EFFECT OF POST HARVEST TREATMENT WITH CERTAIN BIOAGENTS OR ETHANOL ON DECAY OF SNAP BEAN PODS DURING STORAGE

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Abstract

Botrytis cinerea, Sclerotinia sclerotiorum and *Pythium aphanidermatum* are among the pathogenic fungi on snap beans during cold storage. *In vitro* tests showed that *Trichoderma harzianum* resulted in 100% inhibition of mycelial growth in all tested pathogens followed by *Trichoderma viride* and *Trichoderma glioclidium* compared with the control. The effect of ethanol vapor 75% tested for different exposure intervals was assessed against of *Botrytis cinerea* 100%. *In vivo* trials showed that *T. harzianum* reduced (1.50%) disease severity with *Botrytis cinerea* followed by *T. glioclidium* (8.95%) and *T. viride* (12.3%), while ethanol resulted in lower effect (20.5%) compared with the control after 4 weeks storage season 2009. On the other hand, Ethanol vapor (75% / 5 min) complete inhibition with the two fungi *Sclerotinia sclerotiorum* and *P. aphanidermatum*. Generally, bioagent gave effective control for the three pathogens. The tested post harvest treatments prolonged the shelf life of snap beans at 22±2°C. All treatments reduced the loss in fresh weight of snap beans pods during the cold storage at 10°C for 4 weeks. On the other hand post harvest treatments increased peroxidase activity in snap bean pods. While, decreased polyglactronase activity in naturally and artificially infected with the three pathogens compared with untreated

Key words: Post harvest treatments, Bioagent, Ethanol, snap beans, *Botrytis cinerea, Sclerotinia sclerotiorum, Pythium aphanidermatum* and Quality.

INTRODUCTION

Snap bean pods (*Phaseolus vulgaris* L.) decay during storage, transport, marketing or export by a variety of fungi mainly: *Botrytis cinerea, Pythium aphanidermatum* and *Sclerotinia sclerotiorum*, which are pathogens on more than 200 species of vegetables during storage (Catherine *et al*, 2000, Siviero and Motton, 2000, Naffa and Rabie, 2006).

Organic grape growers are prohibited from using sulfur dioxide, and ethanol could be considered as an alterative to avoid the common bleaching and rachis injury to grape berries caused by sulfur dioxide as well as sulfur residues (Luvisi, 1992 and Austin, 1997) Post harvest treatments with ethanol vapor and some salts increase

quality measurements of cantaloupe or cucumber fruits during cold storage (Saltveit and Sharaf, 1992, Atta –Aly *et al.*, 1999 Naffa, 2001 and Naffa and Rabie, 2006).

Many organisms can provide protection against disease infection such as *Trichoderma viride* Pers., Fr. (Borras and Agnilay, 1990). Isolates of *Trichoderma spp.* are used most often in biocontrol studies and are generally applied as conidial or chlamydospore preparations (Coley – Smith. *et al*, 1991). *Trichoderma spp.* (*TR*-5) controlled damping off of cucumber caused by *Pythium aphanidermatum* and, also, inhibitory to *Sclerotinia sclerotiorum*, *Pythium knmningense* and *Phytophthora sp. (*Xue *et al.,* 1995) *Trichoderma spp.* have proved to be useful in the control of phytopathogens affecting different crops (Benitez *et al.,* 2004)

The aim of this work was to study the effect of post harvest treatments of snap bean pods with *Trichoderma harzianum T. glioclidium* and *T. viride* or ethanol vapors in protecting pods from storage rots caused by *Botrytis cinerea, Sclerotinia sclerotiorum,* or *Pythium aphanidermatum* and maintaining quality of fruits during storage.

MATERIALS AND METHODS

Causal pathogens

Botrytis cinerea Pers., (gray mould), *Pythium aphanidermatum* (cottony mycelium) and *Sclerotinia sclerotiorum (*Lib) de Bary, isolated from decayed snap bean pods were used in this work.

Post harvest treatments

1) Three bioagents: *Trichoderma harzianum, T. glioclidium* and *T. viride* were loaded on sterilized filter paper disks at concentrations 1 x 10^7 / 5 x 10^6/ 33.3 x10^5 and 25 x10^5 *in vitro*, while dipping snap bean pods was made in 1 x 10^7 spore/ml bioagent *in vivo.*

2) Ethanol vapor: Vapor resulting from ethanol of different concentrations (25-50-75%) and three exposure times (1, 3 and 5 min) was evaluated in *vitro* and 75% for 5 min *in vivo* (Naffa, 2001).

