

Biological Control of Squash Downy Mildew under Field Conditions

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The effects of some microorganisms, e.g. *Trichoderma harzianum* (T1), *T. viride*, (T2) mixture of T1+T2, *Bacillus subtilis* and commercial biocide product namely Blight Stop were studied on the incidence and disease severity of downy mildew of squash caused by *Pseudoperonospora cubensis* under field conditions during two successive growing seasons (2013 and 2014). All the biocontrol agents tested caused significant reduction in both disease incidence and disease severity of downy mildew on the five tested squash cultivars. The lowest percentage of disease incidence was recorded on Blonky 180 cv. during the two successive growing seasons. Among the tested bioagents, *B. subtilis* treatment caused significant reduction in both disease severity and disease incidence during 2013. However, the commercial product Blight Stop was the best in this respect during season 2014. All bioagent treatments caused significant increases in some growth parameters, i.e. fruit number/plant, plant height and yield of fruits.

Keywords: *Bacillus subtilis*, biological control, downy mildew, *Pseudoperonospora cubensis*, squash and *Trichoderma harzianum*.

Squash plants are subjected to invasion by several diseases in cultivated area. Downy mildew is a common and serious disease of cucurbit crops in the entire world. This disease occurs in squash plants grown under field conditions. Unfortunately, once the symptoms occur on the plants, there is not much can do for controlling the disease. The yield loss is proportional to the severity of the disease and the length of time that plants have been infected (Mossler and Nesheim, 2005). *Pseudoperonospora cubensis* (Berkeley and Curtis). Rostovtsev, the causal agent of cucurbit downy mildew is responsible for devastating losses worldwide of cucumber, cantaloupe, pumpkin, watermelon and squash. Symptoms usually include yellowing of foliage and the trademark trait of this disease, a soft growth of mildew on leaf undersides. Mildew appears in a variety of colours, including yellow, grey, brown or purple. If this disease is not controlled in a timely manner, symptoms can be severe enough to cause extensive premature defoliation of older leaves and wipe out the crop.

The wide hazardous use of fungicides to control plant diseases had 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 *et al.*, 2003,

Abd El-Moneim *et al.*, 2008). Biological control agents inhibit plant pathogens through one or more of the following mechanisms: mycoparasitism, competition for key nutrients and colonization sites, production of antibiotics, or stimulation of plant defence mechanisms (Oerke *et al.*, 1994). Many investigators reported efficacy of biocontrol agents in plant protection against different air borne pathogens (Harman, 2006 and Lebeda and Yigal 2011). Some investigators explained this protection effect as antibiosis action occurred in infection court (Matei and Matei, 2008). Some other investigators reported changes in plant physiology and chemical components in plants treated with these bioagents (Hafez *et al.*, 2012). The main mechanisms employed by biocontrol agents to control fungal plant pathogens are competition for space and nutrients, mycoparasitism, secretion of bioactive molecules, and stimulation of the plant's defences (De Curtis *et al.*, 2010). Several *Bacillus* spp. including *B. subtilis* are antagonistic to plant pathogens. *Bacillus* species produce at least 66 different antibiotic compounds (Ferreira *et al.*, 1991). The antagonistic effect of *B. subtilis* against many fungi *in vitro* and *in vivo* was reported by Lorito *et al.* (1998), Abd El-Moity *et al.* (2003) and 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-Moneim, 2005 and Hussein *et al.*, 2007). *Trichoderma* spp. act through different mechanisms including mycoparasitism (Abd El-Moity and Shatla 1981 and Benhamoud and Chet 1993), also through production of antifungal substances (Turner, 1971 and Hayes 1992). *Trichoderma* spp. also acts through production of destructive enzymes, *i.e.* chitinase (Pederes *et al.*, 1992 and Bolar *et al.*, 2000).

