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Control of Damping off and / or Sore Shin in Cotton and White Mould in Cowpea Plant Disease(s) by Using a Bio-fungicide *Coniothyrium minitans* Campbell

[1]El-Sayed M. Embaby, [2]Eman M. El-Taher and [3]E.A.M. Gado

[1]Department of Plant Pathology, National Research Centre (NRC),
[2]The Regional Center for Mycology and Biotechnology, Al-Azhar University,
[3]Plant Pathology Department, Faculty of Agriculture, Ain-Shams University, Cairo, Egypt,

Abstract: *In vitro*, mycelial growth rate (s) of *Rhizoctonia solani* (the causal agent of damping-off and / or sore shin disease in cotton plants) and *Sclerotinia sclerotiorum* (the causal agent of white mould disease in cowpea plants) were reduced significantly when it is cultured in the front of the bio-fungicide *Coniothyrium minitans* Campbell. According to time of application, the growth rates of either pathogenic fungi were decreased by increasing the time of inoculation from zero to 24 and 72 hrs, respectively (positive reaction was recorded with the time of application). Under greenhouse condition, a bio-fungicide, *C. minitans,* significantly decreased the percentage of infected cotton and cowpea plants by either fungi comparing with untreated control (free bio-fungicide), as well as significantly increased the percentage of healthy survival plants, grown in soil infested by *R. solani* and *S. sclerotiorum*, respectively. A bio-fungicide (*C. minitans*) gave positive reaction and significantly increased all the growth parameters as root, plant length and fresh weight in both cotton and cowpea plans (in addition flowering percent in cowpea) in comparison to absence the bio-fungicide.

Key words: *Coniothyrium minitans - Rhizoctonia solani - Sclerotinia sclerotiorum*.

INTRODUCTION

Cotton (*Gossypium hirutum* L.) is one of the most fiber and field crops in Egypt. Also, cowpea (*Vigna sinensis* Endl.) is one of the most important vegetable crops and / or plant family of legumes (fabaceae) as source of plant proteins in human food. *R. solani* and *S. sclerotiorum* are the most important soil borne fungi spreading in Egypt as well as in the world. The first fungus (*R. solani*) affecting cotton seedling, causing damping-off and sore shin (root rot) disease[15]. *S. sclerotiorum* is very serious on many crops and widely distributed in Egypt causing white mould disease in bean (*Phaseolus vulgaris* L.) and sunflower (*Helianthus annus*)[3,11,14,4].

Coniothyrium minitans an effective biological control agent of *R. solani* and gave very good control of *S. sclerotiorum* on lettuce[10,22,23,17,21,18,9].

Coniothyrium minitans used as a soil treatment for controlling white mould (*S. sclerotiorum*) in annual legumes and sunflower under greenhouse and field conditions[19]. *C. minitans* applied as soil substrate inocula to soil before planting, significantly reduced infection of lettuce caused by *S. sclerotiorum*[2].

In field trials, application of conidial suspensions of *C. minitans* to a bean crop soon after white mould outbreak lead to a higher percentage of sclerotial infection than later applications[6]. *C. minitans* reduced the growth rate of *Sclerotinia sclerotiorum, Sclerotium rolfsii* and *Sclerotium cepivorum, in vetro.* Under greenhouse condition, a bio-fungicide (*C. minitans*) significantly increased the percentage of healthy plants of bean lead to great increase of growth parameters in comparison to its absence[4].

The present study was aimed to test the fungus of *C. minitans* as a bio-fungicide on mycelial growth of the pathogenic fungi and time of application, also, examined the role of *C. minitans* in diseases control as showing the percentage of the healthy survival and infected plants as well as on growth parameters of cotton and cowpea plants.

MATERIALS AND METHODS

Source of Fungal Used: Two pathogens in different fungi, i.e. *Rhizoctonia solani* which caused damping-off, sore shin and / or root rot disease(s) in cotton (*Gossypium*) and *Sclerotinia sclerotiorum* causing white mould (rot) in cowpea (*Vigna sinensis*), in addition a

Corresponding Author: El-Sayed M. Embaby, Department of Plant Pathology, National Research Centre (NRC),

bio-fungicide *Coniothyrium minitans* Campbell were obtained from Plant Pathology Department, National Research Centre (NRC), El-Dokki, Egypt.

