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A Protocol Suggested for Managing Tomato Early Blight

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Application of five fungicides, *i.e.* Consento, Flent, Score, Sereno and Tridex 8% as well as five antioxidants, *i.e.* Bion, calcium chloride, lithium sulphate, potassium mono-hydrogen-phosphate and salicylic acid, caused significant reduction in the linear growth of *Alternaria solani*, the causal of tomato early blight, compared with check treatment. This reduction was gradually increased by increasing the incorporated concentration. Also, these compounds resulted in significant reduction in the artificial infection by the disease with considerable increase in the foliage fresh weight of tomato plants compared with check treatment. Spraying tomato plants with the tested fungicides, antioxidants and the alternation between them in field experiments during 2007 and 2008 growing seasons revealed that fungicides were the most efficient in managing the natural infection of the disease and resulted in producing the highest fruit yield compared with antioxidants as well as the alternation between them.

Keywords: Antioxidants, early blight, fungicides, Tomato.

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important solanaceous crops in Egypt either for local consumption and exportation. Tomato is considered as one of the highest nutritional crops because of its high contents of vitamin C as well as many chemical compounds and elements which are not found in the other solanaceous crops. The cultivated area during the year of 2007 reached to about 461,351 feddan which yielded about 7,270,196 ton of fruits (Anonymous, 2008). Under Egyptian conditions tomato plants are vulnerable to infect by bacterial, fungal and viral diseases (Gomaa, 2001; Abdel-Sayed, 2006 and Abada *et al.*, 2008b), in addition to physiological disorders and nematode infections. However, fungal diseases, especially early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, is the most threatening one (El-Abyad *et al.*, 1993; Gomaa, 2001; Abdel-Sayed, 2006 and Abada *et al.*, 2008b), which causes great reduction in the quantity and quality of fruit yield. In addition, the disease is favoured by warm temperature and extended periods of leaf wetness from dew, rain fall and crowded plantation. The plants are more susceptible to infection by the disease during fruiting period (Cerkauskas, 2005 and Momel and Pemezny, 2006). It is well known that tomato fruits are mostly consumed freshly, thereby spraying fungicides just before harvesting resulted in high fungicide residue in the fruits, which cause great hazard to the human health.

Therefore, this work was mainly planned to evaluate the efficiency of the alternation of spraying some fungicides and antioxidants on reducing the natural infection of tomato early blight.

Materials and Methods

Effect of some fungicides and antioxidants on the linear growth of Alternaria solani:

Five fungicides, *i.e.* Consento (fenomen 7.5 + prmamocarp HCl 37.5%), Flent (trifloxytrobin), Score (difenoconazole), Sereno (mancozeb 50% + Femamidone) and Tridex 80% (mancozeb) as well as five antioxidants, *i.e.* Bion (bezal 1,2,3 thiadiazole,7 carbothioic acid 5-methylester), calcium chloride ($CaCl_2$), lithium sulphate (Li_2SO_4), potassium mono-hydrogen-phosphate (K_2HPo_4) and salicylic acid ($C_7H_6O_3$), were kindly provided from Prof. Kahiry A. Abada, Plant Pathol. Dept., Fac. Agric., Cairo Univ., and evaluated for their effects against the *in vitro* linear growth of *A. solani* (Ellis & Martin) the causal of tomato early blight.

Different concentrations of the tested fungicides, *i.e.* 10, 25, 50, 100, 250, 500, 750 and 1000 ppm (depending on their active ingredient) and the tested antioxidants, *i.e.* 5, 10, 20, 30, 40, 50 and 75 mM, were tested. Certain volumes of the prepared fungicides and antioxidants were added to the proper amounts of V_8 medium (200ml vegetable juice, 20.0g Agar and 800ml distilled water) just before solidification to obtain the proposed concentrations, and then poured into sterilized Petri-dishes (9-cm-diam.). All dishes were inoculated in the centre with 5-mm-discs taken from the edge of 8-day-old *A. solani* cultures. Five replicated dishes containing V_8 only or inoculated with *A. solani* were used as check. All dishes were incubated at 30±1°C for 7 days (Gomaa, 2001), then the fungus linear growth was measured and the average was calculated.

