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Biological Control of Black Scurf and Dry Rot of Potato

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Black scurf and dry rot of potato caused by *Rhizoctonia solani* and *Fusarium sambucinum*, respectively are a major yield-limiting factor for potato tuber production. Pathogenicity tests of the two isolated potato pathogens, using some potato cultivars indicated that potato cv. Hermis was the most susceptible, followed by cvs. Picasso, Sponta, Cara and Diamont. Some fungal and bacterial bioagents as well as an isolate of actinomycetes isolated from soil rhizosphere samples of healthy potato plants were screened for their antagonistic effect against these two pathogens. *Trichoderma harzianum*, *Epicoccum* sp., *Streptomyces endus* and an actinomycetes isolate were the most effective bioagents in suppressing the radial growth of the two pathogens. In general, these bioagents were less effective in retarding growth of *R. solani* if compared with *F. sambucinum* growth. The biological control showed the possibility of controlling black scurf and dry rot of potato by certain bioagents as *T. harzianum*, *Epicoccum* sp., *S. endus* and an isolate of actinomycetes under greenhouse (*R. solani* and *F. sambucinum* infested soil) conditions. These treatments significantly decreased pre-and post-emergence damping-off (killing developing potato sprouts), infected yield and increased each of survival plants, chlorophyll content and potato tuber yield compared with the untreated seed pieces. The treatment of seed tubers with *T. harzianum*, *Epicoccum* sp. was more efficient than the actinomycetes isolate and *S. endus* in most of estimated parameters. The effects were similar in most cases to those of Rizolex fungicide, which increased emergence, chlorophyll content of potatoes leaf, potato tuber yield and reduced black scurf and dry rot severity. Results of this study suggest that *T. harzianum*, *Epicoccum* sp. are effective bioagents in controlling black scurf and dry rot of potato and could be considered as promising alternative to chemical products.

Keywords: biological control, *Fusarium sambucinum*, potato tuber, *Rhizoctonia solani* and rhizosphere.

Potatoes are widely cultivated, and could contribute to reducing worldwide food shortages (Han *et al.*, 2005). However, potato plants are susceptible to devastation by various diseases, such as black scurf caused by *Rhizoctonia solani* and dry rot caused by *Fusarium sambucinum* (Agrios, 1997). Black scurf and dry rot diseases have limiting effects on tuber yield. In the same time, *R. solani*-infected plant-lets may develop crown rot, root rot, or stem canker which often leads to wilting and plant death in the severe cases. Similarly, Fusarium dry rot of seed tubers can reduce establishment by killing the developing potato sprouts. Also, both diseases can greatly affect tuber quality, and, therefore, can severely reduce its market value (Wolf and Verret, 1999; Yao *et al.*, 2002; Grosch *et al.*, 2005 and Wharton and Kirk, 2007). Unfortunately, all the commonly grown potato cultivars are susceptible to *R. solani* and *F. sambucinum*, although some are less susceptible than others but several breeding lines have been reported to have a higher degree of resistance to dry rot Wharton and Kirk (2007). Control of *R. solani* and *F. sambucinum* is difficult because these pathogens survive for many years as sclerotia in soil or as mycelium in organic matter under several environmental conditions (Hyakumachi and Ui, 1982). Furthermore, these pathogens have extremely wide host range (Ogoshi, 1987). Historically, control of Rhizoctonia and Fusarium diseases has commonly relied on cultural practices and on the use of chemicals. However, cultural practices alone are not efficient and, at the present time, no effective fungicides are available, although some chemicals are recommended (Yao *et al.*, 2002). In the recent years, an environmentally friendly and sustainable alternative to protect plants against soil-borne pathogens is the biological control using antagonistic microorganisms as bioagents (Eckwall and Schottel, 1997; Munimbazi and Bullerman, 1998; Emmert and Handelsman, 1999; Weller *et al.*, 2002; Yao *et al.*, 2002; Han *et al.*, 2005; Grosch *et al.*, 2005; El-Kot and Belal, 2006). Several researchers have already proved fungal microorganisms to suppress diseases caused by *R. solani* (Lewis and Larkin, 1998; Ross *et al.*, 1998; El-Kazzaz *et al.*, 2000; Ahmed *et al.*, 2003 and Van den Boogert and Luttikholt, 2004). Additionally, antagonistic plant-associated bacteria and actinomycetes are another important group of beneficial microorganisms for the control of soil-borne plant pathogens (Mao *et. al.*, 1997; El-Kazzaz *et al.*, 2002; Weller *et al.*, 2002; Ahmed *et al.*, 2003; Han *et al.*, 2005; Grosch *et al.*, 2005 and El-Kot and Belal, 2006). Therefore, the present investigation was designed to investigate the potential of seed tubers treatment with the bioagents to reduce the diseases of black scurf and dry rot of potato.

