PREVENTIVE EFFECT OF SOME BIO PREPARATIONS, NATURAL COMPOUNDS AND CHEMICAL SALT AGAINST POWDERY MILDEW OF STRAWBERRIES

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Abstract

Two different beneficial microorganisms namely *Bacillus subtilis* and *Trichoderma harzianum*, two commercial natural compounds namely soft guard and Chitocare (Chitosan) were used as foliar spray to study their effects on strawberry powdery mildew disease، compared with Calciven (calcium chloride) preparation at deferent rates (concentrations). Disease incidence, percentage of disease severity were determined under greenhouse conditions and field conditions, in addition to weight of crop and the shelf life period of the harvested fruits were determined under field conditions.

Treatments were implemented in greenhouse and field under natural infestation condition for two successive seasons 2007 - 2008 and 2008 - 2009.

Data of greenhouse show that treatments using *Bacillus subtilis* or soft guard or chitocare led to the best disease control and give 69.2% and 61.5% reduction of disease incidence, respectively، followed by *Trichoderma harzianum and* Calcivin gave 53.80% reduction of disease incidence. Data also show that positive correlation between concentration of different preparations and plant protection were noticed.

Results obtained from field experiments were in harmony with those obtained from greenhouse regarding plant protection. Under field conditions, all treatments led to increase in weight of the crop compared with control treatment. All treatments led to increase of shelf life of fruits to 7 days compared with 3 days in control treatment.

Key words: Biological control, Powdery mildew, Strawberry, *Bacillus subtilis, Trichoderma harzianum*, Chitosan, Calcium chloride.

INTRODUCTION

Strawberry plant *(Fragaria X ananassa)* is one of the most important exported agriculture products in Egypt. This is due to the good characteristic of Egyptian strawberries, early appearance in markets and also for high economic value (Abd- El-Moity, 2001).

Powdery mildew caused by *Sphaerotheca macularis* f.sp. *fragariae* is a fungal disease that attacks strawberry foliage, flowers and fruit, it's spores prefer intermittently moist conditions and do not germinate in free- standing water. Foggy

cool nights and worm days are the favorable condition for disease incidence (Martin and Born, 2007).

The wide spread use of fungicides to control plant diseases has led to an increase of health hazards due to their phytotoxic residues and pollution effects. Therefore, using some other means for disease control instead of agrochemical is strongly encouraged. Biological control of plant pathogenic fungi has received considerable attention as an alternative strategy (El-Rafai Ilham *et al.*, 2003,Abd El-Moneim Maisa *et al.*,2008). Several *Bacillus* spp. include *B. subtilis* are antagonistic to plant pathogens. *Bacillus* spp. produces at least 66 different antibiotic compounds (Ferreira *et al.* 1991). The antagonistic effect of *B. subtilis* against many fungi *in vitro* and *in vivo* reported by Abd El-Moity *et al.,* 2003, Abd El Moneim Maisa ,2005and Hussein *et al.,* 2007.

The use of *Trichoderma* spp. as a tool in the biological control of many plant diseases has been a subject of many studies (Osman *et al.*, 2001, Abd El-Moity *et al.,* 2003, Abd El-Moneim Maisa, 2005 and Hussein *et al.,* 2007).

Trichoderma spp. act through different mechanisms including mycoparasitism (Abd El-Moity and Shatla, 1981, Martin and Hancock,1987 and Benhamous and Chet,1993), also through production of antifungal substances (Turner, 1971 and Hayes, 1992). *Trichoderma* spp. also act through production of destructive enzymes *i.e.* chitinase (Pederes *et al.,* 1992 and Bolar *et al.,* 2000).

Chitosan a given name to a deacetylated form of chitin is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps, whose main attributes corresponds to its polycationic nature.

Chitosan as a natural compound has been proven to control numerous pre and post harvest diseases on different crops. (Bautista-Banos *et al.*, 2006), chitosan as safe material has antifungal activity against plant pathogens (El-Mougy Nehal *et al.*, 2006). Chitosan induce downy mildew disease resistance and promote growth (Sharathchandra *et al.*, 2004).

