Egypt. J. Phytopathol., Vol. 34, No.1, pp. 41-50 (2006)

Induction of Systemic Acquired Resistance against Common Blight of Bean (*Phaseolus vulgaris*) Caused by *Xanthomonas campestris* pv. *phaseoli*

K.A.M. Abo-Elyousr

Plant Pathol. Dept., Fac. of Agric., Assiut Univ., Egypt.

Xanthomonas campestris pv. *phaseoli* was isolated from naturally blighted leaves of bean plants grown in Assiut governorate. The effect of Bion and BioZell-2000 B against common blight of bean plant was tested. *In vitro* studies, both tested compounds exhibited no inhibitory effect against the pathogen. Under greenhouse conditions, bean variety "Red Kidney" treated by Bion and BioZell-2000 B resulted in marked disease suppression. A high decrease of the disease rate of 68% and 50% was correlated with a reduction in the bacterial population up to 50 % and 45%, respectively.

Key words: Bean common blight, Bion, BioZell-2000 B, SAR and *Xanthomonas campestris* pv. *Phaseoli*.

Legume crops play an important role in human nutrition. Bean (*Phaseolus vulgaris*) is one of the most important legumes in the world due to its high commercial value, extensive production, consumers use, and nutritional value, *i.e.* carbohydrates, protein, minerals, and vitamins (Coyne *et al.*, 1965). It is traditionally a basic food crop in many developing countries, and it serves as a major plant protein source in rural and urban areas. Diseases are important constraint affecting bean yields. Among such diseases, common bacterial blight, caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) has a particular importance. It is one of the most destructive bean diseases especially when environmental conditions are favourable for the pathogen (Yoshii *et al.*, 1978 and Webster *et al.*, 1983).There is no adequate chemical control for such disease. However, management practices such as use of bacteria-free seed, rotation and ploughing of infested straw have been utilized successfully in the United States of America (Suchuster and Coyne, 1981). Limited success has been achieved with fungicides application such as Bordeaux mixture, copper oxychloride, copper sulphate, and antibiotics (Schwartz and Galvez, 1989 and Saettler, 1989). Cost, potential chemical residues, and resistance among *Xcp* strains are the known drawbacks of chemical applications. Thus, the use of resistant bean cultivars to *Xcp* is economically and technically the most practical method for effective management of common blight (Coyne *et al.*, 1973; Yoshii *et al.*, 1978 and Pastor-Corrales and Abawi, 1988).

Recently, several workers suggested that Bion is a systemic signal responsible for induction of systemic acquired resistance against certain bacterial, fungal and viral infection (Anfoka, 2000; Yuying *et al.*, 2001; Boksh *et al.*, 2003; Anith *et al.*, 2004 and David and Howard 2005). Also, BioZell-2000 B was used as a resistance inducer against fire blight disease in Germany. (Zeller and Laux 2002 and Abo-Elyousr *et al.*, 2004).

The present work was conducted to isolate and identify the causal agent of common blight disease of bean plants grown in Assiut Governorate and availability of controlling the disease by using Bion and BioZell-2000 B as resistance inducers.

Materials and Methods

Isolation of causal organism:

Naturally diseased bean plants showing common blight symptoms were collected from different localities of Assiut Governorate. They were surface sterilized by soaking in 2% sodium hypochlorite solution for 2 minutes, rinsed twice in sterile water, then small portion of the diseased tissues were macerated in 0.06% Na Cl solutions, after 10 minutes, a loopful of the resulting suspension was streaked over the surface of the nutrient agar (NA) plates. Plates were incubated at 27°C for 3-5 days and examined for colony development. The single colony technique was adopted to obtain pure cultures.

Pathogenicity test:

Inoculum was prepared from early log-phase cells which were obtained by growing the bacterial culture in nutrient yeast extract broth, incubated at 25°C on an orbital shaker at 200 rpm for 24 h. Bacteria were subsequently pelleted by centrifugation at 15000 rpm for 5 min and washed in 0.1% saline solution. Their concentration was adjusted to 10^8 colony-forming units (CFU) by dilution to give suspensions with an OD 660 of 0.2. The middle leaf vein was injected by 0.1 ml bacterial suspension (Klement *et al.*, 1990). Check plants were treated similarly with bacteria-free of 0.1% saline solution. Polyethylene bags covered inoculated plants for 48 hrs at 25-27°C in the greenhouse and examined daily for disease symptoms.