1- Antagonistic Test: (*In vitro*); potato dextrose agar (PDA) medium was used to increase a bio-fungicide (by putting 0.5 g as granules structure) in sterilized Petri-dishes (9 cm diam.)[12]. Plates were inoculated with 4 mm disks from 7 days old culture of the antagonistic fungus in one side of the plate and in the other side host fungi were applied *C. minitans* at different intervals, i.e. zero time, 24 h. and 72 h.

Other plates were inoculated with the only pathogenic fungi as control treatment. Three Petri-dishes for every particular treatment were used as replicates. All plates were incubated at 24 ± 2°C[4]. Percentage of reduction in mycelial growth rate as affected by a bio-fungicide according to the time of application were calculated and recorded when the control plates were filled by growth of the pathogenic fungi as follow:-

$$\text{Reduction \%} = \frac{R_1 - R_2}{R_1} \times 100$$

Where: R_1 = growth in control plates (without antagonism)

R_2 = growth in the presence of the a bio-agent

2- Greenhouse Experiments: Biological control experiment(s) were carried out under greenhouse condition in NRC during 2005 / 2006 season. Clay loamy soil were sterilized with formalin solution 2 %, then covered with plastic sheets for three weeks. Plastic sheets were removed and soil was left for one month for formalin evaporation. Sterilized soil was killed in plastic pots (20 cm diam.) each containing 1.5 kg soil / pot. *Coniothyrium minitans* (the bio-agent) was grown on sand / barley / water medium (1:1:2 w/w/v), then used for treating soil at rat 3% (inoculum mixed thoroughly with soil), irrigated and left for one week to activate the inocula[4]. Pathogenic fungal inocula were prepared in autoclaved corn meal sand water substrates (1:1:2, w/w/v) in flasks 250 ml. Each flask (containing 100 ml. medium) was inoculated with a 4mm diam. disk of pathogenic fungi which taken from 7 days old culture of PDA, then incubated at 24 ± 2°C for 3 weeks. After one week later from soil had been infested with bio-agent. First group of potted soil was infested by inocula of each pathogen separately at rates 50 sclerotia / kg soil for *S. sclerotiorum* and at rate 5% w/w of *R. Solani* then irrigated and left for one week. Second group was left free bio-agent (infested with pathogenic fungi only) as comparison. After 7 days from infestation, the prepared potted soil were sown with the host plant of healthy cotton and cowpea seeds, then, their surface had been sterilized, at rate 5 seeds / pot in both two potted groups. Four pots were used as replicates for each treatment. Pre and post emergence damping-off were recorded after 2-4 weeks for *R. Solani* in cotton and 4-8 weeks for *S. Sclerotiorum* in cowpea. All survived plants were uprooted and the percentage of diseases incidence were recorded. Also, some morphological characters such as root and plant length (centimeter) hypocotyl, fresh weight (gram) were measured and recorded between treated and untreated (control).

RESULTS AND DISCUSSIONS

***In vitro* Studies:** The effect of bio-agent *Coniothyrium minitans* on the growth rate (mm) of *Rhizoctonia solani* (the causal agent of sore shin and root rot of cotton plants) and *Sclerotinia sclerotiorum* (the causal agent of white mold in cowpea plants) were recorded in Table (1). Data presented that, the growth rates of either fungi i.e. *R. solani* and *S. sclerotiorum* were significantly reduced in the presence of a bio-fungicide (*C. minitans*) comparing with untreated control. No significant diffrences were recorded between the tested fungi i.e. *R. solani* and *S. sclerotiorum*. Concerning the time of application: the growth rates of both tested fungi were significantly decreased by increasing the time of application period comparing with the same (zero) time of application. On the other hand, growth of third period (72 hr. of application) was greatly affected comparing with other periods. Data in the same table show that; *R. solani* strongly affected under this condition than *S. sclerotiorum*. Mycelial growth of *R. solani* were decreased from 70.30 mm to 60.10 and 20.30 mm with 18.9, 32.2 and 74.4 reduction percent by increasing the time of inoculation from zero, 24 and 72 hr. respectively. Also; the mycelial growth rate of *S. sclerotiorum* was decreased from 70mm in zero time of inoculation to 50.90 and 30 mm by increasing the time of application to 24 and 72 hr. with 22.2%, 34.4% and 66.7% of reduction respectively.