Some chemicals were reported as resistance inducers against plant disease. Calcium applications have been used to delay aging or ripening to reduce post harvest decay and control of many diseases in fruit and vegetables (Poovaiah, 1986, Conway *et al.,* 1994 and El –Gamal Nadia *et al.,* 2007). Foliar application of calcium chloride has been also reported to delay ripping and control mould diseases in strawberries (Cheour *et al.,* 1991). In addition to calcium is responsible for formation of strong cell walls (Suzuki *et al.*, 2003,Abd El-Moneim Maisa, 2005 and Abd El-Moneim Maisa *et al.,* 2008).

The present investigation aimed to study the effects of some different bio-agents, chitosan and calcium chloride on controlling powdery mildew of strawberries.

MATERIALS AND METHODS

Different preparations:

Different bio control agents isolates *Trichoderma harzianum*, *Bacillus subtilis,* in addition to the Calciven (calcium chloride preparation 15%) were obtained kindly from Central lab of Organic Agriculture, ARC, Giza. Whereas natural products of chitosan were used from commercial products soft Guard (Chitosan oligosaccharin) Leiliy Agro chemistry Co. LTD., China and chitocare (Chitosan oligomers) Sentic company.

Trichoderma harzianum was grown in liquid gliotoxin fermentation medium (G.F.M.) developed by (Brain and Hemming ,1945) for nine days. The suspension of *T. harzianum* was prepared by adjusting number of *Trichoderma* propagules in the suspension to be $30X10^6$ cfu / ml. *B.subtilis* was grown on nutrient glucose broth (NGB) developed by (Dowson, 1957) for 48h. The bacterial suspension was also adjusted to be contained $30X10^6$ cfu / ml. Calciven (Calcium chloride) preparation 15 % was used at the rate of 1 liter /150 liter of water where as bio preparation and chitocare were used at 1: 100liter of water. Softguard was used at the rate of 1 liter / 200 liter water.

Greenhouse Experiments:

For all greenhouse experiments unless otherwise mentioned, new pots 25 cm in diameter were used. Pots were filled with 2 kg soil. One strawberry seedlings variety Camarosa was transplanted in each pot and one hundred replicates were used for each treatment. These experiments were carried out in two growing seasons (2007-2008 and 2008-2009). Under natural infestation condition.

1- Evaluation of different preparations on control of Powdery mildew in strawberry plants in greenhouse:

All previous preparations were sprayed every 2 weeks. A group of strawberry, plants were sprayed with water only served as control. Different treatments were examined periodically and different parameters including percentage of disease incidence, disease severity and percentage of reduction in disease incidence and severity were measured to evaluate the effect of different preparations on disease control. Disease severity was determined according to the scale reported by Horsfal and Barratt (1945).

2- Effect of some different bio-agents at different concentrations against powdery mildew in strawberry plants in greenhouse:

Different bioagents (*T. harzianum* and *B. subtilis*) preparations were used as suspension at two different rates (1 / 100 and / 200 liter water). Plants were sprayed with water only act as control. Disease assessment was recorded as previously mentioned in (1) to find out the most effective concentration of these bio-agents in controlling powdery mildew on strawberries.

3- Effect of some preparations at different concentrations in control of powdery mildew in strawberry plants in greenhouse.

Soft guard as commercial chitosan product was used as suspension in water at different concentration e.g. 1:200, 1:400, 1:600 and 1:800, on the other side chitocare as another commercial chitosan product was used as suspension in water at different rate e.g. 1:100, 1:200 and 1:300.

Calciven (Calcium chloride) preparation was used as solution at two different rates 1:150 and 1:250. Plants received water only act as control. Disease was recorded as previously mentioned in (1) to find out the most effective concentration of theses preparations.

Field Experiments:

This study was carried out for two successive seasons (2007/2008 and 2008/2009) under natural infestation condition in one feddan area at Mashtol-El-Sharkia governorate. The experiment area was divided into three plots:

- One plot was used to evaluate all used treatments (bio-agents, Calciven, soft guard and chitocare) on controlling powdery mildew of strawberry (Kamarosa variety).
- The second plot was used to study the effect of different concentrations of different bio-agent treatment on controlling powdery mildew of strawberries.
- The third plot was used to study the effect of different concentrations of chitosan (soft guard and chitocare) and calcium chloride treatment on controlling powdery mildew of strawberries.

