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EFFECT OF SOME ORGANIC ACIDS ON ANATOMICAL, PHYSIOLOGICAL CHANGES AND POST-HARVEST DISEASES OF SNAP BEAN PODS.

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ABSTRACT

Grey mold and cottony rot caused by *Botrytis cinerea* and *Pythium aphanidermatum*, respectively, were the most serious post-harvest diseases attacking snap bean. Five organic acids, i.e. ascorbic, citric, boric, salicylic and acetic, were tested for controlling both fungal diseases attacking snap bean pods of cvs, Xera and Valentino *in vivo*. The tested organic acids were sprayed on green bean plants of the two varieties for three times, at blooming stage, and repeated every 10 days as time interval between sprays till 5 days before harvesting, during seasons 2012 and 2013. The harvested snap bean pods were kept in perforated polyethylene consumer bags or in a type of modified atmosphere package (Xtend® films Easy-Tear bags). Valentino pods of control treatment showed higher susceptibility to both diseases compared with Xera pods.

Pre-harvest spray of the tested acids inhibited completely the decay development of naturally infected pods of both snap bean varieties during storage at $7\pm 1^{\circ}\text{C}$ and 90-95% RH for 18 days except acetic acid on cv. Xera at the lower concentration 0.1%, which controlled the both post-harvest diseases with efficacy about 70%. Boric, acetic and ascorbic acids showed minimum decay caused by *B. cinerea* at the high concentration (1, 0.2, 2%, respectively) compared with those treated with citric and salicylic acids. As for cv. Valentino, citric acid at 2% was the most effective treatment against fungal decay of artificially inoculated snap bean pods with *B. cinerea* as well as naturally infected pods. However, boric, citric and ascorbic acids with

all tested concentrations inhibited the cottony rot on snap bean pods artificially inoculated with *P. aphanidermatum*.

Pre-harvest spraying of snap bean with organic acids incited anatomical changes in cuticle and epidermis of pods. In this respect, spraying boric acid on green bean plants cv. Xera was the most effective treatment in increasing the thickness of epidermis of snap bean pods. The cuticle of Xera pods harvested from sprayed plants with tested organic acids were thicker than the control. Meanwhile, spraying organic acids on green bean plants cv. Valentino did not affect the epidermis layer thickness of pods. While, salicylic, acetic and citric acids sprays increased cuticle layer thickness. However, the anatomical changes either in epidermis and/or cuticle induced by pre-harvest organic acid sprays was recorded.

Phytoalexins content was detected in naturally and artificially infected snap bean pods of pre-sprayed bean plants with tested organic acids post 72 hr of harvest. The highest concentrations of coumestrol and 6- α -Hydroxyphaseollin were detected in artificially infected Valentino pods with *B. cinerea* pre-spraying with salicylic acid. Also high levels of coumestrol, kievitone as well as 6- α -Hydroxyphaseollin in snap bean pods cv. Valentino infected with *B. cinerea* and sprayed with boric acid. The highest concentration of 6- α -Hydroxyphaseollin (5048.63 $\mu\text{g}/\text{kg}$ fresh weight) was recorded in snap bean pods cv. Valentino infected with *P. aphanidermatum*. Salicylic acid increased the phaseollidin concentration in both tested varieties artificially infected with *P. aphanidermatum* than the control. Phaseollin was highly detected in artificially inoculated Xera pods with *B. cinerea* than in Valentino pods.

Keywords: *Botrytis cinerea*, Cuticle, Epidermis, Modified atmosphere packaging, Organic acids, Phytoalexins, Pre-harvest, *Pythium aphanidermatum*, Snap bean and Storage,

INTRODUCTION

Snap bean pods decay is caused by *Botrytis cinerea* during the growth in the field, storage, transportation, marketing or exportation. *Botrytis cinerea* is a pathogen on more than 200 species of vegetables during storage (Siviero and Motton, 2000 and Fahiem, 2010). Also, *Pythium* pod rot or “leak. Basal pods *Pythium aphanidermatum* is a great constraint to snap bean production (Damicone *et al.*, 2012).

Fields with a low level of *Pythium* leak may be harvested, but the disease often increases dramatically in bulk containers used for transit and storage.

Many attempts were made to protect snap bean pods against certain post-harvest diseases during marketing and storage. Some natural products including acetic acid are active antimicrobial agents and have been widely used in the management of fungal rotting of fruits and vegetables, thereby prolonging shelf life (**Pramila and Dubey, 2004**). Boric acid and calcium chloride alone or in combination as foliar sprays gave the best results in controlling *B. cinerea* on apple fruit (**Hafez and Haggag, 2007**).

Foliar application of salicylic acid or methyl jasmonate on common bean plants caused anatomical changes. The significant anatomical changes were as increase in thickness of leaflet blade, thickness of palisade and spongy parenchyma as well as thickness of midrib region of the leaflet in addition to change in the dimension of vascular bundles (**Farouk and Osman (2011)**).

Phytoalexins are compounds produced as a defense mechanism of the plant due to fungal infection or when invaded by a parasite. **Cruichshank et al. (1974)** found that the concentrations of two lipophilic pterocarpanoid phytoalexins, phaseollin and phaseollidin, in infection-droplets were influenced by host cultivar, fungal species and length of incubation phase. Phaseollin was the major component with the host cultivar Red Kidney Selection W245. **Rizk et al. (1984)** reported that phytoalexins were produced after inoculation of green bean pods with *Fusarium solani*, *Penicillium patulum* and *Phytophthora megasperma*. They isolated five phytoalexins identified as phaseollin, coumestrol, kievitone, phaseollidin and 6- α -Hydroxyphaseollin. **Van Den Heuvel and Grootveld (1978)** determined phaseollin, phaseollidin, phaseollin isoflavan, 6 α -hydroxyphaseollin in inoculated French bean leaves with three pathogenic and two nonpathogenic isolates of *Botrytis cinerea*. Phaseollin was predominant. **Diego et al. (2002)** found higher phaseollin production in resistant cultivars of Colombian bean cultivars than in susceptible ones to *Colletotrichu lindemuthianum*. **Diego et al. (2013)** evaluated isoflavonoid phytoalexin production in response to the application of salicylic acid in cotyledons of four common bean (*Phaseolus vulgaris*) cultivars. Cotyledons of

anthracnose-resistant cultivars induced by SA produced substantially higher phytoalexin contents as compared to the susceptible ones.

The present work aimed to determine the role of pre-harvest spraying with certain organic acids in minimizing the major post-harvest diseases on snap bean pods. Investigating some anatomical changes in cuticle and epidermis of treated bean plants with tested organic acids. Also, determining the phytoalexin content in treated snap bean pods during cold storage and with using modified atmosphere packaging.

MATERIALS AND METHODS

Source of organic acids and the tested fungal isolates: Five organic acids i.e. ascorbic, citric, boric, salicylic and acetic were bought from El-Gomhoria Chemical CO. One Isolate of *B. cinerea* and one isolate of *P. aphanidermatum* were previously isolated from infected snap bean pods with gray mold or cottony rot and tested for their pathogenicity at Department of post-harvest diseases, Plant Pathology Research Institute, ARC, Giza, Egypt.

Efficacy of pre-harvest organic acids spray on snap bean pods decay during cold storage:

Three sprays of organic acids were applied on green bean plants cvs. Xera and Valentino, at blooming stage, and repeated every 10 days as time interval between sprays till 5 days before harvesting, the trail was done in El-Quanater, private field region, at Qualuobia Governorate during seasons 2012 and 2013. Salicylic and acetic acids were sprayed at concentrations of 0.1 and 0.2%, ascorbic and citric acids at 1.0% and 2.0%, while boric acid was at 0.5 and 1%. The sprayed Green bean plants with plain water served as control. The experimental plot consisted of three rows of plants, each row is 3×0.7 m (12 hill per row and each hill contain two plants). Three plots were used as replicates for each treatment.