Effect of Adding Bio-Agent on Healthy and Infected Plants: Effect of adding bio-agent (*C. minitans*) on the healthy survival and infected plants of either cotton and cowpea plants due to *R. solani* or *S. sclerotiorum* under greenhouse condition were recorded in Table (2) and Fig. (1). Data show that, *C. minitans* gave positive reaction in controlling both fungi, i.e. *R. solani* and *S. sclerotiorum* compared with untreated control. Bio-agent significantly increased the healthy survival plants as well as reduced infection percent than untreated. On the other hand, *R. solani* was the most affected and gave higher increasing percent

Table 1: Effect of a bio-fungicide (*C. minitans*) on the mycelial growth rates(mm) for either *R. solani* and *S. Sclerotiorum.*

	Rhizoctonia solani		*Sclerotinia sclerotiorum*	
Period Time	Mycelial growth (mm)	Reduction %	Mycelial growth (mm)	Reduction %
Zero time	70.3	18.9	70.0	22.2
24 hr.	60.1	32.2	50.9	34.4
72 hr.	20.3	74.4	30.0	66.7
Untreated (Control)	90.0	-	90.0	-

L.S.D 5%	For Fungi	For period	time Interaction
	(A)	(B)	A*B C
	0.529	0.748	1.058
	N.S	Sig.	Sig.

Table 2: Effect of a bio-fungicide (*C. minitans*) on healthy survival and infected cotton and cowpea plants with *R. solani* and *S. sclerotiorum*

		R. solani			*S. sclerotiorum*		
	Period (Weeks)	U%	T%	Reduction	U%	T%	Reduction
H	3	31.3	75.0	58.27	73.3	80.0	8.38
I		68.3	25.0	63.61	26.7	20.0	25.10
H	8	-	-	-	46.7	73.3	36.29
I		-	-	-	53.3	26.7	49.91

L.S.D5%	For Fungi	For treatment	For interaction
	(A)	(B)	A*B C
	0.52	0.52	0.52
	Sig.	Sig.	Sig.

H = Healthy; I = Infected; U – Untreated (Free bio-fungicide); T = Treated (With bio-fungicide)

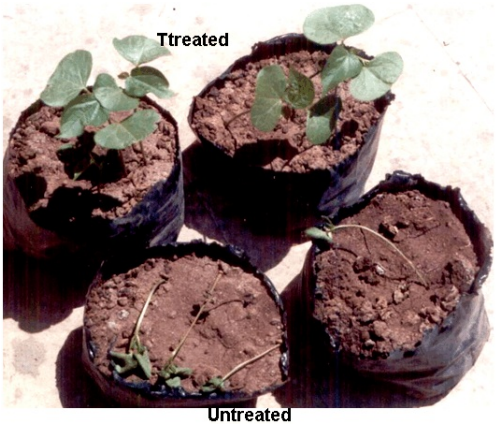

Fig. 1: Effect of a bio-fungicide (*C. minitans*) on cotton plants infected by *R. solani*.

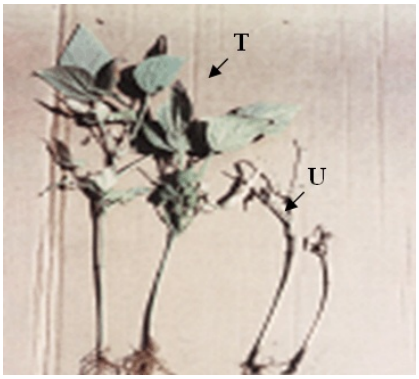

Fig. 2: Effect of a bio-fungicide (*C. minitans*) on growth parameters of Cowpea plants infected with *S. sclerotiorum*.
T = Treated (With bio-fungicide)
U – Untreated (Free bio-fungicide).

58.27% than *S. sclerotiorum*. *C. minitans* increased the healthy of survival plants infected with *S. sclerotiorum* from 73.3 % to 80.0 % with 8.38 % increasing percent after 3 weeks and from 46.7% to 73.3 % with 36.29 % increasing after 8 weeks. Also, increased the healthy survival of cotton plants infected with *R. solani* from 31.3 % to 75.0 % with 58.27 % increasing percent after 3 weeks. Also, data in the same table show that, a bio-fungicide *C. minitans* reduced significantly the

Table 3: Effect of a bio-fungicide (*C. minitans*) on growth parameters of cotton and cowpea plants infected by *R. solani* and *S. sclerotiorum*

