Under shelf life conditions

Healthy green bean pods from each treatment were stored under natural infection and shelf life conditions (18± 1˚C) for 7 days. Postharvest decay were recorded after 7 days of storage.

2. Under refrigerator conditions

Healthy green bean pods from each treatment were stored under natural infection and refrigerator conditions (4 – 7˚C) for 14 days. Posharvest decay was recorded after 7 and 14 days of storage

3. Under refrigeration followed by shelf life conditions

Healthy green bean pods from each treatment were stored under natural infection and refrigerator conditions (4 – 7˚C) for 14 days , then stored under natural infection and shelf life conditions (18± 1˚C) for 4 days. Postharvest decay were recorded after 7, and 14 days of storage under refrigeration and after 4 days under shelf life conditions.

b.Green bean pods stored under artificial inoculation

1. Inoculum preparation

Spore suspension (10^6 spores / ml) of *B. cinerea* was prepared by culturing the fungus on PDA slant in 500 ml bottles for 10 days at 20°C. Spores were released in sterilized water using a brush and filtered through muslin cloth to exclude mycelial growth. Spores were counted using haemocytometer slide. While inoculum of *S. sclerotiorum* were prepared as a mycelial fragments, The fungus was grown on PDB medium at 20˚C for 10 days, and mass of growth was blended. (Soltan, 1993).

2. Inoculation of green bean pods

Healthy green bean pods (cv. Poulista) were artificially scratch (by tooth burst). Wounded green bean pods were sprayed with inoculum of any of the tested fungi using an atomizer at the rate of 100 ml spore suspension (10^6 spores / ml) / 100 bean pods for *B. cinerea*. While, inoculum with *S. sclerotiorum* were used as mycelial fragments at the same rate. The inoculated pods were stored under different storage conditions as follow:

3. Under shelf life conditions

Healthy green bean pods from each treatment were stored under artificial inoculation and shelf life conditions (18± 1˚C) for 7 days. Gray and white moulds were recorded after 7 days of storage

4. Under refrigerator conditions

Healthy green bean pods from each treatment were stored under artificial inoculation and refrigerator conditions (4 – 7 ˚C) for 14 days. Gray and white moulds were recorded after 7 and 14 days of storage.

5. Under refrigeration followed by shelf life conditions

Healthy green bean pods from each treatment were stored under artificial inoculation and refrigerator conditions (4 – 7 ˚C) for 14 days, then stored under artificial inoculation and shelf life conditions (18± 1˚C) for 4 days. Gray and white moulds were recorded after 7 and 14 days of storage under refrigerator and after 4 days under shelf life conditions.

In all experiments, green bean pods were packed in carton plates (23×12 × 4 cm), each plate contained a uniform 50 pods and covered with polyethylene bags to increase the relative humidity. Five replicates were used for each treatment

Statistical analysis

Data were subjected to the proper statistical analysis according to the method described by Snedecor and Cochran (1982). Means were verified according to the Duncan's multiple rang test (1955).

RESULTS

I. Survey of postharvest diseases of green bean pods and Frequency of isolated fungi.

Survey was carried out in different commercial markets *i.e* 6- October, El- Obour, El-Moneeb, El-Lewaa and Badr City at three times during Autumn and Winter growing seasons to record the percentage of green bean pods infection. Results in Table (1) indicated that high percentages of infection were recorded in all surveyed locations. The higher infection was recorded in Autumn growing season as compared with Winter growing season. The mean infection was 25.7 and 18.7% in Autumn and Winter growing seasons, respectively.

Table 1. Survey of green bean pods decay during Autumn and Winter of 2005 / 2006 growing seasons

Markets	% infection of green bean pods diseases	
	Growing seasons	
	Autumn	Winter
6-October	A 26.5 a*	B 21.5 a
El-Obour	A 29.0 a	B 19.6 a
El-moneeb	A ٢٣,٦a	B 17.6 a
El-Lewaa	A 25.0 a	B 12.5 b
Badr City	A 24.5 a	B 22.1 a
Means	A 25.7	B 18.7

Values followed by the same letter are not significantly differed (P= 0.0 5).

***, Small letters to compare between markets and capital letters to compare between growing seasons.**

Frequency of isolated fungi from rotted green bean pods was carried out on samples collected from different commercial markets *i.e* 6- October, El-Obour, El-Moneeb, El-Lewaa and Badr City for identifying and determining the important fungi that attack green bean pods. Illustrated results in Table (2) showed that *S. sclerotiorum* and *B. cinerea* are the most important fungi which recorded higher frequency in all different markets. They recorded 28.5 and 24.9%, respectively .This phenomenon was observed in all surveyed locations. *P. debaryanum* was occupied the third order in its importance. Meanwhile, *A. alternata*, *F. semitictum* and *R. stolonifer* showed the lowest frequency without difference between studied commercial markets.

2. Effect of different treatments on linear growth of (*S.sclerotiorum* and *B.cinerea*) fungi.

Thyme, marjoram, eucalyptus , halfa-gar, artemisia, lemongrass, and soft guard at four concentrations *e.g.* 0.5, 1.0, 2.0 and 3% except that lemongrass at 0.25% in addition to the previous concentrations and chitosan

at 0.02,0.04,and 0.06% were tested to study their inhibitory effect on linear growth of *S. sclerotiorum* and *B.cinerea* fungi. Results indicate that all treatments significantly reduced the linear growth of *S. sclertiorum* and *B.cinerea.* Complete inhibition of linear growth was obtained with thyme , marjoram , eucalyptus , soft guard at concentrations of 3.0% , halfa-gar, artemisia, chitosan at 1.0% and lemongrass at 0.5%. The high reduction was obtained with thyme , marjoram , at concentrations of 2.0%, halfa-gar and artemisia, at 0.5% which reduced the linear growth more than 80.5 and 76.5% for *S. sclerotiorum* and *B.cinerea* respectively . Other treatments showed moderate effect.

Table 2. Frequency of isolated fungi from rotted green bean pods collected from different commercial markets

	Frequency of isolated fungi %						
Markets	***S. sclerotiorum***	***B. cinerea***	***P. debaryanum***	***A. alternata***	***F. semitictum***	***R. stolonifer***	**Others**
6-October	A 29.1bc	A 25.8c	B 19.1b	C 11.2bc	D 5.4b	D 4.5ab	D 4.9a
El-Obour	A 24.7d	A 30.1a	BC 12.8d	B 14.1a	C 7.3a	C 7.2a	C 3.8ab
El-Moneeb	A 33.2a	C 17.3e	BC 20.0ab	D 12.6b	D 7.3a	DE 6.2ab	E 3.4b
El-Lewaa	A 27.2c	A 28.4b	B 21.5a	C 8.9d	CD 7.8a	DE 4.1b	E 2.1c
Badr City	A 28.3bc	AB 23.1d	B 20.4ab	C 10.6c	C 6.9ab	C 5.6ab	C 5.0a
Mean	28.5	24.9	18.7	11.4	6.9	5.5	3.8

Values followed by the same letter are not significantly differed (P= 0.0 5).
***, Small letters to compare between markets and capital letters to compare between fungi.**

3.Pathogenicity test

Data presented in Table (3) revealed that all isolates of *S. sclerotiorum* and *B. cinerea* caused the highest infection in green bean pods under wounded or unwounded techniques.The most sever isolates of *S. sclerotiorum* were isolates of Badr City and El- Obour markets, which caused the highest infection more than 71.8 and 84.3% when applied under wounded or unwounded techniques with Paulista and Bronco cultivars, respectively. Meanwhile, isolate of El-Obour of *B. cinerea* caused 60.8 and 58.0% infection in cvs. Paulista and Bronco respectively when applied under unwounded technique. While, wound technique caused high increase in disease incidence being 75.6 and 68.0% with Paulista and Bronco cultivars, respectively. All isolates of *P. debaryanum* showed moderate infection with Paulista and Bronco cultivars as well as when applied under wounded or unwounded technique. Other tested fungi were less effective.

