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BIOCONTROL OF SOME SCLEROTIA FORMING FUNGI ATTACKING GREEN BEANS PLANT USING TWO FUNGIVORUS MITES AND *Trichoderma harzianum*

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ABSTRACT

Isolation trials were carried out from wilt green bean plants showing typical symptoms of damping-off. Samples of crow ford variety were collected from farms located at Fayoum and Giza governorates, associated with the tow fungivorus mite *Kleemannia kosi* and *Proctolaelaps pygmaeus*

Some of plants diseases may be controlled by using soil mites which are normally considered fungivores mites.

The two fungi *Sclerotium bataticola* and *Rhizoctonia solani*, were isolated from naturally plants of green bean associated with the tow fungivorus mite *Kleemannia kosi* and *Proctolaelaps pygmaeus*

Laboratory studies revealed the ability of two mite species collected from green bean debris, root and soil, *K. kosi* and *P. pygmaeus*, to feed and propagate on the growth of the two different fungal species. Soil application of each mite species alone or together beside fungus *Trichoderma harzianu* was successful as a biocontrol technique against green bean root-rot and seedling-off disease.

INTRODUCTION

Damping-off and root-rot disease caused by fungi are the most serious disease affecting Lentil (lens culinaris Med) plants grown under Egyptian conditions (Omar. *et al* ,1988; Abo-Zeid. *et al*, 1990; Hamdi. *et al* ,1991; Hamdi. *et al*, 2002; El-Shaer, 2003 and Morsy, 2005).

Both *Rhizoctonia solani* and *Sclerotium bataticola* are among the most destructive fungi attacking legume crops in Egypt (Nofal *et al* , 1982; Sabet *et al* , 1998 and Morsy, 2005). These sclerotia forming fungi are often very tolerant to unfavorable environmental conditions and to several fungicides (Garrett, 1970).

The biological control of pathogen populations by microbial antagonists is a goal among the proposed avenues for minimizing damage caused by pathogens. Side by side several bioagents are usually found along with the pathogens, which show an antagonistic reaction. For instance, most species belonging to the genus *Trichoderma* are able to antagonize many plants pathogenic fungi and dome times give equal control effects with those of certain fungicides (Sivan and Chet, 1989; Ibrahim *et al.*, 2001; Rasmy, 2002; El-Abbasi *et al.*, 2003 and Nawar, 2005). In addition, there are many published reports concerning the possible use of fungivorus mites as fungal feeders and ingest spores for many soil-borne fungi (Mabrouk, 1983; 1988 and 1992; El-Bishlawy andAbada, 1989 ; Moustafa and Mabrouk, 1997 and El-Hady and Mabrouk, 2001).

Therefore, the main objectives in this study were to examine the possibility of using the two fungivorus mites *Kleemannia kosi* and *Proctolaelaps pygmaeus* beside fungus *Trichoderma harzianu* to control the Damping-off disease of green bean incited by *Sclerotium bataticola* and *Rhizoctonia solani.*

MATERIALS AND METHODS

Isolation and identification of the associated fungi :

Samples of plants showing typical symptoms of root-rot were collected from farms located at Fayoum and Giza governorates. Soil samples where these plants grown were also taken and put in polyethylene bags. Both plant and soil samples were kept in an ice tank during transportation. Isolation trials were carried out from plant samples on P D A medium.

Source of mite:

The soil fungivorous mites namely *Kleemannia kosi* and *Proctolaelaps pygmaeus* were collected from debris taken from green bean fields. Mites were extracted by using modified Tullgren funnel for24 hours. The fungivorous mites were reared on small plastic cells (2.8 cm in diameter and 2cm in depth). The bottom of the cell was covered with a thin stratum of plaster of Paris and charcoal, Small squares (2x2 cm) of the tested fungi grown on P D A medium, were transferred to these cells. Twenty-five individuals of each mite species were transferred into single cell. After 15 days of incubation at 25°C, the numbers of survived mites were counted.

Isolation from mite:

Isolation trials were carried out from unsurface sterilized mites by transferring any of the two mites separately to P D A plates. Plates were then sealed using a Para film and incubated at 25°C, for four days.

