

Determinations of the entomopathogenic fungus *Beauveria brongniartii* on three sugar beet pests

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Abstract: The entomopathogenic fungus *Beauveria brongniartii*, were evaluated against three sugar pests, *Phyllotreta cruciferae*, *Pegomyia hyoscami*, *Cassida vittata*, under laboratory and field conditions. Under laboratory conditions, LC50 recorded 100×10^4 , 120×10^4 and 131×10^4 spores/ml after *P. cruciferae*, *P. hyoscami* and *C. vittata* treated with different concentrations of *B. brongniartii*. The entomopathogenic fungus *B. brongniartii* against sugar beet insect pests, showed that the number of eggs laid /female were significantly decreased to 22 ± 1.7 , 33 ± 8.9 , and 35 ± 2.6 individuals for *P. cruciferae*, *P. hyoscami*, and respectively as compared to 266 ± 8.7 individuals in the control. The egg hatching for the corresponding pests were, 4,7,9 as compared to 99 % in the control that the weight of sugar beet were significantly increased to 2345 ± 54.66 , 2337 ± 66.11 and in the plots treated with the fungi for *P. cruciferae*, *P. hyoscami*, and *C. vittata* respectively as compared to 1788 ± 55.43 ton/feddan in the control during season 2012. The corresponding data obtained during 2013 were 2567 ± 67.91 , 2443 ± 54.98 , and 2436 ± 22.87 as compared to 1227 ± 45.09 ton/feddan in the control the percentage of the sugar beet pests significantly decreased during both two successive season 2012 and 2013 after fungi treatments.

Keywords: *Beauveria brongniartii*, *Phyllotreta cruciferae*, *Pegomyia hyoscami*, *Cassida vittata*

1. Introduction

Sugar beet is considered one of the most important crops in Egypt because it was used in the production of sugar as it contains 15-20% sugar. The sugar beet has currently been infested with many insect pests which cause high losses in the crop yield and decrease its sugar content [1], [2]. Among these insects, *Cassida vittata* (Vill) (Coleoptera: Chrysomelidae); *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) and *Pegomyia hyoscami* (Witt) (Diptera: Anthomyiidae). Chemical insecticides were used to control these insect pests, but they were always causing a lot of pollution to the environment. Thereafter microbial control agents were advocated to be used against such pests [3]; [4]; [5] and [6]. [7,8,9] control three sugar beets pests by *B. bassiana*. The entomopathogenic fungi have long been known to cause epizootics among certain insect pests under laboratory and field conditions [1]; [10]; [7]. [10], reported that the fungi is very effective in controlling many insect pests. Also, the use of many plant derivatives gave promising results for controlling many insect pests [11]; [12].

This study aims to evaluate the effect of the entomopathogenic fungus *Beauveria brongniartii*, on *C.*

vittata; *P. hyoscami* and *P. cruciferae* pests under laboratory conditions and to explore how far they can protect the crop in the field and increasing its yield.

2. Materials and methods

2.1. Laboratory studies.

The sugar beet insects *C. vittata*; *P. hyoscami* and *P. cruciferae* were reared under laboratory conditions (26 ± 2 C° and 60 ± 5 %RH) in cages 50X 50X 60 cm per each. The third larval stage was used in the experimental work .

2.2. Cultivation of the fungi

The fungus *Beauveria brongniartii*, was kindly obtained from Prof. Dr Alain Vey, Mycology unite, National De La Recherche Scientifique, Univ. Montpellier. (Apopka strain 97 and reproduced in Microbiology Dept., N. R. C. Cairo, Egypt. The fungi were primarily purified using the mono-spore technique. They were propagated in Petri-dishes (10cm) on potato dextrose agar medium (PDAM) enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 C°. Seven-days old cultures with well developed spores were harvested by washing with 10 cc sterilized water then added

3ml, Tween-80 and completed to 100 ml water and used as stock suspension with known spore concentration then kept in a refrigerator at 4 C°, from which the fungi were sub-cultured to be used in laboratory evaluation tests (infectivity and bioassay tests) adjusted as conidiophores concentration of 1×10^8 /ml. Large amount of conidia spores, if needed, were produced by culturing the fungus on liquid medium in 1L cell culture glass bottles according to [13].