Harvested snap bean pods of each variety were divided into three groups. The first group was used for naturally infected investigation. The second and third groups were artificially inoculated with spore suspension of 4×10^5 spores/ml of *B. cinerea*, while Inoculum concentration of *P. aphanidermatum* was prepared as propagule suspension and adjusted to about 4×10^5 propagules/ml.

Three replicates were used for such treatment. Each replicate consisted of 27 pods and packed in tested packaging material. Two types of packages were used as modified atmosphere packaging (MAP) type Xtend® films Easy-Tear bags (StePac Ltd.) and in perforated polyethylene consumer bags. Naturally infected and artificially inoculated snap bean pods were stored at $7\pm 1^\circ\text{C}$ and 90 - 95% RH for 18 days. Disease severity (%) of infected pods were recorded according to **Hanounik (1986)** as follows:

$$\text{Disease incidence (\%)} = \frac{\text{number of diseased pods}}{\text{total number of treated pods}} \times 100$$

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

Where:

n = number of infected pods in each category, N = total number of pods, and

4 = maximum of numerical values of symptoms categories and v = numerical values of symptoms category

The categories were determined as follows:

1 = decayed area of the pod ranged 1- 24%, 2 = decayed area of the pod 25- 49%

3 = decayed area of the pod 50- 74%, 4 = decayed area of the pod 75- 100%

Anatomical studies (epidermis and cuticle layers thickness):

The pod samples (natural infected pods) were taken 15 days after the third spray with organic acids (Salicylic and acetic acids were sprayed at concentrations of 0.2%, ascorbic and citric acids at 2.0%, and boric acid at 1 %.) from green bean plant cvs. Xera and Valentino. The samples contained Pod No 2 from the beginning of newest branch in green bean plant. The taken vegetative specimens were killed and fixed in FAA (5 mL formalin 40%, 5 ml glacial acetic acid and 90 ml ethyl alcohol 70%) for at least 72 hours. Specimens to be sectioned are removed with forceps and washed in 50% ethyl alcohol, then dehydrated in series concentrations of ethyl alcohol, 70, 90, 95 and 100%. Dehydrated specimens were infiltrated in xylene, embedded in paraffin wax of melting point $60-63^\circ\text{C}$ in an oven, and then sectioned to 20μ in thickness (**Sass 1951**). Sections were stained using the double

stain method (fast green and safranin), cleared in xylene and mounted in Canada balsam (**Johanson, 1940**). Selected sections were examined and photographed using microscope to determine the anatomical changes in pods (cuticle and epidermis) responses resulted from such treatment. The histology work was carried out in the Regional Center for Mycology and Biotechnology, AL-Azhar Univ.

Phytoalexins extraction: An aliquot of 100 μL suspension of *B. cinerea* or *P. aphanidermatum* at 10^5 spores or propagules/ml were placed in snap bean pods after removal of the seeds for imposing phytoalexins production (**Van Etten and Smith, 1975**). Phytoalexins were extracted from the pods, cleaned up and purified on TLC. Purity of eluted phytoalexins was confirmed by UV spectrum for peak absorbance and concentrations were determined according to **Rizk et al. (1984)** and **AOAC (2000)**. Accordingly, standard solutions of phytoalexins (Phaseollin, Coumestrol, Kievitone, Phaseollidin and 6- β -Hydroxyphaseollin) at 100 $\mu\text{g/mL}$ obtained from the Regional Center for Mycology and Biotechnology, AL-Azhar Univ.

Phytoalexins determination in snap bean pods:

Naturally infected and artificially inoculated snap bean pods with both fungi were used to determine the phytoalexins content. 50 g of each treatment were blended with 100 ml 70% ethanol in water in an explosion-proof high speed blender for 5 min. The mixture was centrifuged at 3000 rpm for 20 min, and the clear supernatant of alcoholic extract was evaporated under vacuum in a rotary evaporator at 45°C until dryness. The residues were re-dissolved up in 100 ml warm distilled water and filtered through Whatman filter paper No. 40. The aqueous solution was extracted by shaking with 50 ml ethyl acetate, three times. Combined organic extract was dried over anhydrous sodium sulfate. The ethyl acetate was evaporated in vacuum, then dry film was re-dissolved in known amount of ethyl acetate. The obtained extracts from each treatment as well as control were used for spotting on TLC plates of silica gel GF254 (Merck). An aliquot of 30 μL of extract was spotted on TLC plates. Aliquots of 30 μL of standard solutions of different phytoalexins were spotted. The used developing solution was chloroform: toluene: acetone (40:35:25) according to **Lyon and Wood (1975)**. The chromatoplates were examined under short wave UV light (254 nm). The separated components appeared as dark mauve spot on a green fluorescent background at the

same R_f of the standards. Amount of detected phytoalexins were determined using SIM Photo Documentation System.

Quantitative determination of phytoalexins:

The positive spots identified under UV light were scanned with the photo documentation system (SIM Documentation System, Bio – Best, 140 A). The concentration of phytoalexins in snap bean pods samples in $\mu\text{g}/\text{kg}$ fresh weight (ppb) was determined according to the instructions of the manufacturer using BIO-ID V.6.10 computer program which is set up to analyze the intensity of the spots in the sample developed on TLC comparing to those of the phytoalexin standards.

Statistical analysis

All obtained data were analyzed using Analysis of Variance (ANOVA) among the treatments. the means were compared by least significant differences (LSD) at $p \leq 0.05$ as described by **Song and Keane (2006)**.

RESULTS AND DISCUSSION

Effect of pre-harvest sprays with organic acids in controlling the post-harvest infection of grey mold and cottony rot of snap bean pods during cold storage:

Ascorbic, citric, boric, salicylic and acetic acids sprays on green bean plants of Xera and Valentino varieties reduced development of grey mold and cottony rot on pods packed post-harvesting in perforated polyethylene consumer bags (PEB) or in modified atmosphere consumer packaging (MAP) and stored at $7 \pm 1^\circ\text{C}$ and 90-95% RH for 18 days during seasons 2012 and 2013 (**Tables 1 and 2, respectively**). Snap bean pods of control treatment (without pre-harvest acid sprays) kept in MAP showed far less decay development either in naturally infected or artificially inoculated pods with *B. cinerea* or *P. aphanidermatum* than when kept in PEB. Xera pods of control treatment kept in MAP or in PEB showed less decay than naturally infected or artificially inoculated Valentino pods with both fungi. This finding referred to higher susceptibility of Valentino pods to fungal infection particularly *B. cinerea* and *P. aphanidermatum* than Xera pods. It was also found that disease incidence of *P. aphanidermatum* was higher than of *B. cinerea*. However, artificial

inoculation of snap bean pods of both varieties with *B. cinerea* or *P. aphanidermatum* caused total disease incidence% when pods were kept in PEB due to pressure of high inoculum and favorable conditions. Disease severity was higher on Valentino pods artificially inoculated with both fungi than Xera pods, but the opposite was found with natural infection. When the pods were kept in MAP, Xera pods showed less disease infection and severity than Valentino pods, except the kept naturally infected Xera pods in MAP.

