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Efficiency of some soil mite species as a biocontrol of *Rhizoctonia solani* damping off and root rot diseases in cotton

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ABSTRACT

The present study attempt to deal with the pathogenic fungus *Rhizoctonia solani* as a nutritional regime for different soil mite species under laboratory conditions. Eight oribatid mite species were chosen viz: *Cilioppia difficilis, Xylobates capucinus, Javacarus kühneltii, Xylobates lophotrichus, Rhysotritia ardua ardua, Epilohmannia pallida aegyptica, Nothrus biciliatus* and one gamasid species (Uropodidae) *Leiodinychus karmeri* for our study. In pre-equipped rearing vials under laboratory conditions, all obtained mite species were tested towards the pathogenic fungus *Rhizoctonia solani* as food item. Two oribatid mite species *X. lophotrichus* and *X. capucinus* showed a successful role as bio-control agents against R. solani damping off and root rot symptoms in cotton in both sterilized and non-sterilized soil. Laboratory results showed that, the two tested mite species were found to reduce significantly the incidence of cotton seedling diseases (damping off, root rot and stem cankers) caused by *R. solani*. A better suppression was occurred in non-sterilized soil than the sterilized one. Incorporation of tested two mite species to soil were found to enhance plant health and cause quantitative changes in fungal populations of *R. solani*, as indicated by the low colonization index of fungal hyphae on roots of cotton plants when cultured on agar.

Keywords:- *Xylobates lophotrichus; Xylobates capucinus*; biocontrol; *Rhizoctonia solani* ; root rot; damping off ; cotton.

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1. INTRODUCTION

It is well known that, *Rhizoctonia solani* is a widely distributed fungal pathogen. This soil borne pathogen play a major role in the development of root-rot disease complexes on many important field crops; resulting in post-emergence damping-off in seedlings or seedling decay, and death of the plants (McCormack *et al.,* 2013). Environmental impacts happened from excessive and unjustifiable use of chemicals for suppressing soil pathogens directed the interest of the world to find an alternative biological control method for use in integrated pest management strategies for these crop diseases (Matloob and Juber, 2013).The composition of the soil fungal community is affected by fungus-feeding soil micro-arthropods, suggesting that fungus-feeding soil animals may affect losses of seeds caused by pathogenic fungus attack.

The objective of the present study was estimating the consumption rate of each mite species, when tested separately on the tested plant pathogenic fungus. Finding out the most efficient mites among the tested ones, that are preferentially feed on *R. solani* in the laboratory. Determining the role of the more fungus feeding mites in suppressing *R. solani* diseases in cotton under semi field condition.

2. MATERIALS AND METHODS

2.1 Soil sampling

Soil samples were collected in July 2011, by steel corer of size (10 ×10 ×5 cm) from soil cultivated with cotton crop, at Kafer-El-Hema village, El-Gharbeia Governorate, Egypt.In the laboratory, from soil samples mites were collected using Berlese' s funnels.Preparation for identification of species undertaken as explained in detail elsewhere Al-Assiuty (1981). Mite identification was accomplished by Balogh and Balogh (1992) for oribatid species, and Hughes (1976) for mesostigmatid species.

2.2 Microcosm experiment for palatability of different genera of soil mites on R. solani

Tested mite species were reared in culture vials pre-equipped prior to the experiment, in which a mixture of Plaster of Paris (9:1) as substrate was used (Goto, 1961). The vialswere then kept moist by adding a few drops of distilled water. Finally, culture vials was covered with a fine layer of muslin minuted with small holes to maintain air circulation.Tested fungus *R. solani* was grown on potato dextrose agar (PDA) media, and presented as small 7days old agar discs of 5 mm Ø to the tested mites in rearing vessels. Five mites were placed to every rearing vial with five replicates. Renew died individuals and food items when necessary (Scheu and Simmerling, 2004). Data obtained after 7 days of rearing, by counting the fecal pellets deposited in close vicinity and inside the hole of fungal agar discs as indication of the amount of food consumed by tested mite species (Schneider and Maraun, 2005). Accordingly; the most efficient mites feeding on *R. solani* diet will be used later in biological control experiment.

2.3 Preparation of R. solani inoculum

Fungal inoculum that will be mixed with tested soil was prepared, by transferring four mycelial plugs of 6 mm diameter were cut from the edge of 7days old *R. solani* culture grown on (PDA) medium into Petri dishes filled with 30 g of sterilized wheat bran mixed with sand (2:1). Then, the wheat bran culture of *R. solani* was incubated at 23–25 ºC for five days, and air-dried for 2 days under a laminar flow hood. Inoculum was passed through disinfected sieve with 1 mm openings and particles larger than 1 mm were not used as inoculum.Prepared inoculum was kept in a paper bag at 4–5 ºC for no more than one month until needed (Papavizas and Lewis, 1986 and Brewer and Larkin, 2005).