So, use of an isolate of *Trichoderma* that has more than one mechanism against the pathogen can increase the potentiality of biocontrol (Stasz *et al.*, 1988). Moreover, using more than one isolate increased the possibility of synergistic effect of different mechanisms against the pathogen (Schisler *et al.*, 1997). In this respect, Cook (1993) stated that several strains of *Trichoderma* should be included *T. viride* Harz. in a formulation to widen the range of control. *Trichoderma harzianum* is unique in that it can promote plant growth, activate biocontrol against air borne pathogens and can induce systemic resistance to foliar pathogens. Combining effective strains of bioagents was also studied by Harman *et al.* (1989); Harman (1990); Janisiewicz (1996) and Schisler *et al.* (1997). Mixing antagonists with each other may lead to antagonistic effect consequently decreases efficacy of treatment or may lead to synergistic effect and increase the efficacy. This increase or decrease is due to harmony and compatibility factors between bioagents. Synergism, antagonism as well as neutral interactions have been reported between fungal antagonists (Ruano Rosa and López Herrera, 2009; Sangeetha *et al.*, 2009 and Xiang Ming *et al.*, 2010). *T. harzianum* is a fungal biocontrol agent that attacks a range of pathogenic fungi. *Trichoderma harzianum* alone or in combination with other *Trichoderma* species can be used in the biological control of several plant diseases (Papavizas, 1985 and Samuels, 1996).

The present investigation aimed to evaluate the susceptibility of five squash varieties to infection by mildew and some growth parameters under field conditions.

Materials and Methods

1. Different preparations:

Different biocontrol isolates, i.e. *Trichoderma harzianum* (T1), *T. viride* (T2), mixture of them (T1 and T2) and *Bacillus subtilis* in addition to the Blight Stop as commercial biocide were kindly obtained from Central Lab of Organic Agriculture, ARC, Giza. *Trichoderma harzianum* (T1) and *T. viride* (T2) were grown in liquid gliotoxin fermentation medium (G.F.M.) developed by Brain and Hemming (1945) for nine days. *B. subtilis* was grown on nutrient glucose broth (NGB) developed by Dowson (1957) for 48h. The concentrations of different biocontrol agents were adjusted to be 30×10^6 cfu/ml.

2. Field experiments:

This study was carried out for two successive seasons (2013/2014) under natural infection in one Feddan area at Anshas Valley -Sharkiya governorate. Seeds of five squash cultivars (Ardendo, Arbika, *Cucurbita pepo*, Blonky180 and Mastra 829) were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt. During the course of the biocontrol evaluation study, squash cultivars were sown at depth of 2 cm in clay soil. The field trial (20 plots) was designed in complete randomized block with three replicates. Each plot was 3x3 m and had four rows of 3m in length and 75 cm in width. The soil was irrigated 7 days before sowing date. Squash cultivars were planted in hills at the rate of 2 seeds/hill at 20 cm space. The efficacy of suspension treatments with *T. harzianum*, *T. viride* and mixture of them as well as Blight Stop used in separated blocks was carried out to evaluate their effects on the disease incidence and severity of downy mildew on squash cultivars. Squash plants sprayed with water only were used to serve as a control.

3. Evaluation of bioagents on controlling downy mildew of squash:

The field trial (20 plots) was designed in complete randomized block with three replicates. Each plot was 3x3 m and had four rows of 3m in length and 75 cm in width. In this study, the bioagents and blight stop as commercial biocide were used as suspensions at the rate of one litre /200 litre water (15×10^4 cfu/ml). All previous preparations were sprayed 15 days after planting. The growing plants were sprayed two times at (15 and 45 days) before flowering period. Different treatments and different parameters including percentage of disease incidence (DI), and disease severity were determined as; $DI \% = [(Number\ of\ infected\ plants / Total\ number\ of\ inspected\ plants) \times 100]$. Disease severity was calculated according to the scale and formula reported by Biswas *et al.* (1992):

$$Disease\ severity\ (\%) = [Sum\ of\ (n \times r)] / N^7 \times 100$$

Whereas: n= Number of infected leaves in each grade;

r= Numerical value of each category;

N= Total No. of infected leaves X Maximum numerical grade.