Greenhouse experiment:

Apparently healthy tomato seedlings (cv. Super strain B), 30-day-old, were transplanted in plastic pots (25-cm-diam.) containing clay soil disinfested by 5% formalin. Three seedlings were transplanted in each pot and three weeks later the plants were sprayed with Consento, Flent, Score, Sereno and Tridex 80% at 250 ml, 20 g, 50 ml, 250 g and 250 g/100 litre water, respectively, meanwhile all tested antioxidants were sprayed at 50 mM. Super film, as sticker and spreader material, was added at the rate of 50ml / l water to the tested fungicides and antioxidants. Three days after chemical treatments, tested plants were sprayed with the tested fungus spore suspension ($1x10^3$ spore / ml) and kept under plastic cages for 48 h to maintain high humidity (Abdel-Sayed, 2006). Two weeks later, the plants received another spray with the tested chemicals. Four pots were used as replicates for each treatment. A set of plants, sprayed with the tested fungus and water only, was kept as check. The disease severity was assessed two weeks after the second chemicals spray. Also, foliage fresh weight of the tested plants was recorded and the average weight/plant was calculated.

Field experiments:

Field experiments were conducted during 2007 and 2008 growing season at Dakahliya governorate under natural conditions of infection by early blight.

The experimental field comprised in plots, each of 6x7m (42 m^2), and received all recommended agricultural practices as irrigation and fertilization. Apparently healthy seedlings (30-day-old) of tomato (cv. Super Strain B) were transplanted

during mid of April of each successive season. The applied treatments were designed as follows:

1. Plants received six sprays, with the tested fungicides at aforementioned doses, at two week intervals.
2. Plants received six sprays, with any of Bion or salicylic acid (the highest efficient antioxidants) at 50 mM, at two week intervals.
3. Plants received three sprays, with any of the aforementioned fungicides, at two week intervals (until formation of pre-mature green fruits) then received another three sprays with any of Bion or salicylic acid at two week intervals.
4. Plants received water only and served as a check treatment.

Three plots were used as replicates for each treatment. Spraying program began one month after transplanting the seedlings. Disease severity assessed one week after each spray and the average was calculated. Also, the number of infected fruits/plot as well as fruit weight (kg/plot) was recorded at each harvesting time and the averages were calculated.

Disease assessment:

The severity of early blight disease was determined using the devised scale (0-7) described by Twnsend and Heuberger (1943) as follow:

$$\text{Disease severity (\%)} = \frac{\text{Sum (n x r)}}{\text{NR}} \times 100$$

Whereas: n= Number of infected leaves on the plant.
N= Total number of leaves on the plant.
r= Numerical rate of infected leaves.
R= Highest numeric rate.

Statistical analyses:

Obtained data were statistically analyzed using either split design method or the completely randomized design according to Snedecor and Cochran (1967). Treatment averages were compared at 0.05 level of probability using L.S.D. procedure (Fisher, 1948).

Results

1- Effect of some fungicides and antioxidants on the linear growth of A. solani:

1-1- Effect of fungicides:

Data presented in Table (1) show that all the tested fungicides, *i.e.* Consento, Flent, Score, Sereno and Tridex 80% caused significant reduction to the linear growth of *A. solani*, when compared with the check treatment. This reduction was gradually increased by increasing the tested concentration.

Flent was the most efficient fungicide, when caused complete inhibition to the fungus linear growth at 50 ppm, followed by Score then Sereno (at 100 and 500 ppm, respectively). Meanwhile, both Consento and Tridex 80% caused complete inhibition at 750 and 1000 ppm, respectively.

Table 1. *In vitro* effect of five fungicides on the linear growth of *A. solani*

Fungicide	Linear growth (mm) at concentration (ppm)								
	10	25	50	100	250	500	750	1000	Mean
Consento	87.0	85.6	80.0	73.2	65.0	47.6	0.0	0.0	57.1
Flent	53.2	21.0	0.0	0.0	0.0	0.0	0.0	0.0	9.3
Sereno	81.6	72.8	64.6	48.0	21.0	0.0	0.0	0.0	36.0
Score	64.0	49.4	20.2	0.0	0.0	0.0	0.0	0.0	16.7
Tridex 80%	88.0	85.0	81.2	73.6	65.8	48.0	17.8	0.0	57.4
Check	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
Mean*	74.8	62.8	49.2	39.0	30.4	19.1	7.2	0.0	-----
L.S.D. at 5% for: Fungicides (F)= 2.4, Concentrations (C)=1.7, F x C= 3.1									

* Mean is not included the check values.