***A-In vitro* experiments**

Effect of ethanol vapors and bioagents control treatments on mycelial growth of *Botrytis cinerea* Pers. Ex Nocea *Pythium aphanidermatum* and *Sclerotinia sclerotiorum* was studied *In vitro.* PDA medium seeded with pathogenic fungus and also untreated medium (control) was poured into Petri dishes in five replicates per treatment. After medium solidification, plates were inoculated with sterilized filter paper discs of bioagent incubated at 20 $\pm$ 2 °C for 7 days. On the other hand, ethanol vapor flow system was used to study the effect of ethanol on the fungus. The percentage of reduction in colony diameter was calculated as follows:

Reduction (%) of colony diameter = (de –dt) / de x 100
Where : de = average diameter of linear growth in control.
dt= average diameter of linear growth in treatment.

***B-In vivo* experiments**

Freshly harvested snap bean pods (Paulista cv) were hand picked from El-Aiatt, Giza for two successive seasons 2009 and 2010, both naturally infected and artificially inoculated with the three pathogens. Pods were wounded with a sterilized needle and sprayed with spore suspension of *B. cinerea*, at a concentration of $1x10^6$ spore/ml, and *Pythium aphanidermatum* at a concentration of $1x10^6$ spore/ml. The inoculation was carried out by *Sclerotinia sclerotiorum* growth disks (3mm) inserted in pods.

The inoculated pods were layed in plastic containers and covered with polyethylene sheets after setting a glass beaker filled with water as continuous support of high relative humidity (about 90-95% RH). The container was incubated at room temperature (22±2°C) for 24 hr to allow establishment of fungal infection. The inoculated pods were removed from the plastic container for bioagents treatment and exposing pods to ethanol vapor was carried out through a modified version (Naffa, 2001) of the air – flow system described by Atta – Aly *et al.*, (1999). Three replicates of artificially inoculated and naturally infected pod sets were not treated served as a control.

Bioagents and ethanol vapor treated as well as untreated snap bean pods were stored after packing in a cold room at 10°C for 4 weeks. At the end of cold storage period, the shelf life was estimated by removing the pods from the cold storage and keeping them at 22±2°C. Shelf-life was determined for all pods, as the 50% of the pods remains healthy without decay symptoms.

Development of snap bean rot after the cold storage as well as during the shelf life period was estimated according to the following categories:

0 = No decay appeared
1 = 1-25% of snap bean pods Decayed
2 = 26-50% of snap bean pods Decayed
3 = 51-75% of snap bean pods Decayed
4 = 75%-100 of snap bean pods Decayed

The infection percentage was determined and so disease severity was calculated according to the formula proposed by Forsberg (1970) as follows:

Disease severity % = Σ (n x v)/ 4N X 100

Where:

n =the number of affected snap bean pods of each category,

V = class or category of affected snap bean pods,

N = Total number of snap bean pods, and

4 =highest category for disease severity.

Snap bean pods Quality parameters

The snap bean pod quality parameters i.e., loss in weight and activity of polyglactorunase and polyphenol oxidase enzymes, were determined in the treated uninoculated pods and inoculated with *Botrytis cinerea, Pythium aphanidermatum* or *Sclerotinia sclerotiorum* after 4 weeks of cold storage.

1-Loss in Weight

Losses in snap bean pods fresh weight% was estimated in the inoculated or uninoculated snap bean of various treatments (average weight of 40 pods for each treatment) according to the following equation:

% L.W = (Initial weight - weight of snap bean at sampling date) x 100/ Initial weight of snap bean pods

2 - Activity of enzymes

The enzymes activity, i.e., polyglactorunase (PG), and polyphenol oxidase (PPO) were determined at the same dates of sampling for snap bean pods inoculated with *B. cinerea, P. aphanidermatum* or *S. sclerotiorum* and uninoculated treated pods.

Samples of 100 grams of snap bean pods of each treatment were blended in 200 ml distilled water for 2 minutes. The mixture was squeezed through several layers of cheesecloth and centrifuged at 3000 rpm for 20 minutes. Supernatants were kept at 5 °C until assaying was made. The measurement of enzyme activity was carried out in the filtrates according to the method of Aneja (2001).

