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### Efficacy of *Cladosporium cladosporioides*, as a Biocontrol Agent for Controlling *Tetranychus urticae* Koch (Acari: Tetranychidae) under Laboratory Conditions

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#### ABSTRACT

The two spotted spider mite *Tetranychus urticae* (Acari, Tetranychidae) is a serious polyphagous plant pest. Laboratorial experiments were conducted to evaluate the efficacy of *Cladosporium cladosporioides* as microbial control agent against *T. urticae* adults and larvae. The current data showed that *C. cladosporioides* revealed very satisfactory efficacy when compared with *B. bassiana*. The results indicated that LC<sub>50</sub> of: 1.37x10<sup>4</sup> and 4.45x10<sup>3</sup> conidia/ml against adults and new hatched larvae, respectively.

**Keywords:** *Cladosporium cladosporioides*, biocontrol, *Tetranychus urticae*

#### INTRODUCTION

The two spotted spider mite *Tetranychus urticae* (Acari, Tetranychidae) is an important polyphagous pest worldwide (Walter & Proctor, 1999). *T. urticae* (Koch.) usually feeds on leaves leading to yellow, brown blotch and accompanied by dry and leaf fall. Severe mite infestation causes significant economic reduction in crop production quality and quantity (Abd El- Rahman *et al.* 2005). Chemical control has been confounded by many environmental and health problems. Moreover, development of resistance in spider mite populations against acaricides. So, searching for more safe alternatives became very necessary.

Entomopathogenic fungi were among the first organisms used for the biological control of pests. Many species of *Cladosporium* were used as a safe alternative of traditional chemical insecticides for controlling many plant-insect pests like aphids and whiteflies. The target of the current study is to investigate the efficacy of *C. cladosporioides* as biocontrol agent for controlling both of larvae and adults of *T. urticae* under laboratory conditions.

#### MATERIALS AND METHODS

##### The tested pest:

Laboratory strain of *T. urticae* was obtained from the Faculty of Agriculture, Mansoura University. The culture of the spider mite was maintained on castor leaves placed upside down in Petri-dishes (20 cm in diameter) filled with cotton-wool, saturated with water and kept under laboratory conditions of 25±2°C, and 75 ±5 RH and 14:10 hours L: D. Infested leaves and tap-water were changed as necessary.

##### Entomopathogenic Fungi:

##### *Cladosporium cladosporioides*:

The culture of *C. cladosporioides* was obtained from Dr. Heba Youssif El-Sayed Ibrahim as pure strain. This strain was previously isolated from *A. craccivora* and was identified by Assiut Univ. Mycological Center (AUMC), Egypt. (Ibrahim, 2012)

The fungal strain of *C. cladosporioides* was cultivated on autoclaved Sabouraud Dextrose yeast extract Agar (10 g/L peptone, 40 g/L dextrose, 10 g/L yeast extract and 20 g/L agar) and incubated at 25± 2°C and 80±5% RH until further growth. *C. cladosporioides* spores were harvested and counted using a haemocytometer Neeubauer. Different concentrations of spore suspensions were prepared.

**Biosect** (Organic Bio technology): a commercial product of *Beauveria bassiana* 32x10<sup>6</sup> conidia/ml to serve as a positive control.

##### Bioassay:

Petri-dishes (20 cm in diameter) were filled with cotton wool, saturated with water. Three discs (2.5 cm in diameter) of washed and sterilized castor leaves were placed upside down on cotton wool in each dish. A thin film of water was left around the edge of the leaf discs to prevent escaping of the spider mite and the cotton pad was moistened daily. Ten individuals (adult females or ten newly hatched larvae in case of treatment of larvae) were transferred on each disc. Then, they were sprayed with different concentrations of tested fungus, *C. cladosporioides*. Another three discs were sprayed with *B. bassiana* different concentrations to serve as positive control. Untreated three discs (negative control) for each experiment were sprayed only with water and 0.05 % aqueous Tween 80. The Petri-dishes were incubated at 25± 2°C, 75 ± 5 %RH. , and photoperiod 14: 10 hs (L: D).Data

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was recorded daily and the experiment continued for seven days.

**Statistical analysis:**

The average of mortality percentages of both larvae and adults of spider mite were determined and corrected using Abbott's formula (1925). The corrected mortality percentages were calculated according to Finney (1971). LC<sub>50</sub>, LC<sub>90</sub>, slope values and toxicity index were estimated by using Sun's equation (1950).

**RESULTS AND DISCUSSION**

Data illustrated in Table 1&2 showed that the cumulative mortality percentages of both *T. urticae* adults and larvae was increased with increasing concentrations and time intervals after treatment. This agreed with Yankovskaya (1999) who tested the pathogenicity of *Paecilomyces fumosoroseus* and *M. anisopliae* against the two-spotted spider mite, *T. urticae* and found that mortality percentage increased with increasing the time elapsed after treatment. *T. urticae* cadavers were turned to a brown or dark green color, then the color turned black. This agreed with Gámez-Guzmán et al. (2019).

Also, it is clear from the obtained data that the mite larvae were more susceptible to both entomopathogenic fungi. Results indicated that *C. cladosporioides* revealed more potentiality than *B. bassiana* on adults of *T. urticae* with LC<sub>50</sub>: 1.37×10<sup>4</sup> conidia/ml, LC<sub>90</sub> 60.79×10<sup>4</sup> conidia/ml and toxicity index at LC<sub>50</sub> of 100%, while, *B. bassiana* showed LC<sub>50</sub> of 1.76×10<sup>4</sup> conidia/ml, LC<sub>90</sub>: 10.59×10<sup>5</sup> conidia/ml and toxicity index: 77.61%.