2.3. Evaluation of the fungi effects on the target insect pests

The fungi, *Beauveria brongniartii*, at concentrations ranged from 1×10^2 to 1×10^8 spores/ml were tested against *C. vittata*; *P. hyoscami* and *P. cruciferae* third instar larvae. Under laboratory conditions ($26 \pm 2^\circ$ C and 65 ± 5 %RH.). Fresh leaves of sugar beet were sprayed with the desired diluted suspension to the point of run off, left to dry, then put in 1 L plastic container(5 containers were used/concentration/ treatment). Twenty newly larvae of each species were placed in each container and covered with muslin. Untreated leaves were sprayed by water only and used as control. The leaves were changed every other day. The experiment was repeated 4 times. The percentages of mortality were calculated after seven days and corrected according to [14] while LC_{50} s were calculated through probit analysis of [15].

2.4. Field trials

The field trials were carried out in the growing sugar beet during the two successive growing seasons 2012 and 2013. Sugar beet was cultivated at Eben-Malek farm at El – Nobaryia farm, N. R. C. The sugar beet was planted in November 15th in an area of about one feddan. The area was divided into plots (each about 40 m²). Four plots were assigned for each treatment and for control as well, two rows of plants were left untreated between plots. Application of the fungi occurred at the rate of 1×10^8 spores/ml. sprayed at the sunset. Four applications were made at 4- weeks intervals during crop growing season. Control plots were left without any treatments. Examinations of 40 plants/plot/treatment were carried out just before the first application and seven days after last application to calculate the average reduction percentages in the target insect infestation percentages which was calculated in each treatment according to [16]. The agricultural practices followed the recommendations of the Ministry of Agricultural.

Twenty tubers were taken from the first 5 rows in each treatment and in the control as well. Sugar (sucrose) content per beet was determined in the Biochemistry Department in the National Research center.

3. Results

Data in table 1, show that the LC_{50} recorded 100×10^4 . 120×10^4 and 131×10^4 spores/ml after *P. cruciferae* , *P. hyoscami* and *C. vittata* treated with different concentrations of *B. brongniartii*.

Table 2 show that the entomopathogenic fungus *B. brongniartii* against sugar beet insect pests, which showed that

the number of eggs laid /female were significantly decreased to 22 ± 1.7 , 33 ± 8.9 , and 35 ± 2.6 individuals for *P. cruciferae*, *P. hyoscami* , and respectively as compared to 266 ± 8.7 individuals in the control (Table 2). The egg hatching for the corresponding pests were, 4,7,9 as compared to 99 % in the control.

Table 3 show that the weight of sugar beet were significantly increased to 2345 ± 54.66 , 2337 ± 66.11 and in the plots treated with the fungi for *P. cruciferae*, *P. hyoscami* , and *C. vittata* respectively as compared to 1788 ± 55.43 ton/feddaan in the control during season 2012. The corresponding data obtained during 2013 were 2567 ± 67.91 , 2443 ± 54.98 , and 2436 ± 22.87 as compared to 1227 ± 45.09 ton/feddan in the control (Table3).

Figure 1 and 2 show the percentage of the sugar beet pests which significantly decreased during both two successive season 2012 and 2013 after fungi treatments.

3.1. The Economic returns from the impact of using mushrooms *Beauveria brongniartii* on the target Pests such as *P. cruciferae* , *P. hyoscami* , *C. vittata* and its influences on the sugar beet production.

Table (2) show That the using of mushrooms *Beauveria brongniartii* on the target Pests such as *P. cruciferae* , *P. hyoscami* , *C. vittata* has many effects, these included the decrease in the number of eggs per Female by about 91% 0.88% 0.87% , The percentage of the cage in the eggs also fell by about 95 % 92 % 90% in compared with the control that represented about 266 egg , and about 99% for each of the three types respectively . The using of fungus has effects on the rate of deaths and damage the all larval, Where the death rate reached a maximum of 71% in the *P. hyoscami* kind with an increase of 10% and 16% in compared with the *P. cruciferae*, *C. vittata* respectively . While its effected on the larval damage was almost constant in the three types . Also the impact on fungus had an effects on the pupae damage with a maximum rate of 79% in pest *C. vittata* , and a minimum rate of 74% in pest *P. hyoscami* .

