EFFECT OF PREHARVEST SPRAYING TREATMENTS OF SOME ECO-FRIENDLY AGENTS WITH MODIFIED ATMOSPHERE TO CONTROL POSTHARVEST ROTS OF MELON FRUITS

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ABSTRACT

Effect of some preharvest spraying treatments such as potassium sorbate (PS), chitosan (CH), propolis (PRO) and benzoic acid combined with modified atmosphere (MA) to control postharvest decay and rots on melon fruits caused by *Fusarium semitectum, Aleternaria alternata,* and *Rhizopus stolonifer* were investigated. *In vitro trials,* potassium sorbate showed the highest direct effect against the mycelial growth of all tested fungi. Chitosan and propolis had the highest effect against A. *alternata* and *R. stolonifer,* respectively. Benzoic acid showed the highest inhibitory effect on the mycelial growth of all tested fungi. Modified atmosphere *in vitro* had low effect at all tested concentrations among all tested alternatives. *In vivo* trials, preharvest spraying treatments combined with modified atmosphere showed a high level of protection against development of postharvest rots of all tested fungi, in which benzoic acid + Modified atmosphere was the most effective treatment. Pretharvest spraying treatments combined with MA showed a low level of activity for both peroxidase and polyphenol oxidase enzymes, as well as significantly maintained the physical and chemical properties.

Key words: Melon fruit rots, Potasium sorbate, Chitosan, Propolis, bezoic acid, Modified atmosphere, *Fusarium semitectum*, *Aleternaria alternata*, *Rhizopus stolonifer*, peroxidase, polyphenol oxidase

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the important vegetable crops grown under protective conditions or open filed in Egypt and all over the world. Generally, melon fruits are a rich source of carbohydrates, vitamin A, vitamin C, folic acid and other micro and macro elements i.e. copper, iron, zinc and potassium. It is a low in calories, sodium, calcium and contains some dietary fibers.

Melon is attacked by many diseases in the filed, transportation and storage. Postharvest decay of melon fruits mainly occurs by *Fusarium* spp., *Rhizopus* spp. and *Alternaria* spp. **(Wang *et al.*, 2010)**. In addition, physiological disorders in storage may also occur such as loss of firmness and over ripening, which result in loss of marketability of melon fruits. Annually, it's estimated that, at least 15-50% of the global fruits and vegetables crops production are lost at the postharvest stage even before reaching the consumers **(FAO, 2011).**

Since the extensive use of fungicides in the management of plant pathogenic fungi resulted in sever negative environmental and agricultural impacts, there are developing international trend toward the utilization of alternative control means as plant products protection without going in fungicide treadmill.

Control strategy has been done by using natural and chemical means, in which all of them recorded as Generally Recognized as Safe (GRAS) substances

i.e. salts antioxidant, chitosan and propolis. In addition, combination of such physical mean *i.e.* modified atmosphere (MA) is involved as well.

Potassium sorbate are easy degradable salt widely used as food additive ingredients and it have been manifested to control many plant pathogenic fungi notably postharvest disease **(palou *et al.,* 2008 and Youssef and Roberto, 2014).** Chitosan a diactyation constitution of chitin abundantly investigated as an alternative control approach against several plant diseases **(Katiyar *et al*., 2015 and El-Guilli *et al*., 2016).** Propolis is a natural mixture constitution produced by honeybees from substances collected from glue trees and investigated to yield antifungal activity against postharvest diseases (**Curifuta *et al.,* 2012**). Source of antioxidants *i.e.* benzoic were investigated as alternative compounds to deter fungal plant pathogenic fungi **(Mahsa *et al*., 2015).** Modified atmosphere technologies are used as preservation mean for control the postharvest decay development beside their ability to nutritional quality and extend shelf-life of many fruit and vegetables **(Sen *et al*., 2012).**

The current work was designed to evaluate the antifungal activity of some alternative abiotic agents to deter the development of postharvest diseasein which could help to reduce the amount of fungicide. In addition, to maintain the quality of stored fruits.

MATERIALS AND METHODS

Tested fruits:

The fruits melon (*Cucumis melo* L. cv. Galia) used in the present investigation were obtained from the growing melon plants under the open filed condition at Sedment El-Gabal region, Fayoum governorate, Egypt**.**

Isolation, purification and identification of the causal organisms:

The associated fungi were isolated from rotted melon fruits collected from three different markets, *i.e.* Fayoum, Beni-Suief, 6 October during 2013 and 2014 seasons. The isolated fungi were purified by hyphal-tip technique **(Brown, 1924)**. The identification of the isolated fungi was carried out in Mycological Research and Plant Diseases Survey Department, Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.

Inoculation technique:

A small plug of mycelium from the slant ager tubes was transferred to PDA plates and incubated at 25°C; 7-8; 9-10 days and 22-28 hours for *Fusarium semitectum, Alternaria alternata* and *Rhizopus stolonifer,* respectively. The artificial inoculation of melon fruits with small plug (5mm) of the fungal mycelium was applied in different ways as follows:

1- A small plug (5mm) of the fungal culture on PDA of each isolate was putted on the fruit surface without pre- inoculation wounding.
2- A small plug (5mm) of the fungal culture on PDA of each isolate was putted on the fruit surface, in a hole made by the mean a cork borer and recovering the hole with the removed fruit pieces after inoculation.
3- A small plug (5mm) of the fungal culture on PDA of each isolate was putted on the shoot end region.

All methods of inoculation were compared with un inoculated control. Three fruits were used in each replicate and 3 replicates were used for each treatment. The fruits treated or untreated were harvested and placed into carton boxes. After 24 h form harvest, melon fruits were surfaced-disinfected using sodium hypochlorite 2% for 2 min **(Yao *et al.* (2003)**, and then washed with sterilized distilled water (SDW). After washing, fruits were left to dry at room temperature for 2 h, then placed again into carton boxes. All of the melon fruits were

inoculated with 5mm disc of the tested fungi grown on PDA medium. The developed symptoms were observed and scored as fruit rotted area in diameter (cm).

Pathogenicity test:

Melon fruits cv galia was used in pathogenicity test. Diseases infection was scrod by estimating of rotted area in diameter.

Proportion of the tested substances and concentrations:

Potassium sorbate ($C_6H_7KO_2$) (99%, PS) at three concentrations 1, 2 and 3 % (w/v) was evaluated. All of the tested concentrations were amended with PDA medium just before pouring in the petri dishes. One promising concentration *i.e* 3% were used *in vivo* trials.

Chitosan stock solution 2% (w/v) was prepared by dissolving 2 g of chitosan powder in 100 mL of 1% glacial acetic acid in distilled water, with agitation overnight as a stock according to *(***Xiao *et al.,* 2011)**. Chitosan stock solution was adjusted to 5.6 pH. *In vitro* trial, the stock soltion used to obtain the main tested three concentrations (0.5, 1 and 1.5 g/l). One promising concentration *i.e.* 1.5 g/l was used *in vivo* trials.

Propolis was hand collected from different areas in Fayoum governorate (Egypt). Twenty g from propolis was dissolved in 70 % ethanol by shaking overnight protected from light. The resulting ethanol extract was filtered three times through filter paper and concentrated to a final concentration of 3, 6 and 9 mg/mL and used for the antifungal assay **(Ozdemir *et al.,* 2010).** One promising concentration *i.e.* 1.5 g/l was used in all *in vivo* trials.