Table 3. Pathogenisity test of different isolated fungi collected from different markets under wounded or unwounded technique

Fungal isolates	Source	Pod infection %			
		Unwounded		Wounded	
		Paulista	Bronco	Paulista	Bronco
S. sclerotiorum	Badr City	71.8 a	72.8 a	98.3 a	84.3 a
	El-Obour	a٧١,٨	70.9 a	91.0 a	83.6 a
	El-Lewaa	51.8 c	49.5 c	63.3 c	54.6 c
	El-Moneeb	49.0 c	42.0 d	53.3 d	48.3 d
	6-October	61.8 b	60.1 b	81.0 b	69.6 b
B. cinerea	Badr City	47.1 c	38.1 de	67.3 c	55.0 c
	El-Obour	60.8 b	58.0 b	75.6 b	68.0 b
	El-Lewaa	58.1 b	37.1 de	67.0 c	48.3 d
	El-Moneeb	41.8 d	29.0 e	51.3 d	38.6 ef
	6-October	41.5 d	37.8 de	61.3 c	58.3 c
P. debaryanum	Badr City	40.0 d	40.1 de	46.0 e	44.3 de
	El-Obour	37.8 d	35.1 e	55.3 d	40.0 e
	El-Lewaa	33.1 de	31.7 e	40.3 e	40.3 e
	El-Moneeb	30.7 e	21.8 f	40.0 e	33.3 f
	6-October	39.6 d	30.8 e	55.3 d	40.6 e
A. alternata	Badr City	12.6 f	11.6 g	13.3 fg	13.0 g
	El-Obour	10.1 fg	8.3 g	12.0 g	10.6 g
	El-Lewaa	9.6 g	8.6 g	11.0 g	9.3 g
	El-Moneeb	12.1 fg	11.0 g	14.0 g	12.0 g
	6-October	16.6 f	13.8 g	19.0 f	15.3 g
F. semitictum	Badr City	13.5 fg	12.5 g	14.6 fg	13.3 g
	El-Obour	12.8 fg	13.1 g	14.0 fg	13.0 g
	El-Lewaa	9.6 g	10.5 g	10.0 g	10.3 g
	El-Moneeb	12.8 fg	13.1 g	19.0 f	16.0 g
	6-October	15.5 fg	13.1 g	14.0 fg	13.0 g
Control	-	-	-	-	-

Values followed by the same letter are not significantly differed (P= 0.0 5).

4. Field experiments

1. Preharvest treatments during Autumn growing seasons (2006 / 2007 and 2007 / 2008)

Thyme, marjoram, eucalyptus and soft guard at concentration of 3.0%, lemongrass at 0.5%, chitosan at 0.06% and halfa-gar and artemisia at 1.0% were tested to study their effect on green bean pods disease incidence as follows:

a.under natural infection

Obtained results in Fig (1) showed that all materials significantly reduced the disease incidence. The pronounced materials are chitosan and lemongrass when applied three times which reduced the green bean pods diseases more than 87.7 , 85.4, 90.5 and 86.3%, respectively during the first and the second Autumn growing seasons as compared with untreated plants. Moderate reductions in disease incidence were obtained from soft guard,

thyme and halfa-gar materials when applied three times which reduced green bean pods diseases more than 78.3 and 76.3% during the first and the second Autumn growing seasons, respectively. Other materials showed moderate effect.

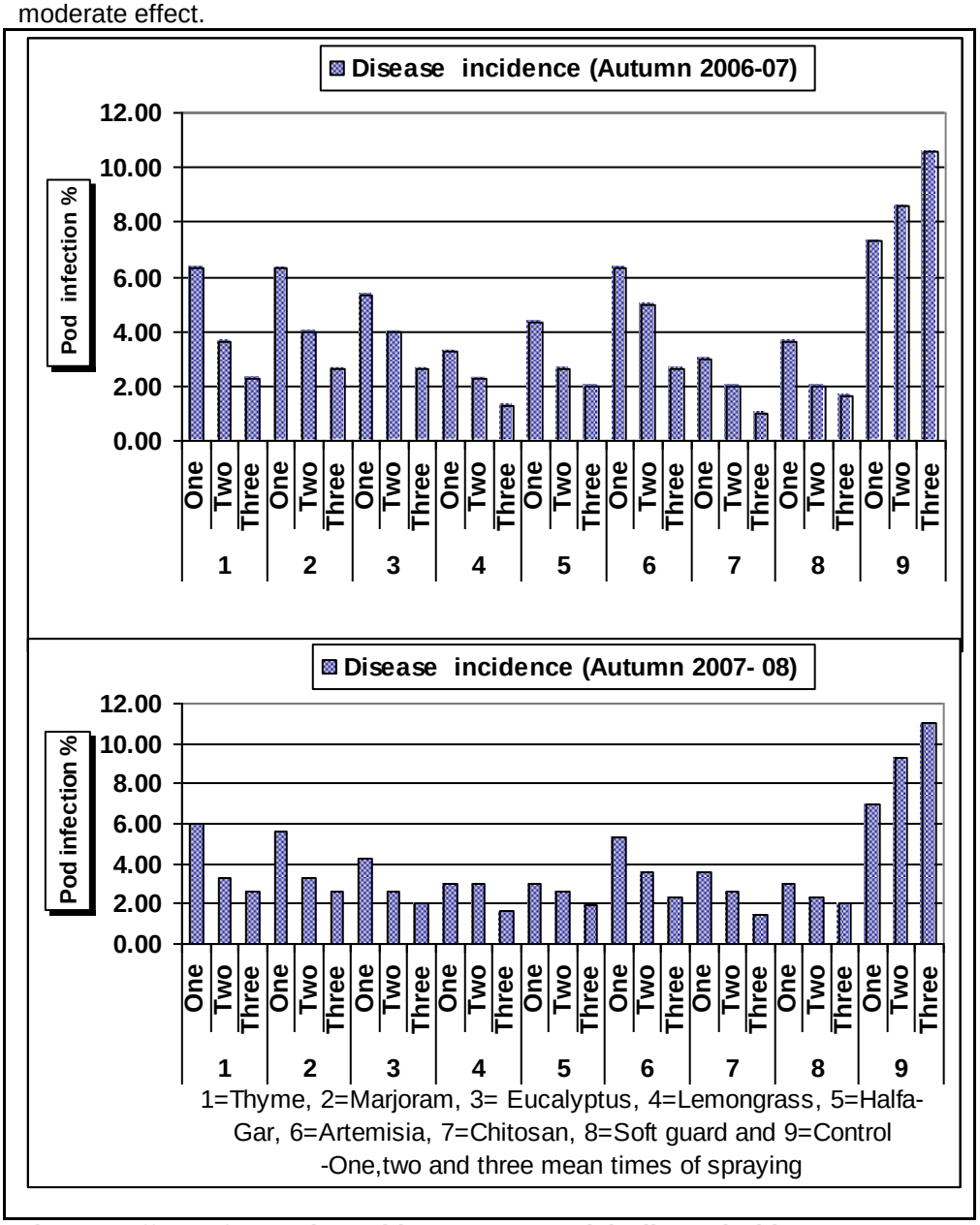

Fig. 1. Effect of spraying with some essential oils and chitosan on natural infection of green bean pods cv.Paulista under field conditions during utumn of 2006/2007 and 2007/2008 growing seasons.