Generally, Xera pods showed less decay than Valentino pods during the both experimental seasons' experiments, except for keeping pods in PEB in season 2013. This changing pattern of decay between both varieties in PEB could be attributed to less values of decay referring to less natural infection of the pods with fungi regardless packaging material. It could be also expected that response of Xera pods toward the modified atmosphere conditions was better than Valentino pods which could be more sensitive to higher CO₂ concentrations.

As for data of seasons 2012 and 2013 on Xera snap bean in **Tables 1 and 2**, all pre-harvest treatments of organic acids then packing either in PEB or in MAP significantly reduced infection percentage and disease severity of pods with *B. cinerea* and *P. aphanidermatum* comparing with the inoculated control pods packed in PEB. However, keeping snap bean pods in MAP enhanced the efficacy of pre-harvest sprays with organic acids to control such decay. More suppressing of fungal decay on kept snap bean pods in MAP was simultaneously obtained when green bean plants were pre-sprayed with organic acids. Higher concentrations of boric acid (1%), ascorbic (2%) and acetic acid (0.2%) combined with MAP were the most suppressive treatments to control grey mold with efficacies 94.6, 93.7, 94.6%, respectively, in reducing the disease severity during season 2012 and 91.9, 91.0, 93.8%, respectively, in reducing the disease severity during season 2013.

When keeping Xera pods in PEB, 2% acetic acid and at 0.2% ascorbic acid reduced grey mold to 24.7% with efficacy of 75.3% and disease severity to about 12.5% with efficacy of about 80% (season 2012). Following season, acetic, ascorbic and boric acids followed by salicylic acids at higher concentrations were the most effective against grey mold. On the other hand, citric acid followed by salicylic acid at higher concentrations were the most effective treatments in reducing

cottony rot incidence with about 90.1 – 88.9% efficacy, respectively, and exceeded 94.6 – 92.7% efficacy, respectively, to reduce disease severity. However, all other acid treatments at different concentrations lowered cottony rot with efficacies over 80%, except for lower concentration of acetic acid. Second season, citric and salicylic acids at higher concentrations also proved their superior efficacy to control cottony rot.

Regarding Xera pods artificially inoculated with *P. aphanidermatum*, acetic, ascorbic and boric acids at both tested concentrations and salicylic acid at the higher concentration totally suppressed cottony rot of pods kept in MAP during seasons 2012 and 2013.

As for naturally infected Xera pods kept in MAP, all treatments completely suppressed the fungal decay, except for acetic acid at 0.1% which achieved efficacies to lower disease infection and severity by 71.4% and 84.1%, respectively (season 2012). Second season, 2013, all acids completely suppressed the fungal decay on Xera pods kept in MAP. During seasons 2012 and 2013, when keeping Xera pods in PEB, only the higher concentrations of the tested organic acids totally suppressed fungal decay development except for acetic acid treatment.

Generally, pre-harvest sprays with ascorbic, acetic and boric acids followed by salicylic acids, especially at their higher concentrations proved to be the most effective treatments to control grey mold and cottony rot caused by *B. cinerea* and *P. aphanidermatum* on Xera pods kept at MAP or PEB during cold storage. This approach of disease control using organic acids was also adopted by **Parida et al. (1991)** who revealed that carbendazim, thiorea, boric acid and potassium metabisulfate were effective in reducing storage decay in tomato fruits. Also, **Pramila and Dubey (2004)** used several chemicals including acetic acid for the management of fungal rotting of fruits and vegetables, thereby prolonging shelf life. Also, **Hafez and Haggag (2007)** also investigated the suppressive effect of pre-harvest treatments of boric acid and calcium chloride on apple trees alone or in combination as disinfectants for *Botrytis cinerea* which the cause of fruit rot during cold storage. Two sprays were adopted to reduce fungal decay and to improve quality of Anna apples.

Table (1): Effect of pre-harvest sprays with organic acids in controlling the post-harvest infection of grey mold and cottony rot of snap bean pods using different packaging types under cold storage at 7±1°C and 90-95% RH for 18 days, season 2012.

Treatment	Conc. %	Xera variety										Valentino variety																		
		Artificial inoculation					Natural infection					Artificial inoculation					Natural infection													
		<i>Botrytis cinerea</i>		<i>Pythium aphanidermatum</i>			DI	EF	DS	EF	DI	<i>Botrytis cinerea</i>		<i>Pythium aphanidermatum</i>			DI	EF	DS	EF	DI	<i>Botrytis cinerea</i>		<i>Pythium aphanidermatum</i>			DI	EF	DS	EF
Modified atmosphere packaging (MAP)																														
Boric acid	0.5	25.9	74.1	13.9	78.1	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	25.9	74.1	12.0	83.1	40.7	59.3	16.4	76.8	0.0	100.0	0.0	100.0	0.0	100.0	
	1	8.6	91.4	3.4	94.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	17.3	82.7	9.0	87.5	23.5	76.5	9.0	87.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
Citric acid	1	29.6	70.4	19.8	70.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	3.7	96.3	1.2	98.3	28.4	71.6	9.9	86.0	0.0	100.0	0.0	100.0	0.0	100.0	
	2	11.1	88.9	6.5	89.8	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
Ascorbic acid	1	14.8	85.2	9.9	84.4	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	23.5	76.5	11.1	84.4	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
	2	8.6	91.4	4.0	93.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	13.3	86.7	4.9	93.1	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
Salicylic acid	0.1	23.5	76.5	12.4	80.5	4.9	95.1	2.5	96.1	0.0	100.0	0.0	100.0	13.6	86.4	6.2	91.4	25.9	74.1	9.0	87.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
	0.2	17.3	82.7	8.0	87.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	13.6	86.4	4.9	93.1	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
Acetic acid	0.1	29.6	70.4	15.7	75.1	16.1	84.0	6.5	89.7	12.3	71.4	4.3	84.1	31.0	69.0	13.6	81.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
	0.2	8.6	91.4	3.4	94.6	7.4	92.6	4.3	93.1	0.0	100.0	0.0	100.0	17.3	82.7	7.4	89.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
Control (MAP)		34.6	65.4	17.8	71.9	42.0	58.0	22.5	64.2	19.8	54.2	10.5	61.4	44.4	55.6	28.1	60.6	51.8	48.2	29.9	57.5	22.2	50.0	14.5	44.0					
LSD at 0.05	T	NS	NS	NS	NS	5.2	2.6	2.6	2.6	2.6	NS	NS	2.6	6.5	3.7	6.5	3.7	7.7	7.7	7.7	7.7	3.7	3.7	NS	NS	NS	NS	NS	NS	
	C	7.4	4.6	4.0	4.0	2.0	2.0	2.0	2.0	1.3	1.3	5.0	5.0	2.9	2.9	6.0	6.0	6.0	6.0	6.0	6.0	2.9	2.9	L6	L6	L6	L6	L6	L6	
Perforated polyethylene consumer bags (PEB)																														
Boric acid	0.5	33.3	66.7	18.2	71.2	21.0	79.0	9.9	84.3	13.6	68.5	5.2	80.9	37.0	63.0	21.3	70.1	57.1	42.9	33.3	52.6	14.8	66.7	6.5	75.0					
	1	27.2	72.8	13.9	78.1	19.8	80.3	8.9	85.9	0.0	100.0	0.0	100.0	24.8	75.2	21.6	69.7	37.0	63.0	19.4	72.4	0.0	100.0	0.0	100.0					
Citric acid	1	40.7	59.3	23.2	63.4	16.1	84.0	7.4	88.3	8.6	80.0	2.8	89.8	23.5	76.5	11.1	84.4	43.2	56.8	25.3	64.0	13.6	69.4	6.2	76.2					
	2	34.6	65.4	17.3	72.6	9.9	90.1	3.4	94.6	0.0	100.0	0.0	100.0	16.1	84.0	6.2	91.4	14.7	85.3	6.8	90.4	0.0	100.0	0.0	100.0					
Ascorbic acid	1	38.3	61.7	24.0	62.1	21.0	79.0	9.6	84.8	11.1	74.2	3.7	86.4	39.5	60.5	22.2	68.8	18.5	81.5	9.3	86.9	11.1	75.0	4.0	84.6					
	2	24.7	75.3	12.7	80.0	19.8	80.3	8.9	85.8	0.0	100.0	0.0	100.0	18.5	81.5	9.0	87.5	18.5	81.5	9.3	86.8	0.0	100.0	0.0	100.0					
Salicylic acid	0.1	39.5	60.5	22.2	64.9	23.5	76.5	10.8	82.9	13.6	68.5	5.9	78.4	30.9	69.1	16.1	77.5	45.7	54.3	27.5	61.0	9.9	77.8	3.4	86.9					
	0.2	28.4	71.6	15.1	76.1	11.1	88.9	4.6	92.7	0.0	100.0	0.0	100.0	16.1	84.0	6.2	91.4	11.1	88.9	4.3	93.9	0.0	100.0	0.0	100.0					
Acetic acid	0.1	49.34	50.6	27.5	56.6	29.6	70.4	15.1	76.0	22.2	48.5	10.5	61.3	45.7	54.3	25.3	64.5	19.8	80.3	9.3	86.8	11.1	75.0	4.6	82.1					
	0.2	24.7	75.3	12.4	80.5	21.0	79.0	9.9	84.3	8.6	80.0	3.4	87.5	21.0	79.0	8.6	87.9	7.4	92.6	2.2	96.9	3.7	91.7	1.2	95.2					
Control (PEB)		100.0	63.3	100.0	63.0	63.0	43.1	27.2	100.0	71.3	27.2	100.0	71.3	27.2	100.0	71.3	27.2	100.0	71.3	27.2	100.0	71.3	27.2	100.0	71.3	25.9				
LSD at 0.05	T	9.1	6.4	5.9	5.2	5.2	5.2	5.2	5.2	3.0	3.0	3.0	3.0	7.6	7.6	3.3	3.3	6.2	6.2	6.2	6.2	3.3	3.3	3.4	3.0					
	C	5.8	4.0	5.0	5.0	3.3	3.3	3.3	3.3	1.9	1.9	7.6	7.6	3.3	3.3	6.2	6.2	6.2	6.2	6.2	6.2	3.3	3.3	3.4	3.0					