Benzoic acid at three concentrations 2, 4 and 6 g/l were evaluated. All of the tested concentrations were amended with PDA medium just before pouring in the petri dishes. One promising concentration *i.e* 9 g/l were used *in vivo* trials.

Effect of the different alternatives on the linear growth of the tested the fungi:

Three different concentrations of all of the substances (mentioned above) were evaluated for its activity *in vitro* against *Fusarium semitectum, Alternaria alternata* and *Rhizopus stolonifer*, each concentration was amended to PDA medium just before pouring in the petri dishes. Treated as well as the untreated media (control) were poured in petri dishes. Three replicates were used for each treatment and inoculated with 5 mm disc of the growing culture of the tested fungi. Plates were incubated at 25°C and the developed radial mycelial growth was measured by the linear growth in (cm) after the full hyphal growth of the control; 7-8; 9-10 days and 22-28 hours for *F. semitectum, A .alternata* and *R. stolonifer,* respectively.

Effect of preharvest spraying treatments combined with modified atmosphere (MA) developed postharvest rots of the fruits of melon:

Field experiment and treatments:

The plants of galia melon cultivar grown under the open filed condition at Sedment El-Gabal region, Fayoum governorate within 2014 and 2015 seasons used to deign the field experiment. The field plot was subdivided into four separate sections. Plants were selected for uniformity of fruit development and absence of evident any symptoms of diseases. Trials were arranged in a block randomized design with 3 lines (replicates) for each treatment and the fruits treated with water served as control.

Treatments were applied two seasons and twice during each season, the first spray was made fourty days after planting and the second spray was made two weeks before harvest. The inoculation of treated and untreated fruits was carried out as mentioned above on shoot end region. Both fruits melon were treated as preharvest as well as the control were putted in plastic pages and welded before injection with 2 % O_2 to 15 % CO_2 under air vacuum conditions

using (AUDIONVAC VMS 123), Then all fruits were stored at 10 ˚C and 95% Rh for 21 days.

Disease assessment

After inoculation of melon fruits and storage for 21 days, the disease severity was determined as the rotted area of melon fruits in diameter (cm).

Fruit quality:

Sample of the melon fruits at harvest and after storage for 21 days were taken to determine physical properties, *i.e.* general appearance and firmness, chemical properties, *i.e.* total soluble solids percentage, titratable acidity percentage and TSS/TA ratio.

General appearance was measured according to **Atress and Attia, (2011).** The firmness of the fruits was measured according to **Abbott, (1976).**
Total soluble solids % was determined according to **Atress and Attia, (2011).**
Titratable acidity percentage % of melon fruits was determined according to **AOAC (1990).**

Enzyme assay:

At harvest and after storage, sample of fruits was taken to determine enzymes activity, *i.e.* peroxidase and polyphenol oxidase enzymes.

The activity of peroxidase was assayed according to (**Biles *et al.*, 2000).**
Polyphenol oxidase Activity was determined as reported by **(Li and Steffens, 2002).**

RESULTS:

Isolation and identification of the causal organisms associated with rotted melon fruits:

Fungi associated with diseased galia melon cultivar fruits collected from Fayoum, Beni-suief, and 6 October markets during 2013 and 2014 seasons are demonstrated in **Figure (1)**. The most frequently encountered fungi (%) during seasons 2013 and 2014 was *Fusarium semitectum* with average of 29.06% and 18.76%, respectively, followed by *Altrernaria alternata* with average of 16.94 % and 17.80 % at 2013 and 2014 seasons, respectively. Meanwhile, *Rhizopus stolonifer* was frequently existed with average of 14.73 % and 15.93 % for two seasons, respectively.

Pathogenicity test of the isolated fungi:

Melon fruits at full-green stage were used in the Pathogenicity test. Data in **Figure (1)** indicated that fruits were infected by all of the tested fungi with shoot end inoculation method. Mostely, *Rhizopus stolonifer*, *Fusarium semitectum*, revealed the most pathogenic response resulting in severe rots with average diameter of the rotted area being 45.3, 7.5, 7 cm, respectively.

***In vitro* trials:**

The complete inhibition in the linear growth was obtained on *R. stolonifer* treated with all concentrations potassium sorbate and 3 % of potassium sorbate on *F. semitectum*. All tested concentrations of chitosan (CH) significantly reduced the mycelial growth of all tested fungi comparing to the control excepted at 0.5 g/l concentration on *R. stolonifer* (**Table 2).**The mycelial growth of *A. alternata* was moderately to high sensitive to CH in which the linear growth inhibited by 47.77 to 75.77% from the low to high tested concentration respectively, followed by *R. stolonifer* in which reaching its maximum inhibition at 1.5 g/l while the radial growth of *F. semitectum* was only decreased by 16.33 to 42%. The tested concentrations of propolis inhabited in moderately to low way the linear growth depending on the used dose. The radial growth of *R. stolonifer* significantly reduced with 62.22, 49.22 and 40.55% at 9, 6 and 3 mg/ml, respectively. The lowest dicreased in the linear growth was obtained at the same concentrations at *F.*

semitectum by 26.66, 12.77 and 6.11% from the high to lower dose respectively, while *A. alternata* decreased by 8.33 to 33.88% at 0.3 and 0.9, respectively.

Results in **Table (2)** also, illustrate the direct inhibitory action of benzoic acid (BEN) on the linear growth of the tested fungi. All tested concentrations of BEN significantly reduce the linear growth of *F. semitectum*, and *R. stolonifer*. The completely inhibition was obtained by 0.4 and 0.6 g/l BEN on *R. stolonifer* and at 0.6 g/l BEN on *F. semitectum.*

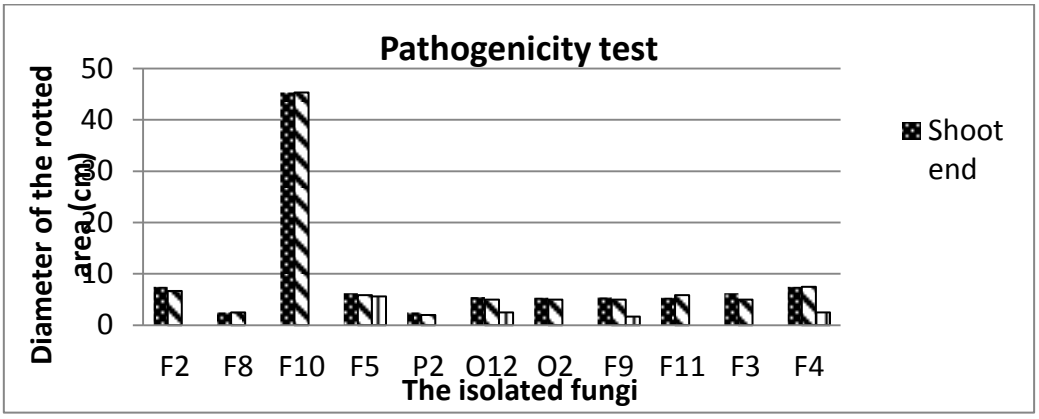


Figure1: Pathogenicity test of the fungi isolated from diseased melon fruits during storage 25 ◦C and 90% RH after 10 days.