Ability of the two extracted mites to feed on *R. solani*, *S. bataticola*, *T. harzianum* and *A. niger*.

The two extracted mite species, i. e. *K. kosi* and *P. pygmaeus* were surface sterilized by immersing each separately in 2% sodium hypochlorite solution for one minute, washed three times in sterilized distilled water, and then transferred aseptically to dry on sterilized filter papers. The living individuals from each of the mite species were singly transmitted by little sterilized brush to small plastic cells (2.8 cm in diameter and 2cm in depth). The bottom of the cell was covered with a thin stratum of plaster of Paris and charcoal. Small squares (2x2 cm in dim) of the tested fungi (*R. solani*, *S. bataticola*, *T. harzianu* and *A. niger*) grown on PDA medium for 10 days, were transferred to these cells.

Soil infestation technique:

A- With the fungi:

Diseased samples were thoroughly washed under running tap water then rotted parts were surface sterilized rotted parts were surface passed sterilized using 2%sodium hypochlorite. The surface sterilized rotted parts were passed through three successive sterilized distilled water then dried between two folds of sterilized filter papers. Pieces were transferred under a

skeptical condition to Petri-dishes containing PDA medium at the rate of 4 pieces /plate. Petri-dishes were thereafter, incubated at 20°C for 4 days. The emerged fungi were picked up and sub cultured into PDA medium to obtain pure cultures.

Inoculums of the tested fungi were prepared by growing each fungus for 4 weeks at 25°C in autoclaved, 0.5 bottles containing 20g sorghum substrate and 30 ml water. The inoculums of each fungus was added and mixed with the sterilized soil at the rate of 5%inoculum.

B- With the fungivorus mites:

Fifty adult females of each mite species, *K. kosi* and *P pygmaeus* were added to each pot two days after the last irrigation.

Biocontrol of green bean root-rot:

Surface sterilized green bean seeds by 2%sodium hypochlorite were sown in pots already containing soil received different combination of fungi and mites. Three seeds were sown in each pot. Five pots were used as replicates for each treatment. Disease assessment was recorded 15 and 45 days after sowing as percentages of pre and post-emergence root-rot disease, respectively. Numbers of mite individual were counted at the end of the experiment. In addition, total counts of fungal colonies in soil for each of the two tested fungi were recorded directly after soil infestation and at the end of each treatment.

RESULTS AND DISCUSSION

Data in Table (I) revealed that different species of fungivorous mites, *Kleemannia kosi* and *Proctolaelaps pygmaeus* were found in different soil samples associated with two soil fungi, *Sclerotium bataticola* and *Rhizoctonia solani* in Giza and El-Fayoum governorates the percentage of diseased green bean Damping-off, 9.6, 7.6% with total 17.1% and 8.5%mean in Giza, and also in El-Fayoum governorates were 8.8, 6.2, 15.1 and 7.6%, respectively.

Table (I): The percentage of root-rot diseased associated with two fungivorous mites and numbers of mite/Kg of soil in two governorates and in different county.

Governorates	County	% root-rot disease	Numbers of mite *K. kosi* /Kg	Numbers of mite *P.pygmaeus*/Kg
Giza	Giza	9.6	12.4	3.9
	Aiat	7.6	8.5	4.7
	Total	17.1	20.9	8.6
	Mean	8.5	10.5	4.4
El-Fayoum	Fayoum	8.8	8.7	4.9
	Sanores	6.2	4.9	3.0
	Total	15.1	13.6	7.9
	Mean	7.6	6.8	3.9

The two different fungi, *Sclerotium bataticola* and *Rhizoctonia solani* were isolated from root-rotted of green bean plants. Concerning the ability of

K. kosi and *P. pygmaeus* to fed on and transmit the two tested fungi, data presented in Table (II) revealed that the number of these mites increased by feeding on any of the tested fungi. Number of *K. kosi increased* from 25 (the initial population) to 268 and 208 when fed on *Sclerotium bataticola* and *R. solani,* respectively. Meanwhile, the number of *P. pygmaeus* increased from 25 individuals to 135and 96 individuals when fed on the aforementioned fungi, respectively. Thus, it could be concluded that *K. kosi* utilized both fungi more actively than *P. pygmaeus*

Table (II), indicated the ability of the tested mite species to digest *Sclerotium bataticola* more than *S. rolfsii* which might contains high nutrition value responsible for decreasing the duration period of mite development. (Mabrouk, 1992 & Moustafa and Mabrouk 1997).