1-2- Effect of antioxidants:

Data in Table (2) indicate that all the tested antioxidants caused significant reduction in the linear growth of *A. solani*, when compared with the check treatment. This reduction gradually increased by increasing the incorporated concentration.

Salicylic acid was the most efficient one in this regard, as it caused complete reduction to the linear growth at 40 mM followed by Bion (at 50 mM), then lithium sulphate (at 75mM.). On the other hand, both calcium chloride and potassium mono-hydrogen-phosphate failed to cause complete inhibition even at 100 mM.

Table 2. *In vitro* effect of five antioxidants on the linear growth of *A. solani*

Antioxidant	Linear growth (mm) at concentration (mM)								
	5	10	20	30	40	50	75	100	Mean
Bion	86.0	76.0	64.6	48.2	18.6	0.0	0.0	0.0	36.7
Calcium chloride	84.2	78.6	65.8	59.4	46.4	34.2	18.0	11.0	52.0
Lithium sulphate	76.8	63.2	47.6	32.2	22.8	12.0	0.0	0.0	30.1
Potassium mono-hydrogen-phosphate	86.6	76.8	66.0	58.2	47.8	34.6	17.6	10.6	49.8
Salicylic acid	78.2	65.4	49.0	20.0	0.0	0.0	0.0	0.0	26.6
Check	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
Mean*	83.2	72.0	58.6	43.6	27.1	16.2	7.1	4.3	-----
L.S.D. at 5% for: Antioxidants (A)= 2.4, Concentrations (C)=1.4, A x C= 2.6									

* Mean is not included the check values.

2- Greenhouse experiments:

Presented data in Table (3) reveal that all the tested fungicides and antioxidants caused significant reduction to the artificial infection by the tested fungus and increased foliage fresh weight compared with check treatment. Moreover, tested fungicides, *i.e.* Consento, Flent, Score, Sereno and Tridex 80%, were more efficient in reducing the disease, being 4.7, 5.0, 2.4, 5.0 and 2.0%, respectively, when

Table 3. Effect of spraying tomato plants with some fungicides and antioxidants on the severity of early blight and foliage fresh weight under greenhouse conditions

Treatment	Disease severity (%)	Foliage fresh weight * (g/plant)
Fungicides:		
Consento	4.6	201.3
Flent	5.5	210.0
Score	2.4	218.6
Sereno	5.0	224.0
Tridex 80 %	2.0	225.0
Antioxidants:		
Bion	12.4	189.0
Calcium chloride	14.6	186.1
Lithium sulphate	13.8	190.9
Potassium mono-hydrogen-phosphate	15.0	186.0
Salicylic acid	10.4	192.4
Check	41.2	118.7
L.S.D. at 5 %	1.8	2.3

* Data were recorded 54 days after planting, when tested plants reached 84 day-old.

compared with the tested antioxidants, *i.e.* Bion, calcium chloride, potassium mono-hydrogen-phosphate and salicylic acid, being 12.4, 14.6, 13.8, 15.0 and 10.4%, respectively. The same trend was found when foliage fresh weight was considered, being 201.3, 210.0, 218.6, 224.0 and 225.0 g / plant for the tested fungicides and 189.4, 186.1, 190.9, 186.0 and 192.4 g/plant, respectively. Check plants, unsprayed with any chemicals, recorded 42.1% disease severity and 118.7 g/plant fresh weight.

3-Field experiments:

3-1- Effect on disease severity and the number of infected fruits:

On base of the results obtained from greenhouse experiments, both Bion and salicylic acid were chosen, for their higher efficiency, to apply them alone or in alternation with the tested fungicides under field conditions. Data presented in Table (4) show that spraying tomato plants, naturally infected by early blight, with the tested fungicides, *i.e.* Consento, Flent, Score, Sereno and Tridex 80%, resulted in high reduction in the disease severity, where it reached, on the average of the two successive growing seasons, 7.2, 4.9, 3.4, 8.7, and 7.7%, respectively. Meanwhile, the number of infected fruits recorded an average of 3.0, 1.2, 0.8, 3.3 and 1.6, respectively. Meanwhile, spraying with any of Bion or salicylic acid recorded average of 21.7 and 20.7% disease severity as well as 7.6 and 7.0 infected fruits, respectively. On the other hand, the alternation between tested fungicides and antioxidants resulted in intermediate figures of disease severity and infected fruits. Check plants recorded 52.5% disease severity and 23.6 infected fruits. No significant differences were detected between the values of disease severity or infected fruits due to the effect of the growing season.