Plant disease index was prepared to illustrate the symptoms in each category:

1= (1-2) spots / leaf, 2= (3-5) spots / leaf, 3= (6-10) spots / leaf, 4= up to 25 % infected area on the leaf, 5= up to 50 % infected area on the leaf, 6= up to 75 % infected area on the leaf and 7=more than 75 % = of leaf area infected.

4. Plant growth and yield parameters:

Random samples of five squash cultivars were collected after 60 days of sowing for each bio-control agent treatment as well as the control plants. The plant growth parameters taken into consideration were number of fruits per plant, plant height (cm) and fresh weight (g) of fruits and total yield.

5. Statistical analysis

Statistical analysis was carried out using the procedures ANOVA (Snedecor and Cochran, 1980).

Results and Discussion

Data presented in Tables (1 and 2) clearly show that all biocontrol agents tested caused significant reduction in the disease incidence of downy mildew of squash on the cultivars tested. It is also obvious from data (Tables 1 and 2) that lowest disease incidence was recorded on Blonky 180 cv. during the two successive growing seasons. The corresponding mean values were 36.93% and 31.40% in 2013 and 2014, respectively. On the other hand, Ardeno cv. appeared to be highly susceptible to infection by downy mildew disease during the two growing seasons (2013 and 2014), being 60% and 61.08 %. Moreover, data in Tables (1 and 2) indicate that in the growing season 2013 the commercial product Blight Stop treatment caused a significant reduction in the disease incidence on all cultivars tested followed by treatment with a mixture of T1 and T2, as the mean values calculated were 37.98 and 40.34%, respectively. In the growing season 2014, *B. subtilis* treatments resulted in great reduction in the disease incidence, as the corresponding mean value was 38.0% (Tables 1 & 2). The other tested bioagents caused also significant reduction in the disease incidence if compared with the control in both seasons (Tables 1 & 2).

Table 1. Effect of biocontrol agents tested on the incidence of downy mildew disease on squash cultivars under field conditions, season 2013

Tested biocontrol agent	Disease incidence (%) on cvs.					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	63.3	25.0	41.7	45.0	30.0	41.0
<i>T. harzianum</i> (T1)	53.3	38.3	36.7	45.0	36.7	42.0
<i>T. viride</i> (T2)	61.7	31.7	36.7	46.7	26.7	40.7
Mixture of (T1+T2)	51.7	40.0	33.3	45.0	31.7	40.34
Blight Stop	50.0	33.3	33.3	43.3	30.0	37.98
Control	80.0	53.3	55.0	60.0	58.3	61.3
Mean	60.0	36.93	43.9	47.0	35.6	
L.S.D at 0.05 for:						
Varieties (V)	1.71					
Bioagents (B)	1.88					
B x V	4.19					

* V1= Cv. Ardeno; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=*Cucurbita pepo*.

Table 2. Effect of biocontrol agents tested on the incidence of downy mildew disease on squash cultivars under field conditions, season 2014

Tested biocontrol agent	Disease incidence (%) on cvs.					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	58.3	20.0	26.7	51.7	33.3	38.0
<i>T. harzianum</i> (T1)	63.3	26.7	33.3	41.7	46.7	42.3
<i>T. viride</i> (T2)	60.0	31.7	31.7	45.0	43.3	42.3
Mixture of (T1+T2)	53.3	30.0	33.3	50.0	40.3	41.4
Blight stop	53.3	30.0	26.6	45.0	41.7	39.3
Control	78.3	50.0	50.0	58.3	51.7	57.7
Mean	61.08	31.4	33.60	48.6	42.8	
L.S.D at 0.05 for:						
Varieties (V)	1.61					
Bioagents (B)	1.76					
B x V	3.93					

* V1= Cv. Ardeno; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

The clear reduction in the disease incidence as a result of using biocontrol agents tested might be attributed to the antagonism occurred between the bioagents and the casual of squash downy mildew. It is well known that *Trichoderma* spp. have certain mechanisms to effect on plant pathogens including mycoparasitism (Benhamoud and Chet, 1993 and Harman, 2006), production of some destructive enzymes such as chitinase and cellulase that degrade pathogen cell wall (Boler *et al.*, 2000) and produce some growth regulators that stimulate plant to tolerate infection or working as resistance producers (Constantinescu *et al.*, 2009 and De Curtis *et al.*, 2010). On the other hand, *B. subtilis* has the ability to produce some antifungal to wide range of plant pathogens.