DI = Disease infection DS = Disease severity MAP = Modified atmosphere packaging PEB= perforated polyethylene consumer bag
 EF = Efficacy Control was infected with *B. cinerea* or *P. aphanidermatum* or without infection and untreated

Table (2): Effect of pre-harvest sprays with organic acids in controlling the post-harvest infection of grey mold and cottony rot of snap bean pods using different packaging types under cold storage at 7±1°C and 90-95% RH for 18 days, season 2013.

Treatment	Conc. %	Xera variety												Valentino variety																	
		Artificial inoculation						Natural infection						Artificial inoculation						Natural infection											
		<i>Botrytis cinerea</i>			<i>Pythium aphanidermatum</i>									<i>Botrytis cinerea</i>			<i>Pythium aphanidermatum</i>														
		DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS	DI	EF	DS
Modified atmosphere packaging (MAP)																															
Boric acid	0.5	22.2	77.8	14.8	77.1	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	22.2	77.8	9.3	86.9	43.2	56.8	17.6	74.7	0.0	100.0	0.0	100.0
	1	11.1	88.9	5.3	91.9	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	16.1	84.0	6.5	91.0	22.2	77.8	9.3	86.7	0.0	100.0	0.0	100.0
Citric acid	1	27.2	72.8	16.7	74.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	4.9	95.1	1.5	97.9	27.1	72.9	10.8	84.5	0.0	100.0	0.0	100.0
	2	14.8	85.2	7.7	88.1	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Ascorbic acid	1	18.5	81.5	10.5	83.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	25.9	74.1	12.3	82.8	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
	2	9.9	90.1	5.9	91.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	11.1	88.9	4.0	94.4	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Salicylic acid	0.1	23.5	76.5	12.4	80.9	7.2	93.8	2.2	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	13.6	82.4	5.6	92.3	28.4	71.6	12.4	82.2	0.0	100.0	0.0	100.0
	0.2	14.8	85.2	7.1	89.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	7.6	4.9	81.8	29.6	70.4	15.4	78.5	0.0	100.0	0.0	100.0	
Acetic acid	0.1	32.1	67.9	16.7	74.3	14.8	85.2	6.2	90.3	12.3	70.6	4.9	81.8	29.6	70.4	15.4	78.5	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
	0.2	11.1	88.9	4.0	93.8	6.2	93.8	2.2	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
Control (MAP)		32.1	67.9	13.9	78.6	40.74	59.3	24.7	61.2	19.8	52.9	10.5	61.4	46.9	53.1	27.5	61.6	49.4	50.6	28.7	58.7	24.7	44.4	12.7	51.2						
	T	NS	NS	NS	4.2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
LSD at 0.05		5.0	5.0	3.3	3.2	3.2	2.6	2.7	2.7	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
	C	5.0	5.0	3.3	3.2	3.2	2.6	2.7	2.7	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
Perforated polyethylene consumer bags (PEB)																															
Boric acid	0.5	32.1	67.9	18.2	71.9	14.8	85.2	6.2	90.3	7.4	82.3	5.8	78.7	34.6	65.4	18.5	74.1	51.9	48.2	29.3	57.8	9.9	76.2	3.7	84.4						
	1	21.0	79.0	12.4	80.9	13.6	86.4	5.9	90.8	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	32.1	67.9	16.7	76.7	37.0	63.0	18.5	73.4	0.0	100.0	0.0	100.0
Citric acid	1	35.8	64.2	19.8	69.5	11.1	88.9	4.0	93.7	4.9	88.2	1.2	95.4	18.5	81.5	9.0	87.5	37.0	63.0	18.5	73.3	11.1	73.2	3.7	84.4						
	2	28.4	71.6	17.0	73.8	7.4	92.6	2.2	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	11.1	88.9	4.0	94.4	11.1	88.9	4.6	93.4	0.0	100.0	0.0	100.0
Ascorbic acid	1	29.6	70.4	12.3	81.0	24.8	75.2	5.9	90.8	7.4	82.3	2.2	92.1	39.5	60.5	21.0	70.7	16.1	84.0	6.5	90.7	7.4	82.1	2.2	90.9						
	2	21.0	79.0	10.8	83.3	13.6	86.4	5.6	91.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	14.8	85.2	6.8	90.5	16.0	84.1	7.1	89.8	0.0	100.0	0.0	100.0
Salicylic acid	0.1	34.6	65.4	20.7	68.1	19.8	80.3	9.9	84.5	11.1	73.5	3.7	86.4	27.2	72.8	14.8	79.3	44.4	55.6	24.1	65.3	8.6	79.2	2.8	88.3						
	0.2	24.7	75.3	13.3	79.5	8.6	91.4	3.4	94.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	17.3	82.7	8.0	88.8	8.6	91.4	2.8	96.0	0.0	100.0	0.0	100.0
Acetic acid	0.1	44.4	55.6	25.0	61.4	23.5	76.5	13.0	79.6	19.8	52.9	8.3	69.3	43.2	56.8	24.1	66.4	14.8	85.2	4.9	92.9	7.4	82.1	2.2	90.9						
	0.2	19.8	80.3	10.8	83.3	16.1	84.0	7.1	88.8	7.4	82.3	2.2	92.1	18.5	81.5	8.0	88.8	4.9	95.1	1.2	98.2	2.5	94.0	0.6	97.4						
Control (PEB)		100.0	64.8	100.0	63.6	42.0	27.2	1.8	3.9	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8					
	T	8.5	5.7	3.6	6.2	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
LSD at 0.05		5.4	5.4	3.6	6.2	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	C	5.4	5.4	3.6	6.2	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