2.4 *Preparations of seeds and soil infestation with R. solani*

Sterilized and non-sterilized soil samples were used in experiments, soil sieved through a 6.6 mm screen and sterilization via autoclaved at 120°C for 1 hr on three successive days (Brewer and Larkin, 2005). Cotton seeds *Gossypium barbadense* were surface-disinfested in an aqueous solution of 5% sodium hypochlorite and 2 % ethanol in an Erlenmeyer flask with continuous shaking for 30 min. Disinfested seeds were rinsed in sterile distilled water and blotted on sterile filter papers. Pathogen inoculum was incorporated to both sterilized and non-sterilized soil at a rate of 4 gram inoculum / kg dry soil (Brewer and Larkin, 2005 and Safiuddin *et al.,* 2011). *R. solani* inoculum was mixed well with the soil in the form of bulk amendment of the fungus grown on wheat bran in a plastic bag with continuous shaking. Mixed pathogen inoculum and soil were incubated in the dark at room temperature for one week before planting (Lartey *et al.,* 1991).

2.5 The experimental design and treatments

Two mite species (*Xylobates lophotrichus* and *Xylobates capucinus*) were chosen for experiments as biocontrol agent. Mites were exposed to starvation for 2 days and become habituated on *R.solani* as fungal diet for one week before incorporating into soil. They were added separately to the experimental pots next day of pots preparation.Five replicates in each plant/fungus/mite treatments provided with 200 mite individuals from each species. The mites were placed into pots manually with a small brush into holes located around each seed. The oribatid treatments consisted of 10 pots per species (sterilized and non-sterilized soil) and 40 pots in total.

The cotton seeds were moistened for 4 days in a Petri dish containing wet cotton swab. Moistened cotton seeds planted in plastic pots (5 cm diam.× 11 cm deep) containing 250 g of soil (sterilized or non-sterilized). Three seeds

were arranged in the pot 1–2 cm below the soil surface; all pots were kept under laboratory condition and watered when needed.

Experimental plots were designed as four treatments for each of sterilized and non-sterilized soil prepared in 5 replicates as follow; (1) untreated control "only host seeds ", (2) pathogen control " host seeds + *R. solani* " , (3) host seeds + *R.solani* + mite species 1 and (4) host seeds + *R.sol*ani + mite species 2.

2.6 Parameters as a criteria for pathogensity

Plants were harvested after 4 weeks until the non-infested and infested soils had fully emerged (Brewer and Larkin, 2005). Germinated seedlings (shoots and roots) were collected, counted, fresh and dry weighted and checked for seedlings damping off. The number of diseased plants, the stem height (from soil line up to the growing tip), and the number of leaves per plant were recorded (Pavloua and Vakalounakis, 2005).

Furthermore, brown-colored and slightly wilted brown lesions on the stem near the soil surface were also monitored; these symptoms which are the signs of the stem cankers disease caused by *R. solani* (Brewer and Larkin, 2005). Disease incidence (%) was estimated as the percentage of infected cotton plants (Pavlou and Vakalounakis, 2005)

Non –germinated cotton seeds were picked from the soil on a sieve. Seeds were surface-sterilized for 1 min in 70% (vol.) alcohol followed by 3 min in 5% (vol.) sodium hypochlorite. Then, seeds were rinsing well in sterile distilled water for 10 minutes, and placed on plates of Potato Dextrose Agar (PDA) with antibiotics (Papavizas and Lewis, 1986). The characteristic of hyphal extension growth (mm) of *R. solani* on seeds was detected on plates after 3-4 days at 23-25°C.

Disease intensity of root rot caused by the fungus was estimated. Roots were separated carefully from the soil and washed in running tap water. Five 1 cm long root segments were selected at random, washed vigorously with distilled water. Washed root segments were plated onto Potato Dextrose Agar (PDA) plates supplemented with chloramifincol antibiotic. Plates were incubated at 23-25°C for 3-4 days, the hyphael extensions that extended from the root segments were observed (Siddiqui *et a*l., 2002).

