

EVALUATION OF SOME BIOLOGICAL AND CHEMICAL TREATMENTS ON THE CONTROL OF MUNGBEAN DAMPING-OFF

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ABSTRACT

Fungicidal effect of Benlate (benomyl) and Monceren (pencycuran), efficacy of two biotic agents; *Trichoderma viridi* and *Bacillus subtilis*, as well as Saponin; a steroidal glycoside extracted from alfalfa seeds, were evaluated for the control of pre- and post-emergence damping-off on three mungbean entries showing different levels of infection to the causal agents; *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Sacc., and *Macrophomina phaseolina* (Maubl); under greenhouse and field conditions at three locations; Sakha, Itay El-Baroud and Shandaweel.

Generally, seed treatment with the aforementioned treatments significantly reduced the percentage of infection of pre- and post-emergence damping-off on the three mungbean entries, consequently increased the crop yield.

Integration of both seed dressing, with either benomyl or pencycuran at a rate of 3 g/kg seed using the less infected entry VC 2719 was the most effective method for controlling mungbean damping-off.

Although bioagents, *T. viridi*, *B. subtilis* and Saponin were less effective than benomyl and pencycuran, they were considered more safety than those fungicides.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is very wide legume crop in southeast Asia countries (Singh, 1988; Abu-Salim *et al.*, 1997 and Ashour, 2000). It is a new introduced legume crop in Egypt (Ashour *et al.*, 1993 and Ashour *et al.*, 1994) and mostly grown for human and animal consumption.

Fungal diseases are one of the major constraints regarding mungbean production all over the world. Damping-off and root rot are of the most important diseases attacking the roots and stem base of mungbean (Bhate *et al.*, 1985 and Anderson 1985).

Various methods have been applied to minimize disease incidence; including varietal resistance, cultural practices, chemical and biological control, (Siddiqui *et al.*, 2001; Elazegui, 1983; Tschen and Kuo, 1985 and Mishra *et al.*, 2001). At the same time, effect of saponin as a plant-derived natural product against fungi was promising for controlling damping-off and root rot (Wolters, 1970 and Omar and Abd El-Halim, 1992).

The present investigation was conducted to study the effectiveness of different seed treatments assigned for controlling pre- and post-emergence damping-off on three entries of mungbean under greenhouse and field conditions at three different locations of Egypt, season 2001.

MATERIALS AND METHODS

Isolation of the causal fungi of mungbean damping-off :

Pathogenic isolates of *Rhizoctonia solani* (Kuhn), *Fusarium solani* (Mart.) Sacc. and *Macrophomina phaseolina* (Muabl.) were isolated from different mungbean seedlings. The hyphal tip and single spore techniques were followed for the isolation and purification of each fungus.

Source of mungbean seeds :

Seeds of three mungbean entries namely Kawmy 1, VC 2010 and VC 2719 were obtained from the Legume Crop Research Department, Field Crops Research Institute, Agricultural Research Center (Giza).

1. Greenhouse Experiments :

Pot experiment was carried out to evaluate the effect of seed treatment of two fungicides [Moncren (Pencycuran) and Benlate (benomyl)] at the rate of 3 g/kg seeds; two biocides (*Trichoderma viridi* and *Bacillus subtilis*) at 2×10^6 spores/ml) and Saponin as a natural compound at 2 g/kg seeds on the control of pre- and post-emergence damping-off of three mungbean entries artificially inoculated with three different fungi.

Pathogenic isolates of the causal organisms viz. *R. solani*, *F. solani* and *M. phaseolina* were used separately throughout the present study.

Seeds of mungbean entries Kawmy 1, VC 2010 and VC 2719 were separately treated with the aforementioned fungicides, biocides and natural compound at the rates indicated, however untreated seeds served as a control. The inoculum of the three pathogens *R. solani*, *F. solani* and *M. phaseolina* were grown on sterilized barley grains medium for 10 days at 25 ± 1 °C. Then, each pathogen was added to previously autoclaved soil at the rate of 5% of soil weight. The infested soil was dispersed in 20-cm pots sterilized with 38% formalin. Each treatment was replicated four times, and 10 treated seeds of each entry were sown in each pot. Non treated seeds were sown in infested soil served as check. Data were taken 25 and 45 days after sowing as percentage of pre- and post-emergence damping-off compared with the control treatment.