While its effects on the exit pests was amounted minimum of 3% of *P. cruciferae* with estimated a decline of 2% and 4% for each of the *P. hyoscami*, *C. vittata*, respectively, Finally its impact was almost fixed to the rate of damaged adults in the three types as illustrated in Table (2) . As shown in Table no. (3) the productivity average per feddan of beet crop during the 2012- 2013 seasons after treatment the fungus target pests *B. brongniartii* amounted of 61.29 tons / feddan, 60.55 tons / feddan, 44.79 tons / feddan for each of the *P. cruciferae*, *P. hyoscami*, *C. vittata* with increased in production quantity of 39.92 tons / feddan, 39.18 tons / feddan, 23.42 tons / feddan in compared with the productivity average of the Republic which reached about 21.37 Tons/ feddan. These lead to increased in productivity the Republic of the beet crop that estimated at 17.1 million tons, or about 16.74 million tons, or about 10 million tons which could contribute to the production of about 1.6 million tons of sugar, or about 1.5 million tons of sugar, or about 0.936 million tons of sugar for each of the three lesions after treatment with the fungus *B. brongniartii* respectively. The increase in production consequent on the shifted Egypt from imported to exported state with about 0.300 million tons, or about 0.200 million tons specially from

the first and second treatment .While the consequences of the third treatment reduced the size of Egyptian imports of sugar, that amounted about 1.278 million tons in 2012 to about 0.342 million tons . This contributes lead to the decreased of the Egyptian imports of sugar bill by about \$ 216 million .It can also lead to save about 2.5 billion cubic meters of irrigation water that resulting from the saving about 328 thousand feddan which are planted with sugar crop, or about 1.25 billion cubic meters of water for irrigation in agriculture that contributes with about 164 thousand feddan which are planted with sugar crop , these can using in the cultivation of crops with less of water.

4. Discussion

The same findings obtained by [7] found that the treatments with the fungi increased the yields and decrease the yield loss. [6] found that the yield loss of the potatoes were significantly decreased in the plots which treated with *B. bassiana* and *B. anisopliae*. Similar results were obtained by, [5and 7], [8] and [9]; [11] and [12]. The same results obtained by [4] who find that the potato tuber moth affected by the different formulations of the *Bacillus thuringiensis* and the fungus *Metarhizium bassiana* causes a higher mortality to the target pests. The same findings recoded by [17, 18] who control *Earias insulana* by the microbial control agents .[18 and 19] could to produce the microbial control agents on the coffee

and Dairy media. Sabbour, [20&21] could to enhance the microbial pathogen by added different additive to the microbial control agents. Sabbour 2001 study the biochemical of the microbial control agents bacteria and fungi against *E. insulana* , [11] control potato tuber moth by the combinations between the microbial control agents and the plant extract. [12] and [6] studied the effect of terpenes and microbial control agents against cotton bollworms can find that the cotton bollworms decreases after treatments in both laboratory and field conditions, [20],[21], [22], [23], [24],[26],[27]. [28] used the microbial control agents with plant extracts. The results obtained [23]. [7 &8], [28], [29], [28] also studding the nanotechnology and microbial control agents against stored products under laboratory and store conditions. [28],[29],[30],[31] found that the chemical additives enhance the microbial control agent against pests under field conditions ,also [31], [33]. [34], [35], [36] used UV to enhance the bacteria *B. thuringiensis* against the potato tuber moth. [8], [9], [33]. (34 and 25) find the same obtains. (37 , 38, 22 and [39]. [40] and [33], [41], [7], [42], [43], [44],[4]). [46] found that the entomopathogenic fungi and bacteria control the three sugar beet insect pests

5. Conclusion

The fungus *B. brongniartii* could decreased the infestation with three serious sugar beets to *P. cruciferae* , *P. hyoscamii* and *P. hyoscamii* under laboratory and field conditions

Table 1: Evaluation of the fungi *Beauveria brongniartii*, on the sugar beet pests under laboratory conditions

Target pest	LC ₅₀	S	V	95% Confidence limits
<i>P. cruciferae</i>	100X10 ⁴	0.1	1.4	88-133
<i>P. hyoscamii</i>	120 X10 ⁴	1.1	1.1	97-145
<i>C. vittata</i>	131X10 ⁴	1.2	1.1	99-155

Table 2: Effect of the entomopathogenic fungus *Beauveria brongniartii* against the target insects biology

Target pest	No of eggs laid/female	% of egg hatching	% of larval mortality	% of malformed larvae	% of malformed pupae	% of emerged adults	% of malformed adults
<i>P. cruciferae</i>	22±1.7	4	61	66	77	3	78
<i>P. hyoscamii</i>	33±8.9	7	71	67	74	5	77
<i>C. vittata</i>	35±2.6	9	55	69	79	7	77
Control	266±8.7	99	-	-	-	100	-
F value	33.4	2	5	5	22	21	21
Lsd5%	11.1	2	3	3	11	11	9