2.7 Data processing

- Differences in the palatability of tested mites when feeding on *R.solani* diet were tested, by comparing means (t-test) of fecal pellets deposited by each mite species , expressed as pellets / individual /day.
- Colonization index (CI): Based on the extension of hyphal growth of *R. solani* from infected cotton roots (1cm.) on the agar surface, they are rated on 0-3 scale; where 0 = no observable hyphae were detected; 1 = < 0.5 cm extended threads of hyphae (low level of infection); 2= 0.5-1 cm extended threads of hyphae (intermediate level of infection); 3= >1 cm extended threads of hyphae (high level of infection) (Lewis *et al*.,1991 and Lewis and Papavizas, 1992).

  These ratings were converted to a disease severity index equation (DI) for each pot by using the formula according

  Jones and Clifford (1978). DI%= [(ni)/N] 100/imax, where;

  n= number of healthy or infected seedlings) in each class, i=numerical value (code) of the class, N= total number of

  plants examined, imax= value of the highest class, which represent 3 in our study
- Stem cankers index: Plants were also rated for stem cankers symptoms on a scale of 0–3 as follows: 0= healthy plant with no brown discoloration; 1= brown discoloration of cankers covering < 25 % of of the stem length; 2= cankers covering 25 - 50 % of the stems length; 3= cankers covering > 50 % of the stem length (Brewer and Larkin, 2005).
- Index of infestation: Infection percentage was calculated as follows = (No. of plants infected by a fungus/Total no. of plants) × 100 (Siddiqui *et al*., 2002).
- One - way analysis of variance (ANOVA): the data were analyzed to test differences between the different plant parameters among different treatments for each soil condition (sterilized and non- sterilized). The dependent variables are; shoot length, root length, fresh and dry weights of both shoots and roots and number of leaves as class factors. Treatments thereafter compared using Fisher's least significant difference (LSD) at P ≤ 0.05 (Sokal and Rohlf ,1995).

3. RESULTS

Eight different species of soil mites of which species are seven oribatid species; *Cilioppia difficilis*, *Xylobates capucinus , Javacarus kühneltii, Xylobates lophotrichus, Rhysotritia ardua ardua, Epilohmannia pallida aegyptica, Nothrus biciliatus* and one mesostigmatid species (Uropodidae) *Leiodinychus karmeri* (Table 1). It is clearly that, *C. difficilis* is the most dominant oribatid mite species occupying the highest density among the extracted oribatids in tested soil samples (31.4 individuals / sample). *X.capucinus* come the next (25.6 individuals / sample), and followed by *X.lophotrichus* (24 individuals / sample). Finally *Nothrus sp*. exhibited the lowest abundance in tested soil (1.5 individuals / sample).

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Table 1: Mite species isolated from tested soil samples (Mean ± Sd).

Mite species	Family	Population density $\bar{X}$ ± Sd
Cilioppia difficilis	Oppiidae	31.4 ± 9.89
Xylobates capucinus	Haplozetidae	25.66 ± 7.18
Xylobates lophotrichus	Haplozetidae	24 ± 5.44
Leiodinychus karmeri	Uropodidae	19.33 ± 5.06
Javacarus kühneltii	Lohmanniidae	15.4 ± 4.59
Rhysotritia ardua ardua	Euphthiracaridae	4.93 ± 3.14
Epilohmannia pallida aegyptica	Epilohmanidae	3 ± 2
Nothrus biciliatus	Nothridae	1.55 ± 0.7

Each value is the mean of 30 replicates ± standard deviation

3.1 Feeding biology of different genera of soil mites on R. solani pathogen.

Figure (1) revealed that, *X. lophotrichus* showed the highest feeding behaviour towards *R.solani* diet, as indicated by the number of fecal pellets deposited in close vicinity to this fungus in their rearing cups (2.0 ± 0.2 pellets / ind / day). Then, *X.capucinus* come the next (1.75 ± 0.2 pellets/ind/day). The mesostigmatid species *L. k*armeri showed the lowest feeding behavior (0.8±0.1pellets / ind /day). It was also cleared that, the oribatid mite species such as, *R. a. a*rdua, *C. difficilis, J. kühneltii, E. pallida aegyptica* and *N. biciliatus* rejected *R. solani* as food item during the rearing experiment (pellets ≤ 0.3 / day).There was no significant difference (t-test P > 0.05) between *X. lophotrichus* and *X.ca*pucinus mites when comparing means of their fecal pellets deposited, while highly significant variation (P ≤ 0.01) was recorded among fecal pellets of *X. lophotrichus* or *X.capucinus* and the rest of all rearing mites. On the other hand, there was no significant variations (P > 0.05) in the number of fecal pellets deposited by *J. kühneltii* and *R. a. ardua* as well as means of pellets of *E. p. aegyptic*a and *N. biciliatus* ,since there was no clear feeding variation among these two group of mites.Then, a highly significant variation in number of fecal pellets could be seen among fecal pellets deposited by *N. biciliatus* and the remaining mites except *E. pallida aegyptica* as mentioned above.