Table 4. Effect of spraying tomato plants with fungicides, antioxidants and their alternations on the severity of early blight and number of infected fruits under field conditions during 2007 and 2008 growing seasons

Treatment	Disease severity (%)		Mean	Number of infected fruits		Mean
	2007	2008		2007	2008	
Fungicides:						
Consento (C)	7.2	7.2	7.2	3.0	3.0	3.0
Flent (F)	4.9	4.8	4.9	1.1	1.2	1.2
Score (S)	3.2	3.5	3.4	0.7	0.8	0.8
Sereno (SE)	8.6	8.7	8.7	3.2	3.1	3.3
Tridex 80% (T)	7.5	7.8	7.7	1.5	1.6	1.6
Antioxidants:						
Bion (B)	21.4	22.0	21.7	7.6	7.5	7.6
Salicylic acid (SA)	20.1	21.2	20.7	7.0	7.0	7.0
Alternation:						
C then B	10.8	11.0	10.9	5.0	5.1	5.1
C then SA	10.0	10.4	10.2	5.0	5.0	5.0
F then B	7.4	7.6	7.5	5.0	5.1	5.1
F then SA	6.9	7.0	7.0	5.0	4.8	4.9
S then B	6.2	6.3	6.3	4.1	3.9	4.0
S then SA	5.9	6.0	6.0	3.7	3.5	3.6
SE then B	11.7	12.0	11.9	6.4	6.3	6.4
SE then SA	11.2	11.6	11.4	6.2	6.0	6.1
T then B	11.0	11.0	11.0	6.1	6.3	6.2
T then SA	10.8	11.0	10.9	6.0	6.1	6.1
Check	51.8	53.2	52.5	2 3.7	23.5	23.6
Mean*	9.7	10.0	-----	4.1	4.1	-----
L.S.D. at 5% for: Treatments (T)=		1.2			1.8	
Seasons (S) =		n.s.			n.s.	
T x S =		1.9			2.2	

* Mean is not included the check values.

3-2- Effect on fruit yield:

Data in Table (5) indicate that the efficiency of the tested fungicides and antioxidants, either alone or in alternation, was reflected on the produced fruit yield. In this regard, plants sprayed with tested fungicides produced the highest fruit yield, on the average, being 259.1, 279.0, 285.8, 254.8 and 265.7 kg/plot for Consento, Flent, Score, Sereno and Tridex 80%, respectively, meanwhile plants sprayed with Bion or salicylic acid produced 189.2 and 191.4 kg / plot, respectively. Meanwhile, the alternation between the fungicides and any of Bion or salicylic acid produced intermediate fruit yield, ranged between 245.0 and 283.5 kg / plot. Check plants recorded, on the average, a small amount of fruit yield, being 98.3 kg/plot. Again, no significant differences were detected between the values of fruit yield due to the effect of the growing season.

Table 5. Effect of spraying tomato plants (cv. Super Strain B), naturally infected by early blight, with fungicides and/or antioxidants on the produced fruit yield under field conditions at Dakahliya governorate during 2007 and 2008 growing seasons

Treatment	Average fruit yield (kg)/ plot (42m²) during 2007	2008	Mean
Fungicides:			
Consento (C)	258.1	260.0	259.1
Flent (F)	280.0	278.0	279.0
Score (S)	285.4	286.1	285.8
Sereno (SE)	254.5	255.0	254.8
Tridex 80% (T)	265.0	266.4	265.7
Antioxidants:			
Bion (B)	185.2	190.1	189.2
Salicylic acid (SA)	190.0	192.7	191.4
Alternation:			
C then B	247.6	248.3	248.0
C then SA	248.5	250.0	249.8
F then B	276.1	277.0	276.6
F then SA	279.0	280.4	279.7
S then B	280.0	279.6	279.8
S then SA	283.0	284.0	283.5
SE then B	243.5	247.0	245.0
SE then SA	245.0	246.5	245.8
T then B	249.0	250.0	249.5
T then SA	251.0	252.8	251.9
Check	98.7	97.8	98.3
Mean*	255.4	256.0	-----
L.S.D. at 5 % for: Treatments (T)= 3.1 , Seasons (S)= n.s. and T x S = 2.7			

* Mean is not included the check values.