DI = Disease infection DS = Disease severity MAP = Modified atmosphere packaging PEB= perforated polyethylene consumer bag
 EF = Efficacy Control was infected with *B. cinerea* or *P. aphanidermatum* or without infection and un-treated

Regarding Valentino pods kept in MAP in season 2012 experiments, citric acid at 2% completely suppressed *B. cinerea* and *P. aphanidermatum* as well as decay on artificially inoculated or naturally infected pods, followed by its concentration 1% with 96.3% efficacy. Boric acid at 0.5% was the least effective pre-harvest acid treatment to suppress cottony rot. On the other hand, all organic acid treatments in combination with MAP completely inhibited decay development on naturally infected pods.

As regard for Valentino pods kept in PEB during season 2012, citric and salicylic acids at 2% were the most effective treatments to suppress grey mold and cottony rot. On naturally infected Valentino pods, boric, citric, ascorbic and salicylic acids at high concentration completely inhibited decay development.

Concerning Valentino variety during season 2013, citric, salicylic and ascorbic acids successfully suppressed grey mold on artificially infected pods and cottony rot. Boric, acetic, ascorbic acids at their higher concentrations successfully suppressed the fungal decay on naturally infected pods.

Generally, it could be concluded through both season experiments, that citric acid at 2% and salicylic acid at 0.2% were the most effective treatments to control grey mold and cottony rot on naturally infected and artificially inoculated pods of Valentino variety.

Effect of pre-harvest sprays with certain organic acids on epidermis and cuticle thickness of snap bean pods:

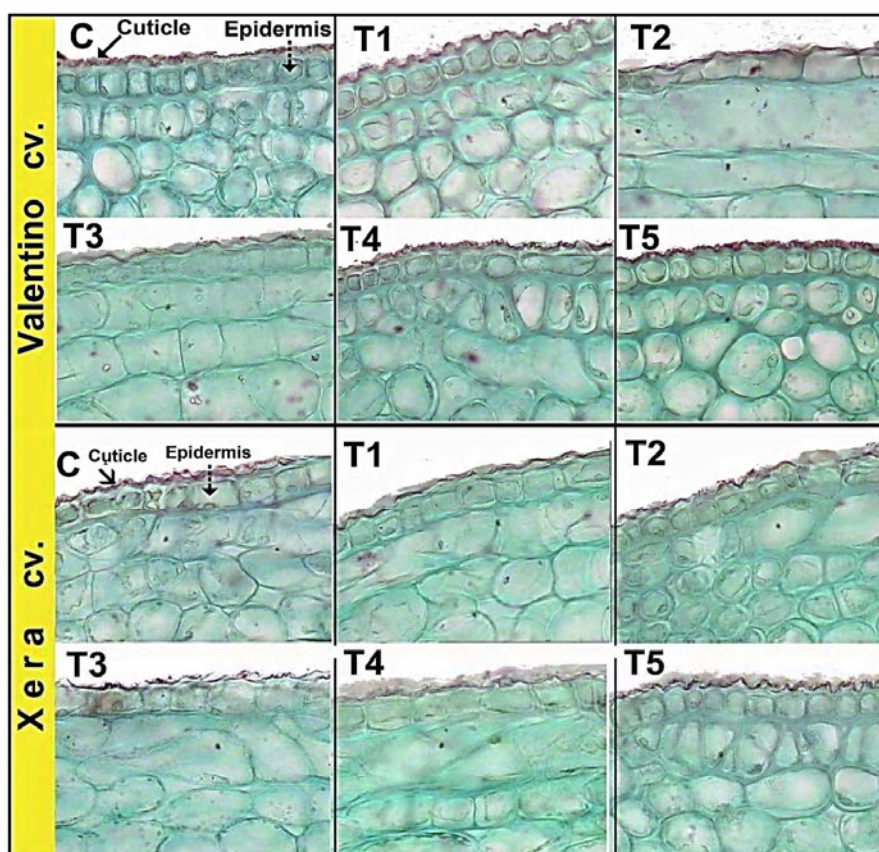
Data presented in **Table 3** and also illustrated in **Figure 1** indicate that application of boric acid as pre-harvest treatment on Xera green bean plants was the most positive treatment to increase the epidermis thickness of the pods causing thicknesses of 18.40 μm comparing with the control (13.24 μm), followed by citric acid and salicylic acid treatments. This increase in epidermis thickness is coincided with the increase in less infection by the two tested pathogens, *B. cinerea* and *P. aphanidermatum*, compared with the disease development in the control treatment. This finding refers to possible increase of snap bean pods resistance to fungal infection by spraying of certain organic acid such as boric, citric or salicylic acids on green bean plants through increasing the epidermis thickness as a mechanical barrier. While ascorbic acid showed less thickness of the epidermis of Xera pods than the control. The pods of Valentino variety collected from such sprayed plants with the tested organic

acids showed less epidermis thickness than the control on contrary to Xera. Citric acid showed the least values of epidermis thickness. So, it could be concluded that the changes in epidermis thickness incited by organic acid sprays is related also to the variety. The cuticle of Xera pods collected from plants of control treatment had more thickness value than that of sprayed with the tested organic acids.

On the other hand, salicylic, acetic and citric acids treatments on Valentino plants produced pods with thicker cuticle than the other organic acids and the control by about 1.3 times. This finding referred to role of some organic acid in increasing the cuticle thickness and decreasing simultaneously the disease infection incited by *Botrytis* and *Pythium* on Valentino pods as obtained in **Tables 1 and 2**. Ascorbic acid sprays did not increase the thickness epidermis and cuticle of Xera and Valentino pods comparing with the control. Boric acid lowered the cuticle thickness of Valentino pods as opposite results of other tested organic acids on Valentino variety. It is worth to mention that cuticle thickness of the Xera control pods was more than that of Valentino pods which may play a role in reducing susceptibility of Xera to fungal infection incited by *Botrytis* and *Pythium* causing postharvest decay. The anatomical changes as a whole in thickness of epidermis and cuticle emphasized a significant role in reducing susceptibility of snap bean pods to decay incited by *B. cinerea* and *P. aphanidermatum*. Anatomical changes by acids were also obtained by **Ismaeil and Bakry (2005)** showed that treating papaya plants with citric acid at 2 g/l increased thickness of epidermis, cortex, phloem zone and xylem zone in petiole flower. On the other hand, spraying green bean plants with salicylic acid at 100 ppm decreased leaf blade thickness due to the decrease in thickness of both palisade and spongy tissues as compared to control (**Mady, 2009**). **Nour et al. (2012)** found that, spraying green bean plants with salicylic acid at 50 and 100 ppm, citric acid at 0.25% and 0.5% increased thickness of leaflet blade, thickness of palisade and spongy, except salicylic acid at 100 ppm that had the opposite effect on these leaflet anatomical characters.

