### Using *Beauveria bassiana* (Bals.) Vuillemin in Spraying and Dusting Applications for Biological Control of Sugar Beet Insect Pests in Egypt

El-Husseini<sup>\*</sup>, M. M.; E. A. Agamy<sup>\*</sup>; A. H. Mesbah<sup>\*\*</sup>; Ola O. El-Fandary<sup>\*\*\*</sup> and M. F. Abdallah<sup>\*</sup>

<sup>\*</sup>Center of Biological Control, Faculty of Agriculture, Cairo University, Giza <sup>\*\*</sup> Agricultural Research Center, Sakha Experiment Station, Kafr El-Sheikh <sup>\*\*\*</sup>Pests and Plant Protection Department, National Research Centre, Dokki, Giza, Egypt (*Received: November 16 and Accepted: December3, 2008*)

#### ABSTRACT

Conidiospores of the entomopathogenic fungus *Beauveria bassiana* were produced in two formulations for spraying and dusting applications in sugar beet fields at Kafr El-Sheikh Governorate, Egypt in 2007/2008 growing season. Different insect pests showed different reduction rates in relation to the application technique. Pests feed by chewing all leaf tissues showed high reduction rates whatever the applied technique, *i.e.*, the noctuid larvae of *Spodoptera littoralis*, *Trichoplusia ni*, *Autographa gamma* and *Syngrapha circumflexa*,. The lepidopteran larvae mining under upper leaf surface leaving an opening or more were highly affected by spraying than dusting due to flow of the conidia with water into their tunnels, *i.e.*, *Scrobipalpa ocellatella* and *Ostrinia nubilalis*. Meanwhile, insects presented most of the time at the lower surface of the sugar beet leaves were poorly affected with spraying but highly reduced in case of dusting, *i.e.*, *Nezara viridula* and *Cassida vittata*, because of the dispersal of conidia reaching all plant parts. In case of the beet fly, *Pegonrya huscami*, it was not affected by spraying and poorly affected by dusting where the latter could infect few adult flies leading to fewer laid eggs on the leaves. Moreover, the larvae mine the leaves in closed tunnels and thus are protected from both sprayed and dusted conidiospores of *B.bassiana*.

Key Words: Beauveria bassiana, Spraying, Dusting, Sugar beet insect pests, Egypt.

#### INTRODUCTION

The sugar beet, Beta vulgaris L., was introduced to Egypt more than two decades ago where now the planted area is expanding and new fabrics for production of the beet sugar are constructed in the new cultivated areas (El-Husseini et al., 2004 and Marei, 2004b). As a new introduced crop in the country, it was attacked by some existing pests of other crops, i.e., the cotton leaf worm, Spodoptera littoralis (Boisd.) and the beet worm S.exigua L. (El-Husseini et al. 2003 and 2004 and El-Khouly, 2006); the European corn borer Ostrinia nubilalis (Hbn.) (Agamy, 2002); the semi-loopers Autographa spp. (El-Husseini et al., 2004); the green stink bug Nezara viridula (L.) (El-Zoghby, 2003); the mole cricket Gryllotalpa gryllotalpa L (Abou Bakr, 2002) and the leaf miner fly Pygomia hyoscami Panz. (Marei, 2004a and El-Khouly, 2006). Furthermore, other insect species that were of no economic importance belonging to the Egyptian insect fauna turned into destructive pests to the sugar beet specially in the newly reclaimed desert land, i.e., the beet rib moth Scrobipalpa ocellatella (Boyd.) (Guirgis, 1985); the tortoise beetle Cassida vittata Vill. (El-Husseini et al.2003; Marei, 2004b and El-Khouly, 2006), and the Temnorhinus brevirostris (Bassouny et al., 1993).

Traditionally, chemical pesticides are in use for controlling all insect pests attacking this crop in Egypt and abroad (Badr *et al.*, 1993 and Ayala and Dominguez, 1996). Concerning the new emerged pests in sugar beet, *e.g.*, *C.vittata*, *S.ocellatalla* and *T.brevirostris*, El-Husseini *et al.* (2003) believed that the intensive use of chemical insecticides in sugar beet fields as introduced crop in Egypt had suppressed the population of natural enemies that were previously keeping them under natural balance in the Egyptian fauna, *e.g.*, the trichogrammatid egg parasitoid, *Monorthochaete nigra* Blood & Kryger reported by El-Agamy *et al.*(1994) and Awadallah (1996) on eggs of *C. vittata*.