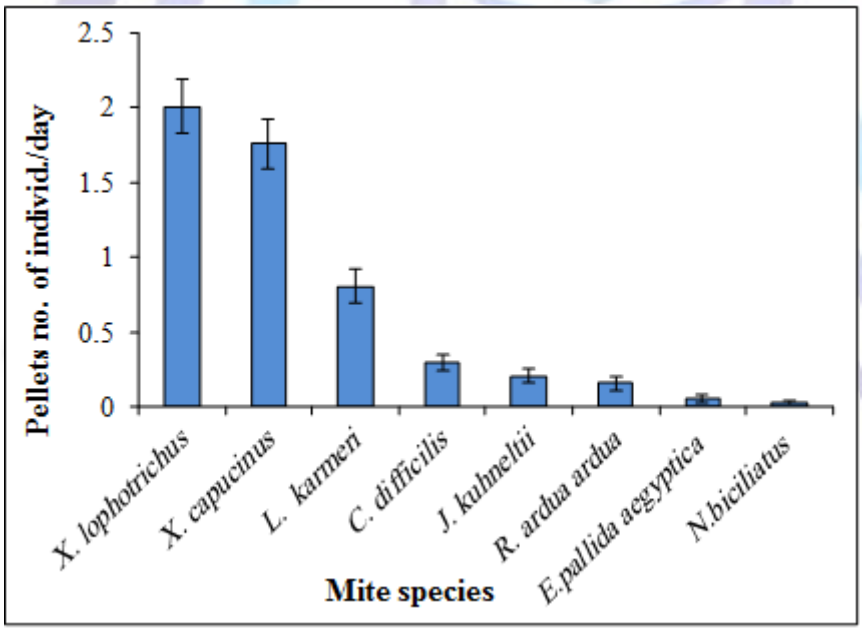

Figure 1: Mean Numbers of fecal pellets deposited by tested mites when feeding on Rhizoctonisolani fungal diet in the microcosm experiment.

Data are the mean of five replicates rearing vessels ± S.D, expressed as pellets /individual /day

It has been found that, reared mite individuals " *Xylobates lophotrichus* and *Xylobates* capucinus " in their nutritional regime were the most attracted mites to *R. solani* as a fungal diet and feed preferentially on it.

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3.2 Influence of pathogenicity of R. solani on growth parameters of cotton seedlings

Table (2) revealed that; sizes of cotton plants are varied among treatments; some treatments reduced significantly plant size when compared to the untreated control. The infested control reduced shoot size due to delayed emergence of plants and the direct deleterious effects of *R. solani* on the plants themselves.

There was a significant differences among plant parameters recorded between treatments "untreated control and pathogen control" ($P \leq 0.05$) in non- sterilized and sterilized soil. In non-sterilized soil, there was no significant difference between the control treatment and the infection and supplemented with *X. lophotrichus* mites in all plant growth parameters except in root fresh and dry weight. Also in non-sterilized soil, cotton shoot length emerged in pots exposed to *Rhizoctonia* infection only have a significantly decreased value (11.8 cm.) versus (14.9 cm.) for cotton plants emerged in soil have the infection and supplemented with *X. lophotrichus* mites. In comparing the two bio-control agents *X. lophotrichus* and *X.capucinus* in suppression of *Rhizoctonia* pathogen, it was clearly that, the second mite species has less potential effect than *X. lophotrichus* mite.

In sterilized soil, cotton shoot length emerged in pots exposed to *Rhizoctonia* infection only have a significantly decreased value at 10.5 cm. versus 14.2 cm. for cotton plants emerged in soil have the infection and supplemented with *X. lophotrichus* mites. As the same, all tested plant parameters were significantly higher ($P \leq 0.05$); e.g., the root fresh weight in soil supplemented with *X. lophotrichus* mites have 0.74 gram versus 0.35 gram in soil have the pathogen only (table 2). Application of *X. capucinus*,the second oribatid mite tested for suppression of *Rhizoctonia* diseases in sterilized soil was also proved to deduce the inoculated infection but with less potential effect than *X. lophotrichus* mites. Root fresh weight was (0.56 g.) significantly higher than the root fresh weight of plants emerged in pots have the pathogen only (0.35 g.). In non sterile soil, there was no significantly difference among the treatments applied with *X. lophotrichus* and *X. capucinus* in all tested plant parameters except for shoot length. In sterile soil, a significant difference between treatments of *R. solani*+ *X. lophotrichus* and *R. solani* + *X. capucinus* in shoot length, root length and shoot fresh weight but the rest of the parameters were not exhibited any significant difference. Furthermore, application of the two bio-control agents *X. lophotrichus* and *X. capucinus* to tested pots revealed to have a role in suppression of *Rhizoctonia* infection and decreased the spread of infection.