	R. solani			*S. sclerotiorum*			
	Root length (cm)	Plant length(cm)	Fresh weight (g)	Root length (cm)	Plant length (cm)	Fresh weight (g)	Flowering (%)
Untreated	1.70	12.50	0.68	1.88	15.38	3.50	1.5
Treated	8.13	15.38	6.52	4.38	23.25	11.60	4.5
Increasing %	79.1	18.70	89.60	57.10	33.9	69.80	66.7

L.S.D. 5 %			
Root length			
For fungi		For treatment	Interaction
(A)		(B)	(AxB---C)
0.93		0.93	1.3
Sig.		Sig.	Sig
Plant length			
For fungi		For treatment	Interaction
(A)		(B)	(AxB---C)
2.4		2.4	3.39
Sig.		Sig.	Sig.
Fresh weight			
For fungi		For treatment	Interaction
(A)		(B)	(AxB---C)
0.668		0.668	0.94
Sig.		Sig.	Sig.

infection percent of cotton plants with *R. solani* from 68.7 % to 25 % with 63.61 % decreasing percent as well as reduced infecting Cowpea plants with *S. sclerotiorum* the causal agent of white mould from 26.7 % to 20.0 % with 25.1 % decreasing percent after the first period time (3 weeks) and from 53.3 % to 26.7% with 49.91 % reduction percent after 8 weeks.

Root length of cotton plants increased from 1.7 to 8.13 cm with 79.1 % increasing of root length; also, plant length (hypocotyl) were increased from 12.5 to 15.38 cm with 18.7 % increasing and fresh weight from 0.68 to 6.52 g with 89.6 % increasing.

The Effect of adding a bio-fungicide on some growth parameters of cotton and cowpea plants infect by *Rhizoctonia solani* or *Sclerotinia sclerotiorum* under greenhouse condition were measured and tabulated in Table (3).

Data presented that a bio-fungicide treatment significantly increased all the growth parameters as root and plant length (cm), fresh weight (g) in both tested fungi, in addition flowering percent with *S. sclerotiorum* on cowpea plants compared with untreated control. Also data indicated that *R. solani* was the most serous fungus than *S. sclerotiorum* specially on root length and fresh weight percent. Bio-agent *C, minitans* increased the growth parameters of cowpea infected with *S. sclerotiorum* as root length cm from 1.88 to 4.38cm with 57.1% increasing, plant length from 15.38 to 23.25cm with 33.9% increasing, fresh weight from 3.5 to 11.6g with 69.8% increasing and flowering percent from 1.5 to 4.5% with 66.75 increasing. Similar results were recorded in cotton plants which infected by *R. solani* when growing in soil infested with a bio-fungicide *C. minitans*. The growth parameters of cotton plant infected by *R. solani* were increased significantly by using a bio-fungicide than untreated control. Bio-fungicide treatment increased.

Discussion: Soil borne plant pathogenic fungi are wide spread and cause considerable losses in many crops. Resistant varieties are not available and chemical control is not effective. Biological control has shown promise as a partial agricultural method for control of plant diseases caused by soil borne fungi, Muker & Grag[16]. Data in this research presented that, bio-fungicide (*Coniothyrium minitans* Campbell) reduced the mycelial growth rates in both tested fungi; *Rhizoctonia solani* the causal agent of sore shin and / or root rot of cotton plants and *Sclerotinia sclerotiorum* the causal agent of white mould of cowpea plants compared with bio-agent free growth, similar results were obtained by Hung & Hoes[8], they reported that, *C. minitans* killing hyphae and sclerotia of *S. sclerotiorum*. Also, Moyuiken, *et al*.[3] reported that, *C. minitans* decreased recovery of *S. sclerotiorum* and increased seed germination of sunflower in agar plate test. Higher reduction percent in the growth rate were

recorded with *R. solani* than *S. sclerotiorum* which recorded 74.4% and 30.0% reduction percent in the mycelial growth rates in either fungi, respectively when the pathogenic fungi were inoculated on media 72 hrs. after inoculation with a bio-agent fungus (*C. minitans*). Muker & Grag[16] reported that in diluted cultures of *S. sclerotiorum* is inhibited by *C. minitans.* Similar results were confirmed by[4], who found that; *in vitro*; growth of sclerotia forming fungi, i.e. *Sclerotinia sclerotiorum, Sclerotium rolfsii* and *Sclerotium cepivorum* were reduced significantly when these fungi were cultured in the front of the bio-fungicide *C. minitans.*