b. Under natural infection and shelf life conditions

Results in Table (4) indicated that preharvest application with all materials significantly reduced the postharvest diseases of green bean pods under natural infection and shelf life conditions. The most effective materials are lemongrass, thyme, chitosan and soft guard when applied at three times which reduced the disease incidence more than 90.5 and 83.3% during the first and the second Autumn growing seasons, respectively. The high reduction was obtained with marjoram, eucalyptus and halfa-gar when applied three times and lemongrass when applied two times which reduced the postharvest diseases more than 82.3 and 78.8 % during the first and the second Autumn growing seasons, respectively. Meanwhile, other materials showed moderate effect.

Table 4. Effect of preharvest treatments with some essential oils and chitosan on postharvest diseases of green bean pods cv. Paulista under natural infection and shelf life conditions

Materials	No. of applications	Autumn growing seasons			
		2006 – 2007		2007 - 2008	
		Disease incidence	Reduction (%)	Disease incidence	Reduction (%)
Thyme	One	5.6 fg	60.0	-*	-
	Two	3.3 jk	78.4	3.0 f	٨٠,٠
	Three	1.3 o	92.3	1.5 g	٩١,٦
Marjoram	One	7.3 e	47.8	-	-
	Two	5.6 fg	63.3	5.0 e	٦٦,٦
	Three	2.6 klm	84.7	3.8 f	٧٨,٨
Eucalyptus	One	6.3 f	55.0	-	-
	Two	4.6 hi	69.9	5.1 e	٦٦,٠
	Three	2.0 mno	88.2	3.5 f	٨٠,٥
Lemongrass	One	4.3 i	69.2	-	-
	Two	2.3 lmn	84.9	3.0 f	٨٠,٠
	Three	1.3 o	92.3	1.5 g	٩١,٦
Halfa-Gar	One	7.3 e	47.8	-	-
	Two	5.6 fg	63.3	6.5 d	٥٦,٦
	Three	3.0 kl	82.3	3.0 f	83.3
Artemisia	One	8.3 d	40.7	-	-
	Two	6.3 f	58.8	8.0 c	٤٦,٦
	Three	4.0 ij	78.4	5.0 e	٧٢,٢
Chitosan	One	4.6 hi	67.1	-	-
	Two	3.3 jk	78.4	5.0 e	٦٦,٦
	Three	1.6 no	90.5	1.5 g	٩١,٦
Soft guard	One	5.3 gh	62.1	-	-
	Two	4.3 i	71.8	6.5 d	٥٦,٦
	Three	1.3 o	92.3	3.0 f	٨٣,٣
Control	One	14.0 c	0.0	-	0.0
	Two	15.3 b	0.0	15.0 b	0.0
	Three	17.0 a	0.0	18.0 a	0.0

Values followed by the same letter are not significantly differed (P= 0.0 5).
***, Data not determined.**

c. Under artificial inoculations and shelf life conditions

Illustrated results in Table (5) indicated that all materials significantly reduced the white and gray mould diseases of green bean pods under artificial inoculation and shelf life conditions.

Table 5. Effect of preharvest application with some materials on postharvest diseases of green bean pods cv. Paulista under artificial inoculation and shelf life conditions during Autumn growing seasons

Materials	No. of applications	Disease incidence			
		White mould		Gray mould	
		2006-2007	2007-2008	2006-2007	2007-2008
Thyme	One	46.6 e	-*	34.3 d	-
	Two	36.6 i	27.5 e	23.3 i	18.0 d
	Three	27.3 lm	21.5 i	20.0 kl	13.0 g
Marjoram	One	46.3 e	-	35.3 c	-
	Two	37.0 i	31.5 d	24.3 h	21.5 c
	Three	28.0 lm	25.0 g	21.6 j	18.0 d
Eucalyptus	One	48.0 d	-	29.6 e	-
	Two	36.6 i	33.0 c	24.6 h	16.5 e
	Three	28.3 l	25.0 g	23.0 i	13.0 g
Lemongrass	One	41.0 gh	-	27.3 g	-
	Two	24.6 n	23.0 h	18.0 m	15.0 f
	Three	21.0 p	21.5 i	13.6 o	10.0 i
Halfa-Gar	One	43.0 f	-	30.3 e	-
	Two	27.0 m	26.5 f	20.6 k	13.0 g
	Three	23.3 o	23.0 h	15.0 n	11.5 h
Artemisia	One	48.6 d	-	33.6 d	-
	Two	31.6 j	31.5 d	23.3 i	21.5 c
	Three	29.6 k	26.5 f	17.6 m	18.5 d
Chitosan	One	40.3 h	-	28.3 f	-
	Two	27.3 lm	26.5 f	20.0 kl	16.5 e
	Three	21.0 p	18.0 k	12.6 p	13.0 g
Soft guard	One	42.0 fg	-	30.3 e	-
	Two	25.0 n	23.0 h	19.3 l	13.0 g
	Three	21.6 p	20.0 j	15.0 n	10.0 i
Control	One	56.3 c	-	45.0 b	-
	Two	60.3 b	48.0 b	51.6 a	36.5 b
	Three	63.6 a	61.5 a	52.3 a	48.0 a

Values followed by the same letter are not significantly differed (P= 0.0 5).
***, Data not determined.**

The most effective materials were lemongrass, chitosan and soft guard when applied three times which caused the lowest white and gray mould incidence reach it 21.0, 21.0, 21.6% and 13.6, 12.6, 15.0%, respectively during Autumn 2006 – 2007 growing season. On the other hand, these materials minimized the disease incidence caused by white and gray mould during Autumn 2007 -2008 growing season, whereas the disease incidence reached it 21.5, 18.0, 20.0% and 10.0, 13.0, 10.0%, respectively. Moderate effect was obtained with lemongrass and soft guard when applied two times as well as thyme and halfa-gar when applied three times which recorded the

lowest white and gray mould, whereas the disease incidence were 24.4, 25.0, 27.3, 23.3% & 18.0, 19.3, 20.0, 15.0% and 23.0, 23.0, 21.5, 23.0% & 15.0, 13.0, 13.0, 11.5%, respectively during the first and the second Autumn growing seasons. While other materials were less effective.

d. Stored in refrigerator followed by shelf life conditions and natural infection

Data presented in Table (6) revealed that all materials significantly reduced postharvest diseases of green bean pods stored under refrigerator conditions followed by shelf life and natural infection.