Table 3: Effect of preharvest spray of organic acids on snap bean pods cuticle and epidermis.

Treatment	Xera variety		Valentino variety	
	Cuticle (μm)	Epidermis (μm)	Cuticle (μm)	Epiderm (μm)
Boric acid	2.50	18.40	2.73	13.41
Citric acid	3.69	15.40	4.06	11.40
Ascorbic acid	2.95	12.49	3.12	12.21
Salicylic acid	3.00	15.13	4.33	11.75
Acetic acid	3.32	13.92	4.10	13.10
Control	4.93	13.24	3.15	16.07

**Figure 1: Effect of preharvest sprays of some organic acids on histological features of pods cell wall (cuticle and epidermis) of Xera and Valentino varieties after 15 days from the last spray.**

C = Control, T1 = Acetic acid at 0.2%, T2 = Ascorbic acid at 2%, T3 = Boric acid at 1%, T4 = Citric acid at 2%, T5 = Salicylic acid at 0.2%. (Magn.= 20X)

Phytoalexins content in snap bean pods naturally infected or artificially inoculated with *B. cinerea* and *P. aphanidermatum* as response to pre-harvest sprays of organic acids:

Concentrations of accumulated phytoalexins ($\mu\text{g}/\text{kg}$ fresh weight) were determined in snap bean pods picked up from sprayed plants with organic acids or let as a control, then inoculated with *B. cinerea* and *P. aphanidermatum* or let for the natural infection, and packed in MAP or in PEB as estimated after 72 hours from harvest and storage at $7\pm 1^\circ\text{C}$ and 90 - 95% RH. Citric and salicylic acids were evaluated to enhance accumulation of phytoalexins as major effective treatments and boric acid was used for comparison beside the control treatment.

Phytoalexins content in pods artificially inoculated with *B. cinerea*:

Data in **Table 4 and Figure 2** show that artificially inoculated Xera pods with *B. cinerea* kept in PEB as a control without organic acid treatment exhibited high amounts of 6- α -hydroxyphaseollin (2668.6 $\mu\text{g}/\text{kg}$), phaseollin (2534.0 $\mu\text{g}/\text{kg}$) and kievitone (1186.6 $\mu\text{g}/\text{kg}$), but low concentration of coumestrol (304.0 $\mu\text{g}/\text{kg}$), while no phaseollidin was detected. On the other hand, less quantity of 6- α -hydroxyphaseollin (1355.5 $\mu\text{g}/\text{kg}$) was produced in control of cv. Valentino pods inoculated with *B. cinerea*, while coumestrol, phaseollidin and phaseollin concentrations were decreased to 536.7, 338.0 and 322.0 $\mu\text{g}/\text{kg}$, respectively, and no kievitone was detected.

However, it could be observed that the total amount of phytoalexins produced in artificially inoculated Xera pods with *B. cinerea* was more than that produced in Valentino pods. This finding was associated with less susceptibility of Xera pods than Valentino pods to the gray mold disease. The phytoalexins produced in comparable high amounts in Xera pods were phaseollin, kievitone and 6- α -hydroxyphaseollin. All of these phytoalexins as individuals or in combination may be responsible of resistance of snap bean pods toward *B. cinerea* infection.

On the other hand, the total amounts of phytoalexins found in Xera pods from treated plants with tested organic acids were less than that of control pods of Xera variety. This finding refers to less role of organic acid sprays to increase the resistance of Xera pods toward *B. cinerea*. On contrary, the total amount of phytoalexins in snap bean pods of Valentino plants sprayed with salicylic acid was 70% more than the control pods,

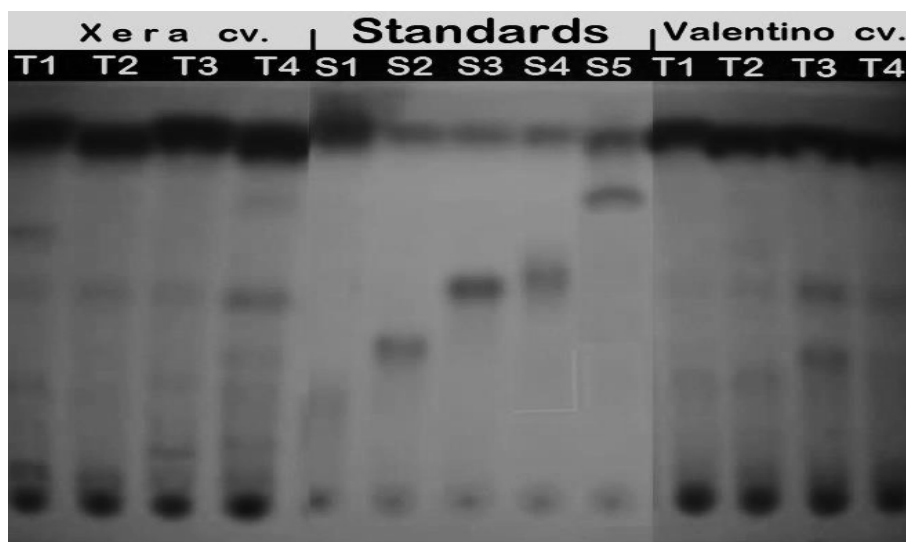
which was concomitantly correlated with high effectiveness against *B. cinerea*. The phytoalexin 6- α -hydroxyphaseollin only was detected in the salicylic acid treatment higher than the control treatment. Regarding the results of *in vitro* evaluation of salicylic acid efficacy against *B. cinerea* as highly suppressive, it could be concluded that salicylic acid may work by different ways against *Botrytis* including eliciting resistance gene expression, or it may be concluded that *Botrytis* has the capability to degrade phytoalexins produced by the plant responding to the infection. **Van Etten et al. (1989)** reported that *Fusarium solani* f. sp. *phaseoli* can detoxify kievitone, phaseollin, phaseollidin and phaseollinisoflavan on bean (*Phaseolus vulgaris*). An extracellular enzymatic system involved in conversion of kievitone to kievitone hydrate. Detoxification of kievitone occurs by hydration of the isopentenyl side chains. In other studies, there were a few fungal pathogens such as *F. solanif. sp. Phaseoli* and *Colletotrichum lindemuthianum*, as well as nonpathogenic isolates of *Septoria nodorum* and *Stemphylium botryosum* had the capability to detoxify phaseollin into other compounds depending on the fungus. Phaseollin was found to be detoxified into several compounds such as α -hydroxyphaseollone by *F. solanif. sp. phaseoli* (**van den Heuvel et al., 1974**), 6 α -Hydroxyphaseollin by *Colletotrichum lindemuthianum* (**Burden et al., 1974**), phaseollinisoflavan by *Stemphylium botryosum* (**Higgins et al., 1974**), and 12,13-dihydrodihydroxyphaseollin by *Septoria nodorum* (**Bailey et al., 1977**). On the other hand, phytoalexins could be degraded to unknown compounds. However, degradation of certain phytoalexins by some fungal isolates refers sometimes to higher pathogenic propensity of these isolates, which could be considered here for *B. cinerea*. Where the metabolites in this study were found in high amounts than phaseollin, it may refer more to degradation activity of produced phytoalexins. Proposed degradation of produced phytoalexins in snap bean pods by *B. cinerea* needs further studies to be elucidated and emphasized. Also, selected organic acids did not elicit phytoalexin production against *B. cinerea* in Xera snap bean where less total amounts were detected in pods collected from sprayed plants with such organic acids, or these sprays controlled the fungus and consequently not enhance phytoalexin production. However, boric acid sprays were associated with higher amounts of phaseollin comparing with other organic acids especially salicylic acid. This finding may refer to reaction of plant toward the fungal infection particularly when less effective treatment against the fungus directly was adopted.

