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MANAGEMENT OF POWDERY MILDEW DISEASE ON SQUASH BY USING BIOLOGICAL AGENTS AND SOME CHEMICAL INDUCERS

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ABSTRACT

Three biocontrol agents *i.e., Bacillus subtilis, Pseudomonas fluorescens, Trichoderma harzianum,* were evaluated for their efficacy in controlling powdery mildew on squash plants. In greenhouse experiment, the highest inhibition of disease severity percentage was achieved by *P. fluorescens, B. subtilis* and *T. harzianum*, and application of Amistar 250 SC on squash plants resulted significant reduction of disease severity. The bacterial bioagents, *i.e. P. fluorescens,* and *B. subtilis* exhibited the highest reduction in disease severity. On the other hand, *T. harzianum* gave the lowest reduction in the disease severity. All resistance inducers (*i.e.*, potassium nitrate (KNO_3), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4 and salicylic acid) treatments resulted in significant reduction in disease severity under greenhouse conditions. Under field conditions, similar reduction in disease severity of powdery mildew was observed in treated plants. All treatments significantly increased squash yield per plant. More than 75% increase in yield was observed in treated plants with (KNO_3 at 100 mM).
Keywords: Squash, *Sphaerotheca fuliginea*, biological control and induced resistance

INTRODUCTION

Squash (*Cucurbita pepo* L.), ranked as one of important vegetable cash crops in Egypt. Total planting area 91054 reached Fadden during 2006 (Department of Agricultural Economic Statistical, Ministry of Agriculture and Land Reclamation, March 2006).

Several fungal diseases are affecting Squash plants in all growing stages causing a considerable reduction in fruit yield per feddan, and the total yield was negatively correlated with disease severity (Al-Raddad, 1993).

Sphaerotheca fuliginea (Schlechtend.: Fr.) Pollacci, is known to be the causal agent of Squash powdery mildew attacked all the squash hybrids in open field and green-houses, reflecting a serious damage for the infected plants are grown, under humid or semiarid climates, with dew or water irrigation, which providing good conditions for disease development (Haggag, 2008).

Infected plants with *S. fuliginea* caused more marked breakdown of leaf photosynthesis and respiration in susceptible varieties (Tomason and Gibson 2006).

Recently, there is a growing need to develop fungicides and bioagents as alternative approaches for controlling plant diseases; one of them is being actively pursued involves the use of induced resistance phenomena. In this regard, many investigators successfully used chemical inducers and/or bioactive substances for controlling powdery mildew (Andrew and William, 2003).

Spraying the recommended dose of azoxystrobin (Amistar 250 SC), was relatively more effective for inhibited powdery mildew disease. (Gandhi and Maheshwari, 2000; Ismail, *et al.*, 2003 and Thind, *et al.*, 2004). Furthermore, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, is an efficient bio-agent that protects cucurbits plants against powdery mildew disease (Bettiol, *et al.*; 19972; Urquhart *et al.*, 1994; Koumaki, *et al.*, 2001; Apablaza, *et al.*, 2002; Levy, *et al.*, 2004; Schmitt, 2006 and Zhang, *et al.*, 2008).

Application of different concentrations of four chemical compounds, *i.e.*, (KNO_3, KH_2PO_4, K_2HPO_4 and salicylic acid) on the induction of resistance against the pathogen was studied *in vivo*. They were sprayed, each alone, on 40-day-old squash plants, 2 days before and/or after inoculation with the pathogen. Commonly used for induction of resistance against powdery mildew disease (Casulli, *et al.*, 2002; Dik *et al.*, 2003 and Abd-El-Kareem, *et.al.*, 2004).

The main objectives of this research are studying the efficacy of using the different concentrations of four chemical compounds for inducting resistance as well as antagonistic isolates against powdery mildew of squash.

MATERIALS AND METHODS

Isolation and identification of the causal pathogen:

Leaves of squash plants showing typical symptoms of powdery mildew were collected from different localities of Beheira Governorate in 2007 growing season. Plants (5 weeks old) of squash, cv. Eskandarani, and infected with *S. fuliginea* were obtainted. The conidia were liberated, by scrapping the surface of the infected leaves with sterilized needle, and suspended in a Petri dish containing 20 ml of sterilized distilled water, then filtered through cheesecloth to remove most of the mycelial fragments (Elad *et al.*, 1989 and Reuveni *et al.*, 1995). The concentration of the spore suspension was determined by aid of a Haemocytometer slide and adjusted to 3×10^5 conidia/ml.