*Fungi: F2 = *Fusarium semitectum*, F8 = *Altrernaria alternata*, F10 = *Rhizopus stolonifer*, F5 = *Trichothecium roseum*, P2 = *Penicillium spp*, O12 =*Fusarium moniliforme*, O2 =*Aspergillus ochraceous*, F9 = *Botrytis cinereaa*, F11 = *Aspergillus niger*, F3 = *Fusarium sporotrichiodios*, F4 = *Fusarium avenaceum*

Table 1: Effect of the tested alternative substances on the linear growth of the tested fungi.

Treatments	concentrations	The linear growth (cm)		
		Fusarium semitectum	***Alternaria alternata***	***Rhizopus stolonifer***
Control	---	9.00 a	9.00 a	9.00 a
Potassium sorbate	1 %	0.00 b	1.56 b	0.00 b
	2 %	0.00 b	1.50 b	0.00 b
	3 %	0.00 b	0.00 c	0.00 b
LSD 5%	**---**	**---**	**1.32**	**---**
Chitosan	0.5 g/l	7.53 b	4.70 b	9,00 a
	1 g/l	6.50 c	4.16 c	7.52 b
	1.5 g/l	5.22 d	2.18 d	3.56 c
LSD 5%	**---**	**0.37**	**0.42**	**0.11**
Propolis	3 mg/ml	8.45 ab	8.25 b	5.35 b
	6 mg/ml	7.85 b	7.15 c	4.57 c
	9 mg/ml	6.60 c	5.95 d	3.40 d
LSD 5%	**---**	**0.72**	**0.68**	**0.74**
Benzoic acid	0.2 g/l	7.03 b	7.70 b	4.66 b
	0.4 g/l	5.46 c	6.46 c	0.00 c
	0.6 g/l	0.00 d	4.99 d	0.00 c
LSD 5%	**---**	**0.94**	**1.10**	**0.46**

Different letters show significant difference at $p<0.05$ according to student's t-test.

Effect preharvest spraying applications of tested alternative substances combinated with modified atmosphere (MA) to control fruit rots:Data in Table (2) Figure (2) indicated the efficacy of combination of modified atmosphere (MA) with preharvest application

of potassium sorbate, benzoic acid, propolis and chitosan on the postharvest fungal decay. Modified control at 21%: 0.03% significantly reduced the rotted area on *F. semitectum* and *R. stolonifer* with approximately 49 and 22% respectively compared to untreated control. In varying level, all tested combination treatments significantly reduce the disease incidence of all tested fungi over untreated and MA control. MA+benzoic acid completely inhibited the development of *R. stolonifer,* while the other two fungi *i.e. F. semitectum,* and *A. alternata* was inhibited with approximately 66 and 62%, respectively. MA+chitosan treatment yielded high inhibition of disease development as compared to control by approximately 78% on *F. semitectum,* and *A. alternata* and79% on *R. stolonifer* in all tested fungi followed by MA+ potassium sorbate application, while the low activity was obtained on PRO+MA treated fruits.

Effect of preharvest spraying combined with modified atmosphere (MA) treatments on peroxidase (POD) and polyphenol oxidase (PPO) activity of galia melon fruits.

Data in **Figure (3)** illustrated that, MA control at 2% O_2: 15% CO_2 as well as all pre-harvest spray combined with MA significantly reduced the activity of POD compared to fruits control stored under the normal atmosphere (21% O_2: 0.03% CO_2). Treatment of MA+PS proved the high highest activity with significant different over MA control and all combined treatment as well. Meanwhile, MA+CH proved the low activity with no significant difference compared to MA. Control, MA+PRO and MA+BEN as well

Table 2: Effect of optimum preharvest treatment of salts, antioxidants, chitosan, propolis in combination with modified atmosphere (AM) 2% O_2:15% CO_2 treatments on diameter of the rotted area (cm) of melon fruits cv. Galia, inoculated with *Fusarium semitectum* (F2), *Alternaria alternata* (F8) and *Rhizopus stolonifer* (F10), after storage at 10 °C and 90-95 RH for 21 days during seasons 2014 and 2015.

Diameter of the rotted area (cm) 2014						
***Treat.**	**Conc.**	**F2**	**F8**	**F10**	**Mean**	**Reduction(%)**
Control	21% O_2: 0.03% CO_2	6.93 a	6.56 a	5.93 a	6.47 a	---
Control+MA	2% O_2:15% CO_2	5.30 b	4.60 b	3.52 b	4.47 b	**30.91**
MA+PS	1%	2.50 d	2.71 d	2.05 d	2.42 d	**62.59**
MA+CH	9 mg/ml	1.82 d	1.50 e	1.30 e	1.54 e	**76.19**
MA+PRO	1.5 g/l	3.83 c	3.52 c	3.02 c	3.45 c	**46.67**
MA+BEN	0.6 g/l	2.50 d	2.31 d	0.00 f	1.60 de	**75.27**
LSD 5%	--	**0.70**	**0.735**	**0.212**	**0.84**	---
VC%	--	**10.08**	**11.43**	**4.43**	**13.96**	---
Diameter of the rotted area (cm) 2015						
***Treat.**	**Conc.**	**F2**	**F8**	**F10**	**Mean**	**Reduction(%)**
Control	21% O_2: 0.03% CO_2	8.48 a	7.43 a	6.86 a	7.59 a	---
Control+MA	2% O_2:15% CO_2	5.97 b	5.10 b	3.9 b	4.99 b	**34.25**
MA+PS	1%	3.53 d	3.21 cd	2.82 c	3.18 c	**58.10**
MA+CH	9 mg/ml	2.70 e	2.13 d	1.75 d	2.19 d	**71.14**
MA+PRO	1.5 g/l	4.20 c	3.9 bc	3.25 bc	3.78 c	**50.19**
MA+BEN	0.6 g/l	3.05 de	2.7 cd	0.00 e	1.91 d	**64.83**
LSD 5%	--	**0.621**	**1.34**	**0.78**	**0.96**	---
VC%	--	**7.33**	**18.11**	**13.99**	**13.46**	---

*Treatments: PS = Potassium sorbate, CH = Chitosan, PRO = Propolis, BEN= Benzoic acid, AM= Modified atmospher Different letters show significant difference at $p<0.05$.

As shown in **Figure (3)** all tested treatments significantly reduced the activity of PPO enzymes compared to control excepted for MA+PS. The low activity was obtained by MA+CH with no significant different compared to MA control. Meanwhile, treatment of MA+PRO and MA+BEN increased the activity much more than MA+control with an significant difference.

Effect of preharvest spraying treatments combined with modified atmosphere (MA) on physical properties of melon fruits:

General appearance (GA):

Results in **Figure (4)** indicated that GA of melon fruits decreased in all applied treatments and untreated control. Such decrease in GA mostly may be due to a slight dryness of the surface fruit, instead of translucency or macroscopic decay.