No significant difference ($P \geq 0.05$) between plant parameters of sterilized and non- sterilized soil of untreated control culture pots. Finally, in treatment of pathogen control (*R. solani* only), there was a significant difference ($P \leq 0.05$) in the plant parameters for sterilized and non- sterilized soil (table 2).

Table 2 : Means (± Sd) of Plant parameters of tested host plant and fungal pathogenicity that recorded for designated experimental sets A,B,C and D at four weeks after fungal inoculation for non sterile and sterile soil..

Treatment	Shoot length(cm)	Root length(cm)	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)	Leaves no.
			Non- sterile soil				
Untreated control (A)	15.53 a	17.33 a	3.037 a	1.28 a	0.54 a	0.195 a	5.29 a
	(±0.74)	(±1.46)	(±0.22)	(±0.20)	(±0.08)	(±0.03)	(±0.51)
R.solani+X.lophotrichus (B)	14.93 a	15.53 ab	2.68 ab	0.93 b	0.435 ab	0.079 b	4.8 a
	(±0.63)	(±1.43)	(±0.31)	(±0.25)	(±0.11)	(±0.02)	(±0.84)
R.solani+X.capucinus (C)	13.2 b	13.6 b	2.3 b	0.71 bc	0.38 bc	0.063 b	4.5 a
	(±0.67)	(±1.56)	(±0.33)	(±0.19)	(±0.11)	(±0.04)	(±0.79)
Pathogen control (*R.solani*) (D)	(11.8 c	7.8 c	1.806 c	0.49 c	0.26 c	0.048 c	3.3 b
	(±1.25)	(±2.08)	(±0.57)	(±0.12)	(±0.10)	(±0.01)	(±0.84)
			Sterile soil				
Untreated control (A)	16.23 a	18.99 a	3.029 a	1.24 a	0.55 a	0.21 a	5.7 a
	(±0.89)	(±1.55)	(±0.16)	(±0.23)	(±0.06)	(±0.02)	(±0.30)
R. solani+X. lophotrichus (B)	14.2 b	15.766 b	2.35 b	0.74 b	0.372 b	0.092 b	4.4 b
	(±0.57)	(±0.88)	(±0.20)	(±0.13)	(±0.10)	(±0.02)	(±0.24)
R. solani+X. capucinus	12.63 c	11.43 c	1.91 c	0.56 bc	0.324 b	0.074 b	4.1 b

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(C)							
	(±0.84)	(±1.05)	(±0.26)	(±0.15)	(±0.11)	(±0.03)	(±0.22)
Pathogen control (*R.solani*) (D)	10.5 d	6.4 d	1.01 d	0.35 c	0.16 c	0.034 c	2.6 c
	(±1.87)	(±1.14)	(±0.31)	(±0.13)	(±0.05)	(±0.01)	(±0.55)

Each value represents the mean number of five replicates, of three cotton seedlings in each cup. Mean values in the same column followed by different letters are significantly different at P ≤ 0.05 significance level according LSD test. Mite1= *X. lophotrichus* & mite 2= *X. capucinus*

Colonization index in Table (3) showed that, cotton plants germinated in sterilized soil inoculated with *R. solani* fungal pathogen (infected control) have more hyphal colonization (CI% = 83.3) on agar (root rot). Culture pots containing non-sterilized soil were exhibited less root rot disease severity index of (CI% = 69.9) on agar than the sterilized ones. Contrariwise, untreated control culture pots which were not inoculated with any fungal pathogen, have healthy cotton plants and no signs of disease symptoms detected (CI % = 0). On the other hand, sterile soil incorporated with *X. lophotrichus* mite as biocontrol agent were exhibited the efficiency of this mite species on suppression of root rot symptoms on cotton plants (CI% = 12.2). On the same approach,more suppression of R. solani root rot symptom appeared in non-sterilized soil culture pots than sterilized ones (CI% = 8.88). Tested mite *X. capucinus*, showed also its potential role in suppression of *R. solani* disease on roots of cotton plants but with slightly less influential effect on reducing root rot symptoms, where, the disease severity index recorded CI% = 13.3 &12.2 for both sterilized and non-sterilized soil, respectively.