Table 4: Effect of organic acids pre-sprays of snap bean plants (cvs. Xera and Valentino) on phytoalexins content of inoculated pods with *B. cinerea* before keeping in MAP or in PEB.

Treatment Acid/package	Phytoalexin ($\mu\text{g}/\text{kg}$ fresh weight)											
	Xera variety						Valentino variety					
	Phaseollin	Coumestrol	Kievitone	Phaseollidin	Hydroxy phaseollin 6- \acute{a} -	Total	Phaseollin	Coumestrol	Kievitone	Phaseollidin	Hydroxy phaseollin 6- \acute{a} -	Total
Citric acid/MAP*	601.4	0	606.6	0	1270.7	2478.7	252.0	282.0	0	0	1327.5	1861.5
Boric acid/MAP	668.0	288.7	538.0	0	1392.0	2886.7	265.4	555.4	0	384.6	1400.6	2606
Salicylic acid/MAP	319.3	274.0	300.6	0	1340.6	2234.5	295.3	1068.6	0	326.0	2664.6	4354.5
Control/PEB**	2534.0	304.0	1186.6	0	2668.6	6693.2	322.0	536.7	0	338.0	1355.5	2552.2

MAP*: consumer modified atmosphere packaging
Control was infected with *B. cinerea* and un-treated.

PEB**: perforated polyethylene consumerbag



T1 = effect of citric acid + MAP	S1 = Kievitone
T2 = effect of boric acid + MAP	S2 = coumestrol
T3 = effect of salicylic acid + MAP	S3 = 6- \acute{a} -Hydroxyphaseollin
T4 = control, fungus inoculated, PEB)	S4= Phaseollidin
	S5= Phaseollin

Figure 2: Phytoalexins concentrations in snap bean pods 72hr after harvest, collected from plants sprayed with citric, boric and salicylic acids, then artificially inoculated with *B. cinerea* before storage at $7\pm 1^\circ\text{C}$ and 90-95% RH.

Phytoalexins content in pods artificially inoculated with *P. aphanidermatum*:

Phaseollin was not detected in Xera pods, either treated with organic acids or only artificially inoculated with *P. aphanidermatum* as shown in **Table 5**. This finding may refer to capability of *P. aphanidermatum* to degrade phaseollin to other chemicals, either phytoalexins or not. The phytoalexins phaseollidin and kievitone were highly increased in Xera of citric and salicylic acid treatments comparing with the control or pods of plants treated with boric acid. while boric acid completely suppressed cottony rot incited by *P. aphanidermatum* on Xera pods. Phaseollidin and kievitone may not play a role in resistance or they do but boric acid control the disease by another way as found in the anatomical changes by increasing the epidermis thickness **Table 3**. The phytoalexin 6- α -hydroxyphaseollin was detected in higher amounts in Xera pods treated with citric and boric acid. Meanwhile, boric acid treatment completely suppressed *P. aphanidermatum* on Xera **Table 1**. citric acid showed less effectiveness. This finding means that the 6- α -hydroxyphaseollin may not have a clear role in resistance to *P. aphanidermatum*. Salicylic acid was the single treatment associated with higher concentrations of kievitone and phaseollidin in Valentino pods comparing to other tested organic acids as well as the control treatment. So, kievitone and phaseollidin may play a role in defense mechanism in Valentino pods, particularly salicylic acid that proved a suppressive effect on *P. aphanidermatum* than other two tested acids.

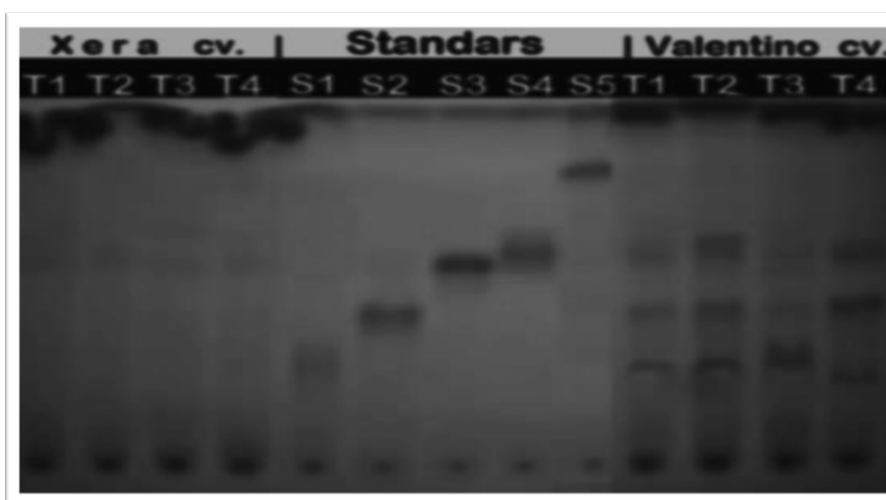
Table 5: Effect of organic acids pre-sprays of snap bean plants (cvs. Xera and Valentino) on phytoalexins content of inoculated pods with *P. aphanidermatum* before keeping in MAP or in PEB.

Treatment Acid/package	Phytoalexin ($\mu\text{g}/\text{kg}$ fresh weight)											
	Xera variety						Valentino variety					
	Phaseollin	Coumestrol	Kievitone	Phaseollidin	Hydroxy phaseollin	6- α -Total	Phaseollin	Coumestrol	Kievitone	Phaseollidin	Hydroxy phaseollin	6- α -Total
Citric acid/MAP*	0	140.6	301.4	1516.6	2664.6	4623.2	0	1020.5	768.0	0	2668.6	4457.1
Boric acid/MAP	0	148.7	0	0	1367.3	1516.0	582.6	1291.3	1741.3	768.0	4724.7	9107.9
Salicylic acid/MAP	0	156.6	602.0	811.3	676.7	2090.0	554.7	2070.0	2154.0	1344.0	1399.4	7522.1
Control/PEB**	0	160.0	152.0	452.5	687.4	1451.9	532.7	2306.0	1842.0	724.7	5048.6	10454.0

MAP*: consumer modified atmosphere packaging

PEB**: perforated polyethylene consumerbag

Control was infected *P. aphanidermatum* and un-treated.



T1 = effect of citric acid + MAP	S1 = Kievitone
T2 = effect of boric acid + MAP	S2 = coumestrol
T3 = effect of salicylic acid + MAP	S3 = 6- α -Hydroxyphaseollin
T4 = control, fungus inoculated, PEB)	S4= Phaseollidin
	S5= Phaseollin

Figure 3: Phytoalexins concentrations in snap bean pods 72hr after harvest, collected from plants sprayed with citric, boric and salicylic acids, then inoculated with *P. aphanidermatum* before storage at $7\pm 1^\circ\text{C}$ and 90-95% RH.