2. Field experiments :

A split-plot design experiments were carried out at three Agric. Res. Stations i.e. Sakha, Itay El-Baroud and Shandaweel, in season 2001, under the stress of naturally infested soil. The three mungbean entries i.e. Kawmy 1, VC 2010 and VC 2719 were sown in the main-plots. While, seed treatments as previously indicated were used as sub-plots, with three replicates in 5.25 m² plots. The recommended agricultural practices were followed, except for the treatments under study. Number of infected plants were counted 25 and 45 days from sowing, and percentage of infection was calculated. Yield of seeds was weighted for each plot.

Statistical Analysis :

The obtained data were statistically analyzed according to Snedecor and Cochran (1967). Treatments averages were compared at 0.05 level of probability (Fisher, 1948).

RESULTS

1. Greenhouse Experiments :

Isolation and Pathogenicity of the Causal Pathogens :

The preliminary studies proved that the used isolates of *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* were pathogenic to mungbean and caused damping-off disease to the local variety Kawmy 1.

Response of mungbean entries to the causal pathogens :

Percentage of survival indicated that the entry Kawmy 1 is more susceptible than the other two entries VC 2010 and VC 2719 for the three inoculated pathogens, Table (1). *M. phaseolina* gave 46.5% survival on Kawmy 1, 54.5% on VC 2010 and 62.3% on VC 2719. Survival percentages as a result of the infection by *R. solani* were 54.8, 59.4 and 66.0% on the three entries, respectively. However, *F. solani* was less pathogenic showing survival percentage of 59.7, 64.3 and 69.2%, respectively, Table (1).

Table (1). Effect of three fungal pathogens on the pre- and post-emergence damping-off of three mungbean entries as an average of 6 seed treatments under greenhouse condition

Entry	Damping-off %								
	<i>R. solani</i>			<i>F. solani</i>			<i>M. phasiolina</i>		
	Pre	Post	Surv. %*	Pre	Post	Surv. %	Pre	Post	Surv. %
Kawmy 1	34.5	10.7	54.8	31.2	9.1	59.7	40.7	12.8	46.5
VC 2010	32.1	8.6	59.4	27.9	7.8	64.3	34.9	10.7	54.5
VC 2719	26.1	8.0	66.0	24.9	6.0	69.2	28.9	8.8	62.3
LSD 5%	0.99	0.55	0.52	0.89	0.39	0.38	0.49	0.71	0.45

* Surv. % = Survival %

Effectiveness of seed treatment on mungbean damping-off :

Significant increase in the survival of mungbean plants was observed for the three inoculated fungi as a result of the effect of the tested fungicides, biocides and natural product as expressed as a mean of the three entries, Table (2).

Higher survival percentages were obtained from pots treated with the two fungicides benomyl and pencycuran followed by the natural product Saponin, finally the two biocides *T. viridi* and *B. subtilis*.

Table (2). Effect of seed treatment on the pre- and post-emergence damping-off of three mungbean entries expressed as an average of three tested entries under greenhouse condition

Treatment	Damping-off %								
	<i>R. solani</i>			<i>F. solani</i>			<i>M. phaseolina</i>		
	Pre	Post	Surv.%*	Pre	Post	Surv.%	Pre	Post	Surv.%
Moncren	22.5	6.7	70.7	20.2	5.6	74.2	27.6	7.8	64.6
Benlate	23.7	4.7	71.6	19.4	4.0	76.6	26.8	6.2	67.1
<i>T. viridi</i>	35.2	10.8	54.1	32.6	7.9	59.6	38.2	11.4	50.4
<i>B. subtilis</i>	35.0	11.3	57.7	31.1	8.6	60.3	38.5	13.1	48.5
Saponin	30.7	8.8	60.5	28.6	8.6	62.8	35.0	11.4	53.6
Control	38.3	12.1	49.6	36.2	11.0	52.9	43.0	14.6	42.4
LSD 5%	1.78	1.62	3.68	3.57	1.98	3.6	3.38	2.7	3.44

* Surv. % = Survival %

The effect of seed treatment of each entry against each of the three inoculated fungi (interaction effect) is shown in Table (3). The highest survival of the entries Kawmy 1 and VC 2719 inoculated with *R. solani* was obtained from seeds treated by benlate with 65.7% and 79.4% survival, compared with 44.87 and 55.8% for untreated seeds, respectively. While for cv. VC 2010 Moncren gave 71.6% survival compared with 48.3% for untreated seeds. Also, seed treatment with benlate for inoculated pots with *F. solani* showed the highest survival percentage as 72.2, 77.1 and 80.6% for the three tested entries Kawmy 1, VC 2010 and VC 2710, respectively, compared with 47.2, 51.5 and 59.9% for the control, Table (3).