1- Evaluation of bio-agents, calcium chloride, soft guard and chitocare on controlling powdery mildew of strawberries:

In this study, the two bio-agents were used as suspension at the rate of one liter / 200 liter water. Calcium chloride at the rate of one liter/ 200 liter water was used. Soft guard was used at the rate of one liter/ 800 liter water, whereas chitocare was used at the rate of one liter/ 200 liter water.

All previous preparations were sprayed every two weeks from 15 September to first May. Plants received only water only act as control treatment. Different treatments were examined periodically and different parameters including percentage

of disease incidence, disease severity and percentage of reduction of both disease incidence and severity as well as the averages weight of crop/fed were also determined.

2- Effect of using some different bio-agents at different concentrations against powdery mildew in strawberry plants.

Different antagonists (*T. harzianum* and *B. subtilis* preparations were used as suspension at different concentrations i.e. one liter /100 or 200 liter of water. Plants only sprayed with water act as control treatment. Disease was recorded as previously mentioned in (1).

3- Effect of some preparations at different concentrations in controlling of strawberry powdery mildew.

Soft guard as commercial chitosan product was used as suspension at four different concentrations the rate of liter/ 200, 400, 600 and 800 liter water, on other side chitocare as another commercial chitosan product was used as suspension at the rate of liter/ 100, 200 and 300 liter water.

Calcium chloride preparation was used as solution at two concentrations of 1 liter / 150 and 250 liter water. Strawberry plants were sprayed with water only act as control. Disease assessment was recorded as previously mentioned in (1) to find out the most effective concentration of theses preparations in controlling powdery mildew on strawberry plants.

4- Effect of different preparations on the harvested strawberry fruits:

Random samples were taking from the yield of strawberries which was produced from plots of evaluation of bio agents, soft guard, chitocare and Calciven (calcium chloride) against powdery mildew.

Collected samples were placed on foam trays and ten replicates were used for each treatment. All trays were incubated at room temperature (25°c), trays were examined periodically and shelf life by days of healthy fruit was determined.

Statistical analysis was carried and means were compared using L.S.D at 5%. (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The effect of different bio-agent, chitosan and Calciven on controlling of strawberry powdery mildew was carried out under greenhouse conditions. Data presented in Table (1) show that, all tested bio-agents soft guard, chitocare and Calciven significantly reduced percentage of disease incidence, percentage of the disease severity compared with control treatment. It is also clear that *Bacillus subtilis* was the most effective treatment in decreasing both percentage of disease incidence

69.2% and disease severity 95%. Soft guard and chitocare followed the *B. subtilis* in their effect and gave 61.5% reduction in disease incidence, 87.5 and 85% in disease severity respectively. *Trichoderma harzianum* and Calciven gave 53.8% reduction in disease incidence and gave 93% and 87.5% reduction in disease severity respectively.

Treatment of *B. subtilis* led to the best reduction of strawberry powdery mildew disease incidence. This reduction might be due to that *B. subtilis* produces antifungal substances as subtilin (Johanson *et al.*, 1960). Data also showed that soft guard and chitocare which contain chitosan as active ingredient occupied the second rank in disease control, this might be due to that chitosan induces a series of defense reactions correlated to enzymatic activity in addition to increase the production of glucanhydrolases, phenolic compounds and synthesis of specific phytoalexins with antifungal activity (Bautist-Banos, *et al.*, 2006 and El- Mougy Nehal *et al.*, 2006). *T. harzianum* produces antifungal and antibiotic substances as gliotoxin, viridin (Abd El moity, 1981).

Table 1. Evaluation of different preparation on controlling of strawberry powdery mildew under greenhouse conditions :

Different treatments	%of disease incidence	%of disease severity	% reduction in disease	% reduction in disease severity
Bacillus subtilis	4.00	0.40	69.20	95.00
Trichoderma harzianum	6.00	0.50	53.80	93.00
Soft Guard	5.00	1.00	61.50	87.50
Chitocare	5.00	1.20	61.50	85.00
Calciven	6.00	1.00	53.80	87.50
Control	13.00	8.00	0.00	0.00
L.S.D at 5%	0.31	0.21	0.45	0.35

Regarding Calciven found that calcium is responsible for formation of strong cell walls this means that this walls play a role in disease reduction in disease incidence (Abd El-moneim Maisa, 2005).