Table 6. Effect of preharvest application with some materials on postharvest diseases of green bean pods cv. Paulista stored in refrigerator followed by shelf life conditions and natural infection during Autumn growing seasons

Materials	No. of applications	Disease incidence			
		2006 / 2007		2007 / 2008	
		Under refrigerator conditions	Under shelf life conditions	Under refrigerator conditions	Under shelf life conditions
Thyme	One	1.0	8.3 cd	-*	-
	Two	0.0	5.6 ef	0.0	3.0 e
	Three	0.0	3.0 gh	0.0	1.5 f
Marjoram	One	1.0	10.0 c	-	-
	Two	0.0	6.6 de	0.0	11.5 b
	Three	0.0	3.6 gh	0.0	6.5 cd
Eucalyptus	One	0.3	9.6 c	-	-
	Two	0.0	8.3 cd	0.0	11.5 b
	Three	0.0	5.6 ef	0.0	6.5 cd
Lemongrass	One	0.3	6.6 de	-	-
	Two	0.0	3.6 gh	0.0	1.5 f
	Three	0.0	2.0 h	0.0	1.5 f
Halfa-Gar	One	0.6	5.6 ef	-	-
	Two	0.0	4.3 fg	0.0	6.5 cd
	Three	0.0	3.3 gh	0.0	3.0 e
Artemisia	One	1.0	7.3 de	-	-
	Two	0.0	5.6 ef	0.0	8.0 c
	Three	0.0	5.0 f	0.0	6.5 cd
Chitosan	One	0.3	5.3 ef	-	-
	Two	0.0	4.0 fg	0.0	5.0 d
	Three	0.0	2.6 h	0.0	3.0 e
Soft guard	One	0.6	8.0 cd	-	-
	Two	0.0	7.0 de	0.0	6.5 cd
	Three	0.0	3.3 gh	0.0	3.0 e
Control	One	1.6	16.3 b	-	-
	Two	2.0	19.0 a	3.0	21.5 a
	Three	4.6	20.3 a	5.0	23.0 a

Values followed by the same letter are not significantly differed (P= 0.0 5).
***, Data not determined.**

Complete inhibition of potsharvest diseases was obtained with all materials when applied two or three times and stored under refrigerator conditions. As for storage under shelf life after refrigerator conditions. Results indicate that the most effective materials were lemongrass, chitosan, soft guard , thyme and halfa-gar when applied three times which recorded the lowest postharvest disease incidence reached it 2.0, 2.6, 3.3, 3.0, 3.3% and 1.5, 3.0, 3.0, 1.5, 3.0%, respectively during Autumn 2006 - 2007 and 2007 - 2008 growing seasons. Treated bean plants with lemongrass two times were caused the lowest postharvest disease incidence reach it 3.6 and 1.5%, respectively during the first and the second Autumn growing seasons. Other materials showed moderate effect.

d. Stored in refrigerator followed by shelf life conditions and artificial inoculations

Obtained results in Table (7) and (8) showed that all materials significantly reduced the white and gray mould diseases of green bean pods stored in refrigerator followed by shelf life conditions and artificially inoculated. The most effective materials under refrigerator conditions were lemongrass, chitosan and soft guard when applied three times which caused the lowest white and gray mould incidence reach it 5.6, 5.3, 5.6% and 4.6, 3.3, 3.6%, respectively during Autumn 2006 -2007 growing season as showen in Table (7). On the other hand, the same materials minimized the disease incidence caused by white and gray mould during Autumn 2007 - 2008 growing season , whereas the disease incidence were 6.5, 6.5, 8.0% and 5.0, 5.0, 5.0%, respectively as showen in Table (8). As for storage under shelf life after refrigerator conditions, the same previous materials caused the lowest white and gray mould, whereas the disease incidence reached it 25.4, 23.0, 25.2% and 22.0, 20.3, 21.0% respectively during Autumn 2006 -2007 growing seasons (Table 7). Furthermore, during 2007 – 2008 growing season, lemongrass, chitosan and soft guard were the best materials which caused the lowest white and gray mould incidence reached it 36.0, 36.5, 38.0% and 28.0, 26.5, 31.0%, respectively as showen in Table (8). Moderate effect was obtained with thyme and halfa-gar when applied three times which were recorded white and gray mould incidence 34.3, 34.3% and 28.0, 27.3%, respectively during 2006 -2007 Autumn growing season. At the same time the disease incidence of white and gray mould were 36.5, 38.0% and 28.0, 28.0%, respectively during 2007 -2008 Autumn growing season. Other materials were less effective.

f. Effect of preharvest application with different essential oils and chitosan on some quality characters and total green bean pods yield

The results indicated that all materials has positive effects on some quality characters *i.e.* pod diameter and number of malformed pods as showen in Table (9). All tested materials increased pod yield during two Autumn growing seasons. The highest increase was obtained with chitosan and lemongrass which increased the pod yield more than 40.0 and 30.0% respectively. Moderate increase was obtained with thyme and soft guard which increased the green bean pods yield by 20.0% during two Autumn

growing seasons. Other materials showed less effective. The results showed that the highest infections were recorded in Autumn growing season, as compared to Winter growing season

Table 7. Effect of preharvest application with some materials on postharvest diseases of green bean pods cv. Paulista stored under refrigerator followed by shelf life conditions and artificial inoculated during 2006-07Autumn growing season

Materials	No. of applications	Disease incidence					
		White mould			Gray mould		
		Days after storage			Days after storage		
		Under refrigerator conditions		Under shelf life conditions	Under refrigerator conditions		Under shelf life conditions
		7	14	4	7	14	4
Thyme	One	9.3 kl	16.0 e	55.0 f	3.6 q	9.6 g	37.3 h
	Two	8.3 lm	13.3 gh	41.6 m	2.6 rs	7.3 ij	31.3 m
	Three	3.6 tu	9.6 jk	34.3 p	1.3 uv	7.0 jk	28.0 o
Marjoram	One	10.6 i	18.0 d	57.6 d	5.0 no	14.3 d	38.3 g
	Two	9.3 kl	13.3 gh	45.6 i	2.6 rs	11.0 f	34.3 k
	Three	4.3 st	11.0 i	36.6 o	1.6 tu	8.6 h	31.3 m
Eucalyptus	One	8.3 lm	19.0 c	56.6 e	5.0 no	10.6 f	40.3 f
	Two	6.0 pq	15.6 ef	44.0 j	2.3 st	7.0 jk	36.3 i
	Three	3.3 uv	12.6 h	38.3 n	2.0 stu	5.3mn	29.3 n
Lemongrass	One	5.6 q	13.6 g	44.0 j	3.3 qr	8.6 h	38.6 g
	Two	2.6 vw	9.6 jk	39.0 n	1.3 uv	6.3 kl	31.0 m
	Three	1.6 xy	5.6 q	25.4 q	0.6 vw	4.6 op	22.0 p
Halfa-Gar	One	7.6 mn	16.0 e	46.6 h	6.3 kl	10.3 fg	42.0 e
	Two	4.0 stu	13.3 gh	43.3 jk	4.0 pq	8.6 h	35.3 j
	Three	2.6 vw	10.6 i	34.3 p	2.6 rs	7.0 jk	27.3 o
Artemisia	One	10.3 ij	19.3 c	50.3 g	10.6 f	12.6 e	47.3 d
	Two	6.6 op	16.3 e	45.6 i	7.3 ij	10.6 f	37.3 h
	Three	4.6 rs	13.6 g	42.3 lm	4.0 pq	8.0 hi	34.6 jk
Chitosan	One	4.3 st	12.6 h	42.6 kl	3.6 q	8.6 h	35.0 jk
	Two	2.3 wx	7.3 no	37.0 o	1.6 tu	5.6 lmn	30.6 m
	Three	1.0 y	5.3 qr	23.0 r	0.3 w	3.3 qr	20.3 q
Soft guard	One	5.6 q	15.0 f	45.3 i	6.0 lm	10.6 f	37.3 h
	Two	3.3 uv	10.3 ij	39.0 n	3.3 qr	6.3 kl	33.0 l
	Three	1.6 xy	5.6 q	25.2 q	1.3 uv	3.6 q	21.0 q
Control	One	13.6 g	28.3 ab	67.0 c	13.3 e	15.3 c	54.3 c
	Two	16.0 e	27.6 b	71.3 b	13.0 e	17.6 b	58.3 b
	Three	19.3 c	29.0 a	89.0 a	15.0cd	19.6 a	74.0 a