In case of *M. phaseolina*, the highest pathogenic fungus in this experiment, significant differences were obtained from all treated seeds compared with untreated ones. Moncren gave 56.7% survival for the entry Kawmy 1 compared with 34.9% survival for untreated seeds. While, benlate showed the highest survival for VC 2010 and VC 2719 with 71.1 and 75.7% survival compared with 42.4 and 50.0% survival for untreated seeds, respectively, Table (3).

2. Field Experiments :

Results in Table (4) indicated that survival percentages were significantly higher in both VC 2010 and VC 2719 than Kawmy 1 entry in the three test locations as an average of the six seed treatments. Concerning seed yield, no significant difference was found between the three entries at Sakha. While at both of the other locations, i.e. Etay El-Baroud and Shandaweel, VC 2719 entry was significantly higher than VC 2010 and Kawmy 1 entries at both locations.

Effect of seed treatment with different fungicides, biocides and natural compounds as a mean of the three tested entries on pre- and post-emergence damping-off, survival and seed yield are presented in Table (5). Significant differences were found at the three locations between treated seeds and the untreated ones for pre-emergence damping-off, post-emergence damping-off and survival. However, Saponin was not significant in some cases.

Table (3). Effect of different seed treatments on the pre- and post-emergence damping-off of three mungbean entries with three different fungi under greenhouse condition

Entry	Treatment	Average Damping-off %								
		<i>R. solani</i>			<i>F. solani</i>			<i>M. phaseolina</i>		
		Pre	Post	Surv. %*	Pre	Post	Surv. %	Pre	Post	Surv. %
Kawmy 1	Moncren	26.1	8.6	65.3	23.1	7.8	69.1	34.2	9.1	56.7
	Benlate	27.1	7.2	65.7	22.3	5.5	72.2	36.7	8.9	54.4
	<i>T. viridi</i>	37.2	11.3	51.5	35.2	8.2	56.7	42.1	13.3	44.6
	<i>B. subtilis</i>	39.2	12.7	48.0	33.9	10.1	56.0	43.3	13.2	43.6
	Saponin	35.9	10.5	53.6	32.4	10.5	57.1	40.5	14.8	44.8
	Control	41.6	13.7	44.8	40.5	12.3	47.2	47.6	17.5	34.9
VC 2010	Moncren	22.3	6.1	71.6	19.2	5.5	75.3	25.2	7.8	67.0
	Benlate	25.7	4.6	69.7	18.3	4.6	77.1	23.2	5.8	71.1
	<i>T. viridi</i>	37.2	10.5	52.4	32.4	9.2	58.4	40.4	11.2	48.5
	<i>B. subtilis</i>	36.7	10.0	53.4	32.4	7.8	59.8	42.1	14.8	43.2
	Saponin	31.1	8.2	60.7	27.1	9.1	63.8	34.3	11.2	54.6
	Control	39.5	12.2	48.3	37.7	10.8	51.5	44.2	13.5	42.4
VC 2719	Moncren	19.2	5.5	75.3	18.3	3.6	78.1	23.4	6.6	70.0
	Benlate	18.3	2.3	79.4	17.5	1.9	80.6	20.4	4.0	75.7
	<i>T. viridi</i>	31.1	10.5	58.4	30.1	6.3	63.6	32.1	9.8	58.1
	<i>B. subtilis</i>	29.1	11.2	59.7	27.1	7.8	65.1	30.1	11.2	58.7
	Saponin	25.2	7.8	67.1	26.2	6.3	67.6	30.3	8.2	61.5
	Control	33.8	10.4	55.8	30.3	9.9	59.9	37.3	12.8	50.0
	LSD 5%	NS	1.36	1.27	2.19	0.95	0.93	1.19	1.74	1.11