Data recorded in Table (2) show that different concentrations of bio-agents led to different degrees of protection against powdery mildew disease. Positive correlation was observed between the level (concentration) of treatment and control of powdery mildew disease the percentages of reduction in disease severity were determined. This might be due to that treatment with bio-preparation at high level led to increase

secondary metabolites production, including antifungal and antibiotics compared with another concentration (Aly *et al.,* 1995).

Data obtained in Table (3) show that different concentration of soft guard and chitocare led to slight degrees of protection against powdery mildew whereas treatment by Calciven at different concentrations led to the same result of protection. This might be due to that chitosan or calcium chloride affect as resistance inducers or have some effect on cell wall building (Bautist-Banos, *et al.*, 2006 and El Gamal Nadia *et al.,* 2007).

Data in Table (4) show that all used antagonistic microorganisms and different chitosan products and calcium chloride gave positive effect against in powdery mildew of strawberry plants under field conditions. Data revealed that all treatments significantly reduced the percentage of disease incidence and disease severity and increase yield comparing with the control treatment. Also data indicated that *B. subtilis, T. harzianum* and soft guard were the most effective treatment against powdery mildew pathogen since they decreased disease incidence and disease severity by 68.78% and 95.8% respectively for *B. subtilis* and by 62.5 % and 91.6% respectively for *T. harzianum* and soft guard. Effect of *B. subtilis* might be due to the production of antibiotics Iturin and increase surfactin (Asaka and Shoda, 1996, Hwang *et al.,* 1996 and Ryder *et al.,* 1999). *Trichoderma sp.* act through different mechanisms including amycoparasitism (Abd El-Moity and Shatla,1981, Benhamous and Chet,1993), also through production of antifungal substances (Hayes 1992) soft guard as a chitosan product had antifungal activity against plant pathogens (El-Mougy Nehal *et al,* 2006) .Chitocare as a chitosan product and calcium chloride follow bio-agents and soft guard in their effect, they decrease the percentage of disease incidence and percentage of disease severity. All treatments led to increase the yield. *B. subtilis, T. harzianum* and soft guard were the most effective and gave 9.640, 9.360 and 9.365 ton / fed was recorded respectively. This effect could be attributing to some growth regulators produced by these antagonistic microorganisms as mentioned by Abd El-Moity (1981), Hwang *et al.* (1996), Sankar and Jeyarajan (1996) and Reguchender *et al.* (1997).

Table 2. Effect of some different bio agents at different concentrations against powdery mildew of strawberry plants in greenhouse.

Different treatments	%of disease incidence	%of disease severity	% reduction of disease	% reduction of disease severity
Bacillus subtilis				
1 - 100	4.00	0.40	69.20	95.00
1 - 200	5.00	1.00	61.50	87.50
Trichoderma harzianum				
1 - 100	6.00	0.50	53.80	93.00
1 - 200	6.00	0.70	53.80	91.25
Control	13.00	8.00	0.00	0.00
L.S.D at 5%	0.48	0.12	0 .52	0.15

Table 3. Effect of some preparation at different concentrations in controlling strawberry powdery mildew in greenhouse.

Different treatments	% disease incidence	% disease severity	% reduction of disease	% reduction of disease severity
Soft Guard				
1 - 200	5.00	1.00	61.50	87.50
1 - 400	5.00	1.10	61.50	86.25
1 - 600	5.00	1.20	61.50	85.00
1 - 800	5.00	1.50	61.50	81.25
Chitocare				
1 - 100	5.00	1.20	61.50	85.00
1 - 200	6.00	1.30	53.80	83.75
1 - 300	6.00	1.50	53.80	81.25
Calciven				
1 - 150	6.00	1.00	53.80	87.50
1 - 250	6.00	1.00	53.80	87.50
Control	13.00	8.00	0.00	0.00
L.S.D at 5%	0.59	0.22	0.66	0. 25

Table 4. Evaluation of different preparation on controlling powdery mildew of strawberries under field conditions.