Values followed by the same letter are not significantly differed (P= 0.0 5).

Table 8. Effect of preharvest application with some essential oils and chitosan on postharvest diseases of green bean pods (cv. Paulista) stored under refrigerator followed by shelf life conditions and artificially inoculated during 2007 - 08 Autumn growing season

Materials	No.of applications	Disease incidence					
		White mould			Gray mould		
		Days after storage			Days after storage		
		Under refrigerator conditions		Under shelf life conditions	Under refrigerator conditions		Under shelf life conditions
		٧	١٤	٤	٧	١٤	٤
Thyme	Two	8.0 i	13.0 f	41.5 e	1.5 j	8.0 f	31.5 f
	Three	1.5 l	8.0 i	36.5 g	0.5 j	6.5 g	28.0 g
Marjoram	Two	8.0 i	10.0 h	40.0 e	1.5 j	11.5e	33.0 e
	Three	3.0 k	8.0 i	38.0 f	0.5 j	8.0 f	31.5 f
Eucalyptus	Two	3.0 k	16.5 e	41.5 e	1.5 j	8.0 f	36.5 d
	Three	1.5 l	11.5 g	38.6 f	0.5 j	6.5 g	31.5 f
Lemongrass	Two	3.0 k	16.5 e	43.0 d	1.0 j	6.5 g	33.0 e
	Three	1.5 l	6.5 j	36.0 g	0.5 j	5.0 h	28.0 g
Halfa-Gar	Two	3.0 k	13.0 f	45.0 c	1.5 j	8.0 f	35.0 d
	Three	1.5 l	11.5 g	38.0 f	0.5j	6.5 g	28.0 g
Artemisia	Two	8.0 i	11.5 g	45.0 c	3.5 i	11.5e	38.0 c
	Three	3.0 k	10.0 h	41.0 e	1.5 j	8.0 f	36.5 d
Chitosan	Two	3.0 k	8.0 i	38.0 f	1.5 j	8.0 f	28.0 g
	Three	1.5 l	6.5 j	36.5 g	0.5 j	5.0 h	26.5 h
Soft gaurd	Two	3.0 k	10.0 h	43.0 d	1.5 j	6.5 g	36.5 d
	Three	1.5 l	8.0 i	38.0 f	0.5 j	5.0 h	31.0 f
Control	Two	18.0 d	26.5 b	70.0 b	13.0 d	18.0b	56.0 b
	Three	21.5 c	28.0 a	93.0 a	16.5 c	21.5a	78.0 a

Values followed by the same letter are not significantly differed (P= 0.0 5).

Table 9. Effect of preharvest application with different essential oils and chitosan on some qualitycharacters and total green bean ods yield during the first harvest

Materials	Pod quality characters		Green bean pods Yield (kg / m^2)			
			Autumn growing seasons			
	Pod diameter (mm)	Number of malformed pods %	2006 / 07		2007 / 08	
			Yield	Increase %	Yield	Increase %
Thyme	7.3 abc	3.6 bc	1.1 bc	20.9	1.2 ab	20.0
Marjoram	6.9 bc	3.3 c	1.0 c	9.9	1.1 b	10.0
Eucalyptus	6.6 cd	1.6 de	1.1 b c	20.9	1.1 b	10.0
Lemongrass	7.5 abc	1.3 e	1.3 a	42.8	1.3 ab	30.0
Halfa-Gar	6.9 abc	3.3 c	1.0c	9.9	1.1b	10.0
Artemisia	6.7 cd	3.3 c	1.0c	9.9	1.1b	10.0
Chitosan	7.5 abc	2.6 cd	1.3 a	42.8	1.4a	40.0
Soft guard	7.3 abc	3.3 c	1.1 bc	20.9	1.2ab	20.0
Control	5.8 e	7.0 a	0.91 d	_	1.0 c	_

Values followed by the same letter are not significantly differed (P= 0.0 5).

DISCUSSION

The importance of White and gray moulds, caused by *Sclerotinia sclerotiorum* and *Botrytis cinerea* respectively as diseases attack green bean pods, were reported by many investigators (Suzuki *et al.,* 1995; Huang *et al.,* 2000; Huang *et al.,* 2002; Huang *et al.,* 2006 and Xu *et al.,* 2006). There is no vailable resistant varieties against white and gray moulds of green bean pods because resistant beans showed high resistance during the period before flowering, and low resistance at pod formation and maturity stages (Barnaveta, 2004).

In the present study, survey was carried out in different commercial markets *i.e* 6- October, El- Obour, El-Moneeb, El-Lewaa and Badr City at three times during growing season for identifying and determining the important fungi that attack green bean pods . Data of this research indicated that white and gray moulds were more severe in Autumn growing season than Winter growing season. This phenomena is mostly due to the high relative humidity and optimal temperature prevailing during the flowering stage of Autumn growing season, which are higher and more favorable to the diseases than in Winter growing season . In this regards, Panja and Jana (2001) reported that white mould disease of french bean caused by *S. sclerotiorum* attacks the green pods, stalks, pedicels, young branches, and petiole bases, which covered by dense cottony white mycelia under a temperature range of 16 - 20^{o}C coupled with the wetness of aerial parts and high soil moisture. They also added that grayish black to black sclerotia were formed externally on all affected parts except on green pods where they were produced both internally and externally.

Our results showed that *S. sclerotiorum* and *B. cinerea* are the most important fungi, which recorded higher frequency in all different markets, at rate of 28.5 and 24.9%, respectively. This phenomenon was observed in all surveyed locations. Many reports have been published in this concern by Ragab (1980), Wong *et al.* (1980), Huang *et al.* (2002), Vieira *et al.* (2003) and Andrees *et al*. (2006). In this concern, Nelson (1998) reported that once white and gray moulds become established in a field it is very difficult to manage. Also, Bag (2000) reported that sampling of the commercial green bean pods plots showed that 10-85% of the pods infected with *S. sclerotiorum* and rotten, causing severe losses in total production and reducing the market value of the production.

In the present study different essential oils *i.e.* thyme, marjoram, eucalyptus, halfa-gar, artemisia and lemongrass were tested to study their inhibitory effect against *S. sclerotiorum* and *B. cinerea* fungi *in vitro* and for controlling gray and white moulds diseases.