Results indicating the effect of the tested biocontrol agents on the disease severity of squash downy mildew are shown in Tables (3 and 4). It is clear from the data that treating with biocontrol agents caused significant reduction in the disease severity compared with control treatment. During season 2013, out of the tested bioagents, *B. subtilis* treatment caused significant reduction in disease severity compared with the control. The corresponding mean values were 35.3% and 57.3%, respectively. Meanwhile, commercial product Blight Stop was the best in reducing disease severity to 36.7% compared to 58.7% in the control treatment during season 2014. Data (Tables 3 & 4) also indicate that cv. Ardeno recorded the lowest percentages of disease severity during the two seasons compared with the other tested cultivars. Bioagents do not only affect outside the treated plants but also affect the plant metabolism inside plant and lead to changes in plant components which may play an important role in plant resistance (Hafez *et al.*, 2012).

Regarding to the effect of using biocontrol agents on number of squash fruits/plant on different cultivars, data in (Tables 5 and 6) reveal that all treatments by bioagents resulted in significant increase in fruit numbers compared with the control treatment. During season 2013, cv. Arbika recorded the highest mean

Table 3. Effect of the tested biocontrol agents on the disease severity of squash downy mildew on 5 different squash cultivars during season 2013 under field conditions

Tested biocontrol agent	Disease severity (%) on cvs.					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	28.3	35.0	25.0	46.7	41.7	35.3
<i>T. harzianum</i> (T1)	25.0	43.3	35.0	50.0	41.7	39.0
<i>T. viride</i> (T2)	20.0	46.7	35.0	50.0	45.0	39.3
Mixture of (T1+T2)	26.7	38.3	30.0	51.7	40.0	37.3
Blight stop	28.3	40.0	38.3	50.0	43.3	40.0
Control	41.7	60.0	55.0	66.7	63.3	57.3
Mean	28.3	43.9	36.4	52.5	45.8	
L.S.D at 0.05 for:						
Varieties (V)	1.36					
Bioagents (B)	1.49					
B x V	3.33					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

Table 4. Effect of the tested biocontrol agents on the disease severity of squash downy mildew on 5 different squash cultivars during season 2014 under field conditions

Tested biocontrol agent	Disease severity (%) on cvs.					Mean
	V1*	V2	V3	V4	V5	
<i>Bacillus subtilis</i>	20.0	48.3	31.7	50.0	40.0	38.0
<i>T. harzianum</i> (T1)	25.0	43.3	33.3	51.7	45.0	39.7
<i>T. viride</i> (T2)	21.7	40.0	26.7	56.7	45.0	38.0
Mixture of (T1+T2)	20.0	45.0	25.0	56.7	40.0	37.3
Blight stop	21.7	43.3	30.0	53.3	35.0	36.7
Control	36.7	60.0	70.0	63.3	63.3	58.7
Mean	24.1	46.7	36.1	55.1	44.7	
L.S.D at 0.05 for:						
Varieties (V)	1.36					
Bioagents (B)	1.49					
B x V	3.33					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

numbers of fruits/ plant being 9.88. Meanwhile, during season 2014, cv. Ardendo showed the highest value of number of fruits/ plant as the corresponding mean was 11.8 fruits/plant. It is worthy to note that among the bioagents tested Blight Stop gave the best results in this respect if compared with any of bioagents tested (Tables 5 & 6). Obviously the increase in number of fruit/plant occurred as a result of controlling squash downy mildew effectively by the bioagents tested which indirectly increased plant vigour.