Phytoalexins concentrations in naturally infected pods:

The determined phytoalexins contented in naturally infected pods of the control treatment was higher than that in pods of pre-harvest sprayed plants with organic acids (**Table 6 and Figure 4**). This finding indicates that accumulation of phytoalexins was induced by fungal infection and decay development on snap bean pods. When organic acids sprays caused suppression of fungal development, the induction of phytoalexins was reduced as data shown for the pods of treated plants **Table 6**. while the determined amounts of the phytoalexins in artificially inoculated pods with *B. cinerea* and *P. aphanidermatum* were higher as observed in **Tables 1 and 2**, respectively. This finding indicates that phytoalexin accumulation was more accelerated depending on the fungal infection more than eliciting by pre-harvest sprays with organic acid. The less induction of phytoalexins by tested organic acid contrarily to **Anderson (1988)** could be attributed to use of high concentrations of tested organic acids, where **Diego et al. (2013)** found that maximum production of isoflavonoid phytoalexin elicited with salicylic acid at 3.62 mM or below, and increasing the acid concentration up to 7.2 mM declined its concentration. Also, they found that the response of phytoalexin accumulation in different varieties elicited by SA was found to be dose–response of elicitor treatments as well as variety responsive. While the tested organic acid treatments completely suppressed the fungal decay on naturally infected pods, it changing the thickness of epidermis and/or cuticle of snap bean pods **Table 3**. However, under the artificial inoculation conditions of snap bean pods with both fungi, more phytoalexins accumulation was resulted as demonstrated in **Tables 4 and 5**.

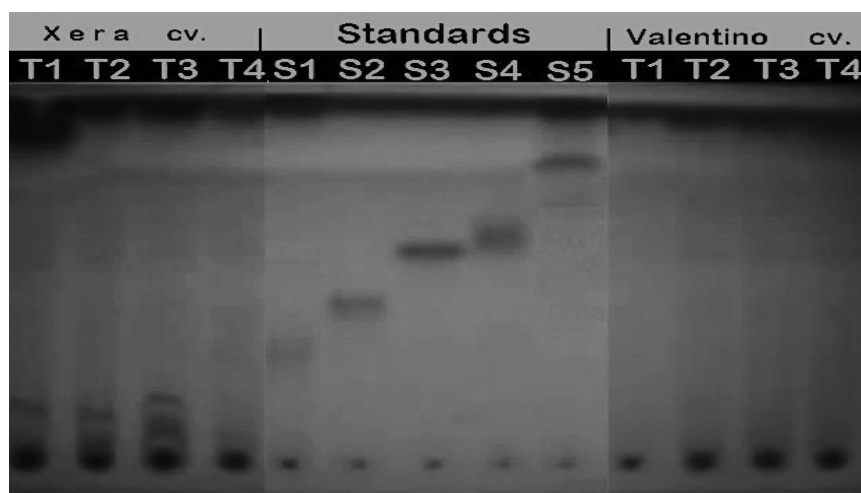
Generally, phytoalexins accumulation was regarded as depending on the fungal infection and snap bean variety more than depending on tested organic acids at tested concentrations.

Table 6: Effect of organic acids pre-sprays of snap bean plants (cvs. Xera and Valentino) on phytoalexins content of naturally infected pods with before keeping in MAP or in PEB.

Treatment Acid/package	Phytoalexin ($\mu\text{g}/\text{kg}$ fresh weight)											
	Xera variety					Valentino variety						
	Phaseollin	Coumestrol	Kievitone	Phaseollidin	6- α -Hydroxyphaseollin	Total	Phaseollin	Coumestrol	Kievitone	Phaseollidin	6- α -Hydroxyphaseollin	Total
Citric acid/MAP*	0	124.5	88.0	316.7	763.3	1292.5	0	0	0	0	0	0
Boric acid/MAP	0	128.7	110.0	259.3	733.4	1231.4	0	275.3	142.0	0	711.4	1128.7
Salicylic acid/MAP	0	85.3	100.6	287.0	688.7	1161.6	0	250.0	149.4	224.8	664.0	1288.2
Control/PEB**	0	111.3	106.0	329.4	820.7	1367.4	0	332.6	132.0	280.6	740.7	1485.9

MAP*: consumer modified atmosphere packaging
Control without infection and un-treated

PEB**: perforated polyethylene consumer bag



T1 = effect of citric acid + MAP	S1 = Kievitone
T2 = effect of boric acid + MAP	S2 = coumestrol
T3 = effect of salicylic acid + MAP	S3 = 6- α -Hydroxyphaseollin
T4 = control, fungus inoculated, PEB)	S4 = Phaseollidin
	S5 = Phaseollin

Figure 4: Phytoalexins concentrations in naturally infected snap bean pods 72hr after harvest, collected from plants foliar sprayed with citric, boric and salicylic acids, then stored at $7\pm 1^\circ\text{C}$ and 90-95% RH.

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تأثير استخدام بعض الأحماض العضوية على التغيرات التشريحية والفسولوجيا وأمراض ما بعد الحصاد فى قرون الفاصوليا الخضراء.

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تتعرض قرون الفاصوليا بعد الحصاد الى العديد من المسببات المرضيه اكثرها خطورة الفطرين بوتريتس سينيريا وبيثيوم أفانيدرماتم. وتهدف هذه الدراسة الى الحد من خطورة هذه المسببات باستخدام بعض الأحماض العضوية كمضادات امنه لأعفان ما بعد الحصاد فى قرون الفاصوليا صنفى أكزيرا وفلانتيو. وقد أستخدم فى هذه الدرلسة خمس أحماض عضوية وهم حمض البوريك والستريك والأسكوريك بتركيزات 0.1 و 0.2 و 0.3%. وقد أظهرت النتائج تحت الظروف الحقلية أن رش نباتات الفاصوليا قبل الحصاد بواسطة البوريك بتركيز 1% وحمض الخليك بتركيز 0.2% وحمض الأسكوريك بتركيز 2% أعطى أفضل مقاومه لأعفان قرون الفاصوليا صنف أكزيرا والمتسببة عن فطر بوتريتس سينيريا. كما أوضحت النتائج أن رش نباتات الفاصوليا صنف فلانتيو بواسطة حمض الستريك بتركيز 2% كان الأفضل بين الأحماض المختبرة فى التثبيط الكامل لأعفان القرون المتسببة عن الفطر بوتريتس سينيريا وتحت ظروف العدوى الطبيعية وذلك بعد الحصاد والتخزين باستخدام نظام الجو الهوائى المعدل. كما وجد أن رش نباتات الفاصوليا بواسطة حمض البوريك والستريك والأسكوريك قد ثبط تماماً أصابة قرون الفاصوليا صنف فلانتيو بفطر البيثيوم أفانيدرماتم مع كل التركيزات المستخدمة وذلك بعد الحصاد والتخزين تحت ظروف الجو الهوائى المعدل. وأوضحت النتائج أيضاً أن رش نباتات الفاصوليا قبل الحصاد بكل من حمض السيلسيلك أوالخليك أوالأسكوريك قد أدى الى زيادة فى سمك طبقة الكيوتيكل فى قرون الصنف فلانتيو, بينما كان الرش بحمض البوريك هو الأفضل فى زيادة سمك طبقة الأبيديرم فى قرون الصنف أكزيرا. ومن ناحية أخرى , فقد وجد أن رش نباتات الفاصوليا بحمض السيلسيلك كان الأفضل فى زيادة محتوى القرون من الفيتوالكسينات الكميسترول و 6 الفا هيدروكسى فاصولين للصنف فلانتيو والتى سبق عدواها صناعياً بفطر بوتريتس سينيرياز أدى الرش أيضاً بحمض السيلسيلك الى زيادة فى تركيز الفاصولدين فى قرون كلا الصنفين محل الدراسة والسابق عدواها صناعياً بفطر بيثيوم أفانيدرماتم. كما أدى أيضا الرش بحمض البوريك الى زيادة تركيز فيتوالكسينات الكميسترول والكافيتون و 6 الفا هيدروكسى فاصولين فى قرون الصنف فلانتيو والسابق عدواها صناعياً بفطر بوتريتس سينيريا.