In order to minimize the quantities of chemical pesticides used for crop protection within the frame of the strategies of Integrated Pest Management biological control, especially microbial (IPM), control of insect pests, became an important component in such strategies as an effective alternative. Particularly, the entomopathogenic fungi have long been known to cause epizootics among insects under certain conditions. The more common fungi used in biological control of different insect pests in the field are, Beauveria bassiana (Bals.) Vuillemin (Feng et al., 1985; El-Safty & Borai, 1987 and El-Zoghby, 2003) and Metarhizium anisopliae (Metsch.) Sorokin (Siddaramaiah, 1986 and Sosa-Gomez & Moscardi, 1998).

The present study is a trial for evaluating the efficacy of *Beauveria bassiana* against sugar beet

insect pests using two different application techniques, *i.e.*, spraying and dusting under field conditions in season 2007/2008 at Kafr El-Sheikh governorate in Egypt.

#### MATERIALS AND METHODES

#### **Entomopathogenic Fungi**

The tested *B.bassiana* was previously isolated at the Center of Biological Control and IPM, Faculty of Agriculture, Cairo University by El-Husseini et al. (2003) using the trapping technique from soil samples with larvae of the greater wax moth Galleria mellonella L. described by Zimmermann (1986). The fungus was propagated on potato dextrose agar medium (PDA) enriched with 1% peptone, 4% glucose and 0.2% yeast at 26°C. Production of large amounts of conidiospores for field application was done according to Rombach et al. (1988) in 1L conical flasks; followed by production of B.bassiana on PDA mixed with 100g crushed soy bean/L (El-Husseini et al., 2004). The conidiospores were harvested by washing in distilled water with the addition of 0.03% Tween-80 and adjusted at a concentration of 1x10<sup>6</sup> spores/ml for field application using the high volume technique (600L-motor).

For dusting, *B.bassiana* was produced on autoclaved half-cocked rice grains in 2L- glass containers at room temperature. Completing sporulation, the whole medium was grinded in an electrical blender and spore count/g for the initial stock was carried out through successive dilutions in sterilized distilled water followed by culturing on PDAM in Petri-dishes of  $\phi 10$  cm. Soybean meal was added to the initial stock as filling material to reach the concentration of 1 X 10<sup>6</sup> spores /g used for dusting by a back-motor with 15 kg tank.

#### **Field Application and Pests Monitoring**

An area of 3 feddans cultivated with sugar beet located at Abou El-Enein village, Desouk, Kafr El-Sheikh governorate, was selected for the experiment. Two feddans were treated by *B. bassiana;* one by spraying and the other by dusting technique; the  $3^{rd}$  feddan was left as an untreated control. Different stages of different insect pests attacking leaves of sugar beet were monitored by the beginning of November 2006. The  $1^{st}$  application took place on the  $7^{th}$  of November 2007 and the  $2^{nd}$  on  $25^{th}$  of March 2008 followed by three successive applications at one week interval. Just before each application took place, random samples each of 20 plants from the treated as well as the control fields were examined *on sito* for the presence of insects which were counted and recorded. Monitoring was carried out regularly one week after application.

The Formula of Hendreson and Tilton (1955) was used to calculate the reduction rate among populations of the sugar beet insect pests reflecting the efficacy of *B.bassiana* as a bio-control agent.

#### **RESULTS AND DISCUSSION**

# 1. The Cotton Leafworm, Spodoptera littoralis (Boisd.):

Data presented on Table (1) show that the 1<sup>st</sup> application of *B.bassiana* reduced the population of *S.littoralis* (young larvae  $L_1$ - $L_3$ ) by 23% after 20 days from spraying compared to 33% in case of dusting among the so called autumn generation, when the plant leaves were still small in size. During the late winter and early summer generations, four successive applications by one week intervals were carried out (Table 1).