Table 5. Effect of different bioagents on fruits number of five squash cultivars during 2013 growing season under field conditions

Tested biocontrol agent	No. fruits/plant					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	9.00	3.70	9.00	8.33	7.67	7.40
<i>T. harzianum</i> (T1)	10.7	3.33	9.00	7.67	7.00	7.54
<i>T. viride</i> (T2)	8.00	2.70	8.00	12.67	7.67	7.80
Mixture of (T1+T2)	11.30	5.00	10.00	8.67	9.33	8.86
Blight stop	11.00	4.33	8.00	13.67	10.00	9.40
Control	8.00	2.00	6.00	7.67	6.00	5.93
Mean	9.70	3.51	8.33	9.88	7.81	
L.S.D at 0.05 for:						
Varieties (V)	0.33					
Bioagents (B)	0.36					
B x V	0.69					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

Table 6. Effect of different bioagents on number of squash fruits/plant of five squash cultivars during 2014 growing seasons under field conditions

Tested biocontrol agent	No. fruits/plant					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	10.0	5.0	10.0	7.7	10.0	8.5
<i>T. harzianum</i> (T1)	13.0	3.0	11.7	9.0	9.3	9.2
<i>T. viride</i> (T2)	10.7	2.7	9.7	7.7	8.0	7.7
Mixture of (T1+T2)	13.7	2.7	10.3	9.7	10.3	9.3
Blight stop	14.0	3.3	12.0	10.3	10.3	10.0
Control	9.3	2.0	7.0	7.3	6.3	6.4
Mean	11.8	3.1	10.1	8.61	9.4	
L.S.D at 0.05 for:						
Varieties (V)	0.28					
Bioagents (B)	0.31					
B x V	0.69					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

Biocontrol agents tested had also significant effect in increasing plant height of all squash cultivars tested (Tables 7 and 8). Generally, noticeable increases in plant height were obtained when Blight Stop was used. On the other hand, treatments by bioagents caused noticeable increase in means of plant height of both Master 829 and Blonky cultivars during season 2013 and 2014, respectively. These results might be due to the effect of Blight Stop on suppressing the pathogen and thus increasing plant vigour.

Table 7. Effect of the tested bioagents on improving plant height (cm) of squash cultivars during 2013 growing season under field conditions

Tested biocontrol agent	Plant height (cm)					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	34.7	64.0	53.3	41.3	52.0	49.1
<i>T. harzianum</i> (T1)	41.0	50.0	46.0	50.3	50.0	47.5
<i>T. viride</i> (T2)	39.3	42.7	42.7	45.7	46.0	43.3
Mixture of (T1+T2)	64.7	60.7	70.3	48.0	62.3	61.2
Blight stop	68.0	61.3	71.0	50.0	64.3	62.9
Control	34.7	39.0	39.7	36.3	38.0	37.5
Mean	46.95	51.3	53.8	45.3	52.1	
L.S.D at 0.05 for:						
Varieties (V)	1.41					
Bioagents (B)	1.54					
B x V	3.45					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

Table 8. Effect of the tested bioagents on plant height (cm) of squash cultivars during 2014 growing season under field conditions

Tested biocontrol agent	Plant height (cm)					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	46.3	63.3	52.7	42.0	51.3	51.1
<i>T. harzianum</i> (T1)	44.3	54.7	42.0	46.0	44.3	45.9
<i>T. viride</i> (T2)	44.0	55.7	48.0	49.3	36.0	46.6
Mixture of (T1+T2)	62.0	69.0	65.7	46.0	62.3	61.0
Blight stop	71.3	69.7	73.3	51.7	61.7	65.5
Control	41.3	42.3	34.3	36.0	40.7	38.9
Mean	51.5	59.1	51.2	45.0	49.4	
L.S.D at 0.05 for:						
Varieties (V)	1.54					
Bioagents (B)	1.58					
B x V	3.76					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

Plant data in Tables (9 and 10) indicate that these bioagents caused significant increases compared with the control treatment during seasons 2013 and 2014. It was also noticed that the increase in fruit yield was more pronounced during season 2014 as a result of using which gave the highest yield of fruit/plot during the two seasons (Tables 9 & 10). On the other hand, cv. Ardendo during seasons 2013 showed the highest mean of fruit yield/plot, being 7.9 kg/plot. However during season 2014 cv. Arbika was the highest one in this respect (8.0 kg/plot).