Fungal and bacterial bioagents:

Three bacterial isolates, *i.e. Bacillus subtilis* (2 isolates) and *Pseudomonas fluorescens* (one isolate) and fungi, *i.e. Trichoderma harzianum* (3 isolates), were obtained from the stock cultures collection of the Bacterial and Biological Control Dept., Plant Pathology Research Institute, Agriculture Research Center Giza, were used as biocontrol.

Pathogenicity tests:

Different isolates of *S. fuliginea* collected from various localities, from different localities of Beheira Governorate, were used in this study. Spore suspensions of these isolates were prepared, as mentioned before, ($3x10^5$conidia/ml). Three weeks old squash seedlings cv. Eskandarani grown in pots (25 cm in diam.) were sprayed with the spore suspensions using an automizer, and incubated under greenhouse conditions (25±5°C and 75-90% R.H.) for 10 and 20 days, then the aggressiveness of the tested isolates was determined by calculating the disease severity.

Biological control:

In vivo evaluation of antagonistic microorganisms:

The highly antagonistic isolates of *B. subtilis*, *P. fluorescens* and *T. harzianum* were used against *S. fuliginea* on squash plants. Three seeds of squash cv. Eskandarani, were sown in each pot (25 cm in diam.) filled with sandy-clay soil (1:1 w/w). Squash plants (40-days-old) were sprayed with each of the bacterial suspensions alone at the concentration of 10^8cfu/ml. The growing plants were sprayed with the tested bioagents, and then sprayed after three days with *S. fuliginea* spore suspension (3 x10^5 conidia/ml).

The fungal isolates were used at concentration of 5x10^8 cfu/ml prepared from 10-days-old cultures grown on PDA. The bioagents were sprayed, each alone, on squash plants by using a hand atomizer. Bioagents were applied at the same time of inoculation and/or three days before inoculation with the pathogen. The foliar fungicides (Topase-100; Afugan and Amistar 250 SC) were used for comparison purpose with the biocontrol agents in controlling the disease incidence. The experiment was repeated twice under greenhouse condition in 2007 and 2008. Pots (25-cm-diameter) were filled with autoclaved clay soil. Three replicates were used. Each replicate consisted of four pots and each pot consisted of 2 plants. Disease assessment was recorded at 10 and 20 days after inoculation.

Tests of induced resistance:

The effect of different concentrations of four chemical compounds, *i.e.*, potassium nitrate (KNO_3 25, 50 and 100 mM), potassium phosphate monobasic (KH_2PO_4 25, 50 and 100 mM), potassium phosphate dibasic (K_2HPO_4 25, 50 and 100 mM) and (salicylic acid 3, 5 and 10 mM) on the induction of resistance against the pathogen was studied *in vivo*. They were sprayed, each alone, on 40-day-old squash plants, 2 days before and/or after inoculation with the pathogen. The experiment was repeated twice under greenhouse conditions, with 3 replicates. Each replicate consisted of four pots and each pot consisted of 3 plants. Disease severity of each treatment was recorded after 10 and 20 days from inoculation date.

Greenhouse experiments:

The effects of above chemical inducers were evaluated on incidence of squash powdery mildew diseases under artificial inoculation conditions with the causal fungi under greenhouse conditions. Squash seeds cv. Eskandarani, were sown in plastic pots (25-cm-diam.) containing loamy soil at rate 3 seeds/pot. Ten pots were used for each treatment. Traditional agricultural practices, *i.e.* irrigation and fertilization, were carried out as needed. KNO_3 25, 50 and 100 mM, KH_2PO_4 25, 50 and 100 mM K_2HPO_4 25, 50 and 100 mM and salicylic acid 3, 5 and 10 mM were applied for testing their efficacy against powdery mildew disease severity. The prepared concentrations were sprayed on the grown plants at the first true leaf growth stage 2 days before and/or after inoculation with the causal fungi. Plant inoculation was carried out at the second true leaf growth stage by spraying of squash leaves with spore suspension (3x10^5conidia/ml) of *S. fuliginea* the causal of powdery mildew. Plants sprayed with tap water served as check. Assessment of disease severity according to (Descalzo *et al.*, 1990) was carried out at the second true leaf of squash plants after 10 and 20 days of inoculation.