Table (II): Effect of the two fungi, *Sclerotium bataticola* and *Rhizoctonia solani* as food on the population increase of the two mite species *Kleemannia kosi* and *Proctolaelaps pygmaeus* after 15 days at 25°C.

Fungi used in feeding	Total individuals of mite species namely	
	Kleemannia kosi	*Proctolaelaps pygmaeus*
S. bataticola	268	135
R. solani	208	96

However, Table (III) revealed that *K. kosi* alone or in combination with *P. pygmaeus* reduced the percentage of diseased green bean seedling with the pathogenic fungi. The results also showed that the two fungivorus mite species decreased the percentage of the green bean root-rot diseased.

Data in table (III) showed that the total number of *K. kosi* increased to 736, 305 &544 individuals when fed on *S. bataticola*; *R. solani* and *S. bataticola* + *R. solani,* respectively. Meanwhile, number of *P. pygmaeus* increased to492, 165&407, individuals respectively. From the previousresults, it may be concluded that the possibility of biological control agent of the green bean damping-off disease by using certain biocontrol agent such as fungivorus mite *K. kosi* and *P. pygmaeus.*

Data in table (III) indicate that adding *T. harzianu* with *K. kosi* decreased the percentages damping-off disease from 40.57 to 17.70% and *P. pygmaeus* decreased the percentages to 20.37%.

Data in table (III) clearly indicate that adding *T. harzianu* either alone or with *K. kosi* and *P. pygmaeus* strongly decreased the percentages of both stages of lentil damping-off disease incidence irrespective to the two pathogenic fungi tested. It is also noticed from results obtained (table III) that both of the two fungivorus mite tested played a role in decreasing the percentages of damping-off disease caused by any of the two sclerotia forming fungi, i.e. *R. solani, S. bataticola.*

The authors conclude that reducing percentages of lentil damping-off disease as a result of adding each of the two fungivorus mite or both besides application of *T. harzianu* might be attributed to large amount of the causative fungi grazed by mites bit lings which predispose these sclerotia to

be invaded by *T. harzianu* hyphae and to be more sensitive to considerable amounts of toxic metabolites secreted by *T. harzianu.*

Table (III): *Kleemannia kosi* and *Proctolaelaps pygmaeus* as biocontrol agent of Damping-off disease caused by *Sclerotium bataticola* and *Rhizoctonia solani.*

Treatment used in soil infestation	Final population of mite /pot	% Damping-off	
		Pre*	Post**
bataticola	00	40.57	32.69
olani	00	45.33	20.84
arzianu	00	0.00	0.00
bataticola + R. solani	00	52.11	40.25
bataticola + T. harzianu	00	22.45	11.60
olani + T. harzianu	00	26.14	21.95
bataticola + R. solani +T. harzianu	00	31.18	27.10
S. bataticola +K. kosi(1)	736	30.00	28.96
R. solani +K. kosi(1)	305	33.12	18.95
T. harzianu +K. kosi(1)	217	0.00	0.00
S. bataticola + R. solani+K. kosi(1)	544	37.22	28.47
S. bataticola + T. harzianu+K. kosi(1)	678	17.70	10.04
R. solani + T. harzianu+K. kosi(1)	396	18.00	11.50
S. bataticola + R. solani +T. harzianu +K. kosi(1)	694	14.26	5.66
S. bataticola + P. pygmaeus(2)	492	34.80	24.70
R. solani + P. pygmaeus(2)	165	36.70	20.75
T. harzianu + P. pygmaeus(2)	94	0.00	0.00
S. bataticola + R. solani+ P. pygmaeus(2)	407	43.20	31.20
S. bataticola + T. harzianu+ P. pygmaeus(2)	496	20.35	14,30
R. solani + T. harzianu+ P. pygmaeus(2)	179	21.08	14.00
S. bataticola + R. solani +T. harzianu+ P.pygmaeus(2)	504	17.50	8.90
S. bataticola +K. kosi(1) + *P. pygmaeus*(2)	418(1)+231(2)	28.10	22.00
R. solani+K. kosi(1) + *P. pygmaeus*(2)	196(1)+ 96(2)	30.60	15.83
T. harzianu+K. kosi(1) + *P. pygmaeus*(2)	135(1)+64(2)	0.00	0.00
S. bataticola + R. solani+K. kosi(1) + *P. pygmaeus*(2)	306(1)+199(2)	31.20	25.60
S. bataticola + T. harzianu+K. kosi(1) + *P. pygmaeus*(2)	365(1)+246(2)	12.65	10.06
R. solani + T. harzianu+K. kosi(1) + *P. pygmaeus*(2)	210(1)+85(2)	11.14	8.50
S. bataticola +R.solani +T. harzianu+K. kosi(1 + *P. pygmaeus*(2)	314(1)+269(2)	5.28	7.75