Materials and Methods

Pathogens:

Samples of potato tubers and plants showing typical symptoms of black scurf and dry rot were collected from different locations at Kafr El-Sheikh and El-Gharbiya governorates, Egypt. Sample pieces were kept in a moist chamber for 48h. then, hyphal-tips of each isolate from the surface growing fungus was transferred into Potato Dextrose Agar plates (PDA). After 2-3days of incubation at 25-28°C, the isolates were purified according to Booth (1977) and the pure cultures were maintained on PDA slants at 4°C for further experiments. Cultural, morphological, microscopical and pathological properties were considered to identify the isolated pathogens according to Burgess *et al.* (1994). Pathogenicity tests were performed on potato cultivars Hermis, Cara, Sponta, Diamont and Picasso using the methods described by Khalifa (1991).

Screening and isolation of antagonists:

Antagonists were isolated from soil rhizosphere samples of healthy potato plants producing areas at Kafr El-Sheikh and El-Gharbiya governorates, Egypt. The used bioagents were isolated on selected media according to the methods recommended by Anonymous (1984), Burgess *et al.* (1994) and Turner *et al.* (1998).

Identification of the bioagent isolates:

The isolated microorganisms (fungi, bacteria and actinomycetes) were divided each into groups or types according to their morphological and microscopical characteristics, rate of growth,... etc. (Parry *et al.*, 1983). accordingly. 17 fungal. 7 bacterial isolates and 5 actinomycetes isolates were selected for further study.

In vitro study:

The antagonistic effects of the used bioagents were performed according the methods adopted by Bell *et al.* (1982); Ibrahim *et al.* (1987) and Ferreira *et al.* (1991).

Biological control of the tested pathogens on potato plants:

An aqueous suspension at the concentration of 10^6 from all of *T. harzianum* and *Epicoccum* sp., and 10^8 from all of *S. endus* and the actinomycetes isolate were prepared. The four antagonists were used to control the tested pathogens in pot experiments. Pots, 40 cm in diameter, which used in the two successive seasons (2005/2006 and 2006/2007) were filled with clay soil and Hermis potato cultivar seed tuber were planted (3 seeds/pot). Inocula of the pathogens were prepared according to Khalifa, (1991). Soil infestation was carried out one week before planting at the rate of 3% (Khalifa, 1991). The infested soil was mixed thoroughly and moistened every other day. The antagonists were applied at the time of planting as seed treatment. Seeds were immersed in each tested antagonist for 45 min. and then dried. Seeds were then sown in each pot (3 seeds/pot). Rizolex T50, the most used fungicide for controlling soil borne diseases was applied for comparison as a seed treatment at the recommended dose (3 g/kg seeds). Each treatment was consisted of 4 replicates. The pots were kept in the greenhouse and watered when needed. The degree of disease incidence was recorded as percentages of pre-and post-emergence damping-off, percentage of survival plants as well as chlorophyll content [chlorophyll content of the relative green colour of one most recently matured leaf per potatoes plant was measured by a chlorophyll meter (SPAD-502, Minolta Co., Ltd., Japan) and reported as the SPAD (measurement of photon capture) value, the infection rate of yield tubers and yield tuber production (Grosch *et al.*, 2005).

Statistical analysis:

The obtained data were statistically analysed according to the method of Gomez and Gomez, (1984).