1-polyglactorinase (PG) was assayed viscometrically

2-polyphenol oxidase (PPO) was determined using spectrophotometer, as described by Malik and Singh (1980).

Statistical analysis. All data obtained were subjected to the proper statistical analysis. Using the MSTAT statistical software and comparison was made following Fishers. L. S. D (0.05).

RESULTS AND DISCUSSION

Effect of *Trichoderma spp* against growth of *B. cinerea* and *S. sclerotiorum* and *P aphanidermatum*

Data in Table (1) Showed that all bioagents, i.e. *Trichoderma viride, Trichoderma harzianum* and *Trichoderma glioclidium* decreased the linear growth of *B. cinerea, P aphanidermatum* and *S. sclerotiorum* compared with the control. It was obvious that higher concentrations showed more suppressive effect on growth of the three pathogens. *Trichoderma harzianum* was most inhibitory against the three tested pathogens.

The data also, indicated that *Trichoderma viride,* at all concentrations, was significantly effective against *P aphanidermatum* and *S. sclerotiorum* on PDA medium followed by *Trichoderma glioclidium* in reducing growth of the three fungi.

Elad (1996) stated that the mechanisms of the antagonism of *Trichoderma spp.* against different pathogens may involve mycoparasitism, competition and antibiosis.

Table 1. Effect of certain bioagents on the linear growth (A) in mm and reduction percentage (B) of *B. cinerea P. aphanidermatum* and *S. sclerotiorum* on PDA medium at 20°C after 7 days

Bioagent	Cons.	*B. cinerea*		*P aphanidermatum*		*S. sclerotiorum*	
		(A)	(B)	(A)	(B)	(A)	(B)
T. viride	1×10^7	0.0	100	0.0	100	10.0	88.8
	5×10^6	0.0	100	3.0	96.6	15.0	83.3
	33×10^5	70.0	22.2	10.0	88.8	16.0	82.2
	25×10^5	80.0	11.1	14.0	84.4	18.0	80.0
	mean	37.5		6.75		14.75	
T. harzianum	1×10^7	0.0	100	0.0	100	0.0	100
	5×10^6	0.0	100	0.0	100	0.0	100
	33×10^5	0.0	100	0.0	100	0.0	100
	25×10^5	0.0	100	10	88.8	0.0	100
	mean	0.0		2.5		0.0	
T. glioclidium	1×10^7	0.0	100	0.0	100	18.0	80.0
	5×10^6	0.0	100	5.0	94.4	20.0	77.7
	33×10^5	30.0	66.6	11.0	87.7	22.0	75.5
	25×10^5	50.0	44.4	15.0	83.3	23.0	74.4
	mean	20		7.75		16.25	
Control	0.0	90.0	0.0	90.0	0.0	90.0	0.0
L,S.D at 0.5%							
Bioagents (B)		1.92		0.96		1.09	
Cons. (C)		2.49		1.24		1.40	
B x C		4.31		2.15		2.44	

Effect of ethanol vapor against growth of *B. cinerea* and *S. sclerotiorum* and *P aphanidermatum*

The inhibitory effect of different concentrations of ethanol vapor (25,50 and 75%) at the three exposure times 1, 3 and 5 min. showed that *B. cinerea* could not grow on media supplemented with ethanol vapor of 75% ethanol at the three

exposure intervals; however, complete inhibition of *B. cinerea* growth was observed for 50% ethanol at 5 min. exposure (Table 2)

Generally, ethanol vapor at all concentrations reduced the linear growth of the three pathogens. The high conc. (75 %) of ethanol vapor was effective in reduction of colony diameter of *B. cinerea P. aphanidermatum* and *S. sclerotiorum* by 100, 83.3 and 66.6%, respectively at the 5min exposure.

Ethanol vapor 80 % was effective in reducing the percentage of decayed tomato fruits (Atta –Aly *et al.*, 1999) and Gabler *et al.*, (2005) found that ethanol (70% wt/ vol) was the most effective to control *B. cinerea* on grape fruits.