The second *B.bassiana* application (25/3/2008) caused reductions of 54 and 63% among larvae of *S.littoralis* (L<sub>4</sub>-L<sub>5</sub>) one week after application for spraying and dusting, respectively, that remained nearly stable after the third application (1/4/2008) recording respective control rates of 50 and 60%. After the fourth application (13/4/2008), the larval population decreased by 75 and 87% for the two treatments, respectively (Table1). The 5<sup>th</sup> application was not necessary as larvae turned to pupae at that time, but it was necessary for controlling other

Table (1): Controlling the cotton leafworm, *S.littoralis* with *B.bassiana* in sugar beet, Sakha, Kafr El-Sheikh governorate, season 2007/2008 with spraying (S) and dusting (D) techniques.

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Sampling	Num	bers of	larvae	Control%		Dates of	Numb	Numbers of larvae			Control %		
Dates	S	D	С	S	D	Field visits	S	D	С	S	D		
7/11/2007*	18	17	19	-	-	25/3/2008*	9	8	12	25	33		
2/12/2007	10	9	13	23	30	1/4/2008*	5	4	11	54	63		
17/1/2008	3	3	7	57	57	13/4/2008*	5	4	10	50	60		
26/1/2008	2	1	6	66	83	20/4/2008*	2	1	8	75	87		
19/2/2008	3	1	4	25	75	27/4/2008	0	0	2	-			
2/3/2008	5	4	7	28	43	11/5/2008	0	0	0	-			
-						1	*						

C = control,

= application dates

peaked insect pests like the sugar beet moth, the green pentatomid bug, and the tortoise beetle. Successful laboratory tests for controlling S.littoralis, Spodoptera spp. and by entomopathognic other noctuid species fungi were recorded (Fargues and Rodrigues-Rueda, 1980; Maniana and Fargues, 1984 and Aponte and Uribe, 2001). The present field those results agree with reported by & Machlowicz Napiorkowska (1986). Siddaramaniah et al. (1986) and El-Husseini et al. (2003).

#### 2. The Semi-looper Pests:

They belong to more than one species, *i.e.*, Trichoplusia ni, Syngrapha circumflexa. and Autogapha gamma; which are not differentiable from each other during the larval stage. They were considered one group of larvae as "semi-loopers". As shown in Table (2), the 1<sup>st</sup> treatment (7/11/2007) with B.bassiana resulted in reductionl rates of 42 and 57% for spraying and dusting, respectively on 2/12/2007. One week after the second spraying, the respective rates of reduction were 25 and 75%. The larval populations remained suppressed by the two treatments by 40 and 60%, respectively. No references concerning use of B.bassiana against the semi-loopers could be obtained, but Napiorkowska and Machlowicz (1986) recorded a natural infection with B.bassiana in larvae of A.gamma reaching 5.7% in fields of cabbage and sugar beet in Poland.

# 3. The European corn borer, Ostrinia nubilalis (Hbn.):

In the last few years, infestation with the European corn borer was noticed in sugar beet fields. It is a major pest on corn and few other crops; and is of a potential economic damage for sugar beet in the future. The feeding larvae damage the base of core leaves, and the wounds spill large amounts of sap allowing growth of the black fungus (sooty mold). The feeding location of larvae on the upper surface of the leaf petiole facilitates its contact with the sprayed/or dusted conidiospores of the entomopathogenic fungi.

Both application techniques caused complete control (100%) among larval population of O.nubilalis in sugar beet fields (Table3); these results confirmed those previously recorded by Bartlett (1943) and Agamy (2002) for the laboratory showing bioassay experiments the high pest the tested susceptibility of this to entomopathogenic fungus. Early records of natural infection with B.bassiana in larvae of O.nubilalis were reported by Lefebvre (1931) and with *M.anisopliae* by Wallengren (1930). The high susceptibility of this pest to entomopathogenic fungi led to successful trials for colonization of corn, *Zea mays* by *B.bassiana* (Wagner and Lewis, 2000) in USA.