* Surv. % = Survival %

Table (4). Pre- and post-emergence damping-off and seed yield of three mungbean entries evaluated at three locations under natural infection conditions of field experiments as an average of 6 seed treatments, season 2001

Entry	Damping-off %											
	Sakha				Etay El-Baroud				Shandaweel			
	Pre	Post	Surv. %*	Kg/plot	Pre	Post	Surv. %	Kg/plot	Pre	Post	Surv. %	Kg/plot
Kawmy 1	7.6	4.9	86.7	0.912	9.5	5.6	83.9	0.775	12.5	6.8	79.8	0.748
VC 2010	7.6	3.4	88.7	0.895	7.5	4.6	86.6	0.843	7.7	5.1	85.7	0.817
VC 2719	6.9	3.8	88.5	0.885	7.1	3.4	88.3	0.885	6.3	4.2	88.5	0.872
LSD 5%	0.45	0.27	0.36	NS	0.38	0.21	0.35	0.02	0.68	0.45	0.33	0.03

* Surv. % = Survival %

Benomyl showed the highest survival percentage as 93.2, 91.5 and 92.4% at Sakha, Etay El-Baroud and Shandaweel, respectively. While Saponin was the least effective treatment showing 85.3, 84.2 and 81.8% compared with the untreated plots with 79.5, 77.7 and 73.7% at the three locations, respectively. Table (5).

No significant differences were found at Sakha locations among the six treatments for the seed yield, while, significant increase was obtained from fungicides and biocides treated seeds comparing with Saponin and untreated seeds at both of Etay El-Baroud and Shandaweel, Table (5).

Table (5). Effect of mungbean seed treatment on pre- and post-emergence damping-off and seed yield at three locations under natural infection field conditions as an average of 3 tested entries, season 2001

Entry	Damping-off %											
	Sakha				Etay El-Baroud				Shandaweel			
	Pre	Post	Surv. %*	Kg/plot	Pre	Post	Surv. %	Kg/plot	Pre	Post	Surv. %	Kg/plot
Moncren	4.4	2.6	92.5	1.14	5.2	3.4	90.0	1.03	6.3	4.8	87.3	1.0
Benlate	3.96	2.0	93.2	1.15	4.6	2.6	91.5	1.13	4.4	2.5	92.4	1.0
<i>T. viridi</i>	7.5	3.1	88.8	0.91	8.3	4.4	86.1	0.78	9.3	3.6	85.5	0.68
<i>B. subtilis</i>	7.2	4.1	88.1	0.90	7.0	3.6	88.3	0.86	7.7	4.3	87.2	0.92
Saponin	8.3	5.7	85.3	0.68	9.5	5.2	84.2	0.69	9.7	7.3	81.8	0.69
Control	12.6	6.6	79.5	0.61	13.5	7.9	77.7	0.52	15.4	9.7	73.7	0.55
LSD 5%	2.29	1.69	3.8	NS	2.77	1.61	3.18	0.23	2.02	2.51	4.45	0.21

* Surv. % = Survival %

Data of the interaction effect of each treatment on each entry at each location are presented in Table (6). At Sakha location, the highest survival percentage was obtained from VC 2719 seed treated by Pencycuran with 94.9% followed by Kawmy 1 and VC 2010 seed treated by benlate with 94.3 and 93.1%, respectively, compared with 79.6, 77.8 and 81.1% for untreated seeds, respectively.

At Etay El-Baroud and Shandaweel locations seed treated by Benlate gave highest survival percentage as 92.2, 92.0 and 90.4% on VC 2010, VC 2719 and Kawmy 1, respectively at Etay El-Baroud, while, as 98.0, 92.7 and 86.4% on VC 2719, VC 2010 and Kawmy 1, at Shandaweel compared with 78.7, 79.1 and 75.3% for the untreated seed at Etay El-Baroud and with 77.2, 73.3 and 70.6% for the control at Sandaweel, (Table 6).

In case of the seed yield, data in Table (6) revealed that significant differences were found among the three entries and the six different treatments at all locations. The highest seed yield was obtained from fungicide treated seeds at all locations.