Table 3: Assessments of damage caused after treatment with the entomopathogenic *Beauveria brongniartii*

Target pest	Season 212	Season 213
	Wt of suger beet (Ton/ feddan)	Wt of suger beet (Ton/ feddan)
<i>P. cruciferae</i>	2345± 54.66	2567±67.91
<i>P. hyoscamii</i>	2337±66.11	2443±54.98
<i>C. vittata</i>	2266± 66.71	2436±22.87
Control	1788±55.43	1227±45.09

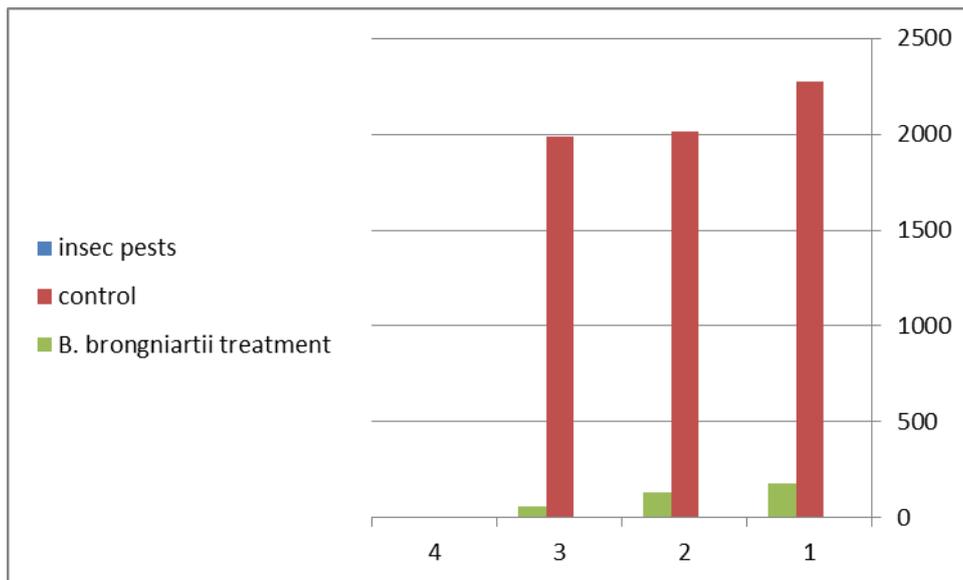


Figure 1: infestation percent during 2012 after fungi treatments

1. *P. hyoscamii* 2. *C. vittata* 3. *P. cruciferae*

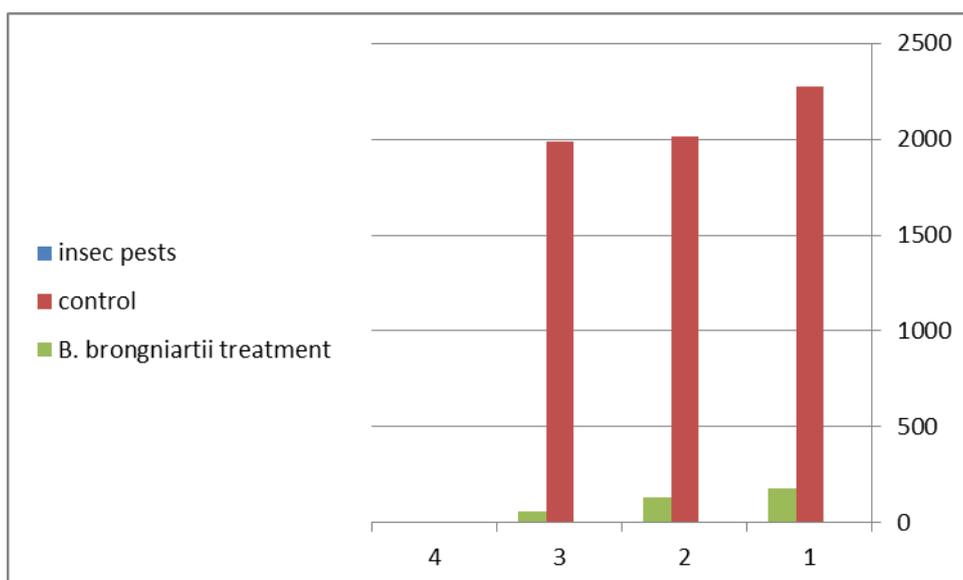


Figure 2: infestation percent during 2013 after fungi treatments

1. *P. hyoscamii* 2. *C. vittata* 3. *P. cruciferae*

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