Field experiment:

Three concentrations of four chemical compounds, i.e., potassium nitrate (KNO_3 25, 50 and 100 mM), potassium phosphate monobasic (KH_2PO_4 25, 50 and 100 mM), potassium phosphate dibasic (K_2HPO_4 25, 50 and 100 mM) and (salicylic acid 3, 5 and 10 mM) were applied to evaluate their effect on the severity of powdery mildew diseases, in addition to squash yield under field conditions. Fungicide, (Amistar 250 SC) was used as comparison treatment. Field experiment was carried out in the Nubaryia region. A field experiment consisted of plots (14x12.5m) each comprised of 8 rows and 25 hills / holes / row conducted in a randomly complete block design with three replicated plots for each particular treatment as well as a check. Squash seeds (cv. Eskandarani) were sown in all plots at the rate of three seeds/hill. Plots received all the traditional agricultural practices. All chemicals were applied twice as foliar spray at, *i.e.*1-2 true leaf and 7-8 leaf growth stage. In addition, (Amistar 250 SC) was sprayed twice every 15 day started after 20 days of sowing date. Application with tap water was used for check treatment. Assessment of powdery mildew disease severity was carried as mentioned before using disease scale described by Descalzo *et al.* (1990). Obtained fruit yield of each plant was weighed after each harvesting and the average of each particular treatment (25 plants) was calculated.

Statistical analysis:

The obtained results were statistically analyzed according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Pathogenicity tests:

Results illustrated in Table (1) and Fig. (1), indicate that all the tested isolates of *S. fuliginea* were able to infect squash plants causing typical powdery mildew symptoms with different degrees of disease severity. Data indicate that isolates Nos. N2, W2 and W3 were highly pathogenic and caused the highest disease severity. Isolate No. N1 exhibited the lowest disease severity on squash plants. Other tested isolates, Nos. W1 and N3 showed moderate infection. Results are in agreement with those of Bardin *et al.*,1997, which examined, strains of *S. fuliginea*, a causal agent of powdery mildew of cucurbits, for differences in virulence. Results are in agreement with those of Velkov and Masheva, 2002; Block and Reitsma, 2005; and Kristkova, *et al.*, 2007)

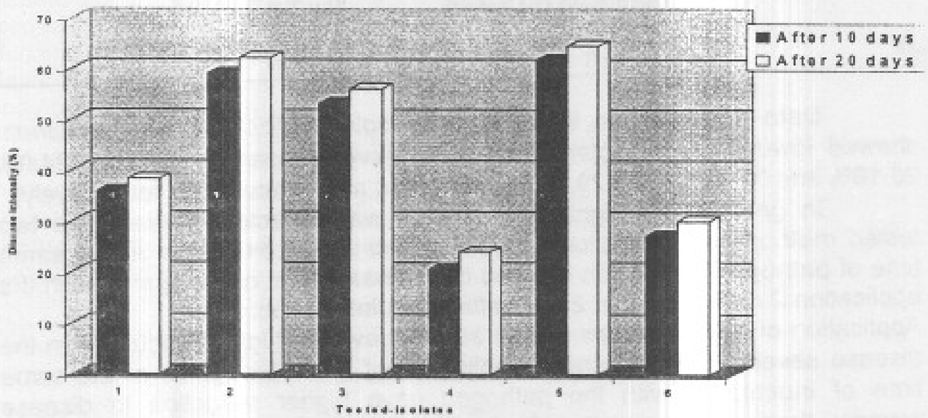
Effectiveness of certain microorganisms in controlling powdery mildew on squash:

Data presented in Table 2 show that all tested bioagents and Amistar 250 SC fungicide treatments significantly reduced powdery mildew of squash plants when any of the two tested methods of application of bioagents was used as compared with control plants. The highest significant reduction in disease severity was observed in case of *P. fluorescens* treatment. Application of *T. harzianum* (1, 2 & 3) showed the lowest reduction in diseases severity

Table (1): Pathogenic variations among *S. fuliginea* isolates on squash c.v.Eskandarani hybrid under greenhouse conditions

Tested isolate		Disease severity(%)		
Location	Isolate No.	10days	20days	Mean
WadiEl-Natron	W1	35.9	38.3	37.1
	W2	59.3	62.3	60.8
	W3	53.3	56.1	54.7
El-Nubaryia	N1	20.7	24.0	22.4
	N2	62.0	64.6	63.3
	N3	27.3	30.1	28.7
Conrol *		00.0	00.0	00.0
Mean		36.9	39.3	38.2
L.S.D at 5% for		Isolates (I) = 1. 20, Time(T) = 0.73, I x T = 2.08		

* Plants were sprayed with water only and served as control.