Identification of the pathogenic bacteria:

The isolated bacterial culture providing to be pathogenic and cause blight symptoms to bean plants were identified according to their morphological, cultural and physiological characteristic as a stated in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

Plant material:

Greenhouse-grown bean variety "Red Kidney" was used for all experiments. Plants were grown in 20 cm pots in a soil mix containing sand, and a slow-release fertilizer 1 % N.P.K (12: 4: 6) in a conditioned greenhouse at 25+5°C with 68-80% RH. 5000-14000 lux light and watered when necessary.

Agar diffusion test:

For agar diffusion test, King's Medium (KB) was used according to Zeller and Brulez (1987). A suspension of *Xcp* isolate No. 1 was spread over agar surface, after drying 0.05% of Bion and BioZell-2000 B were pipetted into 9 mm punches. After 2 days from cultivation at 27°C, inhibition zones were measured. Four replicates were used for each treatment.

Application of Bion and BioZell-2000 B:

BioZell-2000 B (50% etheric oil of thyme (*Thymbra spicta*), 20% oil of *Zea mays*, 20% oil of anise and 10% oil of sesame (*Sesamum indicum*) was used at a concentration of 0.05% (diluted with tap water) as inducing agent by spraying on leaves 48 h before inoculation. Bion (50% active ingredient in a wettable powder formulation) was dissolved in distilled water. Aqueous solutions of Bion was prepared at concentration of 0.05% and then sprayed onto the whole plants.

Disease index:

Disease index was determined 30 days after inoculation according to Louws *et al.* (2001). Plants were scored on the basis of a five-grade disease index as follows:

0= no symptoms; 1= a few necrotic spots; 2= many necrotic spots caused 10-20 leaf area affected; 3= 20-50 leaf area affected and 4= leaf collapsed-plants.

The experiment was repeated twice with 3 replicates. Each replicate consisted of four pots and each pot consisted of 2 plants. Percentages protection was calculated for each treatment according to Godard *et al.* (1999).

Effect of Bion and BioZell-2000 B on pathogen multiplication in vivo:

Bacterial colonies were recovered from inoculated bean plants tissues, treated with Bion, BioZell-2000 B and water 2 days before inoculation, by removing 5-mm-diameter leaf discs by using a cork borer aseptically from the region of inoculation. Excised discs were homogenized in 1 ml of sterile 0·06% NaCl solution, and serially diluted. Aliquots of alternate dilutions (0·1 ml) were plated onto KB medium agar plates. Plates were incubated at 26°C for 48 h, and emerging colonies were counted on all dilution plates showing bacterial growth. Each dilution from each leaf disc was duplicated.

Statistical analysis:

All data were subjected to statistical analysis and means were compared using L.S.D. test (Gomez and Gomez, 1984).

Results

1- Isolation and pathogenicity tests:

Three pure bacterial isolates were obtained from naturally diseased bean leaves. Pathogenicity of isolated bacteria was tested on bean plants. Data illustrated in Fig. (1) show that all three isolates were pathogenic to bean plants cv. Red Kidney and varied in their pathogenicity. Isolates No. 1 caused the highest disease index followed by isolates No. 2. Isolate No. 3 caused the lowest one.

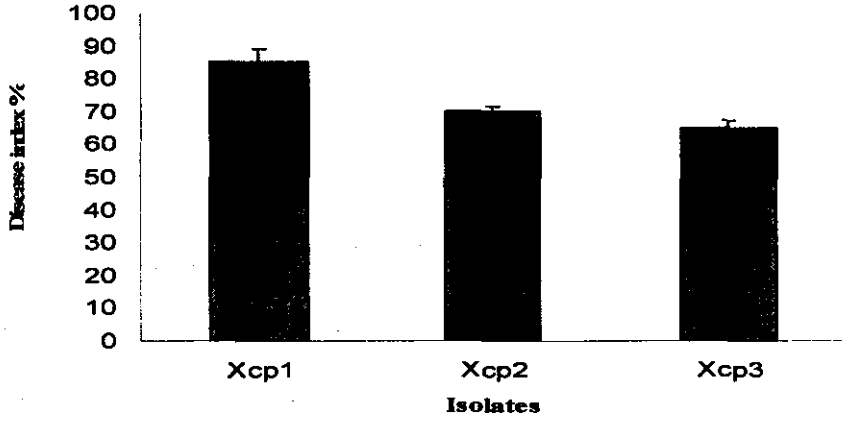

Fig. 1. Pathogenicity tests of three isolate of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) on bean plants. Bars indicate the standard deviation.

