

**FIRST ISOLATION OF THE ENTOMOPATHOGENIC FUNGI, *STACHYBOTRYS* SP. FROM NATURALLY INFECTED TORTOISE BEETLE, *Cassida vittata* VILL (COLEOPTERA: CHRYSOMELIDAE) IN SUGAR BEET FIELDS IN EGYPT**

**Bazazo, K.G.I.\*; Amira Sh. M. Ibrahim \*\* and R. A. S. El-Shafey\*\*\***

\* Plant Protection Res. Dept., Sugar Crops Res. Inst., Agric. Res. Center.

\*\* Economic Entomology Dept., Fac. Agric., Kafr El-Sheikh Univ.

\*\*\* Rice Diseases Res. Inst., Rice Res. and Training Cent., Agric. Res. Center.

**ABSTRACT**

The current study was carried out during 2010/2011 sugar beet season at the Experimental Farm of Sakha Agricultural Research Station for monitoring population fluctuations of *Cassida vittata* and isolation the entomopathogenic fungi, *Stachybotrys* sp. from this insect pest. Data indicated that the insect density gradually increased towards the end of the season to exhibit the highest density (175 individuals/20 plants) on 25 April-10 May. In a laboratory test, 17 adults of *C. vittata* out of 24 ones died because of the fungus, and the accumulated mortality, in a duration of 24 days, was calculated as 70.83%. This obviously show the virulence of *Stachybotrys* sp. in killing adults of *C. vittata*. In a field test, the suspension of *Stachybotrys* sp. ( $5 \times 10^4$  spores/ml water) was sprayed on sugar beet plants against *C. vittata*. Mortality values of the insect one, two and three weeks post-treatment were 12.00, 12.50- and 11.76%, respectively. This investigation shows that this isolated fungus may be promising as an entomopathogenic against this beetle. However, further studies concerning biosafety and the effect of fungus applications in sugar beet fields on natural enemies are required.

**INTRODUCTION**

Sugar beet, *Beta vulgaris* L. (Family: Chenopodiaceae) ranks second as a source of sugar in Egypt and allover the world, providing about 40% of world sugar production. Thus, Ministry of Agriculture and Land Reclamation in Egypt is planning to expand the cultivated sugar beet area in the coming decades to reduce imports and, if possible, to achieve self-sufficiency of sugar.

In 2009/2010 season, the total area cultivated with sugar beet reached 248,871 feddans from which 40% was cultivated at Kafr El-Sheikh Governorate (Anonymous, 2011),

Increase in sugar production needs horizontal and vertical expansion of sugar beet production. Horizontal development means larger cultivated areas of the crop, while vertical development means greater yield per unit area. One of the main constraints limiting vertical development is insect pests which cause considerable losses in sugar beet production (Abo-Saied Ahmed 1987; Mesbah, 2000; Shalaby, 2001 and El-Mahalawy, 2011). The tortoise beetle, *Cassida vittata* Vill. (Coleoptera: Chrysomelidae) proved to be one of

the most destructive insects in sugar beet plantations. The larvae and adults of this chrysomelid feed upon sugar beet leaves causing reduction in foliage and root size, which reflects lower sugar production. Gurguis (1985) estimated the attacked sugar beet plants as 11-39%. Abd El-Ghaffar (1993) and Abo El-Ftooh (2002) indicated that the infestation by this insect pest reduces yield quantity and quality, and may be destructive. The occurring losses in sugar beet yield due to this pest were pointed out by El-Khouly (1998) as considerable decrement in plant foliage, root weight and root-sucrose content. Bazazo (2010) reported that this beetle appears in a high density (887 adults/130 plants) during April. El-Mahalawy (2011) measured the consumed area of sugar beet leaf as 23.5 cm<sup>2</sup> per one larva and one adult.

Strategies of insect pest control in sugar beet depend on applying Integrated Pest Management (IPM) Programs to avoid, or minimize, the use of insecticides particularly sugar beet is a food crop.