Results and Discussion

Isolation, identification and pathogenicity tests:

The isolated organisms were consisting of 21 fungal isolates belonging to the genus *Rhizoctonia* and *Fusarium* as shown by preliminary microscopic examination. The isolated *Fusarium* isolates were identified as *F. sambucinum* and the isolated *Rhizoctonia* isolates were identified as *R. solani.* Data presented in Table (1) and Figures (1) and (2) show that the two isolated potato pathogens, *i.e. R. solani* and *F. sambucinum* were pathogenic to all used potato cultivars. This finding is in agreement with those of Wharton and Kirk (2007), since they reported that all the

Table 1. Pathogenicity tests of *Rhizoctonia solani* and *Fusarium sambucinum* on potato cultivars under greenhouse conditions

Tested potato cultivar	Disease incidence (%)[a]					
	Rhizoctonia solani			*Fusarium sambucinum*		
	Pre-*	Post-**	Sur.***	Pre.	Post.	Sur.
Hermis	7.6 a	12.3 a	80.1 e	10.6 a	8.7 a	80.7 e
Cara	3.4 c	5.9 d	90.7 b	4.5 c	6.2 d	89.3 b
Picasso	4.2 b	7.3 b	88.5 d	6.7 b	7.5 b	85.8 d
Sponta	4.3 b	6.8 c	88.9 c	6.5 b	6.9 c	86.5 c
Diamont	1.8 d	2.4 e	95.8 a	3.2 e	2.9 e	93.9 a

[a] Values with the same alphabetical letters within column are not significantly different ($P<0.05$).
* = Pre-emergence damping off, **= post-emergence damping off and ***= Survival plants.

commonly grown potato cultivars are susceptible to *R. solani* and *F. sambucinum*. In the same time, cv. Hermes was the most susceptible one followed by Picasso and Sponta, while, on the other hand, cv. Diamont was the less susceptible one. Results also indicate that *R. solani* was highly pathogenic to potato cultivars than *F. sambucinum*.

Data presented in Tables (2 and 3) indicate that *R. solani* and *F. sambucinum* have negatively affected chlorophyll content (SPAD) and tuber yield of tested potato cultivars. Also, these two pathogens infected the tuber yield of all potato cultivars with variable degrees (Table 4). In the meantime, *R. solani* has highly infected tubers than *F. sambucinum*. Tuber yield of potato cv. Diamont was the least infected one compared with the other tested cultivars.

Table 2. Effect of *Rhizoctonia solani* and *Fusarium sambucinum* on total chlorophyll content (SPAD) of potato cultivars

Treatment	Tested cultivar[a]				
	Hermis	Cara	Picasso	Sponta	Diamont
R. solani	30.6 b	32.5 c	33.9 c	34.7 c	37.2 b
F. sambucinum	30.3 c	36.9 b	35.7 b	36.3 b	37.3 b
Control	31.2 a	38.3 a	36.4 a	37.1 a	41.2 a

[a] As described in footnote of Table (1).

Table 3. Effect of *Rhizoctonia solani* and *Fusarium sambucinum* on tuber yield (g/pot) of potato cultivars

Treatment	Tested cultivar[a]				
	Hermis	Cara	Picasso	Sponta	Diamont
R. solani	305 c	325 b	335 b	300 a	450 c
F. sambucinum	308 b	312 c	270 a	290 b	470 b
Control	347 a	354 a	369 a	340 c	505 a

[a] As described in footnote of Table (1).

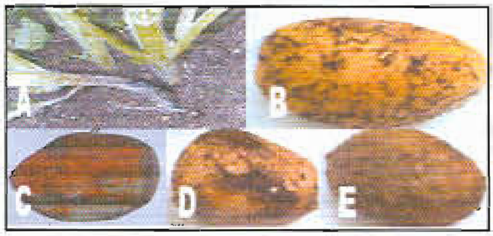

Fig. 1. Symptoms of *Rhizoctonia solani* (artificial inoculation) on potato plants, A= neck rot , B&C= black scurf, D= canker and E= green tuber.

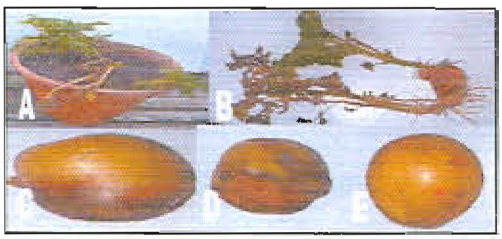

Fig. 2. Symptoms of *Fusarium sambucinum* (artificial inoculation) on potato plants, A&B= Emergence, root rot, C&D= Dry rot and E= Healthy tuber.