Discussion

The effect of five fungicides, *i.e.* Consento, Flent, Score, Sereno and Tridex 80% as well as five antioxidants, *i.e.* Bion, calcium chloride, lithium sulphate, potassium mono-hydrogen-phosphate and salicylic acid, were *in vitro* evaluated for their inhibitory effect on the linear growth of *Alternaria solani*, the causal of tomato early blight. Also, they were evaluated under greenhouse conditions for their efficiency in managing the artificial inoculation with the causal of the disease. Moreover, these fungicides and the most efficient antioxidants in the greenhouse experiments, *i.e.* Bion and salicylic acid, were applied under field conditions in alternations to manage the natural infection of the disease.

It is well known that using the fungicides is considered as the shortest way to obtain efficient results of disease management. However, due to the hazard effect, on the human and/or animals health, of the fungicides residue in the produced plant products especially vegetables which are consumed after a short time of fungicide application, alternative methods of disease management, rather than fungicides, are needed and/or make a schedule or a program of using fungicides before fruit harvesting by a long period until occurring degradation to toxic component of the sprayed fungicides. But, the period after spraying the fungicides needs another safety trial of disease management to lowering disease hazard, in order to obtain adequate results of disease management and to obtain plant products free from fungicides residue. Thus, antioxidants are resumed as an alternative safety chemicals in this regard.

Obtained data revealed that all the tested fungicides and antioxidants caused significant reduction in the linear growth of *A. solani*. This reduction was gradually increased by increasing the incorporated concentration to V_8 medium. Furthermore, tested fungicides were more efficient than the tested antioxidants in this regard. Flent and Score as well as Bion and salicylic acid were the most efficient in inhibiting the linear growth of the tested fungus. The inhibitory effect of most of these fungicides to the growth of *A. solani* was reported by many researchers (Lauws *et al.*, 1996; Gomaa, 2001; Patil *et al.*, 2001; Abdel-Sayed, 2006 and Abada *et al.*, 2008b). Also, the inhibitory effect of some antioxidants to the growth of *A. solani* was investigated by Tofali *et al.* (2003); Abdel-Sayed (2006) and Abada *et al.* (2008b). The high inhibitory effect of the tested fungicides to *A. solani* is expected since these fungicides are commercially recommended for managing tomato early blight. On the other hand, the inhibitory effect of the tested antioxidants may be due to their direct toxic effect and/or their interaction with the component of V_8 medium that suppressed the absorbance of the nutrient substances from the medium (Abada *et al.*, 2008b).

Greenhouse experiments indicated that spraying any of the tested fungicides and antioxidants resulted in significant reduction in the artificial infection by early blight with considerable increase in the foliage fresh weight of tested tomato plants. However, the tested fungicides were more efficient than antioxidants in this regard. Moreover, Score was the most effective fungicide against early blight disease, followed by Flint. Also, the same trend was observed in the field experiments. These results are in a harmony with those recorded by many researchers (Singh *et al.*, 2000; Gomaa, 2001; Patil *et al.*, 2001; Tofali *et al.*, 2003 and Abdel-Sayed, 2006).

It is worth to note that, when Bion or salicylic acid was sprayed on tomato plants (three times in two week intervals) after the application of tested fungicides (three times in two week intervals), the reduction in the natural infection of the disease and the fruit yield were, to some what, lower than that recorded in case of application of the fungicides alone, but it was still higher than that of the application of antioxidants alone. This procedure gives a chance to the degradation changes of the sprayed fungicides to another safety substances and/or lowering their residue to the permitted concentration in the formed fruits.