Table 3 :Effect of applications of tested oribatid mites as biocontrol agents on thedisease incidence of Rhizoctonia root rot symptom on cotton seedlings

	Disease index (0-100)	
Treatment	Sterilized soil	Non-sterilized soil
untreated control	0	0
R. solani+X. lophotrichus	12.22 b	8.88 b
R. solani+X. capucinus	13.33 b	12.22b
Pathogen control (*R. solani*)	83.33 a	69.99 a

Colonization index for cotton root rot symptoms on a scale of 0-3, values are means for five replicate pots

0= healthy root system

1= < 0.5 cm extended threads of hyphae; low level of infection

2= 0.5-1 cm extended threads of hyphae; intermediate level of infection

3= >1 cm extended threads of hyphae; high level of infection

Values in the same column followed by different letters are significantly different at P ≤ 0.05 (LSD test)

There is no statistically significant difference (P > 0.05) in the incidence of root rot symptoms on cotton seedlings of sterilized and non-sterilized soil of both treatments (plant+ pathogen + *X. lophotrichus* and plant + pathogen + *X. capucinus*) as shown in table (3). On the contrary, there is a significant difference in the incidence of root rot symptoms on cotton plants of disease incidence of both treatments (plant + pathogen + *X. lophotrichus* and plant + pathogen + *X. capucinus*) and treatment (plant + pathogen) for both sterilized and non-sterilized soil (P < 0.05) indicatingthe effective roles of *X. lophotrichus* and *X. capucinus* on *Rhizoctonia* root rot disease. Without biocontrol agent, there is no significant variation (P > 0.05) in the incidence of *Rhizoctonia* root rot symptom on cotton plants of sterilized and non-sterilized soil of treatment (plant + pathogen) (Table 3).

3.3 Effect of tested mites on suppression of R.solani damping off symptom

A marked drop (46.7) of the survival ratio of cotton seedlings could be seen in culture pots infected with pathogen only in non-sterilized soil, this corresponds to 60% in sterilized soil (Table 4).

All of the cotton seeds germinated in the untreated control pots has no symptoms of the disease. Some cotton seeds that were not germinated due to some distortions.In contrast, pre and post- emergence of cotton seeds planted in soils treated with *X. lophotrichus* was 13.3 % in non-sterilized soil. In contrast,under sterilized soil tested two mites shown a variable pre-emergence and survival values (table 4).

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Table 4 :Effect of tested mites on suppression of cotton seedlings damping off percentage caused by *R.solani*.

	Non- Sterilized soil		
	Pre-emergence damping off (%)	Post-emergence damping off (%)	Survival plants (%)
plant+pathogen+mite1	13.33	13.33	73.33
plant+pathogen+mite2	11.33	15.33	73.33
plant+pathogen	20	26.67	53.33
	Sterilized soil		
	Pre-emergence damping off (%)	Post-emergence damping off (%)	Survival plants (%)
plant+pathogen+mite1	6.67	20	73.33
plant+pathogen+mite2	13.33	20	66.67
plant+pathogen	20	40	40

Mite1, *X. lophotrichus*; mite2, *X. capucinus*

Values are meansfor five replicate pots of three cotton seeds for each treatment.

3.4 Effect of tested mites on suppression of Rhizoctonia stem cankers symptom

Values of the disease severity index (Table 5) showed that cotton plants germinated in sterilized soil infested with *R. solani* fungal pathogen suffered from brown discoloration (stem cankers) on stems of the plant (DI% = 63.3). Culture pots containing non-sterilized soil were exhibited less stem cankers ratio and have disease severity index (DI% = 39.9).Contrariwise, untreated control culture pots which were not inoculated with any fungal pathogen have healthy cotton plants (DI% = 0). In sterile soil which was incorporated with *X. lophotrichus* as biocontrol agent against the *R. solani* exhibited the role of this mite species on suppression of stem cankers symptoms on cotton plants (DI% = 12.2). This value corresponds to 6.6% in non-sterilized soil culture pots.On the other hand, the second species *X. capucinus* showed also a real potential in suppression of *R. solani* disease on stems of cotton plants where the reducing stem cankers symptoms were DI% = 16.6 & 8.8 for sterilized and non-sterilized soil, respectively.Statistically on each mite species, there is a significant variation in the reduction of *Rhizoctonia* stem cankers due to soil sterilization and type of treatment" (t-test $P \leq 0.05$). However, no significant difference ($P > 0.05$) between the two mite species in sterilized and non-sterilized soil could be seen.