2- Identification of pathogens:

Identification of isolated pathogenic bacteria was carried out using the following characters: shape of cells, motility, gram staining reaction, aerobiosis, spore forming, pigmentation, hydrolysis of casein, gelatin liquefaction, acetyl methyl carbinol production (VP test), reduction of methyl red (MR), nitrate reduction, starch hydrolysis, levan production, hydrogen sulfide production, indole production, utilization of carbon compounds (glucose, fructose, sucrose, lactose, galactose, arabinose, mannose and raffinose).

Morphological, physiological properties of the three pathogenic isolates are presented in Table (1). Data show that all tested isolates were rod shape, motile, gram negative, aerobiosis, did not produce spore, pigmentation, levan positive, produce hydrogen sulfide and did not produce indole, did not hydrolise starch, negative V.P. test, were able to liquefy gelatin, negative M.R. test, did not reduce nitrate, starch hydrolysed and negative urease. Results also showed that all tested isolates produced acid from glucose, fructose, sucrose, lactose, galactose, arabinose, mannose and raffinose

On the basis of the morphological cultural physiological and pathological characteristics of the isolated bacteria and according to those reported by Krieg and Holt (1984). It was concluded that all the tested isolates could be identified as *Xanthomonas campestris* pv. *phaseoli.*

Table 1. Morphological and physiological characteristics of the isolated pathogenic bacteria

Test	Bacterial Isolate			Harby, 2000 as references
	Xcp 1	*Xcp* 2	*Xcp* 3	
Shape of cell	Rod	Rod	Rod	Rod
Motility	+	+	+	+
Gram staining	-	-	-	-
Spore forming	-	-	-	-
Aerobiosis	+	+	+	+
Pigmentation	NDYP	NDYP	NDYP	NDYP
Gelatin liquefaction	+	+	+	?
Urea test	-	-	-	?
V.P.	-	-	-	-
M.R.	-	-	-	-
Nitrate reduction	-	-	-	-
Starch hydrolysis	+	+	+	+
Levan production	+	+	+	+
H_2S production	+	+	+	+
Indole formation	+	+	+	+
Fermentation of carbon compounds:				
Sucrose	Acid	Acid	Acid	?
Glucose	Acid	Acid	Acid	Acid
Fructose	Acid	Acid	Acid	Acid
Lactose	Acid	Acid	Acid	Acid
Galactose	Acid	Acid	Acid	Acid
Raffinose	Acid	Acid	Acid	Acid
Mannose	Acid	Acid	Acid	Acid
Arabinose	Acid	Acid	Acid	Acid

(+)= Positive reaction; (-)= Negative reaction; (NDYP)= Non diffusible yellow pigment and (?)= Not done.

3- Effect of Bion and BioZell-200 B on common blight causal organisms:
In vitro:

Data in Table (2) show that Bion and BioZell-2000 B did not inhibit the *in vitro* growth of *Xanthomonas campestris* pv. *phaseoli*. However, streptomycin showed an inhibitory effect to the pathogen.

In vivo:

Data in Table (3) show that Bion and BioZell-2000 B have significantly reduced the number of the *Xcp* cells as compared with the control. Data also indicate that after 4 days, Bion and BioZell-2000 B have shown the highest reduction in multiplication of the pathogen within the host tissues compared with the control check plants 50 and 45 %, respectively. In general, Bion caused the highest reduction in multiplication of the pathogen followed by BioZell-2000 B after all tested inoculation periods. The inhibitory effect was first observed 4 days after inoculation and was remarkable monitored till the end of the experiment.