Figer (1) Pathogenic variations among *S. fuliginea* isolates on squash (c.v. Eskandarani) under greenhouse condations.

Data presented in Table (2) show significant differences between the control treatments and all tested bioagents. Among four tested antagonistic microorganisms, *Pseudomonas fluorescens* gave the highest protection against the disease when applied either 2 days before or 2 days after inoculation (disease severity averaged 11.50 and 19.00%, respectively), followed by *B. subtilis* (1) (disease severity averaged 15.66 and 20.5%, before and after inoculation, respectively, and *B. subtilis* (2) (disease severity averaged 16.45 and 22.97%, before and after inoculation, respectively).

Table (2): Effect of some antagonistic microorganisms and Amistar 250 SC, fungicide, and time application dates on squash powdery mildew disease under greenhouse conditions

Tested bioagent	Disease severity %							
	2 days before inoculation				2 days after inoculation			
	10 days	20 days	Mean	Efficacy (%)	10 days	20 days	Mean	Efficacy (%)
T. harzianum (1)	26.54	27.41	25.66	36.29	30.88	29.40	31.41	27.4
T. harzianum (2)	24.96	25.83	24.08	40.08	37.82	26.45	28.83	24.08
T. harzianum (3)	26.75	27.33	26.16	35.78	35.75	27.24	29.16	25.33
Bacillus subtilis (1)	17.75	19.83	15.66	57.39	53.57	19.75	20.50	19.00
B. subtilis (2)	17.47	18.50	16.45	58.06	48.71	21.82	22.97	20.68
Pseudomonas fluorescens	15.21	18.91	11.50	62.77	56.91	18.33	19.00	17.66
Fungicide (Amistar 250 SC)	9.54	12.25	6.83	77.10	80.42	8.33	9.00	7.66
control	41.66	40.66	42.66	--	--	42.54	43.58	41.50
Mean		19.98	18.77	36.29			22.72	20.36
L.S.D. at 1% for	Bioagents (B) = 2.73 Application (A) = 3.46 Time (T) = 3.46 B x A x T = 3.46				B x A = 3.03 B x T = 3.03 A x T = 2.65			

Data also indicate that the three isolates *Trichoderma harizianum* showed lowest control potential (disease severity averaged, 25.66, 24.08, 26.16% and 31.41, 28.83, 29.16%, before and after inoculation, respectively).

In general, the significant different was detected between the two tested methods of application and the application of bioagents at the same time of pathogen inoculation showed the highest effect as compared with the application 2 days before or 2 days after inoculation inoculation.

Application of *P. fluorescens and B. subtilis* gave the highest reduction in the disease severity. In most cases application of these bioagents at the same time of inoculation with the pathogen gave higher reduction in disease severity than the application 2 days before and after inoculation. The obtained results are in agreement with those obtained by many other researchers, Bettiol, *et al.*, 1997; Urquhart *et al.*, 1994; Koumaki, *et al.*, 2001; Apablaza, *et al.*, 2002; Levy, *et al.*, 2004; Schmitt, 2006 and Zhang, *et al.*, 2008)

Greenhouse experiment:

Evaluation of some chemical inducers effect on powdery mildew incidence of *S. fuliginea* on squash plants was carried out under greenhouse conditions. Data shown in Tables (1&2) indicate that all treatments have significantly reduced severity of powdery mildew diseases. Chemical inducers applied resulted in superior effect. Data also show that the lowest percentages of powdery mildew disease severity were observed with the application of salicylic acid 3, 5 and 10 mM 10 & 20 days after the artificial inoculation with the conidia of the causal fungus. Moreover, chemical inducers treatment at all used concentrations showed significant effect when applied. These results are in harmony with those reported by Mosa (1997).

Who found that cucumber powdery mildew infection was significantly reduced by 92% when the plants were treated with 50mM K2HPO4 three days after inoculation. This observation is also consistent with the report of Abood *et al.* (1992).