The entomopathogenic agents integrate with other IPM elements to manage insect pests. El-Husseini (1981) reported that the entomopathogenic fungi were effective microbial control agents in crops with vegetation contributes to the presence of high relative humidity in the micro climate within plants as occurring in sugar beet fields. Wipps and Lumsden (2001) emphasized the importance of insect pathogenic organisms, particularly fungi. They indicated that about 100 genera and 750 species have been reported as entomopathogenic agents, many of which are fungi that produce toxins which act as poisons for the insects. El-Husseini *et al.* (2004) isolated the entomopathogenic fungi; *Metarhizium anisopliae*, *Paecilomyces lilacinus* and *Beauveria bassiana* from soil samples of different governorates. They recorded reductions of 7.4, 10.2, 9.5 and 5.8% in *C. vittata* adult populations one week after four successive applications in sugar beet fields. Bazazo (2010) isolated the entomopathogenic fungus, *Fusarium scirpi* Lambotte and Fautrey from the adults of *C. vittata*. In a laboratory test, 84% of *C. vittata* adults, enclosed in Petri dishes with fungus conidiospores were killed and compared to only 10% mortality in the check treatment. In a field evaluation, 24% of *C. vittata* adults sprayed with *F. scirpi* conidiospores were killed.

The current investigation was undertaken to isolate entomopathogenic agents associating with the tortoise beetle, *C. vittata* in sugar beet plantations. In addition, the possibility of using some surveyed agents in insect control in the field was studied.

## **MATERIALS AND METHODS**

The current investigation was carried out at the Experimental Farm of Sakha Agricultural Research Station during 2010/2011 season. The experimental sugar beet field (about ½ feddan) was sown with Raspoly cultivar on mid-October, and received all recommended cultural practices, but without any pesticides.

**1. Monitoring population fluctuation of *Cassida vittata*:**

The population fluctuations of *C. vittata* were monitored in the experimental field from 10 February to 10 May, 2011. Biweekly examinations were conducted, as 20 plants per examination. Visually, and by the aid of lens, eggs, larvae and adults of the insect were counted and recorded in the field on 20 plants. These fluctuations were monitored to find out the duration of high insect population, thus, the tortoise beetle could be observed to find out the occurrence of entomopathogenic agents. On the other hand, field tests using these entomopathogenic agents, could be tested against *C. vittata* adults. Thus, the mortality of the insect adult, due to the applied fungi could be considered as promising result to apply the fungi as biocontrol agent to manage the pest.

**2. Entomopathogenicity:**

**2.1. Isolation of entomopathogenic fungus:**

During studying the population fluctuations of *C. vittata*, it was observed that some adults are diseased and dead, with fungal hyphae on their exoskeleton. Accordingly, during March and April, 2011 when the insect population density was high, the D-Vac machine was used to collect insects, from sugar beet plots. Each time, the catch was emptied in a glass jar, and transferred to the laboratory, where it was examined, and *C. vittata* adults were picked up, and separately kept.

The dead ones were individually kept in Petri dishes (9 cm diameter) provided with moistened filter paper and incubated at 28°C under continuous fluorescent light for three days to stimulate the growth of pathogens on the cadaver cuticle. Ten diseased insects were kept individually in Petri dishes (9 cm diameter) having water agar medium, and incubated for additional three days under 28°C and continuous fluorescent light. A piece of agar with mycelial growth was obtained using a sterilized needle and inoculated individually into ten Petri dishes having Potato Dextrose Agar (PDA) medium. The dishes were incubated for a week under 28°C and continuous fluorescent light.

**2.2. Spore isolation and identification:**

The Petri dishes having sporulated fungi were washed with distilled water to exclude the fungus spores. The spores were kept, and identified to its fungal causal by Dr. Rabei El-Shafey, Plant Pathology Dept., Rice Research and Training Center, Agricultural Research Center, Egypt. The spores were used to formulate a spore suspension that was adjusted to  $5 \times 10^4$  spores/ml water, using the micrometer slide, to be used in field studies against *C. vittata*.

**2.3. Entomopathogenicity test:**

*Cassida vittata* adults were obtained from sugar beet plots using the D-vac machine. The alive adults were picked up from the catch, and kept individually into Petri dishes with a piece of sugar beet leaf for feeding for ten days. Twenty-four *C. vittata* adults, that proved to be healthy, were introduced into Petri dishes and fed upon sugar beet leaves treated with the spore suspension of *Stachybotrys* sp. Another set of 24 healthy adults were kept in Petri dishes, and fed upon sugar beet leaves untreated with the fungus. This test was conducted to find out the virulence of *Stachybotrys* sp. in infecting *C.*

*vittata* adults. The adult mortalities were recorded one and three days, and one, two, three and four weeks after feeding of the insect adults on sugar beet leaves, treated or untreated with the fungus suspension.