Table 5: Effect of applications of oribatid mites as bio-control agents on incidence of Rhizoctonia stem cankers symptom on cotton seedlings

	Disease index (0-100)	
Treatment	Sterilized soil	Non-sterilized soil
Untreated control	0	0
R. solani+X. lophotrichus	12.22 b	6.66 b
R. solani+X. capucinus	16.66 b	8.88 b
Pathogen control (*R. solani*)	63.33 a	39.99 a

Disease index for cotton stem cankers symptoms on a scale of 0-3, values are means for five replicate pots;

0=healthy plant

1= cankers covering < %25of the stem length; low disease symptoms

2= cankers covering %25-%50 of the stem length; intermediate disease symptoms

3= cankers covering >%50 of the stem length; highly disease symptoms

Values in the same column followed by different letters are significantly different at $P \leq 0.05$ (LSD test)

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4. DISCUSSION

Our laboratory data revealed that, there was a remarked difference in the palatability of tested mites when feeding on fungal diet of *Rhizoctonia solani*. Oribatid species *Xylobates lophotrichus* showed the highest feeding preferences towards *R. solani*; *Xylobates capucinus* come the next towards diet preference. Mite feeding activity could describe in the deposited fecal pellets in the rearing cell. Nakamura *et al*. (1991), who tested the plant pathogenic fungus *Rhizoctonia solani* as food for four oribatid mites; one species, *Protoribates agricola* grazed preferentially and reproduced on *R. solani.* The mesostigmatid species *Leiodinychus karmeri:* Uropodidae was of intermediate preference towards *R. solani* fungal diet. Hagrass *et al.* (2011) denoted that, the feeding habits of most species of the family Uropodidae are unknown.They also recorded that, *R. solani* accelerated the development period of tested uropodid species when feeding on it rather than other tested fungi. In the present experiment the oribatid mites *Rhyzotritia a.ardua,Ciliopia difficilis, Javacarus kühneltii, Epilohmannia p. aegyptica andNothrus biciliatus* rejected *R.solani* as fungal food item during the rearing experiment. With regard to the rejection of tested *Nothrus species* to *R. solani* food, Hartenstein (1962) had reported that, *Rhizoctonia solani* and *Fusarium oxysporum* discs were repelled by *Nothrus biciliatus* and did not attract it at all although a highly grazing on dematiaceous fungus *Cladosporium cladosporioides*.Siepel (1990) recorded that, *Nothrus silvestris* is a true panphytophage mite. Likewise, Anderson (1971) stated that*, R. ardua* adults are inhabiting rotten wood and twigs, and those woody tissues mainly of dark brown colour, were preferred to those yellowish colour oribatids.

The highly grazing of *X. lophotrichus* and *X. capucinus* mites on the pathogenic fungus *R. solani*, and the rejection of other tested mites to that fungus may be related to direct and /or indirect effects. Maraun *et al*. (2003) indicated that, preferentially of animals to such fungal food item may back to a direct effect ; profit of the animal from high food quality and nutritious components of hyphae (Martin, 1979 and Maraun *et al.,* 1998), or indirectly from a fungus-mediated increase in the quality of food substrates (litter or leaves). Other effects related to preference or rejection of fungal food items includes:

i) Soil fungi may be toxic to some decomposer animals, the fungal production of mycotoxin or release of secondary metabolite contents (Maraun et al., 2003 and Scheu and Simmerling, 2004) may be the reason why some fungi are edible or rejected as food item.

ii) Beside the nutritional aspects of preferred fungi, differences in chemical (toxic components) and physical (morphological structure of the individual fungal species) can also be critical for the feeding preferences of micro-arthropods (Taylor and Alexander, 2005). iii) The digestive capabilities of the mite species (Bowman, 1984 and Hubert *et al*., 2001), iv) The present laboratory microcosm experiment suggested that, morphology and structure of the fungus may be an important factor for being grazed by some species of mites and rejected by others. In other words, the multinucleate hyphal cells of *R. solani* and its thick wall of hyphae that have 4–15 µm wide (Wiese,1987), may be such factors that require individual mouth parts suitable for sucking nuclei of cell contents or chewing the thick cell wall hyphae.

Latorre (2004) reported that, *R. solani* is one of the most important phytopathogens that attack tomatoes cultivated under greenhouse conditions, causing root rots.Many studies of Lootsma and Scholte (1997a, b) reported that, Rhizoctonia solani is preferred by many collembolans in food selection experiments. Enami and Nakamura (1996) found that; root rot of radish, caused by *R. solani* was controlled by adding the oribatid mite *Scheloribates azumaenisis* to pots with infested soil in a greenhouse experiment.