It is frequently mentioned that using of antioxidants as resistance inducers become known as a safety method of reducing the severity of many diseases including early blight of tomato (Reuveni *et al.*, 1993 and 1997; Kessmann, *et al.*, 1994; Spletzer and Enyedi, 1999; Abdel-Sayed, 2006; Gado, 2006 and Abada *et al.*, 2008b). Reuveni *et al.* (1997) reported that a single spray of 100 mM solution of K_2HPO_4, KH_2PO_4, $Na_4P_2O_2$ and Na_3PO_4 on the upper surface of the first true leaf of cucumber plants, 2h before inoculation with the conidial suspension of *Sphaerotheca fuliginea* induced systemic protection to powdery mildew. Also, Abada *et al.* (2008a) found that, in greenhouse experiment, spraying cucumber plants with any of Bion, calcium chloride, lithium sulphate, potassium bicarbonate, potassium monophosphate and sodium phosphate as resistance inducers, caused significant reduction to powdery mildew with significant increase in the foliage fresh and dry weight/plant compared with control treatment. They added that spraying any of calcium chloride or lithium sulphate in field experiments caused also significant reduction in powdery mildew with significant increase in the produced fruit yield compared with control treatment. Laun (1998) mentioned that Bion via changes in plant biochemistry leading to resistance against the plant pathogens. Moreover, Dean and Kuc (1985), Kuc and Rush (1985) and Doubrava *et al.* (1988) mentioned that induced acquired resistance by antioxidants is persistent and nonspecific for a pathogen. They added that acquired resistance can be induced by simple chemical substances as well as by biotic agent. However, Lancake (1981) indicated that unlike elicitors of phytoalexins accumulation, elicit at the site of application, and may be responsible for localized protection, inducer of systemic resistance and/or sensitize the plant to respond rapidly after infection. These responses induced phytoalexin accumulation and lignifications (Dean and Kuc, 1985 and Kuc and Rush, 1985) and induced or enhance activities of chitinase and glucanse (Metranx and Boller, 1986). Furthermore, Fouly (2004) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable, broad spectrum disease management and could be used preventatively to blaster general plant health, which resulted in long lasting protection. Apart from this, Metranx and Linthorst (1991) reported that salicylic acid resulted in induces many proteins similar in nature to the pathogenesis related proteins. Chen *et al.* (1993) and Chandra *et al.* (2001) cloned and sequenced that salicylic acid binding protein which exhibited catalase activity and elevated amounts of H_2O_2 by inhibiting catalase activity.

The use of antioxidants in alternation with the fungicides could be recommended, especially for freshly plant products, in order to produce high quality products due to disease control with no or low fungicide residues.

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بروتوكول مقترح لمكافحة مرض الندوة المبكرة في الطماطم

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أوضحت الأختبارات المعملية ان المبيدات الفطرية كونسنتو ، فلينت ، سرينو ، سكور ، ترايدكس ٨٠% ومضادات الأكسدة بيون ، كلوريد الكالسيوم ، كبريتات الليثيوم ، فوسفات البوتاسيوم الأحادية ، حامض السلسيلك أحدثت انخفاضا معنويا للنمو الطولي للفطرالترناريا سولاني مسبب مرض الندوة المبكرة في الطماطم مقارنة بمعاملة الكونترول. وكان هذا النقص يتزايد بزيادة التركيزات المضافة من هذه المواد لبيئة في-ايت. أيضا فإن هذه المركبات تسببت في حدوث انخفاض معنوي للإصابة الصناعية بهذا المرض تحت ظروف الصوبة مع إحداث زيادة ملحوظة للمجموع الخضري لنباتات الطماطم مقارنة بمعاملة الكونترول.

وعند رش نباتات الطماطم بالمبيدات الفطرية أو مضادات الأكسدة وكذلك تعاقب الرش بينهما في تجارب حقلية خلال موسمى ٢٠٠٧ ، ٢٠٠٨ وجد أن المبيدات الفطرية كانت الأكثر فعالية في مكافحة الإصابة الطبيعية بالمرض مع إنتاج أعلى محصول للثمار وأن الرش بالمبيدات الفطرية ثم الرش قبل الحصاد بمضادات الأكسدة كانت أقل فعالية من رش المبيدات بمفردها ولكنها كانت أكثر فعالية من رش مضادات الأكسدة بمفردها.