### 4. Sugar Beet Moth, Scrobipalpa ocellatella (Boyd.):

Table (4) show high efficacy of both treatments (spraying and dusting) against the larvae of S.ocellatella; being 100% after 20 days post the 1<sup>st</sup> treatment (1x10<sup>11</sup> spores/feddan) as well as one week after the  $2^{nd}$ ,  $3^{rd}$ ,  $4^{th}$  and  $5^{th}$  treatments with spraying technique. On the other hand, dusting technique resulted in less control of the larvae being 84-89% reduction in larvae after the other 4 applications. The reason for better control of S.ocellatella by spraying than dusting technique may be related to two factors. The first is the high susceptibility of the larvae to the applied fungus; and the second is the location of feeding of larvae on the upper surface of the leaf petiole of plant heart, that facilitates contacting the larvae with fungal spores of the sprayed material in their still superficial opened tunnels with water carrying the conidiospores of the entomopathogenic fungus, while the spores remain on plant surface but not in the heart leaves when dusted.

## 4. The Green Pentatomid Bug, Nezara viridula :

Although N.viridula showed high susceptibility to B.bassiana and M.anisopliae in laboratory tests when directly dipped in conidiospore suspensions (Leite et al., 1988 and El-Zoghby, 2003), the present results of field application presented in Table (5) show no such high efficacy when the fungal spores were applied with spraying technique. Such a result might be firstly due to the low mobility of nymphs and adults; and secondly, the feeding of both stages (nymphs and adults) on the lower surface of leaves most of the time. Thus, the insects do not receive or be contacted with enough conidiospores from the sprayed suspensions. On the other hand, dusting technique could carry the dusted material loaded with the conidiospores to all parts of the plant vegetation including the lower surfaces of the sugar beet leaves, and thus contaminating the insects found on such location. The present results confirmed this hypothesis. Table (5) shows a reduction rate of 22% in numbers of the green pentatomid bug 20 days after spraying (6/11/2007) with B.bassiana opposed to 55% in case of dusting technique. The pest population increased in March, and the 2<sup>nd</sup> application on 25/3/2008 resulted in a reduction rate of 32 and 70% one week post

Sampling	Nun	Numbers of larvae			ol%	Sampling	Nun	nbers of	f larvae	Control%	
Dates	S	D	C	S	D	Dates	S	D	С	S	D
7/11/2007*	5	4	6	16	33	25/3/2008*	3	1	3	0	66
2/12/2007	4	3	7	42	57	1/4/2008*	3	1	4	25	75
17/1/2008	0	0	2	-	-	13/4/2008*	3	1	4	25	75
26/1/2008	2	1	2	0	50	20/4/2008*	3	2	5	40	60
19/2/2008	4	3	7	42	57	27/4/2008	3	2	5	40	60
2/3/2008	4	3	6	33	50	11/5/2008	0	0	0	-	-
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Table (2): Controlling the semi-loopers larvae with *B.bassiana* in sugar beet, Sakha, Kafr El-Sheikh governorate, season 2007/2008 using spraying (S) and dusting (D) techniques.

C = control,

= application dates

Table (3): Controlling the European corn borer, *O. nubilalis* with *B.bassiana* in sugar beet, Sakha, Kafr El-Sheikh governorate, season 2007/2008 using spraying (S) and dusting (D) techniques.

Sampling	Numb	pers of	larvae	Control%		Sampling	Numbers of larvae			Control%		
Dates	S	D	C	S	D	Dates	S	D	C	S	D	
7/11/2007*	4	4	4	0	0	25/3/2008*	3	2	3	0	33	
2/12/2007	0	0	1	100	100	1/4/2008*	0	0	3	100	100	
17/1/2008	0	0	0	0	0	13/4/2008*	0	0	3	100	100	
26/1/2008	0	0	0	0	0	20/4/2008*	0	0	3	100	100	
19/2/2008	0	0	0	0	0	27/4/2008	0	0	4	100	100	
2/3/2008	0	0	0	0	0	11/5/2008	2	0	4	50	100	
			*		. 1.							

C = control,

Table (4): Controlling the sugar beet moth, *Scrobipalpa ocellatella* (Boyd.)with fungi in sugar beet, Sakha, Kafr El-Sheich governorate, season 2007/2008 with spraying (S) and dusting (D) technique.