The present study revealed that, tested *X. lophotrichus* and *X. capucinus* mites have a clear role in reducing the inoculum density of *R. solani* and suppressed its influence on host plant, as deduced from disease severity index of root rot and damping off symptoms. These results come in agreement with similar previous studies carried in greenhouse cultures conducted by Curl *et al.* (1985); El Titi and Ulber (1991); Nakamura *et al*. (1992) they indicated that, asignificant suppression of *Rhizoctonia solani* and *Fusarium oxysporum* causing cucumber diseases were associated with the feeding activity of collembolans. Other related study results in that aspect was recorded by (Lewis and Papavizas, 1993; and Wicks *et al*., 1995).

From the present study, it could be observed that, seeds planted in soils provided with *X. lophotrichus* or *X. capucinus* were germinated earlier by 4 days than those planted in non-provided pots (post emergence damping off). Yedidia *et al.* (2001) have reported similar results where a 30% increase in cucumber seedling emergence was observed up to 8 days after sowing when soil was amended with *Trichoderma harzianum* as a bio-control agent for *R.solani*. Kleifeld and Chet (1992) who confirmed the ability of *Trichoderma harzianum* to inhibit the activity of minor pathogens in the soil rhizosphere that are responsible for seed rots and pre-emergence damping off of seedlings. Our study revealed a significant decrease of seedlings damping off caused by *R. solani* on cotton plants in both sterilized and non-sterilized soil when cotton seed treatment was combined with the mite *X. lophotrichus* and *X. capucinus*, respectively.

Cotton seedlings grown in soils supplemented with *X. lophotrichus* and *X. capucinus*, were recorded higher values of plant heights and fresh and dry weights than the infected control ones. This agrees with the experimental studies of Kleifeld and Chet (1992) who reported that, dry weights of cucumber plants grown in sandy loam soils treated with a conidial suspension (CFU / gm soil) of *Trichoderma harzianum* was increased by 43 % than in non treated soil.

The remarkable increased growth of cotton plants grown in pots supplemented with *X. lophotrichus* and *X. capucinus* may be related to the large increase in root area and root lengths after pathogen suppression. Yedidia *et al*. (2001), who recorded *Trichoderma harzianum* as biocontrol agent resulted in large increase in root area and root lengths, followed by a significant increase in dry weight of plants, shoot lengths and leaf area.

In the present study, we noticed more successful control of *R. solani* by tested mites under non-sterilized conditions than the sterilized ones, as indicated by the disease severity of cotton seedlings. Non-sterilized soil in the present study was observed to have the saprophytic fungus *Trichoderma viridi*. Benítez *et al.* (2004) had deduced that, fungi of the genus *Trichoderma* were proved to be important biocontrol agents of several soil borne phytopathogens. Furthermore, Montealegre *et al*. (2010), achieved the biocontrol of *Rhizoctonia solani* in tomato plants cultivated under greenhouse and field conditions using the *Trichoderma harzianum.* Clapperton *et al*. (2002) reported that, in soil, pathogenic fungi interact with numerous other organisms beside their host plants, some of these organisms have a positive influence on the pathogens, while others are antagonistic. Influence of *Trichoderma* on *R.solani* arise from; mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes (Howell, 2003 and Benítez *et al.* 2004). In addition to stimulatory effect of *Trichoderma* on plant growth (Naseby *et al*., 2000). These assumptions are consistent with previous studies of Curl *et al*. (1988), who revealed that, rhizosphere-inhabiting collembolans, *Proisotoma minuta* and *Onychiurus encarpatus*, were grazed preferentially upon the cotton-seedling pathogen *Rhizoctonia solani* in the presence of three well-known biological control fungi, Laetisaria arvalis , *Trichoderma harzianum* and *Gliocladium virens.* Furthermore, Lewis and Papavizas (1993) had proved that, the Marine fungus *Stilbella aciculosa* successfully reduced damping off diseases caused by *R. solani* on cotton, sugarbeet and radish.

It concluded that the remarked role of *X.lophotrichus* and *X.capucinus* mites when preferentially grazed on the pathogenic fungus *R. solani*, may reflect the role of these mite species as biological control agents against that pathogenic fungus in field situations.It is evident that, the effectiveness of tested oribatid mites as natural bio-control agents against *R. solani* diseases is related to size of the mite population presented to rearing pots in conjunction with the inoculum density of the pathogen supplemented to the soil, as well as the obligate feeding and long habituation of the biocontrol agent (tested mites) on pathogen inoculums in the laboratory, as the only available food material before incorporating into soil, it is necessary for successful biocontrol method.

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