Table 2. Effect of Bion and BioZell-2000 B on growth of *Xanthomonas campestris* pv. *phaseoli in vitro*

Treatment	Inhibition zone (cm)
BioZell-2000 B	0.0
Bion	0.0
Streptomycin	0.6
Control	0.0
L.S.D. 5%	0.11

Table 3. Effect of Bion and BioZell-2000 B on the number of *Xanthomonas campestris* pv. *phaseoli* in bean leaves

Days after inoculation	Control	BioZell-2000 B	Reduction (%)	Bion	Reduction (%)
1	0.30×10^7	0.25×10^7	16.66	0.20×10^7	33.33
2	1.20×10^7	0.80×10^7	33.33	0.60×10^7	50.00
4	6.00×10^7	3.30×10^7	45.00	3.00×10^7	50.00
6	8.30×10^7	5.30×10^7	36.14	4.30×10^7	48.19
7	9.00×10^7	7.00×10^7	22.22	6.40×10^7	28.88
L.S.D. 5%	1.04				

4- Effect of Bion and BioZell-2000 B on incidence of bean common blight disease under greenhouse conditions:

Data in Table (4) indicate that untreated check plants have shown a significantly faster common blight symptoms development for all tested days from inoculation. At all period tested post inoculation treatments of Bion and BioZell-2000 B reduced the disease index 68 and 50%, respectively. In general, Bion showed the highest reduction in disease index followed by BioZell-2000 B after all tested inoculation periods.

Table 4. Effect of Bion and BioZell-2000 B on common blight disease index of beans

Days after inoculation	Control	BioZell-2000 B	Protection (%)	Bion	Protection (%)
4	11.00 *	10.00	9.09	3.50	68.18
7	25.00	24.00	4.00	9.00	64.00
10	52.00	32.00	38.46	23.00	55.76
12	72.00	36.00	51.00	30.00	58.33
14	85.00	50.00	41.17	35.00	58.82
L.S.D. 5%	4.48				

* Disease index (%).

Discussion

The bacterium, *Xanthomonas campestris* pv. *phaseoli* causes serious damage to bean plants causing severe blight symptoms in the field in Assiut governorate. The present work was undertaken to gain some information that may lead to compute the problems that are caused as a result of infection by such disease.

Results reported herein indicate that the three bacterial isolates obtained from naturally diseased bean leaves collected from different localities of Assiut governorate proved to be pathogenic and able to infect bean leaves causing blight symptoms. They were identified as *Xanthomonas campestris* pv. *phaseoli.* These results are in agreement with those reported by several workers (Yoshii *et al.*, 1978, Webster *et al.*, 1983 and Harby, 2000).

From our first findings, no direct effect of Bion and BioZell-2000 B on the pathogen, *in vitro*, which served as a first indication for using such compounds to induce resistance in bean plants. These results are agreed with those reported by Zeller & Zeller (1999) and Abo-Elyousr *et al.* (2004). They found that no inhibitory effect caused by BTH and BioZell-2000 B against the *Erwinia amylovora.*

Results clearly confirm that application of Bion and BioZell-2000 B induces resistance in bean plants. Similar enhanced disease resistance were induced by Bion in bean plants against potential fungal, bacterial and viral disease agents (Louws *et al.*, 2001, Buonaurio *et al.*, 2002 and Anfoka, 2000).

Bion and BioZell-2000 B had an obvious effect on multiplication of bacteria within plants. In generally, Bion showed the highest reduction in multiplication of the pathogen followed by BioZell-2000 B after all tested inoculation periods. Reduced bacterial growth was also reported in tobacco, pepper, tomato and apple plants treated with ASM (Cole, 1999; Buonaurio *et al.*, 2002 Baysal *et al.* 2003; and Abo-Elyousr *et al.* 2004). This effect may be due to their indirect effect on decreasing nutritional substance available to growth of the bacteria. A low nutrient concentration in the intercellular spaces could be a limiting factor for the growth of pathogens (Goodman *et al.*, 1986). Also, Zeller (1998) suggested that this effect may be caused by an accumulation of anthocyanins hindering bacterial pathogenic enzyme as a physiological barrier in xylem parenchymas.