Table 2. Effect of Ethanol vapor on the linear growth (A) and reduction percentage (B) of B. cinerea P. aphanidermatum and S. sclerotiorum on PDA medium after incubation at 20oC for 7 days

Conc.%	Time (min)	*B. cinerea*		*P. aphanidermatum*		*S. sclerotiorum*	
		(A)	(B)	(A)	(B)	(A)	(B)
25	1	37.0	58.8	55.0	38.8	85.0	5.5
	3	25.0	72.2	40.0	55.5	70.0	22.2
	5	15.0	83.3	30.0	66.6	50.0	44.4
50	1	30.0	66.6	50.0	44.4	75.0	16.6
	3	20.0	77.7	35.0	61.1	65.0	27.7
	5	0.0	100	20.0	77.7	40.0	55.5
75	1	0.0	100	40.0	55.5	50.0	44.4
	3	0.0	100	30.0	66.6	45.0	50.0
	5	0.0	100	15.0	83.3	30.0	66.6
control	0.0	90.0	0.0	90.0	0.0	90.0	0.0
L.S.D at 0.5%							
Ethanol (E)		2.59		7.0		4.73	
Time (T)		2.60		7.0		4.73	
E x T		4.50		12.13		8.32	

Post harvest treatments significantly reduced the severity of pod rots in artificially inoculated snap bean pods after cold storage at 10 ° C for 4 weeks, (Tables 3 and 4). The data showed that ethanol vapor treatment reduced severity without affecting the infection percentage with *P. aphanidermatum* and *S. sclerotiorum* (0.01 – 0.006) and (0.021 – 0.015) during seasons 2009 and 2010, respectively; however, *B. cinerea* disease severity was less affected. On the other hand, the lowest severity% was obtained by *T. glioclidium* (0.024) in case of *P. aphanidermatum* and by

Trichoderma harzianum, (1.50) with *B. cinerea* and *T. viride* (0.018) with *S. sclerotiorum* during season 2009. The same trend was observed in 2010 season.

Various mechanisms are suggested to clarify the role of antagonistic organisms in suppression of fungal growth that could be through antibiosis (Ghisalberti and Rowland, 1993), and / or mycoparasitism (Haran et al, 1996).

Table 3. Disease severity % and infection% of Ethanol vapor and some bioagents treated snap beans stored at 10 o C and 90%R.H. for 4 weeks in season 2009

Treatments	Fungi					
	B. cinerea		*P. aphanidermatum*		*S. sclerotiorum*	
	Dis	inf	Dis	inf	Dis	inf
Ethanol 75%/5min	20.5	100	0.01	100	0.006	100
T.viride10^7 spore/ml	12.3	100	3.70	100	0.018	100
T.harzianum10^7spore/ml	1.50	100	3.64	100	0.313	100
T.glioclidium10^7spore/ml	8.95	100	0.024	100	0.190	100
Control	90.43	100	83.33	100	80.02	100
L.S.D at 0.5%	3.09		2.53		4.20	

Dis = Disease severity % inf = infection%

Table 4. Disease severity % and infection% of Ethanol vapor and some bioagents control snap beans rots during cold storage at 10 o C and 90%R.H. for 4 weeks in season 2010.

Treatments	Fungi					
	B. cinerea		*P. aphanidermatum*		*S. sclerotiorum*	
	Dis	inf	Dis	inf	Dis	inf
Ethanol 75%/5min	22.6	100	0.021	100	0.015	100
T.viride10^7 spore/ml	13.2	100	3.61	100	0.021	100
T.harzianum10^7spore/ml	1.53	100	3.58	100	0.187	100
T.glioclidium10^7spore/ml	9.25	100	0.027	100	0.307	100
Control	92.59	100	86.42	100	77.16	100
L.S.D at 0.5%	0.84		2.16		1.63	

Dis = Disease severity % inf = infection%

Naturally infected snap bean pods stored at 10 ° C for 4 weeks during seasons 2009 and 2010 showed 29.63- 37.04% infection, respectively. (Table 5) The exposure of snap bean pods to ethanol vapor was the most effective treatment to suppress rot development during the cold storage for 4 weeks. (3.7 -7.41) followed by *T. glioclidium* (7.41 – 11.11) while, *T. harzianum* and *T. viride* resulted in infection of 22.22 -20.63% and 25.92 – 18.52% during seasons 2009 and 2010, respectively. Infection in the control was 29.63 – 37.04% in the respective seasons.