Pre* after 15 days of sowing
Post after 45 days of sowing**

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المقاومة الحيوية لبعض الفطريات المكونة لإجسام حجرية والتى تصيب نباتات الفاصوليا الخضراء باستخدام اثنان من الأكاروسات فطرية التغذية وفطر تريكودرما هيرزيانم

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نتج عن عمليات العزل من نباتات الفاصوليا الخضراء المصابة طبيعيا بأعفان الجذورفى محافظتى الجيزة و الفيوم عن وجود اربعة انواع من الفطريات الممرضة لنبات الفاصوليا الخضراء هم سكلوروشيم بتاتكولا ، ريزوكتونيا سولاني،اسبرجلس نيجر، تريكودرما هيرزيانم. وكان مصاحبا لهما نوعين من الأكاروسات فطرية التغذية هما كليمانيا كوساى ، بروكتوليلابس بيجمايس .

لذلك أستهدف البحث

١- دراسة إمكانية استخدام تلك الأكاروسات في مقاومة الفطريات المسببة للإصابة في نباتات الفاصوليا الخضراء في محاولة لتقليل النسبة المئوية للإصابة .

٢- دراسة إمكانية استخدام تلك الأكاروسات بلاشتراك مع الفطر تريكودرما هيرزيانم المهاجم للفطريات الممرضة في مقاومة الفطريات المسببة للإصابة في نباتات الفاصوليا الخضراء .

وكانت أهم النتائج المتحصل عليها هي:

١-قدرة أثنين من الاكاروسات فطرية التغذية المعزولة من مخلفات الفاصوليا الخضراء هما كليمانيا كوساى ، بروكتوليلابس بيجمايس علي التغذية والتكاثر علي أنواع الفطر السابقة و التي تلعب الدور الأساسي في الأمراض التي تصيب نباتات الفاصوليا الخضراء.

٢-أدت عمليات المقاومة الحيوية باستخدام كلا من نوعى الأكاروس سواء منفردا أو معا آلي الحصول علي نتائج ايجابية في مقاومة أمراض موت البادرات في نبات الفاصوليا الخضراء حيث وجد أن:-

ا) زيادة أعداد الأكاروس المتحصل عليها في كلا النوعين الاكاروسيين محل الدراسة بعد نهاية التجربة.

ب) انخفاض النسبة المئوية لموت البادرات في جميع المعاملات التي تم فيها أضافت الأكاروس بنوعية.

٣- أدت عمليات المقاومة الحيوية باستخدام كلا من نوعى الأكاروس سواء منفردا أو معا الي جانب الفطر تريكودرما هيرزيانم آلي الحصول علي نتائج عاليةالايجابية في مقاومة أمراض موت البادرات في نبات الفاصوليا الخضراء حيث لوحظ انخفاض النسبة المئوية لموت البادرات في جميع المعاملات التي تم فيها أضافت الأكاروس بنوعية الي جانب الفطر تريكودرما هيرزيانم.