In greenhouse experiments, severity of common blight symptoms development in plants after application of Bion and BioZell-2000 B were recorded. After 10 days from treatment by Bion and BioZell-2000 B, disease index was reduced greatly than the control check plants. These results agree with those reported by Louws *et al.* (2001) who mentioned that Bion was evaluated for management of bacterial spot caused by *X. axonopodis* pv. *vesicatoria* and bacterial speck of tomato caused by *P. syringae* pv. *tomato* in field experiments. Bion applied at 35g/ha, reduced foliar disease severity in 14 of the 15 bacterial spot and all 7 bacterial speck experiments. Disease control was similar or superior to that obtained using a standard copper bactericide program. Also, Baysal *et al.* (2003) mentioned that the disease index of bacterial canker of tomato plants was suppressed by BTH over periods of 2 weeks.

References

Abo-Elyousr, K., Zeller, W. , Laux, P. , Sallam, M.A. and Hassan, M. H. 2004. Induction of systemic acquired resistance against fire blight caused by *Erwinia amylovora. The 4th Scientific Conf. Agric. Sci.*, Assiut, Egypt.

Anfoka G.H. 2000. Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum* Mill. cv. Volledung) to cucumber mosaic virus. *Crop Protection*, **19**: 401–5.

Anith, K.N.; Kloepper, J.W.; Morois, J.J.; Olson, S.M. and Jones, J.B. 2004. Efficacy of plant growth promoting Rhizobacteria, Acibenzolar-S-methyl and soil amendment for integrated management of bacterial wilt on tomato. *Plant Dis.*, **88**: 669-673.

Baysal, O.; Soylu, E.M. and Soylu, S. 2003. Induction of defence-related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* sp. *michiganensis*. *Plant Pathol.*, **52**: 747-753.

Boksh, A.I.; Morris, S.G. and Deverall, B.J. 2003. Effect of benzothiadiazole and acetyl salicylic acid on ß-1,3 glucanase activity and disease resistance in potato. *Plant Pathol.*, **52**: 22-27.

Buonaurio, R.; Scarponi, L.; Ferrara, M.; Sidoti, P. And Bertona, P. 2002. Induction of systemic acquired resistance in pepper plants by acbenzolar-S-methyl against bacterial spot disease. *European J. Plant Pathol.*, **108**: 41-49

Cole, D.L. 1999. The efficacy of acbenzolar-S-methyl, an inducer of systemic acquired resistance against bacterial and fungal disease of tobacco. *Crop Protection*, **18**: 267-273.

Coyne, D.P.; Schuster, M.L. and Harris, L. 1965. Inheritance, heritability, and response to selection for common blight (*Xanthomonas phaseoli*) tolerance in *Phaseolus vulgaris* field bean crosses. *Proc. Amer. Soc. Horti. Sci.*, **86**: 373–379.

Coyne, D.P.; Schuster, M.L. and Hill, K. 1973. Genetic control reaction to common blight bacterium in bean (*Phaseolus vulgaris*) as influenced by plant age and bacterial multiplication. *J. Amer. Soc. Horti. Sci.*, **98**: 9499.

David, H.G. and Howard, F.S. 2005. Management of Xanthomonas leaf blight of onion with a plant activator, biological control agents and copper bactericides. *Plant Dis.*, **89**: 631-639.

Godard, J.P.; Ziadi, S.; Monot, C.; Le Corre, D. and Silue, D. 1999. Benzothiadiazole (BTH) induces resistance in cauliflower (*Brassica oleracea* var. *botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica*. *Crop Protection*, **18**: 397–405.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research.* 2nd Ed. John Willey & Sons Inc., New York, 680 pp.

Goodman, R.N.; Kiraly, Z. and Wood, K.R. 1986. *The Biochemistry and Physiology of Plant Diseases*. University of Missouri Press, Columbia, USA. 433pp.

Harby, M.A. 2000. Bacterial common blight on bean plants. *Assiut J. Agric. Sci.*, **31**: 255-284.

Klement, Z.; Rudolph, K. and Sands, D.C. 1990. *Methods in Phytobacteriology. Akademiai Kiodo*. Budapest. 568pp.

Krieg, N.R. and Holt, J.G. 1984. *Bergey's Manual of Systematic Bacteriology*. Vol. 1. Williams and Wilkins Company, Baltimore Md., U.S.A. 469pp.

Louws, F.J.; Campbell, H.L.; Cuppels, D.A.; Jones, J.B.; Shoemaker, P.B.; Sahin, F. and Miller, S.A. 2001. Field control of bacterial Spot and bacterial speck of tomato using a pant activator. *Plant Dis.*, **85**: 481-488.