Illustrated results showed that complete inhibition of linear growth of *S. sclerotiorum* and *B. cinerea* was obtained with chitosan at 0.06% and thyme, marjoram, eucalyptus and soft gaurd at 3.0%, halfa-gar and artemisia at 1.0% and lemongrass at 0.5%. On the other hand, chitosan, thyme, eucalyptus, lemongrass and soft gaurd at the same concentration caused

100.0% and more than 72.8% inhibition in spores and sclerotial germination of *B. cinerea* and *S. sclerotiorum*, respectively.

The effect of chitosan or essential oils on the growth of several phytopathogenic fungi has not been fully elucidated, but several hypotheses have been postulated, first: its polycationic nature, it is believed that chitosan or essential oils interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (Leuba and Stossel, 1986). Second: the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis (Hadwiger and Loshke 1981). Third: the chelating of metals, spore elements and essential nutrients. Forth : the interaction of chitosan or essential oils with fungal DNA and RNA (Hadwiger and Loshke, 1981) . Fifth: Malformation of fungal mycelial. In this respect, Cheah *et al.* (1997) reported that *Sclerotinia sclerotiorum* treated with chitosan showed inexcessive mycelial branching, abnormal shapes, swelling and hyphae size reduction. Also, chitosan is not only effective in halting the growth of the pathogen but also induces marked morphological changes, structural alterations and molecular disorganization of fungal cells (El Ghaouth *et al.,* 1999 and Ait *et al.,* 2004). Moreover, El Hassni *et al.* (2004) explained that chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea*. Furthermore, Banos *et al.* (2006) cleared that by microscopic observation of fungi treated with chitosan, it can affect on the morphology of the hyphae. Effect of chitosan for inhibition of pathogenic fungi was reported by several investigators (Cheah *et al.,* 1997 and Ait *et al.,* 2004).

Essential oils have been shown to possess antifungal activity against some important plant pathogens (Isman, 2000; Meepagala *et al.,* 2002 and Kordali *et al.,* 2005). In this respect, Saleh *et al.* (2006) found that the antifungal activity of artemisia were associated with two major volatile compounds *i.e* carvone and piperitone isolated from the fresh leaves of the artimisia plants. They added that their antifungal activity was effective against *Penicillum citrinum* and *Mucor rouxii*. Moreover, Pawar and Thaker (2006) examined 75 different essential oils for their inhibitory effect on hyphal growth and spore formation of *A. niger* an opportunistic human pathogen and a strong air pollutant. They found that cinnamon, cinnamon (leaf), cassia, clove and lemongrass were the top five essential oils which demonstrated marked inhibitory effect against hyphal growth and spore formation of *A. niger*. Fungal growth inhibition may be related to lemongrass major component. In this regard, Edris and Mahmoud (2003) found that the major component of hydrocarbon and the oxygenated fractions of lemongrass oil were myrecene and citral based on fraction weight. On the other hand, Adegoke and Odesola (1996) showed that antimicrobial activity and preservative of lemongrass oil are believed to be associated with phytochemical components of lemongrass powder like alkaloids, tannins and cadiac glycosides.

Also, halfa-gar has antifungal activity against several pathogenic fungi. In this respect, Hoballah (2006) reported that the most effective extracts against *B. subtilis, E. faecalis, M. roseus, and S. lutea* were halfa-gar and

artimisia, whereas artimisia and halfa-gar showed effects against *E. carotovora, E. chrysanthem* and *X. campestris* as plant pathogen microorganisms. On the other hand, the anti-pathogenic fungal effects of the investigated medicinal plant extracts showed high active potency toward *Fusarium solani, A. flavus, A. niger* and *P. digitatum* by halfa-gar extract.

The inhibitory effect of eucalyptus against plant pathogens was reported by Hmamouchi *et al.* (1990), Harbrone and Barberan (1991) and Byron and Hall (2002). In this respect, Singh and Tripathi (1999) reported that essential oils from eucalyptus hybrid inhibited the growth of *Fusarium* spp. *Colletotrichum* sp., *Aspergillus* sp. and *Alternaria alternata.*

Regarding to thyme and its major component, thymol, Zambonelli *et al.* (1996) reported that the fungicidal activity of thyme oil against the pathogenic fungi is probably a result of chitin penetration of the hyphal wall which damages the lipoprotein cytoplasmic membrane and leading to escape of cytoplasm.

In the present study preharvest application with chitosan and or essential oils at three times during Autumn and Winter growing seasons resulted in reducing the postharvest diseases of gray and white moulds when stored under different storage conditions. In this sense, spray of chitosan at 10 days before harvest on strawberry plants reduced gray mould during fruit storage (Bhaskara *et al.,* 2000). Likewise, postharvest control was reported on table grapes treated with chitosan, under field conditions. Furthermore, Abdel-Khair and Haggag (2007) reported that lemongrass and marjoram leaves extracts reduced mycelial growth and spore germination of *Phytophthora infestans* and *Alternaria solani* the causal agents of late and early blight diseases, respectively. They added that aqueous extract of lemongrass leaves was the best one in controlling both late and early blight under field conditions. On the other hand, the effect of leaf extracts from eucalyptus for controlling soil-borne fungi was tested on the cotton wilt pathogen, *Fusarium oxysporum*, eucalypts leaf extract caused complete inhibition of mycelial growth (Kanherkar *et al.,* 2007). Similar results was obtained when soil amendment with leaves, stem, bark and fruit of eucalyptus, at 5% w/w showed significant increase in germination, shoot length, shoot weight, root length and root weight of chick-pea and mung bean plants. They added that the inoculation by *Fusarium* sp., *M. phaseolina* and *R. solani* was also reduced (Shahnaz *et al.,* 2007).

Exogenous materials with chitosan and or essential oils elicitor of host defense responses, including accumulation of chitinases, ß-1,3-glucanases and phenolic compounds, induction of lignification, synthesis of phytoalexins and inhibition of host tissue maceration enzymes (Bhaskara *et al.,* 1999 ; Zhang and Quantick, 1998 and Edris and Mahmoud , 2003).

Our results revealed that chitosan and or essential oils materials increased qualitative and quantitative of green bean pods yield. In this regard, Lafontaine and Benhamou (1996) reported that tomato yield was highly correlated with the concentration of chitosan applied to soil inoculated with *F. oxysporum f. sp. radicis-lycopersici* before seedling transplanting. Abdel-Kareem *et al.* (2002) reported that treated potato plants with chitosan

induced resistance against late and early blight diseases and increased tuber yield under field conditions.

The pronounced results in this research *i.e*. reduction the incidence of gray and white moulds and increased qualitative and quantitative of green bean pods yield my be due to its antifungal activity against pathogenic fungi *S. sclerotiorum,* and *B. cinerea*, reduced the respiration rate, ethylene production, interval O_2 levels and increased the interval CO_2 of green beans pods , increased activities of peroxidase, polyphenol oxidase enzymes and total phenol and significantly reduced the activities of cellulase (Cx), polygalacturonases (PG) and pectin methyl esterase (PME) enzymes produced by *S. sclerotiorum and B. cinerea in vitro* and *in vivo.*

REFERENCES

Abdel-Kareem, F.; Abd Alla, M.A. and EL-Mohamady, R.S.R. (2002). Induced resistance in potato plants for controlling late blight disease under field conditions. Egypt Journal Applied Sc., 17(2):51-66.