Different treatments	%of disease incidence	%of disease severity	% reduction of disease	% reduction of disease severity	Weight of crop / fed
Bacillus subtilis	5.000	0.500	68.750	95.800	9.640
Trichoderma harzianum	6.000	1.000	62.500	91.600	9.360
Soft Guard	6.000	1.000	62.500	91.600	9.365
Chitocare	7.000	1.200	56.250	90.000	8.640
Calciven	8.000	1.000	50.000	91.600	7.930
Control	16.000	12.000	0.000	0.000	7.200
L.S.D at 5%	0.350	0.230	1.530	0.650	0. 250

Soft guard as a chitosan product increased harvested yield. This might be due to its ability to form a semi permeable coating (Bautist-Banos, *et al.*, 2006).

Effect of some bio-agents at different concentrations against powdery mildew strawberry crop in field:

Data recorded in Table (5) show that different concentrations of different bio-agents led to different degrees of protection against powdery mildew disease. Positive correlation was observed between the levels (concentrations) of treatment and reduction of disease and increase of yield if compared with control treatment. This might be due to that treatment with bio-preparation at high level increase secondary metabolites, enzymes and growth regulators compared with another concentration (Abd El-Moity, 1981 and Rodriguez and Cotes, 1999). In addition to the high concentration contain high number of antagonists.

Since there is correlation between the number of antagonist and efficacy of the treatment , so high concentration gave best result in disease control (Abd El-Moity *et al.*, 1997 and Abd El Moneim Maisa, 2005).

Data recorded in Table (6) show the effect of different concentrations of soft guard and chitocare as a chitosan products and Calciven (calcium chloride) salt in controlling strawberry powdery mildew. Different all tested treatments concentrations gave the same result in reduction of disease incidence. Whereas positive correlation was observed between the levels (concentrations) of different tested treatments and

percentage of reduction of disease severity and weight of yield/ fed. This might be due to chitosan has antifungal activity against plant pathogens (El-Mougy Nehal *et al.*, 2006). In addition chitosan induce structural barriers and synthesis of specific phytoalexins with antifungal activity (Bautist-Banos, *et al.*, 2006). In addition to chitosan increased harvested yield due to its ability to form a semi permeable coating (Bautist-Banos, *et al.*, 2006).

On the other hand, high level of calcium chloride increase the efficacy of control the disease and increase the yield. This finding might be due to that calcium is responsible for formation of strong cell walls. Calcium deficiency in plasma membranes caused cell collapses so spray with calcium chloride improve the cell structure and prevent the disease incidence and finally increase the yield (Suzuki *et al.*, 2003 and Abd El Moneim Maisa, 2005).

Table 5. Effect of some different bio agent at different concentration against powdery mildew of strawberries in field:

Different treatments	%of disease incidence	%of disease severity	% reduction of disease	% reduction of disease severity	Weight of crop / fed
Bacillus subtilis					
1 - 100	5.00	0.50	68.75	95.80	9.640
1 - 200	6.00	1.00	62.50	91.60	8.940
Trichoderma harzianum					
1 - 100	6.00	1.00	62.50	91.60	9.360
1 - 200	8.00	1.10	50.50	90.80	8.920
Control	16.00	12.00	0.00	0.00	7.200
L.S.D at 5%	0.30	0.20	0. 39	0.26	0.17

Table 6. Effect of some preparation at different concentration in control powdery mildew of strawberries in field.

Different treatments	%of disease incidence	%of disease severity	%reduction of disease	%reduction of disease severity	Weight of yield / fed
Soft Guard					
1 - 200	6.000	1.000	62.500	91.600	9.365
1 - 400	6.000	1.200	62.500	90.000	9.340
1 - 600	6.000	1.500	62.500	87.500	9.310
1 - 800	6.000	2.000	62.500	83.330	9.300
Chitocare					
1 - 100	7.000	1.200	56.250	90.000	8.640
1 - 200	7.000	1.500	56.250	87.500	8.610
1 - 300	7.000	2.000	56.250	83.330	8.600
Calciven					
1 - 150	8.000	1.000	50.000	91.600	7.930
1 - 250	8.000	1.200	50.000	90.000	7.920
Control	16.000	12.000	0.000	0.000	7.200
L.S.D at 5%	0.380	0.570	0. 390	. 630	0.940

Effect of different preparations on the harvested strawberry fruits:

Data obtained in Table (7) show that all treatments both different bioagent and natural compounds as chitosan and natural salt as calcium chloride led to increase the shelf life to 7 days if compared with control has 3 days shelf life. This may be due to that bio-agents increase the dry matter fruits and reduce the water content (Abd El moity, 2001). Where as chitosan minimize the rate of respiration and reducing water loss in treated fruits (Bautist-Banos *et al.*, 2006). Calcium chloride improve the cell structure and led to increase the shelf life (Suzuki *et al.*, 2003 and Abd El moneim, 2005).