Concerning the effect of preharvest spraying treatments combined with modified atmosphere (MA), data showed that there were significant differences between of preharvest spraying treatments combined with modified atmosphere (MA) treatments and untreated control on GA after 21 days of storage. However, melon fruits obtained from plants treated with CH +MA, BEN +MA, PRO +MA and PS +MA were the most effective treatments for maintaining GA at harvest and at the end of stories period (21 day) compared with untreated control. In another word: Control +MA treatment had low score of appearance fruit compared with anther treatments.

Firmness:

Result in **Figure (5)** showed that the highest value of fruit firmness was observes at harvest in all treatments and untreated control, however, the lowest one was observed after 21 days of storage. Concerning the effect of preharvest spraying treatments combined with modified atmosphere (MA) on firmness of melon fruits, data revealed that melon fruits treated with CH +MA, PRO +MA and PS +MA were the most effective treatments for maintaining fruit firmness with no significant differences between them followed by BEN +MA at harvest and after 12 days of storage. While the lowest values were with control +MA treatment followed by untreated control at harvest and after 21days of storage at 10 ^{o}C.

Effect of preharvest spraying treatments combined with modified atmosphere (MA) on chemical properties of melon fruits.

Total soluble solids % (TSS):

Data in **Figure (6)** revealed that in general the highest value of TSS % in all treatments and control were shown in the initial storage, however, the lowest one was gave after 21 days of storage. Regarding the effect of some preharvest spraying treatments combined with modified atmosphere (MA) on TSS %, data in illustrated that there were significant different in TSS % between treatments and untreated control after 21 days storage, however, melon fruits treated with CH +MA, PS +MA and BEN +MA gave the highest value of TSS content with no significant differences between them at harvest and had significant differences between them after storage for 21 days at 10 ^{o}C , while the lowest one was control+MA followed by untreated control at harvest with no significant differences between them and had significant differences between them after storage for 21 days at 10 ^{o}C.

Titratable acidity % (TA):

Results in **Figure (7)** reveled that, in general the highest value of TA content in all preharvest treatment and untreated control were sown in the initial storage (at harvest), however, the lowest one was gave after 21 days of storage. Concerning the effect of some preharvest spraying treatments combined with

modified atmosphere (MA) ,data revealed that at harvest, there were significant differences in TA content between treatments and untreated control, however, melon fruits treated with BEN +MA and CH +MA gave the highest value of TA content with no significant differences between them, fruits obtained from PS +MA and PRO+AM were less effective in this respect while the lowest value of TA with control +MA treatment and untreated control. However, after 21 days of storage showed that there were significant differences between treatments and untreated control also between them.

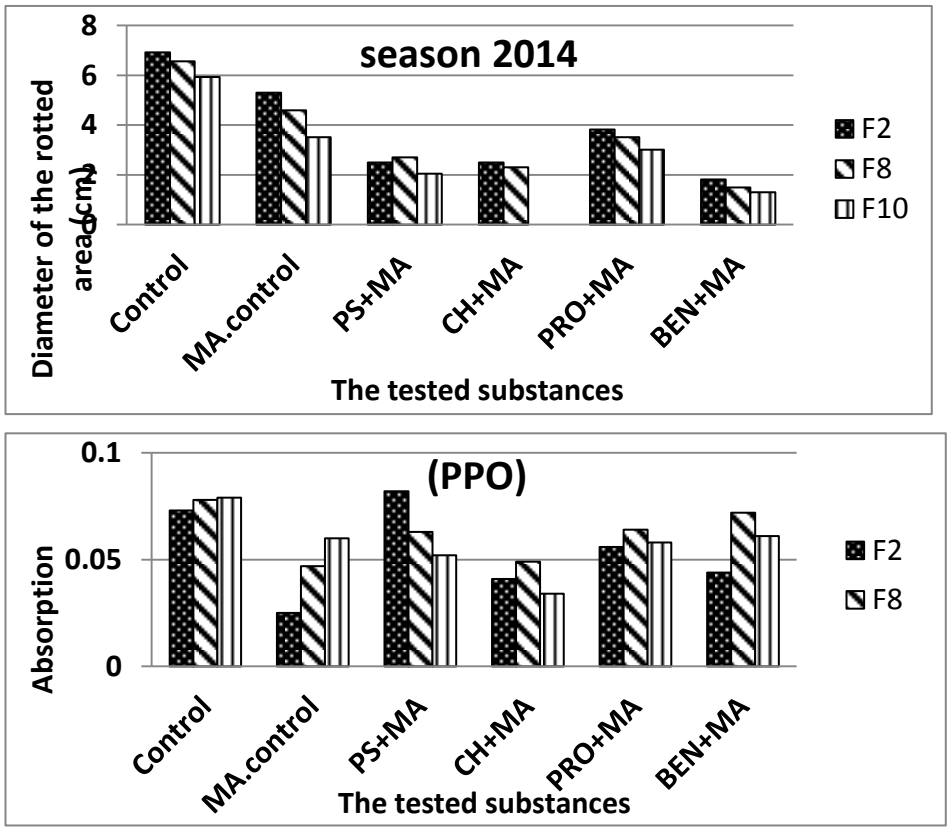


Figure 2: Effect of optimum preharvest treatment of salts, antioxidants, chitosan, propolis in combination with modified atmosphere (AM) $2\%O_2$:$15\%CO_2$ treatments on diameter of the rotted area (cm) of galia melon fruits, inoculcated with *Fusarium semitectum* (F2), *Altrernaria alternata* (F8) and *Rhizopus stolonifer* (F10), after storage 21 days at 10 °C and 90-95 RH during **2014 and 2015** seasons.

*Treatments: PS = Potassium sorbate (1%), CH = Chitosan (1.5 g/l), PRO = Propolis (9 mg/ml), BEN= Benzoic acid (0.6 g/l), AM= Modified atmosphere.