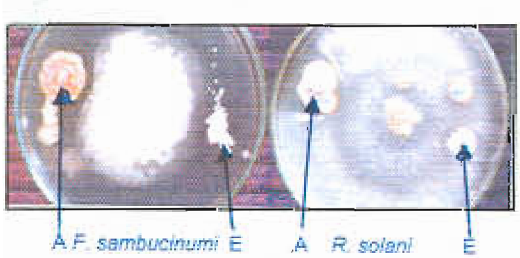

Fig. 3. Effect of *Epicoccum* sp. (E) and an actinomycetes isolate (A) on *Fusarium sambucinum* and *Rhizoctonia solani*.

Table 4. Effect of *Rhizoctonia solani* and *Fusarium sambucinum* on the infection rate of yield tuber of potato cultivars

Treatment	Tested cultivar[a]				
	Hermis	Cara	Picasso	Sponta	Diamont
R. solani	30.7 a	13.8 a	12.9 a	13.2 a	7.2 a
F. sambucinum	25.4 b	12.5 b	12.3 b	12.7 b	6.4 b
Control	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c

[a] As described in footnote of Table (1).

Screening and identification of bioagents:

The initial screening of more than 17 fungal, 7 bacterial and 5 actinomycetes isolates originated from different rhizosphere-soil samples of healthy potato plants, resulted in the isolation of 12 isolates exhibiting obvious antagonistic action on plates against one or more of the two isolated potato pathogens. The *in vitro* antagonists showed that two fungal isolates identified as *Trichoderma harzianum* and *Epicoccum* sp., only one isolate identified as *Streptomyces endus* and unidentified actinomycetes isolate were the most effective bioagents in suppressing the radial growth of the two pathogens, in general. Yet, they were less effective in retarding growth of *R. solani* as compared with *F. sambucinum* for example Figure (3).

Biological control of black scurf and dry rot of potato:

The selected antagonists exhibited their efficacy to control the damping-off, black scurf and dry rot of potato cv. Hermis during the growth seasons of 2005/2006 and 2006/2007 under the greenhouse conditions. Data presented in Tables (5, 6, 7 and 8) show that treatment of seed tubers with *T. harzianum*, *Epicoccum* sp., *S. endus* and the actinomycetes isolate were significantly increased emergence of potato sprouts grown in soil infested with each of *R. solani* and *F. sambucinum*. On the other hand, the infection rate of potato tuber yield with black scurf and dry rot was significantly decreased compared with untreated seed pieces. Interestingly, these effects were similar to or less than those obtained with Rizolex fungicide. Results also indicated that *T. harzianum* and *Epicoccum* sp. were the best seed treatments, since they increased survival plants from 77.0-97.1, 77.0-96.9 and 76.6-97.9, 76.6-97.2 under soil infestation with *R. solani* and from 79.2-93.8, 79.2-93.7 and 78.8-94.7, 78.8-94.3 under soil infestation with *F. sambucinum* respectively, during seasons 2005/2006 and 2006/2007. Also, they significantly decreased the infection rate of yield tuber with black scurf from 32.9-1.9, 32.9-1.6 and 35.1-1.4, 35.1-2.1 and dry rot from 31.9-2.6, 31.9-2.8 and 34.1-3.1, 34.1-2.9, respectively during the seasons of 2005/2006 and 2006/2007. On the other hand, chlorophyll content (SPAD) and yield of potato tuber were also significantly increased as a result of using these treatments and tuber yield were significantly increased during the two seasons as a result of using these treatments specially *T. harzianum* and *Epicoccum* sp. The efficiency of the antifungal isolates *T. harzianum*, *Epicoccum* sp., *S. endus* and Actinomyces to protect potato tuber

Table 5. Effect of seed tuber treatment with the antagonists on disease incidence, chlorophyll content (SPAD), tuber yield and infection rate of tuber yield by *Rhizoctonia solani* during 2005/2006 season

Treatment	Disease incidence (%)			SPAD** (leaves)	T.Y.***	I.R.****
	Pre-	Post-	Sur.			
T. harzianum	2.9 e*	0.0 e	97.1 a	45.9 a	860.4 b	1.9 e
S. endus	4.2 c	2.2 b	93.6 e	36.1 d	528.5 e	17.2 b
Epicoccum sp.	3.1 d	0.0 e	96.9 b	41.2 b	880.2 a	1.6 f
Actinomyces	4.8 b	1.2 d	94.0 d	32.3 f	550.7 d	16.3 c
Rizolex	3.2 d	1.8 c	95.0 c	36.7 c	780.9 c	5.8 d
R. solani	12.3 a	10.7 a	77.0 f	35.5 e	501.3 f	32.9 a

* As described in footnote of Table (1).
** = Chlorophyll content, ***= Tuber yield and ****= Infection rate.