Table (3): Effect of resistance-inducing chemicals on disease severity under greenhouse conditions

Tested inducer resistant	Concentration	Disease severity (%)			
		After 10 days	After 20 days	Mean	Efficacy (%)
KNO_3	25mM	16.5	20.5	18.5	53.0
	50mM	14.9	15.8	15.4	60.9
	100mM	10.4	11.0	10.7	72.8
KH_2PO_4	25mM	20.0	29.8	24.8	37.0
	50mM	18.9	28.4	23.7	39.8
	100mM	10.8	22.3	16.6	57.9
K_2HPO_4	25mM	15.8	19.7	17.8	54.8
	50mM	11.5	13.5	12.5	68.2
	100mM	9.9	10.0	9.95	74.7
salicylic acid	3mM	22.6	35.5	29.1	26.1
	5mM	26.0	34.9	30.45	22.6
	10mM	27.0	33.9	30.45	22.6
Amistar 250 SC	50ml/100L.	5.9	6.3	6.1	84.5
Water	--	36.82	41.92	39.36	--
L.S.D. at 1%	Treatment (T) = 3.97,Conc. (C)= 4. 36, TxC=4.67.				

Field experiment:

The efficacy of foliar application with some chemical inducers, *i.e.* potassium nitrate (KNO_3 25, 50 and 100 mM), potassium phosphate monobasic (KH_2PO_4 25, 50 and 100 mM), potassium phosphate dibasic (K_2HPO_4 25, 50 and 100 mM) and (salicylic acid 3, 5 and 10 mM) in addition to the fungicide (Amistar 250 SC) as a comparison treatment, on powdery mildew disease severity of squash plants was evaluated under field conditions. Data presented in Table (--) indicate that all treatments have significantly reduced powdery mildew severity. The highest reduction in disease severity was obtained by K_2HPO_4 at 50 & 100 mM, which reduced the disease more than 83.9 and 84.6 %. Moderate effect was obtained with KNO_3 at 50 and 100mM; KH_2PO_4 at 100mM 71.9, 79.4 & 69.6% respectively. Amistar 250 SC, which reduced the disease severity more than 82.6 % compared with untreated plants. Meanwhile, KH_2PO_4 at 25mM and salicylic acid at 3mM was the least effective treatment.

Concerning the efficacy of the tested chemical inducers, under field conditions, on squash yield was also recorded. Data shown in Table (5) indicate that all treatments have significantly increased squash yield per plant. The highest increase in the yield was obtained in treated plants with K_2HPO_4 at 50, & 100 mM and KNO_3 at 100 mM. They increased squash yield more than 70.1 & 92.7 & 76% respectively. The moderate yield increase was as much over check treatment and obtained by K_2HPO_4 at 25mM, KNO_3 at 100 mM and Amistar 250 SC 66.7 & 50 & 54.2% respectively. Meanwhile, treatment with salicylic acid at 3mM was the least effective in this concern.

Table (4): Effect of application with some resistance-inducing chemicals on disease severity of powdery mildew of squash plants in response to under field conditions

Tested inducer	Concentration	Disease severity (%)	Reduction (%)
KNO_3	25mM	30	50.7
	50mM	17.1	71.9
	100mM	12.7	79.4
KH_2PO_4	25mM	35.1	42.4
	50mM	27	55.6
	100mM	18.5	69.6
K_2HPO_4	25mM	15.9	73.9
	50mM	9.8	83.9
	100mM	9.4	84.6
Salicylic acid	3mM	34.8	44.1
	5mM	28	54.02
	10mM	20	67.2
Amistar 250 SC	50 cm/100 L. Water	10.6	82.6
Water (check)	0.0	60.90	--

The obtained results obviously indicate that the reduction in disease severity was reflected on the obtained yield. These results are in harmony with those reported by Al-Raddad (1993) He stated that powdery mildew reduced the yield of squash, and the total yield was negatively correlated with disease severity, whereas the fruit yield dropped sharply when the percentage of leaf area infected by the pathogen reached 45% or more. Also, Dik, *et al.* (2003) found that the reduction in cucumber yield was due to breakdown of leaf photosynthesis and respiration in susceptible varieties to powdery mildew. The effective concentration does of chemical inducers not cause any symptoms of phytotoxicity, provided that spraying is not done at high irradiation conditions. Bicarbonates have been submitted for the Dutch list of exceptions on the Pesticide Law.