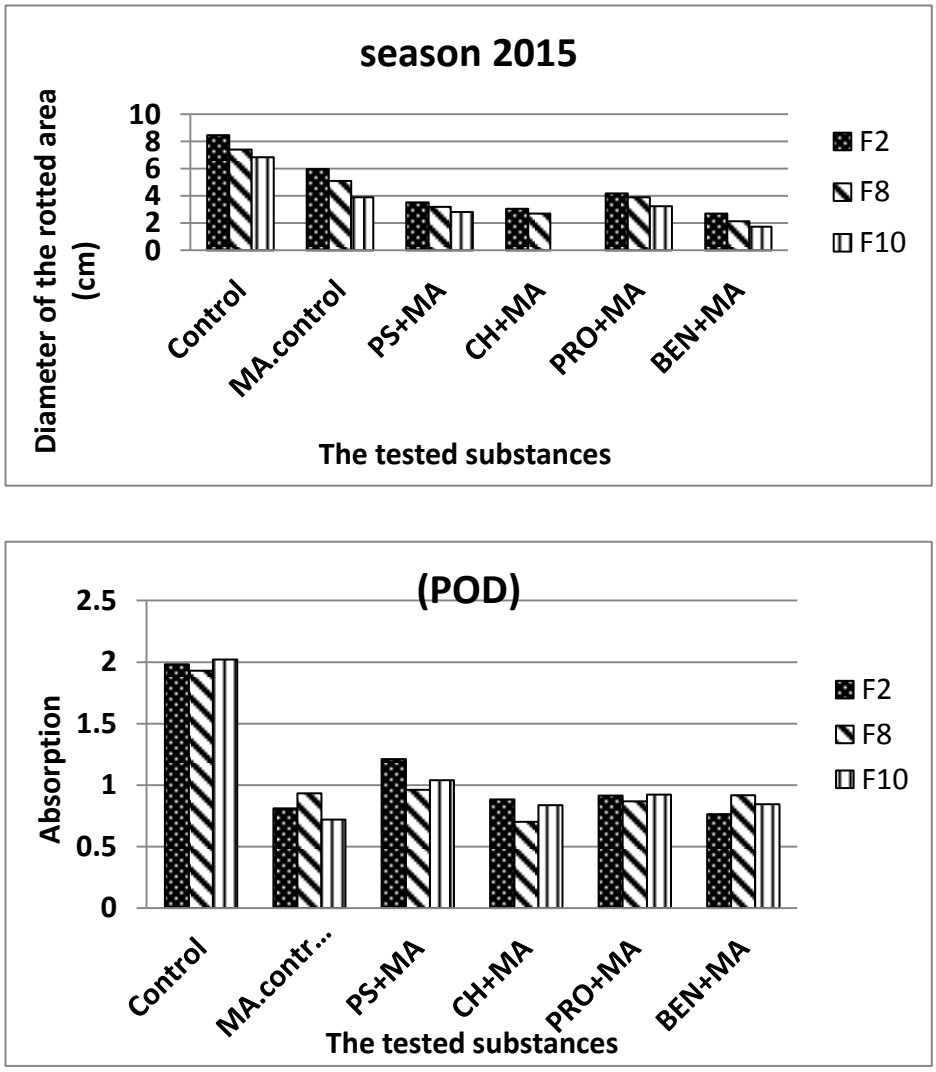
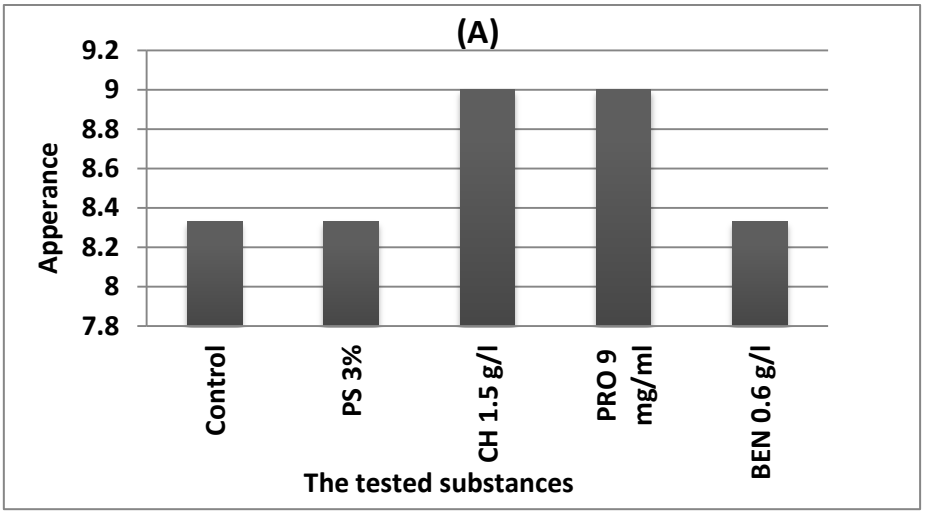


Figure 3: Effect of optimum preharvest spraying treatment combinated with modified atmosphere (AM) 2%o_2:15%co_2 treatments on peroxidase (POD) and polyphenol oxidase (PPO) activity of galia melon fruits, inoculcated with *Fusarium semitectum* (F2), *Altrernaria alternata* (F8) and *Rhizopus stolonifer* (F10), stored at 10°C and 90-95 RH for 21 days.

*Treatments: PS = Potassium sorbate (1%), CH = Chitosan (1.5 g/l), PRO = Propolis (9 mg/ml), BEN= Benzoic acid (0.6 g/l), AM= Modified atmosphere.



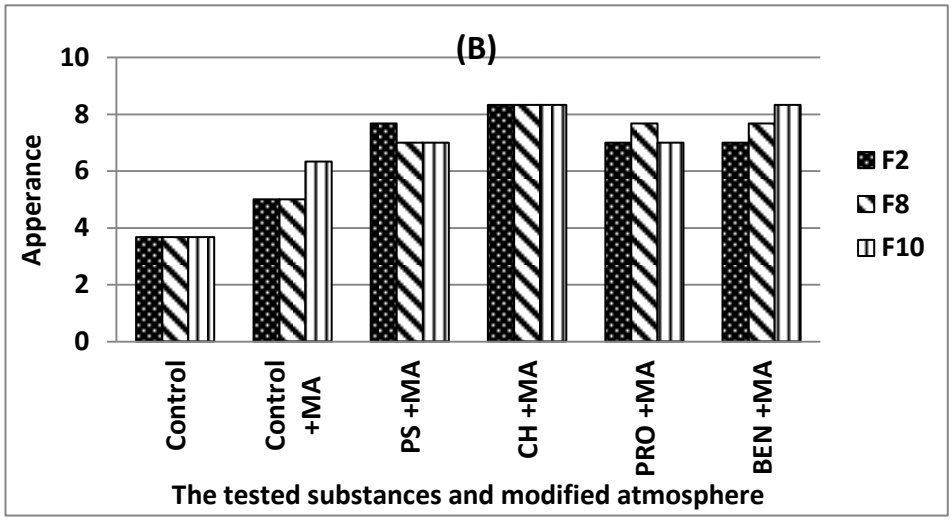


Figure 4: Effect of preharvest spraying in combination with modified atmosphere (MA) at concentration 2% O_2:15 % CO_2 on general appearance of galia melon fruits, inoculcated with *Fusarium semitectum* (F2), *Altrernaria alternata* (F8) and *Rhizopus stolonifer* (F10), stored at 10°C and 90-95 RH for 21 days.

**Treatments: PS = Potassium sorbate (1%), CH = Chitosan (1.5 g/l), PRO = Propolis (9 mg/ml), BEN= Benzoic acid (0.6 g/l), AM= Modified atmosphere.