Bio-fungicide *C. minitans* gave positive reaction between the time of application and reduction percent of the mycelial growth rate(s) with either pathogenic fungi. The most effective time of application was noticed when add 72 hrs before pathogenic fungi, whereas the least effective was observed when added the bio-fungicide *C. minitans* with the pathogenic fungi at the same time (zero time). These results are agreement with Gerlagh, *et al*.[6]. *Coniothyrium minitans* was screened for antagonism to *S. sclerotiorum* in a Petri dish bioassay. Antagonisms expressed as a reduction in the rate of colonization by *S. sclerotiorum* occurred whether *C. minitans* was co-inoculated at the same time, one day before or after *S. sclerotiorum* but was slightly restricted when *S. sclerotiorum* was given a lead of one day. Similar results were also reported by Stewart *et al*.,[20]; Benuzzi & Albonetti[1]; Gerlagh, *et al*.,[6]; Gerlagh, *et al*.,[7] and Embaby,[4].

The obtained data increased reduction percent in growth rate of the pathogenic fungi by increasing the period time of applied *C. minitans* before inoculated fungal pathogen(s), this means increased reduction of growth rate percent from 18.9 % to 32.2 % and 74.4 % with *R. solani* at zero time, 24 hrs and 72 hrs, respectively and from 22.2 %, 34.4 % and 66.7 % with *S. sclerotiorum* at the same period time of application. Similar results were obtained by Embaby,[4], who reported that, delaying of inoculation the sclerotia forming fungi by 72 hrs after the dishes had been inoculated by the bio-fungicide greatly reduced growth of sclerotia forming fungi were carried out in the same time (zero).

Under greenhouse bio-fungicide *C. minitans* was act as effective biological control agent to protect the cotton root system infected with damping-off and/or sore shin causing by *R. solani* as well as cowpea roots infected with *S. sclerotiorum,* the causal agent of white mould disease and gave positive reaction in controlling both fungi compared with untreated control. It was significantly increased percentage of healthy survival plants and it's reduction as well as decreased the infection percent, similar results were obtained by Budge *et al*.,[2]. they reported that; bio-agent (*C. minitans*) applied to soil before planting, significantly reduced infection of lettuce cased by *S. sclerotiorum*.

Lynch, *et al*[10]. pre-planting soil applications of *C. minitans* gave very good control of *S. sclerotiorum* on lettuce. Sabet, *et al.*[17]: found that; *Pseudomonas lindbergii* and *C. minitans* (as bio-control agents) were more effective against groundnut damping-off and pod rot organisms as *Rhizoctonia solani*, *Fusarim oxysporum* and *Sclerotium rolfsii* (*Corticium rolfsii*).

Also, Westerdijk[21] reported that, the use of antagonist *C. minitans* applied at the pre-germination date and planting date to control *S. sclerotiorum* and *S. minor*. Bio-agent *C. minitans* increased the healthy of cowpea plants infected with *S. sclerotiorum* from 73.3% to 80% after 4 weeks and from 46.7% to 73.3% after 8 weeks as well as increased the healthy of survival cotton plants infected with *Rhizoctonia solani* from 31.3% to 75% with 58.27 increasing percent after 4 weeks. Similar results were obtained by Embaby[4].

Bio-fungicide (*C. minitans*) treatment increased all growth parameters, i.e. root and plant length (cm) and fresh weight (g) than untreated control and enhanced the plant growth. Bio-agent increased the growth parameters of cotton plants infected by *R. solani* as root length from 1.7 to 8.13 cm with 79.1% increasing, plant growth from 12.5 to 15.38 cm with 18.7% increasing and fresh weight from 0.68 to 6.52 g with 89.6% increasing.

Similar results were recorded in cowpea plants infected by *S. sclerotiorum* when treated with a bio-fungicide *C. minitans* pre-planting. Treated cowpea showed an increase in the root length from (0.88 to 4.38 cm with 57.1% increasing, plant length from 15.38 to 23.25cm with 33.9% increasing and fresh weight from 3.5 to 11.6g with 69.8% increasing, in addition increased flowering percent from 1.5% to 4.5% with 66.7% increasing. Similar results were obtained by Embaby 2006, who reported that; the presence of bio-fungicide in soil infested with sclerotia forming fungi led to great increase of growth parameters in comparison to its absence. It significantly increased root and plant length and fresh weight of survival plants.

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