The mechanisms involved in Trichoderma are induction of resistance in plants (Yedidia et al, 1999).The mode of action of ethanol in terms of counteracting ethylene biosynthesis and action may explain the delays of tomato fruit ripening obtained with ethanol vapor application (Saltveit and Sharaf, 1992 and Atta –Aly et al., 1999)

Table 5. Disease severity % and efficacy of Ethanol vapor and some bioagents development natural infected snap bean pods.

Treatments	Seasons			
	2009		2010	
	Infection %	efficacy	Infection %	efficacy
Ethanol 75%/5min	3.7	87.51	7.41	79.99
T.viride10^7 spore/ml	25.92	12.52	18.52	50.0
T.harzianum10^7spore/ml	22.22	25.01	20.63	44.30
T.glioclidium10^7spore/ml	7.41	74.99	11.11	70.03
Control	29.63	0.0	37.04	0.0
L.S.D at 0.5%	0.69		3.04	

Effect of ethanol vapor and some bioagents on snap beans pods quality under natural infection and artificial inoculation

Data in Table(6). Show the shelf life of untreated snap bean pods at 22 ± 2 °C of either artificially inoculated with *B. cinerea P. aphanidermatum* and *S. sclerotiorum* or left to natural infection was (0 – 3 days), respectively when kept at 10 ° C for 4 weeks . The shelf life was prolonged to 11 -14 days in artificially and natural infection, respectively as a result of the different treatments. Post harvest treatments kept snap bean pods for long shelf life when cold stored 4 weeks were also the remarkable treatments that prolonged the shelf life. Naffa, (2001) showed that the bio products plant guard and Rhizo-N increased shelf life of cantaloupe fruits either naturally or artificially infected with *F. semitictum, C. herbarum* and *A. alternata* .

Table 6. Shelf life of snap bean pods in days at 22+2oC for artificially or naturally infected after storage at 10oC and RH 90-95% for 4 weeks.

Treatments	Artificially inoculation			Natural infection
	B. cinerea	*P. aphanidermatum*	*S. sclerotiorum*	
Ethanol 75%/5min	3	7	10	14
T.viride10^7 spore/ml	5	9	11	13
T.harzianum10^7spore/ml	9	8	7	10
T.glioclidium10^7spore/ml	3	10	8	12
Control	0.0	0.0	0.0	3

Data in Table (7) indicate that post harvest treatments reduced the loss in fresh weight of snap bean pods during the cold storage at 10 ° C and 90% RH for 4 weeks.

T.harzianum were the most effective treatments to reduce the weight loss of the naturally infected (0.56 – 1.30) and artificially inoculated with *B. cinerea* (0.71 – 1.01) during the cold storage.

The most probable mode of action of ethanol is on the penetration of proteins, particularly those of mitochondrial membranes (Larson and Morton, 1991). Smilanick (1995) found that ethanol controlled green mold without injury to fruit.

Table 7. Effect of Ethanol vapor and some bioagents on percentage weight loss of snap bean pods in natural or artificially infected after cold storage at 10 o C and 90% RH for 4 weeks storage.

Treatments	percentage of weight loss							
	Artificially infected with						Naturally infected	
	B. cinerea		*P .aphanidermatum*		*S. sclerotiorum*			
	Storage (weeks)							
	2	4	2	4	2	4	2	4
Ethanol 75%/5min	1.90	3.30	2.86	3.67	0.91	2.96	2.86	4.52
T.viride10^7spore/ml	1.23	2.43	1.59	3.23	3.83	5.72	1.57	2.92
T.harzianum10^7spore/ml	0.71	1.01	1.25	1.93	3.79	5.34	0.56	1.30
T.glioclidium10^7spore/ml	1.16	2.66	3.48	4.96	3.51	5.07	3.63	4.27
Control	3.69	4.12	5.03	6.88	4.12	6.41	4.36	6.85

Data in Table (8) indicate the activity of polygiacturnase enzymes (PG) in snap bean pods stored with post harvest treatments in naturally or artificially inoculated pods compared with the untreated control. Higher values of activity of (PG) after 30 min were recorded in artificially infected fruits compared with the untreated control. Generally, lower values were obtained for reduction in viscosity with *Trichoderma glioclidium* than other post harvest treatments except for samples infected with *S. sclerotiorum* .