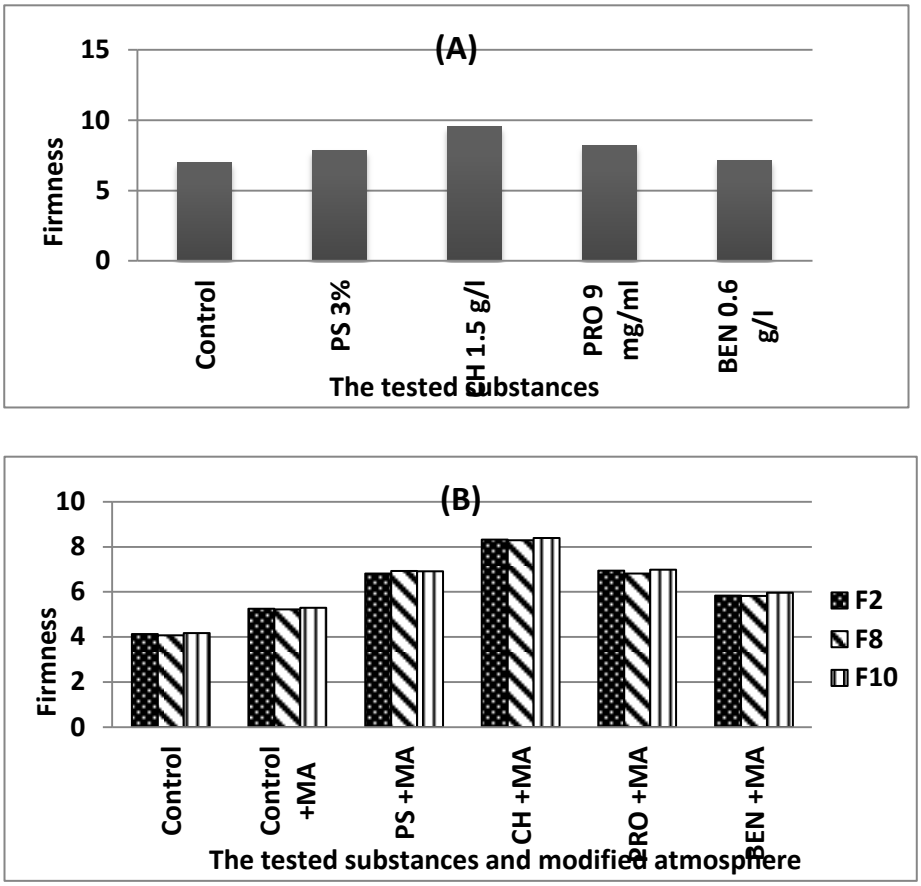


Figure 5: Effect of preharvest spraying in combination with modified atmosphere (MA) at concentration 2% O_2:15 % CO_2 on firmness of galia melon fruits, inoculcated with *Fusarium semitectum* (F2), *Altrernaria alternata* (F8) and *Rhizopus stolonifer* (F10), stored at 10°C and 90-95 RH for 21 days.

*Treatments: PS = Potassium sorbate (1%), CH = Chitosan (1.5 g/l), PRO = Propolis (9 mg/ml), BEN= Benzoic acid (0.6 g/l), AM= Modified atmosphere.

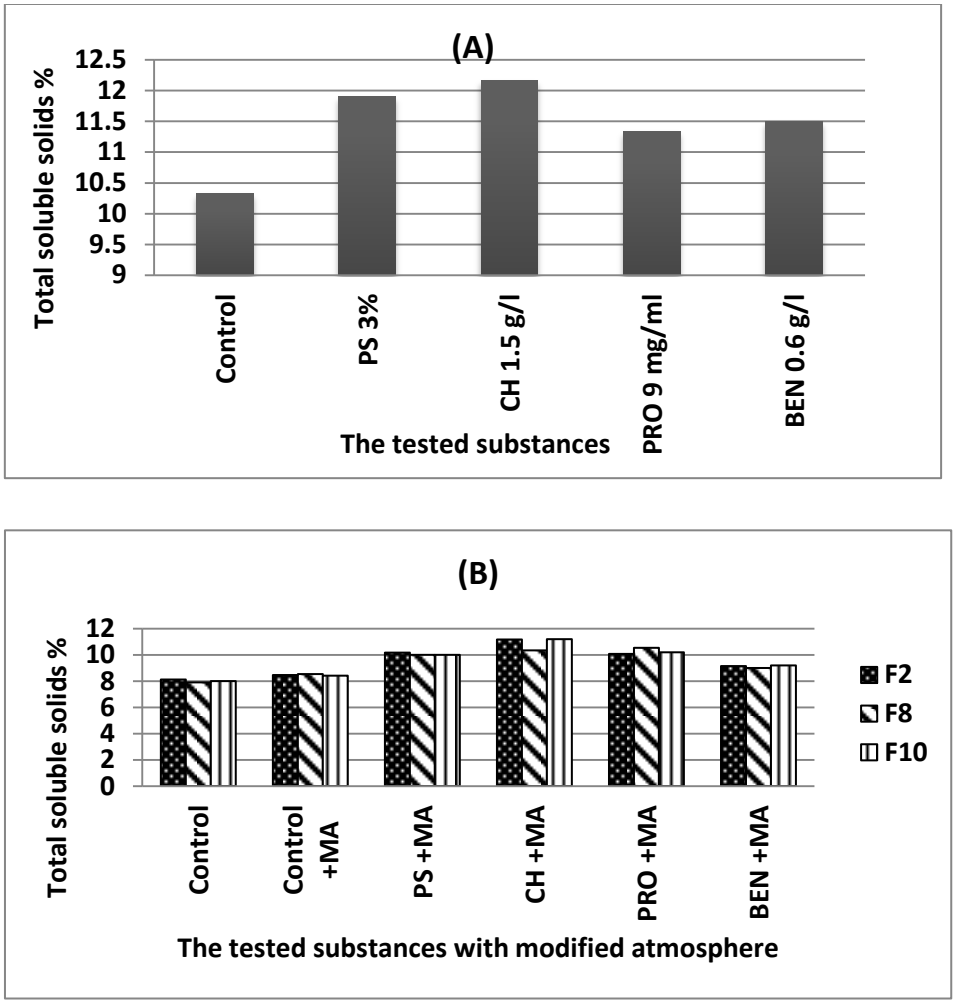


Figure 6: Effect of preharvest spraying in combination with modified atmosphere (MA) at concentration 2% O_2:15 % CO_2 on total soluble solids % (TSS) of galia melon fruits at harvest (A), inoculcated with *Fusarium semitectum* (F2), *Altrernaria alternata* (F8) and *Rhizopus stolonifer* (F10), stored at 10°C and 90-95 RH for 21 days (B).

*Treatments: PS = Potassium sorbate (1%), CH = Chitosan (1.5 g/l), PRO = Propolis (9 mg/ml), BEN= Benzoic acid (0.6 g/l), AM= Modified atmosphere.