#### **2.4. Field test:**

Three sugar beet plots (20 m<sup>2</sup> each) were assigned to test the efficiency of *Stachybotrys* sp. spore suspension ( $5 \times 10^4$  spores/ml water) against the tortoise beetle. The suspension was sprayed using hand sprayer (2 L volume) onto sugar beet plants on 10 April, 2011, when the highest insect population density was detected. One week post-treatment, the D-vac machine was used to collect adults of *C. vittata*/10 sugar beet plants from the first plot. The sampling was conducted in the second plot two weeks post-treatment, and in third plot three weeks post-treatment. Each catch was examined, and the total number of collected *C. vittata* adults was recorded. The diseased dead adults were counted, and thus, the mortality due to the fungus infection was calculated.

## **RESULTS AND DISCUSSION**

### **1. Monitoring population fluctuations of *Cassida vittata*:**

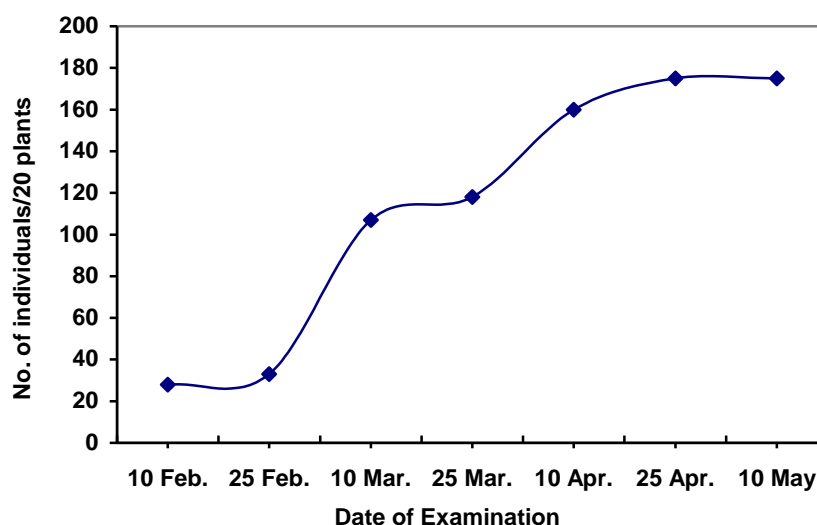
Data presented in Table (1) and in Figure (1) show the population fluctuations of the tortoise beetle, *C. vittata* in sugar beet fields, from February up to May, 2011. Per 20 sugar beet plants, 28 individuals (eggs + larvae + adults) were encountered on 10 February, increased to 33 individuals, two weeks later. An abrupt increase in insect population was recorded on 10 March, as 107 individuals/20 sugar beet plants. The insect density gradually increased towards the end of the season to exhibit the highest density (175 individuals/20 plants) on 25 April-10 May, 2011.

**Table (1): Population fluctuation of *C. vittata* attacking sugar beet fields at the Experimental Farm of Sakha Agricultural Research Station, during 2010/2011 season, using direct visual examination.**

Date of examination	No. of individuals/20 plants			Total	Mean of individuals /plant
	Eggs	Larvae	Adults		
10 Feb.	23	0	5	28	1.40
25 Feb.	20	6	7	33	1.65
10 Mar.	32	50	25	107	5.35
25 Mar.	30	38	50	118	5.90
10 Apr.	20	60	80	160	8.00
25 Apr.	26	74	75	175	8.75
10 May	35	40	100	175	8.75

In addition, Table (1) indicated that the mean of individuals (eggs + larvae + adults) per plant ranged between 1.40-8.75. Thus, it is obvious that the high population density of *C. vittata* occurred during the maturity stages of sugar beet plants. This reflects the danger of this insect pest, as the nutrients in sugar beet leaves, that should be assimilated into sugar in the last stages of sugar beet growth, are highly impaired by the heavy insect infestation. The

reductions in quantity and quality of sugar beet plants due to this insect has been previously shown by Abd El-Ghaffar (1993) and Abo El-Ftooh (2002). However, Bazazo (2010) estimated higher insect population density than that recorded herein, that could be attributed to variable environmental conditions.