Pastor-Corrales, M.A. and Abawi, G.S. 1988. Bean accessions with resistance to *Rhizoctonia solani* under field conditions in Columbia. *Turrialba*, **38**: 87–92.

Saettler, A.W. 1989. Common bacterial blight. Pages: 261–283. In: *Bean Production Problems in the Tropics*. Schwartz, H.F. and Galvez, E.E. (eds.). Centro Internacional de Agricultura Tropical, Cali, Colombia, SA.

Schwartz , H.F. and Galvez, M. 1989. *Bean Production Problems in the Tropics*. Schwartz, H.F. and Pastor-Corrales, M. (eds.). CIA Centro International de Agriculture Tropical. 654pp.

Suchuster, M.L. and Coyne, D.P. 1981. Biology, epidemiology, genetics and breeding for resistance to bacterial pathogens of *Phaseolus vulgaris* L. *Horticultural Rev.*, 3: 28–58.

Webster, D.M.; Temple, S.R. and Galvez, G.E. 1983. Expression of resistance to *Xanthomonas campestris* pv. *phaseoli* in *Phaseolus vulgaris* under tropical conditions. *Plant Dis.*, 67:394–396.

Yoshii, K.; Galvez, G.E. and Alvarez, G. 1978. Screening bean germplasm for tolerance to common bacterial blight caused by *Xanthomonas phaseoli* and the importance of pathogenic variation to varietal improvement. *Plant Dis. Reptr*, **62**: 343–347.

Yuying, S., David, W. and Leng, M. 2001. Elevation of extracellular ß-1,3-glucanase and chitinase activities in rose in response to treatment with Acibenzolar-S-methyl and infection by *D. rosae. J. Plant Physiol.*, **158**: 971-976.

Zeller, W. 1998. Einsatz von Pflanzenstärkungsmitteln zur biologischen Bekämpfung des Feuerbrandes. Habilitationsschrift, Universität Hannover, Germany.

Zeller, W. and Brulez, W. 1987. Change in the phenol metabolism of ornamental shrubs (*Cotoneaster* species) infected with *Erwinia amylovora*. Proc. 6th. Inernat. Conf. Plant Pathol. Bacteriol., Beltsville, Maryland, U.S.A.

Zeller, W. and Laux, P. 2002. Newest Results on the Biocontrol of fire blight in Germany. *Acta Horticulturae*, **590**: 243-246.

Zeller, W. and Zeller, V. 1999. Control of fire blight with the plant activator Bion. *Acta Horticulturae*, **489**: 639-645.

(Received 08/01/2006;
in revised form 10/03/2006)

أستحثاث المقاومة الجهازية ضد مرض اللفحة العادية فى نباتات الفاصوليا والمتسبب عن البكتريا زانثوموناس كامبسيترس الطراز المرضى فاصولاى

كمال أحمد محمد أبوالعيسر
قسم أمراض النبات- كلية الزراعة جامعة أسيوط- أسيوط- مصر.

تم عزل ثلاث عزلات من البكتريا زانثوموناس كامبسيترس الصنف المرضى *فاصولاى* (*Xcp*) من أوراق الفاصوليا المصابة باللفحة العادية تحت ظروف محافظة أسيوط وكانت العزلة رقم *Xcp* 1 هى أشد العزلات فى القدرة المرضية والعزلة *Xcp* 3 أقل العزلات فى القدرة المرضية.

وقد تم دراسة تأثير مادتى البيون والبيوزيل ٢٠٠٠ ب على نمو البكتريا فى المعمل وقد ظهر من النتائج أنه لا يوجد تاثير للمادتين على نمو البكتريا فى المعمل بالتركيز المستعمل وهو ٠,٠٥%.

وبدراسة تاثير هذه المواد على نسبة الأصابة ثبت أن هذه المواد تعمل على خفض نسبة الاصابة فى النباتات تحت ظروف الصوبة بنسبة تتراوح من ٦٨- ٥٠% بينما ادى استخدام هذه المواد إلى خفض أعداد البكتريا فى النباتات بنسبة تراوحت من ٥٠-٤٥%.