Table 6. Effect of seed tuber treatment with the antagonists on disease incidence, chlorophyll content (SPAD), tuber yield and infection rate of tuber yield by *Rhizoctonia solani* during 2006/2007 season

Treatment	Disease incidence (%)			SPAD** (leaves)	T.Y.***	I.R.****
	Pre-	Post-	Sur.			
T. harzianum	2.1 f *	0.0 e	97.9 a	46.2 a	875.2 b	1.4 f
S. endus	3.4 c	1.3 c	95.3 d	40.1 c	603.4 e	15.2 b
Epicoccum sp.	2.8 d	0.0 e	97.2 b	40.8 b	890.1 a	2.1 e
Actinomyces	5.2 b	2.4 b	92.4 e	34.3 e	607.2 d	14.3 c
Rizolex	2.3 e	1.1 d	96.6 c	36.1 d	802.6 c	6.8 d
R. solani	11.9 a	11.5 a	76.6 f	36.2 d	511.7 f	35.1 a

* As described in footnote of Table (5).

Table 7. Effect of seed tuber treatment with the antagonists on disease incidence, chlorophyll content (SPAD), tuber yield and infection rate of tuber yield by *Fusarium sambucinum* during 2005/2006 season

Treatment	Disease incidence (%)			SPAD** (leaves)	T.Y.***	I.R.****
	Pre-	Post-	Sur.			
T. harzianum	6.2 bc *	0.0 d	93.8 a	46.1 a	903.4 b	2.6 e
S. endus	7.1 b	2.2 c	90.7 b	35.7 c	632.6 d	15.3 c
Epicoccum sp.	5.3 c	1.0 d	93.7 a	42.0 b	982.3 a	2.8 e
Actinomyces	6.0 bc	4.1 b	89.9 b	31.2 d	590.7 e	18.3 b
Rizolex	5.1 c	4.0 b	90.9 b	41.5 b	830.5 c	5.6 d
R. solani	11.5 a	9.3 a	79.2 c	31.4 d	520.7 f	31.9 a

* As described in footnote of Table (5).

Table 8. Effect of seed tuber treatment with the antagonists on disease incidence, chlorophyll content (SPAD), tuber yield and infection rate of tuber yield by *Fusarium sambucinum* during 2006/2007 season

Treatment	Disease incidence (%)			SPAD** (leaves)	T.Y.***	I.R.****
	Pre-	Post-	Sur.			
T. harzianum	5.3 c *	0.0 e	94.7 a	44.8 a	916.2 a	3.1 c
S. endus	6.9 b	1.2 d	91.9 b	36.2 d	704.6 c	12.4 b
Epicoccum sp.	5.7 c	0.0 e	94.3 a	43.1 b	950.8 a	2.9 c
Actinomyces	7.1 b	3.6 b	89.3 c	35.3 e	630.9 c	16.2 b
Rizolex	5.4 c	2.8 c	91.8 b	40.6 c	850.2 b	4.9 c
R. solani	12.3 a	8.9 a	78.8 d	32.1 f	531.3 d	34.1 a

* As described in footnote of Table (5).

plants against damping-off and tuber rot has been clearly proved through the present study. This can be explained in the light of results recorded by Abd El-Moity (1981), who stated that *T. harzianum* work through different mechanisms, *i.e.* production of gliotoxin. mycoparasitism and growing very fast and act as barrier between susceptible plant tissues and virulent pathogens. Also, attempts had been successfully carried out using antagonists to control soil-borne pathogens on potato tuber plants (Jager and Velvis, 1985; Liu *et. al.*, 1995; Eckwall and Schottel, 1997; Hoitink and Boehn, 1999; Neeno-Eckwall *et al.*, 2001 and Van den Boogert and Luttikholt, 2004). The antagonistic activity of these used bioagents was recognized by many investigators. Many species of genus are known to be potent producers of many antibiotics against soil-borne pathogens (Belal *et. al.*, 1996; Ross *et. al.*, 1998; Ahmed *et. al.*, 2003 and Han *et. al.*, 2005). The obtained results of this study suggest that the tested microorganisms proved to be an effective bioagents in controlling the tested potato pathogenic fungi and could be considered an alternative to chemical products and hence it can reduce the environmental pollution resulting from using fungicides in controlling plant diseases.