Also, *C. cladosporioides* revealed less potential activity against *T. urticae* larvae than *B. bassiana* where it showed LC<sub>50</sub>: 1.37×10<sup>4</sup> conidia/ml, LC<sub>90</sub> 60.79×10<sup>4</sup> conidia/ml and toxicity index at LC<sub>50</sub> of 100%, while, *B. bassiana* showed LC<sub>50</sub> of 4.45×10<sup>3</sup> conidia/ml, LC<sub>90</sub>: 35.82×10<sup>4</sup> conidia/ml and toxicity index: 45.41%. It was noticeable that there were pronounced difference of *B. bassiana* virulence against different stages of the same host. It may be due to difference of the chemistry topography of the cuticle of adults and larvae (Sosa-Gomez et al., 1997).

**Table 1. Efficiency of the tested entomopathogenic fungi against adults of two spotted spider mite, *T. urticae* under laboratory conditions of 25 ± 2 C<sup>0</sup>, 75 ± 5% RH**

Entomo-pathogenic fungi	Conc. (conidia/ml)	<i>T. urticae</i> Adults								
		Cumulative mortality % at indicated day after treatment.				LC50 (ppm) and confidence limits at 95%	LC90 (ppm) and confidence limits at 95%	Slope ± SE	X <sup>2</sup>	Toxicity index*
		1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day					
<i>C. cladosporioides</i>	1x10 <sup>4</sup>	0	33.33	40.00	46.67	1.37x10 <sup>4</sup>	60.79x10 <sup>4</sup>	0.78 ± 0.07	100.00	
	1x10 <sup>5</sup>	0	43.33	66.67	73.33					
	1x10 <sup>6</sup>	0	50.00	83.33	93.33	2.05x10 <sup>3</sup> 3.41x10 <sup>4</sup> 20.53x10 <sup>3</sup> 88.93x10 <sup>5</sup>				
	1x10 <sup>7</sup>	0	60.00	96.67	100.00					
<i>B. bassiana</i>	1x10 <sup>4</sup>	0	26.67	36.67	43.33	1.76x10 <sup>4</sup>	10.59x10 <sup>5</sup>	0.72 ± 0.01	77.61	
	1x10 <sup>5</sup>	0	36.67	53.33	70.00					
	1x10 <sup>6</sup>	0	46.67	76.67	90.00	2.63x10 <sup>3</sup> 4.5x10 <sup>4</sup> 31.23x10 <sup>4</sup> 26.99x10 <sup>6</sup>				
	1x10 <sup>7</sup>	0	50.00	96.67	100.00					

**Table 2. Efficiency of the tested entomopathogenic fungi against larvae of two spotted spider mite, *T. urticae* under laboratory conditions of 25 ± 2 C<sup>0</sup>, 75 ± 5% RH.**

Entomo-pathogenic fungi	Conc. (conidia/ml)	<i>T. urticae</i> Larvae								
		Cumulative mortality % at indicated day after treatment.				LC50 (ppm) and confidence limits at 95%	LC90 (ppm) and confidence limits at 95%	Slope ± SE	X <sup>2</sup>	Toxicity index*
		1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day					
<i>C. cladosporioides</i>	1x10 <sup>3</sup>	0	13.33	26.67	36.67	4.45x10 <sup>3</sup>	35.82x10 <sup>4</sup>	0.67 ± 0.07	45.41	
	1x10 <sup>4</sup>	0	23.33	36.67	53.33					
	1x10 <sup>5</sup>	0	56.67	70.00	83.33	1.25x10 <sup>3</sup> 10.6x10 <sup>3</sup> 12.5x10 <sup>4</sup> 22.38x10 <sup>5</sup>				
	1x10 <sup>6</sup>	6.67	60.00	86.67	96.67					
<i>B. bassiana</i>	1x10 <sup>3</sup>	0	16.67	30.00	43.33	2.021x10 <sup>3</sup>	40.82x10 <sup>4</sup>	0.56 ± 0.28	100.00	
	1x10 <sup>4</sup>	0	36.67	53.33	66.67					
	1x10 <sup>5</sup>	3.33	50.00	66.67	80.00	2.67x10 <sup>2</sup> 6.18x10 <sup>3</sup> 119.98x10 <sup>3</sup> 43.75x10 <sup>5</sup>				
	1x10 <sup>6</sup>	10.00	60.00	80.00	96.67					

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### كفاءة فطر *Cladosporium cladosporioides* كعامل حيوي لمكافحة الحلم العنكبوتي ذو البقعتين *Tetranychus urticae* تحت الظروف المعملية

مرفت قاسم جبر الشربيني  
معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى- الجيزة- مصر

يعتبر الحلم العنكبوتي ذو البقعتين *Tetranychus urticae* آفة خطيرة متعددة العوائل النباتية. لذلك فقد تم تصميم تجربة معملية لتقييم كفاءة فطر *Cladosporium cladosporioides* كعامل من عوامل مكافحة الميكروبية لمكافحة كلا من الأطوار البالغة ويرقات الحلم العنكبوتي ذو البقعتين. فأظهرت النتائج أن فطر *C. cladosporioides* قد أظهر كفاءة مباشرة جدا مقارنة بفطر *B. bassiana* حيث سجل تركيزا نصف مميت:  $10^4 \times 1.37$  و  $10^3 \times 4.45$  كونيدة/مللي على كل من الأطوار البالغة واليرقات حديثة الفقس على التوالي.