Sampling	Numl	pers of	larvae	Control %		Sampling	Num	pers of	larvae	Control %		
Dates	S	D	С	S	D	Dates	S	D	C	S	D	
7/11/2007	0	0	0	0	0	25/3/2008	3	8	20	85	60	
2/12/2007	0	0	6	100	100	1/4/2008	0	2	19	100	89	
17/1/2008	8	10	11	27	9	13/4/2008	0	3	25	100	88	
26/1/2008	5	6	6	16	0	20/4/2008	0	3	19	100	84	
19/2/2008	3	4	4	75	75	27/4/2008	0	2	14	100	85	
2/3/2008	8	10	21	62	52	11/5/2008	2	4	28	92	85	

C = control, \* = application dates

Table (5). Results of controlling the green pentatomid bug, *Nezara viridula* with *B.bassiana* in sugar beet, Sakha, Kafr El-Sheikh governorate, season 2007/2008 usin g spraying (S) and dusting (D) techniques.

Sampling	Numbers o	Numbers of Nymphs and Adults		Control %		Sampling	Numbers of Nym		ohs and Adults	Cont	rol %
Dates	S	D	С	S	D	Dates	S	D	C	S	D
7/11/2007*	16	16	16	-	-	25/3/2008*	24	9	24	0	62
2/12/2007	14	5	18	22	50	1/4/2008*	23	8	34	32	70
17/1/2008	15	5	14	7	64	13/4/2008*	20	6	40	50	85
26/1/2008	3	1	3	0	33	20/4/2008*	18	5	44	59	89
19/2/2008	6	2 .	6	0	33	27/4/2008	10	3	44	77	93
2/3/2008	5	2	5	0	40	11/5/2008	14	3	45	69	93
C = control,			* = applicat	ion da	ates						

<sup>=</sup> application dates

Sampling	Num	bers of e	ggs	Contro	ol %	Num	bers of la	arvae	Control %		
Dates	S	D	С	С	D	S	D	С	S	D	
7/11/2007*	33	33	34	-	-	28	26	27	-	-	
2/12/2007	6	5	6	-	17	8	6	9	11	33	
17/1/2008	5	5	6	17	17	7	5	7	0	28	
26/1/2008	11	10	12	8	16	15	10	16	6	37	
19/2/2008	7	7	8	12	12	20	12	20	0	60	
2/3/2008	8	8	8	0	0	5	3	6	16	50	
25/3/2008*	15	15	19	21	21	26	16	26	0	38	
1/4/2008*	35	30	37	5	18	27	15	28	3	46	
13/4/2008*	23	23	25	8	8	24	14	25	4	44	
20/4/2008*	7	7	8	12	12	9	4	9	0	55	
27/4/2008	4	4	5	20	20	0	0	1	-	-	
11/5/2008	-	-	-		-	3	3	4	25	25	
C = contro	* = applic	ation dates.									

Table (6): Controlling the beet with fungi in sugar beet fly, *Pygomya hyoscyami* at Kafr El-Sheikh governorate, season 2007/2008 using spraying (S) and dusting (D) techniques.

Table (7). Results of controlling the tortoise beetle, *Cassida vittata* with fungi in sugar beet, Sakha, Kafr El-Sheich govrnorate, season 2007/2008 using spraying (S) and dusting (D) techniques.

<u> </u>			Eggs				Larv	ae & P	upae		Adults				
Sampling Dates	S	D	С	R	<u>%</u>	S	D	C ·	R		· S	D	C ·		<u>%</u>
7/11/0007*		-			D				<u></u>					<u>_</u> <u>S</u>	<u>D</u>
7/11/2007*	0	0	0	-	-		0	0	_ 0	0	0	0	0	0	0
2/12/2007	3	1	4	25	75	0	0	0		0	2	0	3	33	_100_
17/1/2008	4	2	_ 5	20	60	1	0	1	0	0	3	0	4	25	100
26/1/2008	5	2	5	0	60	1	0	1	0	0	7	2	8	12	75
19/2/2008	8	3	8	0	75	8	2	9	11	77	12	4	13	7	69
2/3/2008	5	2	6	16	66	5	1	5	0	20	20	5	21	5	76
25/3/2008*	72	20	72	0	72	131	22	131	0	98	22	5	22	0	77
1/4/2008*	80	22	87	8	75	330	34	348	10	90	55	15	61	10	75
13/4/2008*	35	11	38	0	71	145	21	152	4	86	25	6	27	7	77
20/4/2008*	41	12	50	18	76	177	33	191	7	83	61	11	68	10	84
27/4/2008	10	3	13	23	77	100	14	125	20	88	95	13	105	14	87
11/5/2008	2	0	2	0	100	7	1	9	22	88	240	26	255	6	90
C = cor	C = control. $R = reduction,$				+	lication									