Table (6). Effect of seed treatment with some chemical, biological and natural compounds on pre- and post-emergence damping-off and seed yield of three mungbean entries tested under natural infection field conditions at three locations, season 2001

Entry	Treatment	Emergence % and seed yield (kg/plot)											
		Sakha				Estay El-Baroud				Shandaweel			
		Pre	Post	Surv. %*	Kg/plot	Pre	Post	Surv. %	Kg/plot	Pre	Post	Surv. %	Kg/plot
Kawmy 1	Moncren	4.7	3.3	91.6	1.15	6.0	4.7	87.7	0.93	8.3	6.8	83.6	0.94
	Benlate	3.8	1.4	94.3	1.28	5.0	3.4	90.4	1.10	8.3	3.8	86.4	0.93
	<i>T. viridi</i>	7.5	4.3	86.9	0.85	10.3	6.0	83.1	0.74	14.0	5.5	79.1	0.61
	<i>B. subtilis</i>	8.0	5.7	86.0	0.94	8.3	4.0	86.4	0.78	10.7	7.2	81.9	0.86
	Saponin	9.0	7.0	83.5	0.67	11.3	7.0	80.7	0.62	13.7	8.5	77.2	0.64
Control	12.8	7.5	77.8	0.58	15.8	8.3	75.2	0.48	19.7	9.0	70.6	0.51	
VC 2010	Moncren	5.7	2.7	90.8	1.10	6.0	3.3	89.4	1.05	6.0	5.7	86.0	0.98
	Benlate	3.3	2.7	93.1	1.12	4.0	2.1	92.2	1.18	3.7	2.7	92.7	0.98
	<i>T. viridi</i>	8.3	2.0	89.4	0.92	7.5	4.3	86.8	0.77	7.0	3.3	87.1	0.69
	<i>B. subtilis</i>	6.0	3.3	90.1	0.86	6.0	4.7	87.9	0.84	5.7	2.8	90.6	0.98
	Saponin	8.7	4.3	86.9	0.73	9.0	5.7	84.6	0.70	8.0	6.0	84.3	0.73
Control	13.3	5.3	81.1	0.64	12.3	7.2	78.7	0.52	15.7	10.3	73.3	0.54	
VC 2719	Moncren	3.0	1.7	94.9	1.17	3.7	2.3	92.8	1.10	4.7	2.0	92.2	1.07
	Benlate	4.7	2.0	92.2	1.05	4.7	1.9	92.0	1.12	1.3	1.1	98.0	1.21
	<i>T. viridi</i>	6.8	2.8	90.1	0.95	7.0	3.0	88.5	0.82	7.0	2.0	90.3	0.74
	<i>B. subtilis</i>	7.7	3.3	88.2	0.90	6.8	2.0	90.5	0.95	6.8	3.0	89.1	0.92
	Saponin	7.3	5.7	85.7	0.62	8.3	3.0	87.4	0.75	7.5	7.3	83.9	0.70
Control	11.7	7.0	79.6	0.62	12.3	8.3	79.1	0.57	10.7	9.8	77.2	0.59	
LSD 5%		1.09	0.66	0.89	0.13	0.93	0.51	0.86	0.05	1.67	1.10	0.82	0.07

* Surv. % = Survival %

DISCUSSION

Results of the present study revealed that relative susceptibility of the tested mungbean entries, are of interest. All of the tested entries are vulnerable to attack with pre- and post-emergence damping-off disease and the infection fluctuated between high and moderate according to the entries and the pathogen as well. Data indicated that cv. Kawmy 1 was the most sensitive entry whereas VC 2010 was relatively less sensitive, while entry CV 2719 was the least infested one. These results are in agreement with those obtained by Ashour (2000) who reported that cv. Kawmy 1 was relatively less tolerant than CV 2719 to *R. solani*.

On the other hand, these results could be attributed to the ability of the tested fungi, i.e. *R. solani*, *F. solani* and *M. phaseolina* which increased the infection and reduced seed yield below healthy ones more than other soil borne fungi. These results are in agreement with those obtained by Bhata (1985) who found that 11 mungbean cultivars were sensitive to *M. phaseolina* and Anderson (1985) who noted that *R. solani* and *F. spp.* caused distinct lesions on roots of mungbean.