Abdel-Khair, H. and Haggag, Wafaa, M. (2007). Application of some Egyptian medicinal plant extracts against potato late and early blights. Journal of Agric.and Biol.Sci., 3(3):166-175.

Abuel-Ela, M.M.G. (1993). Studies on white mould disease caused by *Sclerotinia sclerotiorum* on some vegetable crops. M.Sc. Thesis, Fac. Agric. Cairo Univ., 83pp.

Adegoke, G. O. and Odesola, B. A. (1996). Storage of maize and cowpea and inhibition of microbial agents of biodeterioration using the powder and essential oil of lemongrass (*Cymbopogon citratus*). International Biodeterioration and Biodegradation, 6:81-84.

Ait, B. E.; Eullaffroy, P.;Cle´ment, C. and Vernet, G. (2004). Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Rep., 22:608–614.

Andrees, A.J.L.; Rivera Martinez, A. and Pomar, B. F. (2006). Telluric pathogens isolated from bean plants with collar and root rots in north west spain. Spanish Journal of Agricultural Research, 4(1):80-85.

Angelini, P.; Pagiotti, R.; Menghini, A. and Vianello, B. (2006). Antimicrobial activities of various essential oils against food borne pathogenic or spoilage moulds. Annals of Microbiology, 56(1): 65-69.

Bag, T. K. (2000). An out break of watery pod rot of French bean in the hills of Arunachal Pradesh. Journal of Mycology and Plant Pathol., 30(1):130.

Bagamboula, C.F.; Uyttendaele, M. and Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonni* and *S. flexneri*. Food Microbiology, 89:165-169.

Banos, S. B.; Hernandez, L. A.N.; Velazquez, M. G.; Hernandez, l. M.; Ait, B. E.; Bosquez, M. E. and Wilson, C. L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Protection, 25:108-118.

Barnaveta, E. (2004). Dynamics of pathogenic agents from beans (*Phaseolus vulgaris* L.) crops at the agricultural research and development station of Podu-lloaiei. Cercetari Agronomice in Mouldova, 37/38(1/4): 41-49.

Barnett, H.L. and Hunter, B.B. (1972). Illustrated Genera of Imperfect Fungi. Burgess Publication Co., Minnesota, 214pp.

Bautista, B.S.; Hernandez, L.M. and Bosquez, M.E. (2004). Growth inhibition of selected fungi by chitosan and plant extracts. Mixican Journal Phytopathol., 22:178-186.

Benhamou, N. (1996). Elicitor-induced plant defence pathways. Trends Plant Sci., 1:233–240.

Bhaskara, B.M.V.; Arul, J.; Angers, P. and Couture, L. (1999). Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. Journal Agric. Food Chem., 47:1208–1216.

Bhaskara, B.M.V.; Angers, P.; Castaigne, F. and Arul, J. (2000). Chitosan effects on blackmould rot and pathogenic factors produced by *Alternaria alternata* in postharvest tomatoes. Journal Am. Soc. Hort.Sci., 125:742–747.

Brown, W. (1924). Two mycological methods. II.A method cutting a hyphal tip. Ann. Bot., 38: 404.

Byron, E. M. and Hall, A. M. (2002). Inhibition of common cereal pathogenic fungi by clove oil and eucalyptus oil. The Bcpc Conference, Pests and Diseases, Vol.1and 2:765-768.

Cheah, L.H.; Page, B.B.C. and Sheperd, R. (1997). Chitosan coating for inhibition of sclerotina carrots. N. Z. J. Crop Hort. Sci., 25:89–92.

Dimitra, J.D.; Ziogas, B.N. and Polissiou, M.G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea, Fusarium* sp. and *Clavebacter michiganesis sub sp. michiganesis*. Crop Protection, (22):39-44.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometric, 11:1-42.

Edris, A. E. and Mahmoud, S.Y. M. (2003). Relationship between certain volatile components of lemongrass oil and its antiviral activities against bean yellow mosaic potyvirus. Bulletin of the National Research Center, Cairo, 28(31):289-299.

El-Ghaouth, A.; Smilanick, J. L.; Brown, G.E.; Wisniewski, M. and Wilson, C.L. (1999). Application of *Candida saitoana* and *glycolchitosan* for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. Plant Dis., 84:243–248.

El-Hassni, M.; El-Hadrami, A.; Daayf, F.; Barka, E.A. and El-Hadrami, I. (2004). Chitosan, antifungal product against *Fusarium oxysporum f. sp. albedinis* and elicitor of defence reaction in date palm roots. Phytopathologix Mediterranea, 43(2):195-204.

El-Sherbieny, S. N; Zakey, W. H. and Abdel Ghafar, S. N. (2002). Antifungal action of some essential oils against fungi causing cotton seedling damping-off disease. Annals of Agricultural Science, Cairo, 74 (3):1009-1020.

Ezekiel, W.N.(1930). Modified procedure with the Kiett single spore method. Phytopatgology, 20: 583-586.

Feng, W. and Zheng, X. (2007). Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food Control, (18):1126-1130.

Gilman, J.C. (1957). A manual of soil fungi. The Iowa State College Press Ames. Iowa, 450pp.

Hadwiger, L.A. and Loshke, D.C. (1981). Molecular communication in host-parasite interaction: Hexosamine polymers (chitosan) as regulator compounds in recespecific and other interactions. Phytopathology, 71:756-762.

Harbrone, J.B. and Barberan, F.A. (1991). Ecological chemistry and Biochemistry of plant Terpenoids. Claredod Press Oxford, pp.400-426.

Hmamouchi, M.; Tantaui,E.A.; Es, S.N. and Agoumi,A. (1990). Elucidation of the antibacterial and antifungal properties of the essential oils of Eucalyptus. Plants Med. Phytopath., 24:278-289.

Hoballah, A.M.E. (2006). Antimicrobial compounds in some local medicinal plants. Ph.D.Thesis,Fac. Agric.,Cairo Univ., Egypt, 120pp.

Huang, H.C.; Bremer E.; Hynes, R.K. and Erickson, R.S. (2000). Foliar application of fungal biocontrol of white mould of dry bean caused by *Sclerotinia sclerotiorum*. Biological Control.18 (3) : 270-275.

Huang, H.C.; Mundel, H.H.; Saindon, G.; Erickson, R.S. and Chang, M. (2002). Physiological resistance of dray bean (*Phasiolus vulgaris* L.) to *Sclerotinia sclerotiorum* (Lib) de Bary and *Botrytis cinerea* Pers:Fr. Revista Mexicana de Fitopatologia, 20(2):182-186.

Huang, H.C.; Erickson, R.S.; Phillippe L.M.; Mueller, C.A.; Sun, S.K. and Huang, J.W. (2006). Control of apothecia of *Sclerotinia sclerotiorum* by soil a mendment with S-H mixture or Perlka Reg. in bean, canola, and wheat fields. Soil Biology and Biochemistry, 38(6):1348-1352.

Isman, B.M. (2000). Plant essential oils for pest and disease management. Crop Prot., 19:603-608.

Kanherkar, S. H.; Shahare, N. H. and Pachkhede, A. U. (2007). Efficacy of some plant extracts against *Fusarium oxysporium*. Journal of Plant Disease Sciences, 2(2):224-225.

Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala. A. and Yildirim, A. (2005). Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, A. santonicum* and *A. spicigera* essential oils. Journal Agric. Food Chem., 53:9452-9458.

Leuba, J.L. and Stossel, P. (1986). Chitosan and other polyamines. In:"Antifungal activity and interaction with biological membranes"(Eds. Muzarelli,G.; Jeuniaus,C. and Graham,G.W.),chitin in nature and technology. New York Plenum Press, p., 215-222.

McGrath, M.T. (2001). Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. Plant Dis., 85:236-245.

Meepagala, K.M.; Sturtz, G. and Wedge, D.E. (2002). Antifungal constituents of the essential oil fraction of *Artemisia drancunculus* L. var. *dracunculus*. Journal Agric. Food Chem., 50:6989–6992.

Mishra, A.K. and Dubey, N.K. (1993). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. American Society for Microbiology, 60(4):1101-1105.

Nelson, B. (1998). Biology of Sclrotinia. In: Proceedings of the 10th International *Sclerotinia* work shop. 21 Jan. 1998, Fargo. ND.USA. North Dakota State University Department of Plant Pathology Fargo, ND. pp.1-5.

Nguefack, D. F. ; Dongmo, B.; Zollo, P.H.A.; Leth, V.; Nkengfack, A. E. and Poll, L.(2007). Fungicidal potential of essential oils and fractions from *Cymbopogen citratus*, *Ociumum gratissimum* and *Thymus vulgaris* to control *Atternaria padwickii* and *Bipolaris oryzae*, two seed-borne fungi of rice (*oryza sativa* L.). Journal of Essential Oil Research, 19(6): 581-587.

Ocamb, C.M. (2008). Bean, Snap (*Phaseolus vulgaris*) Gray Mould. Oregon State University Extension. Report on January1.http://plant-disease.ippc.orst.edu/articles.cfm? articale id=39.

Ocamb, C.M. and McReynolds, R.B. (2008). Strategies for controlling white and gray mould in the same field. Oregon State University Extension. Report on April 23.http://plant- isease.ippc.orst.edu/articles.cfm?articale id=39.

Panja, B. N. and Jana, J. C. (2001). White mould disease of *phaseolus vulgaris* L. from Tara region of west Bengal. J. of Interacademicia, 5 (1):121-122.

Pawar, V. C. and Thaker, V. S. (2006). *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. Mycoses, 49(4):316-323.

Ragab, Mona, M. (1980). Some pathological and physiological aspects of *Sclerotinia sclerotiorum*. M.Sc. Thesis, Fac. Agric., Caior Univ.137pp.

Saleh, M.A.; Belal, M.H. and El-Baroty, G. (2006). Fungicidal activity of *Artemisia herba-alba* Asso (Asteraceae). Journal of Environmental Science and Health, 41(3):237-244.

Segvie, K.M.; Kosalee, I.; Mastelie, J.; Pieckova, E. and Pepelijnk, S. (2007). Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against mould from damp dwellings. Appl. Microbiol., 44 (1):36-42.

Shah, D.A.; Dillard, H.R. and Cobb, A. (2002). Alternatives to vinclozolin (Ronilan) for controlling gray and white mould on snap bean pods in New York. Plant Health Progress, (September) : 1-6.

Shahnaz, D.; Younus, S. M.; Marium, T. and Tariq, M.J. (2007). Use of *Eucalyptus* sp., in control of root infecting fungi on mung bean and chick-pea. Pakistan Journal of Botany, 39(3):975-979.

Singh, J. and Tripathi, N.N. (1999). Inhibition of storage fungi of black gram (*Vigna mungo* L.) by some essential oils. Flavour Fragrance Journal, 14:42–44.

Soltan, M.H. (1993). Pathological studies on some diseases of snap bean pods during storage and exportation.M.Sc. Thesis,Fac. Agric. AL-Azhar Univ.117pp.

Somda, I.; Leth, V. and Sereme, P. (2007). Antifungal effect of *cymbopogon citratus, Eucalyptus camaldeulensis* and *Azadirachta indica* oil extract on sorghum seed borne fungi. Asian Journal of Plant Sciences, 6(8): 1182-1189.

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

Suzuki, K.; Nishimura, A.; Sugimoto, K. and Kom, T.Y. (1995). Sensitivity of bean gray mould fungus in Hokkaido to fluaziam. Annals of the Phytopathological Society of Japan, 61(4):399-404.

Vieira, R.F.;Pinto, C.M.F. and Paula, J.T. J. (2003). Chemigation with benomyl and fluazinam and their fungicidal effects in soil for white mould control on dry bean. Fitopatologia Brasileira, 28(3):245-250.

Xu, L.; Zhang, S.; Wang, Y. and Li, X. (2006). pathogenicity of *Botrytis cinerea* on postharvest grape berries. Acta Phytopathologica Sinica, 36(1):74-79.

Zambonelli, A.; Daurelio, A.Z.; Bianchi, A. and Albasini, A. (1996). Effect of essential oil on phytopathogenic fungi. Journal of Phytopathology, 144: 491-494.

Zhang, D. and Quantick, P.C. (1998). Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. Journal Hort. Sci. Biotechnol, 73:763–767.

تأثيرالمعاملة قبل الحصاد ببعض الزيوت الطيارة والكيتوزان لمقاومة مرضى العفن الابيض والرمادى فى قرون الفاصوليا الخضراء

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وجد أن الفطرين سكليروتينيا سكليروتيورم وبوترايتس سينيريا قد سجلا أعلى تواجدًا على قرون الفاصوليا الخضراء المصابة صنف بوليستا بنسبة ٢٨,٥ , ٢٤,٩ % على الترتيب , بينما كان الفطريات الأخري أقل تواجدًا.وكانت نسبة الإصابة في موسم الخريف أعلي منها في موسم الشتاء. وبينت النتائج أن الفطرين سكليروتينيا سكليروتيورم وبوترايتس سينيريا كانا أكثر الفطريات قدرة مرضية على قرون الفاصوليا الخضراء صنف بوليستا وبرونكو المجروحة وغير المجروحة على الترتيب. وعند دراسة التأثير الوقائى والعلاجى للزيوت المختلفة والكيتوزان على مرضى العفن الابيض والرمادى فى قرون الفاصوليا الخضراء صنف بوليستا أوضحت النتائج أن المعاملات زيت الزعتر٣%, والكيتوزان٠,٠٦%, وزيت حشيشة الليمون٠,٥% أدت الى انخفاض فى نسبة حدوث مرضى العفن الابيض والرمادى بمقدار ٨٩,٥، ٨٥,٨%, و ٨٤,٦ ، ٧٩,٠% على الترتيب مما يدل علي امكانية استخدامها كوسيلة للمقاومة لكلا المرضين. وتحت ظروف التخزين المختلفة فان كل المعاملات اختزلت مرض العفن الابيض والرمادى فى قرون الفاصوليا الخضراء صنف بوليستا عند معاملتها قبل او بعد الحصاد وكانت افضل المعاملات هى زيت حشيشة الليمون والكيتوزان والسوفت جارد.

الكلمات الدالة: العفن الابيض - العفن الرمادى - الفاصوليا الخضراء - أمراض مابعد - الحصاد - زيوت طيارة - كيتوزان.