In regarding to the percentage of healthy fruits (un rotted) after 7 days we observed that calcium chloride gave the best result if compared with control. This might be due to that calcium chloride improve the cell structure and led to protect the fruit against any mechanical damage and protect the fruits against saprophytic fungi (Suzuki *et al.*, 2003 and Abd El moneim *et al.*, 2008). *B. subtilis* follows calcium

chloride and gave 70% of healthy fruits after 7 days, this may be due to that *B. subtilis* grows very fast and occupies the court of infection and consumes all available nutrients. These actions prevent pathogen spores to germinate and reach susceptible tissues. Also its effect might be due to competitive for spaces or nutrients (Wolk and Sorkar, 1994). *T. harzianum* follows *B. subtilis* and gave 60% of healthy fruits after 7 days, this may be due to that this genus enhances plant resistance through stimulating defense response (Elad *et al.*, 1998 and Howell *et al.*, 2000). Whereas soft guard and chitocare gave 50% and 30% of healthy fruits after 7 days, respectively. This may be due to that chitosan minimize the rate of respiration and reducing water loss (Bautist-Banos *et al.*, 2006).

Table 7. Effect of different preparation on shelf life periods of the harvested strawberry fruits:

Different treatments	shelf life by days	%of healthy fruits after 7 days	%of rotted fruit
Bacillus subtilis	7.00	70.00	30.00
Trichoderma harzianum	7.00	60.00	40.00
Soft Guard	7.00	50.00	50.00
Chitocare	7.00	30.00	70.00
Calciven	7.00	80.00	20.00
Control	3.00	0.00	100.00
L.S.D at 5%	0.80	1.93	4.12

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التأثير الوقائي لبعض التجهيزات الحيوية و المركبات الطبيعية و ملح كيماوي ضد البياض الدقيقي في الفراولة

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تم رش المجموع الخضري للفراولة بكائنات المقاومه الحيويه. (الباسيلس ستلس و التريكوديرما هارزيانم) ومركبات الشيتوزان التجارية (سوفت جارد و شيتوكير) و ذلك مقارنة برش الكالسيفين (ملح كلوريد الكالسيوم) و ذلك بتركيزات مختلفة لمقاومة مرض البياض الدقيقي في الفراولة و تم تقدير كل من نسبة المرض و شدته وذلك تحت ظروف الصوبة بينما تم تقدير نسبة المرض و شدته بالإضافة الي وزن المحصول و تقدير المدة التي تبقى فيها الثمار سليمة بعد الحصاد وذلك تحت ظروف الحقل.

تم استخدام كل المعاملات السابقة بتركيزات مختلفة. تحت ظروف العدوي الطبيعيه بالصوبة و الحقل وفي موسمين متتاليين (٢٠٠٧-٢٠٠٨ و ٢٠٠٨-٢٠٠٩) .

أوضحت النتائج المتحصل عليها في الصوبة أن المعاملة بالباسيلس ستلس ومركب السوفت جارد اوالشيتوكير أعطت أحسن مقاومة للمرض و ذلك بنسبة خفض للمرض تراوحت بين ٦٩,٢ % للباسيلس ستلس و ٦١,٥ % لمركب السوفت جارد او الشيتوكير يلي ذلك مركب التريكوديرما هارزيانم اوالكالسيفين اعطى نسبه ٥٣,٨% خفض لحدوث المرض . كما أوضحت النتائج ايضا أن هناك علاقة طردية بين درجة وقاية نباتات الفراولة و التركيز المستخدم في كل التجهيزات المختلفة.

اثبتت النتائج المتحصل عليها من تجارب الحقل انها كانت متوافقة مع نتائج الصوبة. فقد أدت كل المعاملات إلى زيادة المحصول الناتج و ذلك مقارنة بالكنترول تحت ظروف الحقل. كما أدت كل المعاملات أيضاً إلى زيادة فترة بقاء الثمار سليمة بعد الحصاد إلى سبعة أيام مقارنة بالكنترول و التي كانت ثلاثة أيام.