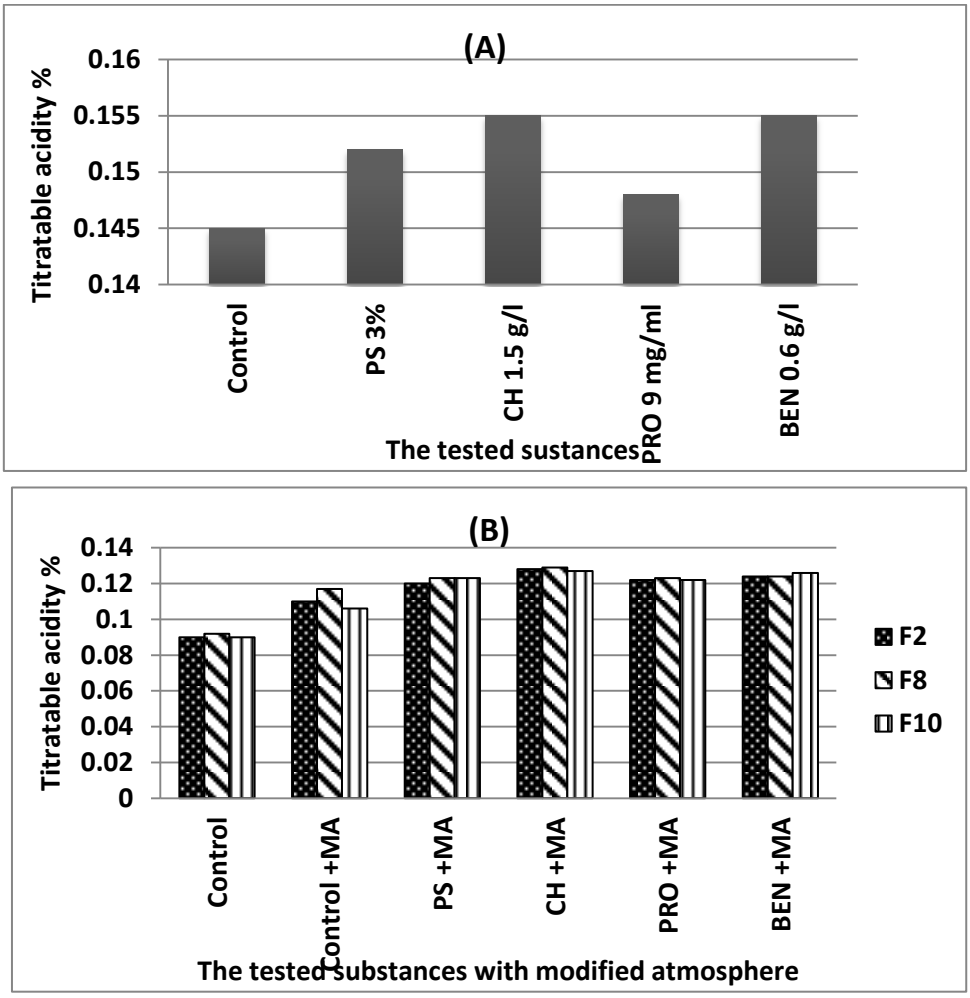


Figure 7: Effect of preharvest spraying in combination with modified atmosphere (MA) at concentration 2% O_2:15 % CO_2 on titratable acidity % (TA) of galia melon fruits at harvest (A), inoculcated with *Fusarium semitectum* (F2), *Altrernaria alternata* (F8) and *Rhizopus stolonifer* (F10), stored at 10°C and 90-95 RH for 21 days (B).

*Treatments: PS = Potassium sorbate (1%), CH = Chitosan (1.5 g/l), PRO = Propolis (9 mg/ml), BEN= Benzoic acid (0.6 g/l), AM= Modified atmosphere.

DISCUSSION

Melon postharvest diseases occurred during transportation, marketing and strorage are mainly caused by *Fusarium semitectum* (*F. semitectum*), *Aleternaria alternata* (*A. alternata*), and *Rhizopus stolonifer* (*R. stolonifer*) **(Yang *et al.*, 2006; Bi *et al.*, 2007; Wang *et al.*, 2010),** in which required an intervention action to maintain marketable produce without going into fungicide treadmill in which increase the negative environmental concerns with the increase of pathogen resistance to the used fungicides (**Secor and Rivera, 2012).**

Many previous research reported that the *in vitro* potential ability of potassium sorbate to inhibit many other fungi **(Mills *et al.*, 2004),** the ability of PS to completely deter the linear growth of such plant pathogenic fungi with respect to the tested concentrations of this results are quite similar with previous findings for instance, **Latifa *et al.*, (2007)** reported that potassium sorbate complete inhibited the mycelial growth of *P. italicum* at 2%. The mode of action of salts to detet the mycelial growth of many microorganisms is suggested to be due to the reduction of cell turgor that may causes spore and mycelium celluloses and shrinking, resulting in fungistatic action **(Karabulut *et al.*, 2003)** as well as by alteration of cell transport function and inhibition of fungal enzymes involved in the glycolytic pathway **(Sofos *et al.*, 1986).** Other suggestions indicated that PH plays an important role in the antifungal activity **(Palou *et al.*, 2001).** While other pervious findings proved that pH values of organic and inorganic salts had a minor role in their inhibitory effects **(Mecteau *et al*, 2002).**

The suppressive action of chitosan against the mycelial growth of *F. semitectum, A. alternata* and *R. stolonifer* were comparable to the control. These data support previous studies that have proved the *in vitro* activity of chitosan on linear growth of *A. alternata* (**Sánchez-Dómínguez et al., 2011),** *Rhizopus* spp. **(García-Rincón *et al.*, 2010),** and many other plant pathogenic fungi (**Yang *et al.*, 2012 and Freddo *et al.*, 2014).** The present results are convergent with **Feliziani *et al.*, (2013a)** who evaluated the *in vitro* activity of chitosan and other alternative agents against the mycelial growth of four postharvest fungi such as *Monilia laxa, B. cineria, R. stolonifer and A. alternata* , found that *R. stolonifer* was the less sensitive one to chitiosan among the tested fungi in which the linear growth reduced with 61.25%, while no mycylial growth was obtained with *A. alternata and Monilia laxa* at the same concentration. The mode of action of chitosan against plant pathogenic fungi and other microorganisms has been suggested to be due to the neutralized the plasma membrane, which under certain conditions may result in membrane destabilization **(Chio *et al.*, 2001),** and/ or by the penetration of the cell wall causes intracellular affectations **(Palma-Guerrero *et al.*, 2008)**.

The potential ability of propolis against the mycelial growth of plant pathogenic fungi have been investigated in many previous work (**Chaillou and Nazareno, 2009 and Valencia *et al.*, 2012). Curifuta *et al.* (2012)** stated that 0.5% (5mg/ml) of propolis yilded high fungal inhibition ranged from 98 to 100 % against *Penicillium expansum, Trichoderma reesei* and *Ulocladium,* while about 60% of the mycelial growth of *Alternaria alternata* and *Botrytis cinerea* were decreasing and *Fusarium* sp. growth was only decreased with 15-20 %. The mode of action of propolis back to the greater activity of their one or more of their components notably flavonoids and phenolics (**Samara de Lira *et al.*, 2012**).

The *in vitro* fungicidal activity of the tested antioxidants showed that BEN was the most effective among all tested substances at the same concentrations with complete inhibition in *F. semtictium* and *R. stlonifier*. These

results are quite similar with **Lattanzio *et al.* (1996)** they found that, BEN completely inhibited the mycelial growth of *R. stolonifer and B. cinerea*.