Table (5): Squash yield in response to application with some resistance-inducing chemicals under field conditions

Tested inducer resistant	Concentration	Squash yield (kg/plot)	Increase (%)
KNO_3	25mM	71.0	47.9
	50mM	72.0	50
	100mM	84.5	76
KH_2PO_4	25mM	65.0	35.4
	50mM	67.5	40.6
	100mM	67.5	40.6
K_2HPO_4	25mM	80.0	66.7
	50mM	82.0	70.1
	100mM	92.5	92.7
salicylic acid	3mM	60.0	25
	5mM	63.5	32.3
	10mM	65.5	36.5
Amistar 250 SC	50 cm/100 L. Water	74.0	54.2
Water (check)	0.0	48.0	--

The present study clearly demonstrates the efficiency of some resistance inducing chemicals for controlling powdery mildew and diseases of squash plants under greenhouse and field conditions. Foliar application of KNO_3, KH_2PO_4, K_2HPO_4 , and salicylic acid at different concentrations, either pre- or post-artificial inoculation or under natural infection conditions, reduced severity as well as the number of *S. fuliginea* on squash leaves.

These results support earlier reports, which indicated that solutions of some salts could induce systemic and local resistance in various plants against some pathogens Abd-El-Kareem *et al.*, 2001).

Initial powdery mildew colonies treated with K_2HPO_4, 10 days after inoculation, lost their white reflective appearance and superficial fungal growth (Reuveni *et al.,* 1995). The complete underlying mechanism of phosphate salts for controlling powdery mildew has not clearly determined (Mucharromah and Kuc, 1991), as well as all tested antagonistic microorganisms, gave the protection against the disease when applied either 2 days before or 2 days after inoculation . Their low cost, low toxicity to the man and environmental pollution make them ideal foliar application for disease control under field conditions.

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مكافحة مرض البياض الدقيقى على الكوسة باستخدام عوامل بيولوجية ومستحثات المقاومة

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أجريت هذه الدراسة بهدف عزل وتعريف المسبب المرضى و إجراء اختبار القدرة المرضية لتلك العزلات على إصابة نباتات الكوسة تحت ظروف الصوبة وكذلك دراسة فاعلية بعض عوامل المكافحة سواء الحيوية أو غير الحيوية كمستحثات للمقاومة الطبيعية للنبات.

ويمكن تلخيص أهم النتائج فيما يلى:

- أمكن الحصول على ستة عزلات مختلفة من الفطر سفروسيكا فلاجينا و ذلك من نباتات كوسة تظهر عليها أعراض الأصابة بمرض البياض الدقيقى تم جمعها من حقول فى مناطق مختلفة موجودة فى منطقة النوبارية محافظة البحيرة وقد أختلفت هذة العزلات معنويا فى قدرتها على أحداث الاصابة بمرض البياض الدقيقى فى نباتات الكوسة. وقد اختلفت العزلات فى قدرتها على إحداث الإصابة وتراوحت بين عزلات ضعيفة، متوسطة وعالية الإصابة
- ظهر اختبار التضاد لعديد من الكائنات الحية الدقيقة) هى عزلات بكتريا باسيليس ستيليس (٢ عزلة) سيدوموناس فلوروسينسس (عزلة واحدة) و الفطر تراكوديرما هاريزيانم (٣ عزلة) أن هذة العزلات لها القدرة على تثبيط شدة الممرض تحت ظروف الصوبة حيث أظهرت العزلات سيدوموناس فلوروسينسس قدرة عالية على خفض شدة الإصابة بالمرض ويليها البكتريا باسيليس ستيليس ثم عزلة فطر تراكوديرما هاريزيانم عند المعاملة بعد و قبل الحقن بالفطر المسبب على التوالى. إستخدلم عوامل المقاومة الحيوية السابقة قبل العدوى بالمسبب المرضى كان أفضل من استخدامها بعد يومين من العدوى بالمسبب المرضى.
- ظهر ان اختبار بعض (محفزات المقاومة الكيماوية) مثل فوسفات البوتاسيوم ثنائية القاعدية ، نترات البوتاسيوم، فوسفات البوتاسيوم احادية القاعدية و حمض السلسليك، على حث العائل لمقاومة المرض تحت ظروف الصوبة بين أن المعاملة بأى من هذه المواد قد أدى إلى خفض شدة المرض و زيادة محصول الكوسة عن النباتات الغير معاملة .حيث ان فوسفات البوتاسيوم ثنائية القاعدية ، نترات البوتاسيوم كانت اكثر فاعلية فى خفض شدة المرض وزيادة المحصول.