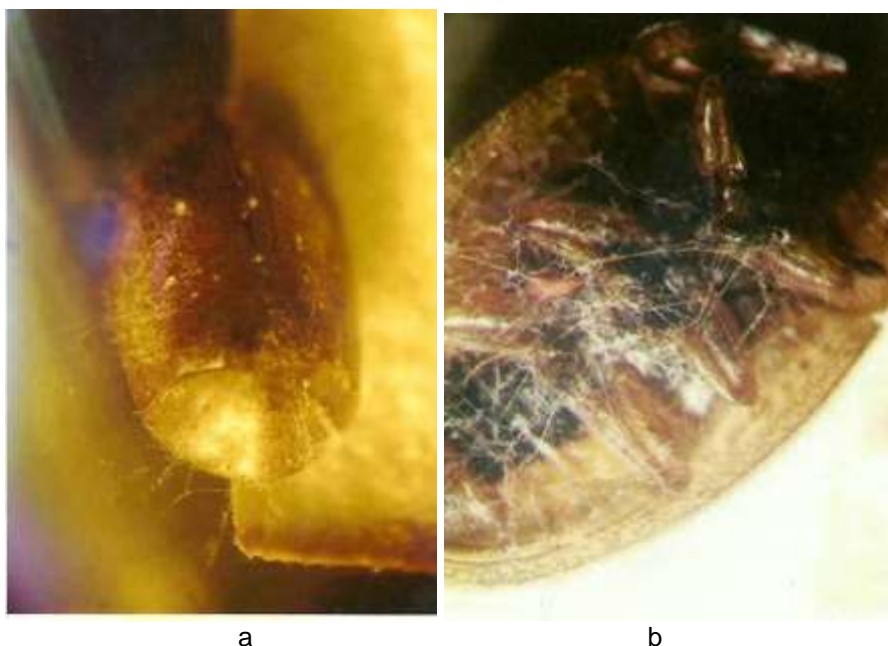
**Fig. (1): Population fluctuation of *C. vittata* attacking sugar beet fields at the Experimental Farm of Sakha Agricultural Research Station, during 2010/2011 season, using direct visual record.**

## **2. Entomopathogenicity**

### **2.1. Spore isolation and identification:**

Samples of dead adults of *C. vittata* were collected using D-vac machine. The hyphae and spores occurring on the exoskeleton of such adults were isolated. This fungus was identified as *Stachybotrys* sp.

Figure (2) illustrates the adults of *C. vittata* collected from sugar beet fields, with the fungal hyphae growing on the insect cuticle, dorsal (a) and ventral (b) views. As the author aware, this is the first record to this fungus as an entomopathogen to *C. vittata* in Egypt. However, *Stachybotrys* sp. was recorded as an entomopathogen to the wheat aphid, *Sitobion avenae* (Homoptera: Aphididae) (Humber and Hansen, 2005). In Iraq, Al-Swuidy (2003) identified *Stachybotrys* sp. as a pathogen against Ghobar mite, *Oligonychus afasiaticus* (Acari: Tetranychidae) which infests date fruits in Iraq.



**Figure (2): *Cassida vittata* adults collected from sugar beet fields, with fungal hyphae growing on the insect cuticle.**  
a)Dorsal view  
b)Ventral view

## 2.2. Entomopathogenicity test:

Mortality of *C. vittata* adults due to the fungus infection was evaluated in a laboratory test (Table 2). Twenty-four *C. vittata* adults were enclosed in Petri dishes and fed upon sugar beet leaves inoculated with the spore suspension ( $5 \times 10^4$ ) of *Stachybotrys* sp.

No mortality in *C. vittata* adults occurred up to the third day of exposing the tortoise beetle adults to the suspension spores of the fungus. Seven days after infection, 4.20% mortality was recorded, increased to 34.78% after 14 days from the treatment. The highest mortality (46.16%) was detected after 28 days from the beginning of the experiment. Since 17 adults, out of 24 ones died because of the fungus, the accumulated mortality, in a duration of 24 days, was calculated as 70.83%. This obviously shows the virulence of *Stachybotrys* sp. in killing the adults of *C. vittata*.

## 2.3. Field test

The three plots (20 m<sup>2</sup> each) sprayed with *Stachybotrys* sp. spore suspension to test the efficiency of the fungus in killing *C. vittata* adults in the sugar beet field were sampled. The mortalities of the tortoise beetle adults were almost the same one, two and three weeks after-treatments, with values of 12.00, 12.50 and 11.76% mortality, respectively. Thus, the mortality in the adults of this insect pest averaged 12.03% throughout the experimental period, that elapsed to three weeks. The lower mortality levels of *C. vittata* adults recorded in the field test compared to those recorded in the laboratory

test could be attributed to the behaviour of the insect adult, as the adults are usually located on the lower surfaces of sugar beet leaves. This phenomenon was previously indicated by El-Husseini *et al.* (2004) who recorded lower mortalities in *Nezara viridula* L., *Cassida vittata*, jassids and whitefly which all inhabit the lower leaf surfaces.