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المقاومة الحيوية للقشرة السوداء والعفن الجاف للبطاطس

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يعتبر مرضى القشرة السوداء والعفن الجاف لدرنات البطاطس والمتسببين عن الفطرين ريزوكتونيا سولانى وفيوزاريوم سامبوسينوم من أهم العوامل المحددة لإنتاج محصول البطاطس.

تم عزل المسببين المرضيين السابقين من درنات ونباتات بطاطس مصابة وتم اختبار قدرتهما المرضية على عدد من أصناف البطاطس مثل الصنف هيرمس(Hermis) والصنف كارا(Cara) والصنف بيكاسو (Picasso) والصنف سبونتا (Sponta) والصنف ديامونت(Diamont) وقد أثبتت النتائج أن جميع الأصناف المختبرة قابلة للإصابة بكلا المسببين المرضيين، وكان الصنف هيرمس (Hermis) أكثرها قابلية للإصابة يليه الصنف بيكاسو (Picasso) بنما كان الصنف ديامونت (Diamont) أقلها قابلية للإصابة .

تم عزل عزلات مضادة فطرية وبكتيرية مختلفة من عينات تربة الرايزوسفير لنباتات بطاطس سليمة و تم اختبار قدرتها التضادية للمسببين المرضيين السابقين. أوضحت النتائج أن العزلات الفطرية المضادة والتي عرفت على أنها تريكودرما هاريزيانم (*Trichoderma harzianum*) ونوع من الإيبيكوكم (*Epicoccum* sp.) و كذلك عزلة ستربتوميسس إندس (*Streptomyces endus*) وعزلة من الأكتينوميسيتس كانوا أكثر العزلات قدرة تضادية للمسببين المرضيين تحت الدراسة حيث أنهم حددوا وبدرجة كبيرة من نمو المسببين المرضيين على البيئة الغذائية ، كما أنهم كانوا أكثر قدرة تضادية للفيوزاريوم عن الريزوكتونيا.

أوضحت نتائج المقاومة الحيوية لكل من المسببين المرضيين ريزوكتونيا سولانى (*Rhizoctonia solani*) وفيوزاريوم سامبوسينوم (*Fusarium sambucinum*) تحت ظروف العدوى الصناعية للتربة فى الصوبة الزجاجية أن العزلات المضادة المستخدمة والسابقة الذكر قد قللت وبدرجة معنوية من حدوث سقوط لبادرات البطاطس (البراعم النابتة) وكذلك قللت من إصابة محصول الدرنات بالمرضين وذلك نتيجة معاملة تقاوي البطاطس قبل زراعتها بمعلق خلايا أو جراثيم العزلات المضادة. بينما سببت هذه المعاملات حدوث زيادة معنوية فى النباتات الباقية (السليمة) والكلوروفيل الكلى للنبات وكذلك محصول الدرنات مقارنة بالكنترول. كما أوضحت النتائج كذلك أن العزلات الفطرية المضادة والتي عرفت على أنها تريكودرما هاريزيانم (*Trichoderma harzianum*) و نوع من الإيبيكوكم (*Epicoccum* sp.) كانتا أفضل فى التأثير على المسببين المرضيين فى معظم المقاييس التي تم تقديرها ، وكانت هذه التأثيرات مشابهة تقريبا للنتائج التي تم الحصول عليها نتيجة معاملة تقاوي البطاطس قبل زراعتها بمبيد الريزولكس.

أظهرت النتائج المتحصل عليها من هذه الدراسة مدى تأثير العوامل الحيوية المختبرة وخاصة التريكودرما هاريزيانم والإيبيكوكم على مقاومة تلك المسببات المرضية الفطرية والتي يجعل من إستخدامها بديلاً للمواد الكيماوية.