Table 9. Effect of different bioagents used for controlling squash downy mildew on average yield (kg/plot) of squash cultivars during 2013 growing season under field conditions

Tested biocontrol agent	Yield (kg/plot)					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	7.0	6.7	6.3	7.0	7.7	6.9
<i>T. harzianum</i> (T1)	8.3	6.0	7.7	8.0	6.0	7.2
<i>T. viride</i> (T2)	9.0	7.7	6.7	8.7	5.7	7.5
Mixture of (T1+T2)	8.3	6.7	8.0	9.3	6.7	7.8
Blight stop	8.7	6.3	7.0	9.0	7.0	7.6
Control	6.0	5.0	5.7	5.0	4.0	5.1
Mean	7.9	6.4	6.9	7.8	6.2	
L.S.D at 0.05 for:						
Varieties (V)	0.24					
Bioagents (B)	0.26					
B x V	0.58					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

Table 10. Effect of different bioagents used for controlling squash downy mildew on average yield (kg/ plot) of squash cultivars during 2014 growing season under field conditions

Tested biocontrol agent	Yield (kg/plot)					
	V1*	V2	V3	V4	V5	Mean
<i>Bacillus subtilis</i>	7.0	6.3	7.3	7.3	7.3	7.1
<i>T. harzianum</i> (T1)	9.7	6.0	8.0	8.7	6.3	7.7
<i>T. viride</i> (T2)	9.3	6.7	7.0	9.7	6.0	7.7
Mixture of (T1+T2)	10.0	7.3	7.3	8.7	6.3	7.9
Blight stop	9.3	6.0	6.7	8.7	7.7	7.7
Control	6.0	4.7	5.0	5.3	4.3.0	5.1
Mean	8.5	6.2	6.9	8.0	6.3	
L.S.D at 0.05 for:						
Varieties (V)	0.21					
Bioagents (B)	0.23					
B x V	0.51					

* V1= Cv. Ardendo; V2= Cv. Blonky180; V3= Cv. Mastra 829; V4= Cv. Arbika and V5=Cucurbita pepo.

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المكافحة الحيوية للبياض الزغبي على نباتات الكوسة تحت ظروف الحقل

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درست تأثيرات بعض الكائنات الدقيقة المسماة بالتريكودرما هيرزيانم و تريكودرما فيردى و خليط منهما وباسيلس ستلس. و المبيد الحيوى التجارى بليت ستوب وذلك على حدوث الاصابة وشدة الاصابة بمرض البياض الزغبي فى الكوسة المتسبب عن الفطر بسيدبرونوسبورا كبنس تحت ظروف الحقل و استخدمت كل العوامل الحيوية رشا على خمس اصناف منزرعة من الكوسة خلال موسمين متتاليين (2013 و 2014) حيث احدثت جميع هذه المعاملات نقصا معنويا ملموسا فى حدوث وشده الاصابة. وقد كانت نسبة حدوث المرض المسجلة على الصنف المنزرع بلانكى 180 خلال موسمى الزراعة 4 و 31 و 90 و 36 على التوالى. و من بين العوامل الحيوية المختبرة المبيد الحيوى التجارى بليت ستوب الذى اعطى نتائج ممتازة فى هذا المجال خلال موسم الزراعة 2014. وقد ادت جميع المعاملات الحيوية المختبرة الى زيادة معنوية فى بعض صفات النمو مثل عدد الثمار /نبات و ارتفاع النبات و محصول الثمار.