**Table (2):Mortality of *Cassida vittata* adults treated in the laboratory with *Stachybotrys* sp. suspension ( $5 \times 10^4$  spores/ml water)**

Duration after treatment (day)	<i>Stachybotrys</i> sp. treatment				Untreated			
	No. of <i>C. vittata</i> adults			Mortality (%)	No. of <i>C. vittata</i> adults			Mortality (%)
	Total	Alive	Dead		Total	Alive	Dead	
1	24	24	0	0	24	0	0	0.0
3	24	24	0	0	24	0	0	0.0
7	24	23	1	4.20	24	0	0	0.0
14	23	15	8	34.78	24	0	0	0.0
21	15	13	2	13.33	24	23	1	4.2
28	13	7	6	46.16	23	0	0	0.0
Accumulated mortality	24	7	17	70.83	24	23	1	4.2

However, biosafety of using any of entomopathogenic agents should be seriously investigated and considered. Application of *Beauveria bassiana* Vuill. has been reported to cause allergies to humans (York, 1958). Larvae of the beneficial coccinellid, *Cryptolaemus montrouzieri* Mulsant. suffered 50% mortality when fed Boverin-t, a commercial conidiospore preparation of *B. bassiana* (Flexner *et al.*, 1986). Both *B. bassiana* and *Metarhizium anisoplae* (Metsch.) infected *Bombyx mori* L. and also killed honey bees following field applications (Podgwaite, 1986). These examples turn on a red light to be cautious about the field application of entomopathogens. This does not mean necessarily to stop using these entomopathogens, but emphasizes to consider the ecosystem as a whole, when using these agents in integrated pest management programs.

**Table (3):Mortality of *Cassida vittata* adults tested in the field with *Stachybotrys* sp. suspension ( $5 \times 10^4$  spores/ml water)**

Duration after treatment (week)	No. of adults/10 plants		Mortality (%)
	Total	Dead	
1	25	3	12.00
2	32	4	12.50
3	51	6	11.76
Total	108	13	12.03

## ACKNOWLEDGEMENT

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**عزل الفطر الممرض *Stachybotrys* sp. من الأفراد الكاملة للخنفساء السلحفائية *Cassida vittata* Vill في حقول بنجر السكر لأول مرة في مصر**  
**كمال جابر إبراهيم بظاظو\*، أميرة شوقي محمد إبراهيم\*\* و ربيع عبد الفتاح سعد الشافعي\*\*\***

\* قسم بحوث وقاية النبات ، معهد بحوث المحاصيل السكرية ، مركز البحوث الزراعية  
 \*\* قسم الحشرات الاقتصادية ، كلية الزراعة ، جامعة كفرالشيخ  
 \*\*\* قسم بحوث أمراض الأرز ، مركز بحوث الأرز ، مركز البحوث الزراعية

أجريت هذه الدراسة في موسم 2010/2011 على حشرة الخنفساء السلحفائية *Cassida vittata* في المزرعة البحثية لمحطة بحوث سखा الزراعية ، وكذا معمل قسم بحوث المحاصيل السكرية بالمحطة. تم مراقبة تقلبات تعداد الخنفساء ، ومتابعة ظهور أى علامات مرضية على الحشرات الكاملة طوال الفترة من فبراير وحتى مايو 2011. تم تسجيل أعلى تعداد للحشرة خلال الفترة من 25 إبريل وحتى 10 مايو. كما لوحظ وجود أفراد ميتة من الحشرات الكاملة ، ووجود نمو فطري على جليد (كيوتيكل) الحشرة. تم عزل الفطر في معمل أمراض النبات بمركز بحوث الأرز بمحطة البحوث الزراعية بسخا ، وتعرفه على أنه الفطر الحشري الممرض *Stachybotrys* sp. وهذا يعتبر أول تسجيل للفطر كمرض للخنفساء في حقول البنجر في مصر. بحساب نسبة الموت التي يحدثها هذا الفطر للأفراد الكاملة لحشرة *C. vittata* في تجربة معملية استمرت لمدة 24 يوما ، وجد أن النسبة التراكمية للموت كانت 70.83%. وفي تجربة حقلية تم رش المعلق الجرثومي للفطر بتركيز  $5 \times 10^4$  جرثومي/مل من الماء وجد أن نسبة موت الحشرات الكاملة لخنفساء *C. vittata* تراوحت بين 11.76 ، 12.50%. وهذه النتائج تعتبر مبشرة من حيث قدرة الفطر على إحداث العدوى وقتل الأفراد الكاملة لهذه الآفة الضارة بمحصول بنجر السكر.

**قام بتحكيم البحث**

**كلية الزراعة – جامعة المنصورة**  
**مركز البحوث الزراعية**

**أ.د / عادل حسن عبد السلام**  
**أ.د / محمود رمزي شريف**