Table 8. Effect of ethanol vapor and some bioagents on polyglactorunase activity on natural or artificially infected snap beans pods for 4 weeks storage.

Treatments	%Reduction in viscosity				
	Time (min)	Naturally infected	Artificially infected with		
			B. cinerea	*P. aphanidermatum*	*S. sclerotiorum*
Ethanol 75%/5min	15	20	14.63	33.33	11.11
	30	26	34.15	50.0	44.44
T.viride10^7spore/ml	15	2.56	12.56	34.0	20
	30	20	41.03	48.0	26
T.harzianum10^7spore/ml	15	11.1	13.95	14.89	9.09
	30	44.44	41.86	34.04	20
T.glioclidium10^7spore/ml	15	13.15	11.11	11.11	16.6
	30	21.05	31.11	33.33	22
Control	15	20.08	27.08	38.33	34.05
	30	43.90	47.92	60.0	45.24

Data in Table (9) indicated that all post harvest treatments increased the activity of peroxidase in snap bean pods naturally infected or inoculated with the three pathogens compared with the control. The increase of peroxidase activity was associated with increasing resistance against infection by many diseases, through the accumulation of phenolic compounds playing a role in disease resistance (Mitlerass *et al.*, 2006) Polyphenol oxidase activity increased in cucumber fruits inoculated with *Botrytis cinerea* and *Sclerotinia sclerotiorum*. (Naffa and Rabie, 2006)

Table 9. Effect of ethanol vapor and some bioagents on peroxidase activity on natural or artificially infected snap beans fruits for 4 weeks storage.

Treatments	Expressed as change in absorbance / minute/ g fresh weight			
	Naturally infected	Artificially infected with		
		B. cinerea	*P. aphanidermatum*	*S. sclerotiorum*
Ethanol 75%/5min	1.18	1.37	1.60	1.31
T.viride10^7 spore/ml	1.34	1.68	1.57	1.43
T.harzianum10^7spore/ml	1.37	1.95	1.54	1.57
T.glioclidium10^7spore/ml	1.42	1.69	1.50	1.63
control	1.01	1.18	1.20	1.09

Generally, post harvest treatments of snap bean pods reported here in reduced rots during storage.

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تأثير معاملات ما بعد الحصاد ببعض الكائنات الحية أو الايثانول لمقاومة أعفان ثمار الفاصوليا أثناء التخزين

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تتعرض قرون الفاصوليا أثناء التخزين المبرد لعديد من الفطريات أهمهم *Botrytis cinerea,* *Sclerotinia sclerotiorum* and *Pythium aphanidermatum* ولمقاومة هذه الفطريات تمت المعاملة ببعض الكائنات الحية أو الايثانول وأوضحت النتائج أن الفطر *Trichoderma harzianum* أدى إلى اختزال للمستعمرة بنسبة ١٠٠% للثلاث فطريات يليه *Trichoderma viride* ، *Trichoderma glioclidium* مقارنة بالكنترول الغير معامل. وعند استخدام تيار من بخار الايثانول بتركيز ٧٥% على ثلاث فترات ١-٣-٥ دقائق أدى إلى اختزال النمو الميسليومى بنسبة ١٠٠% لفطر *Botrytis cinerea.* وأوضحت النتائج أن *Trichoderma harzianum* أدت الى تقليل شدة الاصابة الى١,٥٠% على الثمار لفطر *Botrytis cinerea* يليه *Trichoderma glioclidium* (٨,٩٥%)، *Trichoderma viride* (١٢,٣%) بينما كان الايثانول أقل تأثيراً ٢٠,٥% مقارنه بالكنترول الغير معامل لمدة ٤ أسابيع عام ٢٠٠٩ .

عموما أعطت المعاملة الحيوية كفاءة أكثر لمكافحة الثلاث المسببات المرضية وحافظت كل المعاملات على جودة الثمار من حيث إطالة عمر قرون الفاصوليا بعد التخزين لمدة ٤ أسابيع في الثلاجة ١٠ °م وأيضا قللت الفقد في الوزن على الوجه الأخر وجد زيادة كبيرة في نشاط الأنزيمات المؤكسدة (البيروكسيديز) وقلت من نشاط البولي جلاكترونيز سواء للقرون الطبيعية بدون عدوى أو القرون المعدية بالثلاث فطريات .