The *in vivo* activity of tested alternative substances of the current work revealed that all preharvest treatments in combination with modified atmosphere was relatively high in term of inhibition of the rotted area disease under storage conditions than untreated and MA+ control, however the MA+control was also significant with untreated plants. Previous results are in convergent with the present findings which prove the role of modified atmosphere application alone or in combination with other biotic or abiotic factors to deter the postharvest disease development under storage conditions **(Serradilla *et al.*, 2013).** All preharvest application proved their ability to enhance the modified conditions to deter the fungal development on stored fruits. Concerning potassium sorbate, many previous results indicated the role of preharvest applications of salts on the development of postharvest fungal decay for instance **Youssef *et al.* (2012)** found that preharvest application of several salts such as PS was more effective than postharvest dipping in the control of storage diseases of citrus caused by *P. digitatium* and *P. italicum* in which in the pre harvest application with all tested salts. Much of previous studies suggested that one of the main components of the direct activity of many salts is back to the role of PH (**Smilanick *et al.*, 2005).** However other many previous researches indicated the ability of many salts to induce resistance (IR) or by creating unfavorable environmental condtions for the pathogen invasion and multiplication (**Vendittie *et al.*, 2005)**. Generally, the motivation of enzyme activities due to potassium sorbet treatment may be not only a reason of the substances itself but may also back to the potassium elements in which it could play an important role in the activation of enzymatic systems **(Ashley *et al.*, 2006 and Feliziani *et al.*, 2013b),** in which resulting in reduce the disease incidence of many pre and post fungal diseases **(Holzmueller *et al.*, 2007; Prabhu *et al.*, 2007).**

Concerning chitosan, the ability of preharvest application of chitosan was investigated in the current work to reduce the rooted area disease caused by tested fungi and in accordance with previous reports that indicated the role of pre-harvest application against fruit decay in different crops **(Meng *et al.*, 2010 and Feliziani *et al.*, 2013a).** The mode of action of chitosan in the term of disease control, beside its dierect activity is mainly during the activation of plant defence system of the plant, by motivation of physiological changes which involved in resistance factors against pathogen infection notably synthesis of PR-proteins, lignifications and callose deposition, oxidative burst, influx and exit of ions such as calcium which involved in signaling pathways **(Bautista-Baños *et al.*, 2006 and Yin *et al.*, 2010).** Also, This results are in accordance with many previous reports that indicate the role of chitosan application increase one or more defence related enzyme **(Sun *et al.*, 2010; Wang and Gao, 2013).**Propolis showed the lowest activity to manage postharvest decay and this could back to the preharvest applications of propolis was not effective enough compared to the other substances and this is because because PRO found to have bore structural and chemical properties that showed light instability **(Meng, 2012).** In addition, the lower activity of PRO could be due to the ability of some fungi to developed counter-defence mechanisms against the flavonoids during the detoxification and / or metabolization of these antimicrobial components by fungal extracellular enzymes **(Pedras and Ahiahonu, 2005).** Concerning to the direct activity of benzoic acid, antioxidant substances such as BEN have been found to be involved in local and systemic resistances **(Durrant and Dong, 2004: van Loon *et al.*, 2006).**

The use of modified atmosphere application with low oxygen (O2) and/or high carbon dioxide (CO2) concentrations alone or in combination with the tested substances have the ability to maintained some physo-cimecal properties in varying level notblay firmness and total solid soluble contents (TSS) compared to stored control. Modified atmosphere down the respiration rate, inhibited ethylene production that induce senescence of fruits maintained fruit flesh firmness and TSS. Preharvest applications of potassium sorbate or other source of potassium were illustrated to motivate the accumulation of soluble solids mantinance of fruit firmness increase titratable acidity **(Mlikota *et al.*, 2010; Kelany *et al.*, 2011).** The present findings proved chitosan as pre application with MA had the highest ability to maintain a certain physical and chemical variables compared to control. This mainly because the ability of chitosan as coatings to provide a semi permeable film around the fruit surface which could modifies the atmosphere by reducing oxygen and releasing of carbon dioxide levels. Therefore, the respiration rate will slow down that maintaining the fruit quality and contributing to longer shelf life **(Shao *et al.*, 2012 and Das *et al.*, 2013).** Preharvest application of propolis in combination with MA also maintained the fruit quality parameters. Meanwile, other previous findings showed that PRO treatment had a little effect on TSS contents of mandarin and cherries fruits during storage **(Zahid *et al.*, 2013; Ali *et al.*, 2015).**

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تأثير بعض معاملات الرش بالمواد الصديقة للبيئة مع الجو المعدل على أعفان مابعد حصاد على ثمار الكانتالوب

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القدرة المحتملة لبعض الوسائل غير الحيوية البديلة مثل أملاح، الشيتوزان (CH)، البروبوليس (PRO)، ومضادات الأكسدة الجو المعدل(MA) قيمت لمكافحة فساد ما بعد الحصاد على ثمار الكنتالوب المتسببة عن*Fusarium semitectum, Alternaria alternata,* and *Rhizopus stolonifer*. في التجارب المعملية ،من بين الأملاح المختبرة، أظهرت سوربات البوتاسيوم أعلى تأثير مباشر ضد النمو الميسليومي لجميع الفطريات المختبرة. أثبتت كلا من المواد الطبيعيه الشيتوزان والبروبوليس تأثير واضح ضد جميع الفطريات عند زيادة التركيز، أسفرال CH عن أعلى تأثير ضد *A. alternata*، بينما البروبوليس أظهر أعلى تأثير على *R. stolonifer*. ومن بين المواد المضادة للاكسدة، أظهر حامض البنزويك أعلى فعل مثبط بشكل خاص على النمو الميسليومي على جميع الفطريات المختبرة. في المعمل كان للجو المعدل تأثير منخفض في جميع التركيزات المختبره من بين جميع البدائل التي تم اختبارها. وأجريت التجارب في الحقل رشا قبل الحصاد ثم جنبا إلى جنب مع الجو المعدل بعد الحصاد. تحت ظروف العدوى الصناعيه، أظهرت البدائل تأثير متغير ضد مسببات الأمراض. في الغالب، أظهرت معاملات الرش قبل الحصاد جنبا إلى جنب مع الجو المعدل أعلى مستوى من الحماية ضد فساد ما بعد الحصاد في كل الفطريات المختبرة، حيث كان حامض البنزويك+الجو المعدل المعامله الأكثر فعالية، حيث يثبط تماما تطور *R. stolonifer*. وقد قيمت قدرة البدائل على تعزيز آلية الدفاع في أنسجة الثمار من خلال قياس نشاط بعض الإنزيمات المرتبطة بالمقاومه في ثمار الكنتالوب مثل البيروكسيديز (POD) والبوليفينول أوكسيديز (PPO). أظهرت معاملات الرش قبل الحصاد جنبا إلى جنب مع رش الجو المعدل تقريبا المستوى الاقل لنشاط كلا من إنزيمات POD وPPO. بالإضافة لذلك، تمت دراسة تأثير البدائل على جودة الثمار(الخواص الفيزيائية والكيميائية) لثمار الكنتالوب بما في ذلك المظهر العام والصلابه، النسبة المئويه للمواد الصلبة الذائبة والحموضة. عموما، كشفت معاملات الرش قبل الحصادجنبا إلى جنب مع الجو المعدل أنها حافظت كثيرا على الخصائص الفيزيائية والكيميائية مقارنة مع الكنترول.

الكلمات الدالة: أعفان ثمار الكانتالوب، سوربات البوتاسيوم، الشيتوزان، البروبوليس، حامض البنزويك والجوالمعدل، *Fusarium semitectum, Alternaria alternata, Rhizopus stolonifer*، البيروكسيديز، البوليفينول أوكسيديز.