Application of fungicides against the disease was also studied. Benomyl was the most effective in decreasing disease and improved the percentage of survival against root-rot disease caused by the previous fungi, consequently reflected in increasing seed yield per plot against infected ones. While Penycuran was in the second rank. These results revealed that fungicidal utilization will continue to be one of the solutions to manage some of the plant diseases among the means of the integrated pest management.

The obtained results are in agreement with those obtained by Siddiqui (2001) who found that benlate was the most effective treatment in the suppression of *R. solani*, *M. phaseolina* and *Fusarium spp.* under field conditions on mungbean, soybean, cotton and sunflower.

Biocontrol agents used in this study as seed treatment with either *Trichoderma viridi* or *Bacillus subtilis* significantly reduced pre- and post-emergence of mungbean (*Vigna radiata*) damping-off and consequently increased survival percentage over the untreated control plots. So, seed yield was significantly increased over the control as a result of seed treatment by the two biocides. These results agree with the findings of Tschen (1985) who found that mungbean damping-off caused by *R. solani* was controlled by adding *B. subtilis* to the soil. He added that counting seed with *B. subtilis* was a simple and effective method of control. In addition, Ehteshamul-Haque et al. (1990) found that seed treatment or soil drenches of *Vigna radiata* (mungbean) with different treatments including *B. subtilis* and *T. harzianum* reduced the infection of root rot caused by *M. phaseolina*, *R. solani* and *Fusarium spp.*

It is reasonable to believe that antagonism is an important factor affecting the population density and survival of pathogenic fungi in the soil (Papavizas et al., 1968). Yehia et al. (1982) added that the purpose of biological control of root-rot was not only to raise the plant growth and yield, but also to reduce density of soilborn pathogens.

Results also indicated the possibility of using certain natural products such as Saponin for the control of mungbean damping-off disease which are in agreement with those obtained by Omar and Abd El-Halim (1992) who stated that Saponin significantly reduced the growth of 25 fungal species.

On the other hand, a minor difference in the infection percentage of mungbean plants between locations used (Sakha, Etay El-Baroud and Shandaweel) could be due to the environmental conditions.

It could be concluded that use of fungicides will be one of the most effective means to control plant diseases. Meanwhile, further studies on the biological control of *T. viridi* and *B. subtilis* or the natural product as Saponin may be extended in future. Furthermore, because the new strategy of plant protection will consider keeping the environmental conditions free from the harmful pollution a priority of further studies, are needed to encourage the use of biological control and natural substances searching for fungicidal substitute. With other control measures such as seed treatment with an effective fungicide or biocide.

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تقييم فاعلية بعض المعاملات الحيوية والكيميائية لمقاومة موت البادرات في فول الماتج

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تم تقييم فاعلية المبيدين الفطريين بنليت ومونسرين وكل من الفطر تريكوثيرمما فيردى والبكتيريا باسيلس ساتلس والمركب الطبيعي سابونين كأحد الإستيرويدات الجليكوسيدية المستخلصة من بذور البرسيم الحجازى فى مقاومة مرض موت البادرات قبل وبعد الإنبات فوق سطح التربة لثلاثة أصناف من المانجين (فول الماتج) ذات مستويات مختلفة من القابلية للإصابة بالمسببات المرضية لهذا المرض وهى الفطريات ريزوكتونيا سولاني ، فيوزاريوم سولاني ، ماكروفومينا فاسيوليتا وذلك تحت ظروف الصوبة والحقل فى ثلاث جينات هي سخا ، إبتاي البارود وشنوبل فى موسم ٢٠٠١ . بصفة عامة وجد أن معاملة التقاوى بالمعاملات السابق ذكرها قللت معنويًا النسبة المئوية للإصابة بموت البادرات قبل وبعد الإنبات للأصناف الثلاثة مما أدى إلى زيادة فى المحصول .

المقاومة المتكاملة باستخدام معاملة البذرة سواء بالبليت أو المونسرين بمعزل ٣ جم/كجم بذرة باستخدام الصنف الأقل فى الإصابة VC 2719 كن أفضل طريقة لمقاومة المرض .

على الرغم من أن استخدام العامل الحيوى سواء التريكوثيرمما فيردى أو بكتيريا الباسيلس ساتلس أو المركب الطبيعي سابونين كانت أقل فى الكفاءة من المبيدات المستخدمة إلا أنها تعتبر أكثر امانًا فى الاستخدام .