treatment for both treatments, respectively among populations of the pest (nymphs and adults). The  $3^{rd}$  application on 1/4/2008 caused a reduction rate of 50% (by spraying) and 85% (by dusting) (Table 4). The last application (20/4/2008) recorded 77 and 69 opposed to 93% one and two weeks post application for spraying and dusting techniques, respectively.

Thus, in the field, entomopathogenic fungi showed a moderate control level against nymphs and adults of *N.viridula* ranging between 7 and 77% by spraying technique and between 33 - 93% by dusting technique. The present results of spraying conidia of *B.bassiana* in sugar beet for controlling *N.viridula* agree with those of El-Zoghby (2003).

#### 5. The Leafminer Fly, *Pygomya hyoscyami* Panz.: As shown in Table (6), the applied fungal spores

did not affect the incidence of this pest in sugar beet. The presence of larvae inside the leaf tissues protects them from getting in contact with the sprayed or dusted conidiospores of B.bassiana. Results revealed slight effect on eggs of the fly after each of the five sprayings. Meanwhile, a slight reduction (11%) was recorded among the larvae 20 days post the 1<sup>st</sup> treatment; followed by 3, 4, zero one week after the  $2^{nd}$ ,  $3^{rd}$ ,  $4^{th}$ , and  $5^{th}$  spray applications, respectively. In case of dusting, these rates were 17, 46, 44 and 55% (Table 6). Thus the use of entomopathogenic fungi is yet not effective in controlling eggs and larvae of the sugar beet leafminer, P. hyoscyami; and the slight reductions in case of dusting technique may be due to infection of the adult flies that die and accordingly fewer eggs were laid on the sugar beet plants. As far, there is no available information about treating P.hyoscami with B. bassiana,

#### 6. The Tortoise Beetle, Cassida vittata:

As shown on Table (7), B.bassiana sprayed in the field resulted in an unsatisfactory control of the beetle's eggs, because they were laid on the underside of the leaves, and accordingly they did not came in contact with the conidiospores. The reduction in eggs found on the treated sugar beet plants one week after the 2<sup>nd</sup> spraying (on 25/3/2008) with B. bassiana was only 25%, while it was 75% in case of dusting. One week after the 2<sup>nd</sup> to the 5<sup>th</sup> applications, the reduction rates in eggs of the beetle ranged between 8 and 23% and 75-77% for spraying and dusting treatments, respectively; unsatisfactory control of the beetle's eggs with B. bassiana sprayed in the field as showed by results presented in Table (6). Death of eggs was associated with developed mycosis.

Concerning the control of *C. vittata* larvae and pupae, the reduction rates recorded one week post the 2<sup>nd</sup> treatment with spraying of *B. bassiana* was 10% and 90% for spraying and dusting treatments, respectively (Table 6); followed by 0, 4, 7 and 20% one week post each of the next sprayings opposed to 90, 86, 83 and 88% for dusting treatment. Less efficacy of spraying technique is related to the presence of the pest insects on the underside of the leaves, so that the sprayed conidiospores did not reach them at this location as in the aforementioned case of the eggs.

Adults of *C. vittata* are more mobile than the larvae, but they prefer feeding on the underside of the leaves. Thus, it could be expected that the treatment with fungi using the spraying technique, may explain the obtained low reduction rate among population of the adult beetles. Reduction rates of 33, 17, 9, 10 and 14% were recorded one week after the 5 successive sprayings. Meanwhile, rates recorded on the same dates in case of dusting technique were 100, 75, 77, 84 and 87%, respectively.

Although the laboratory bioassay of tested fungi proved high efficacy to larvae, pupae and adults of *C. vittata* (El-Husseini *et al.*, 2003), the application in the field by spraying technique was not successful because it does not cover the lower surfaces of sugar beet leaves, whereas it was successful by dusting technique through the air current carrying spores to all parts of the plant.

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