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Efficacy of Some Microbial Control Agents Against Cabbage Pests in Egypt

¹M.M Sabbour and ²A.F. Sahab

¹Department of Pests and Plant Protections,

²Department of Plant Pathology, National Research Center, Cairo, Dokki, Egypt

Abstract: The role of some microbial control agents were tested against the insect pests, which infect the cabbage plants in the laboratory, in the green house and in the field. The results showed that the diamondback moth, the cabbageworm and beet armyworm were very susceptible to the microbial control agents used (*Bacillus thuringiensis*, *Beauveria bassiana* and *Metarhizium anisopilae*). The LC₅₀ recorded were 121, 90 and 61 µg mL⁻¹ after treated the *Piers rapae*, *Plutella xylostella* and *Spodoptera exigua* with different concentrations of *B. thuringiensis*, under laboratory conditions, respectively. In addition, the LC₅₀ recorded were 122, 98 and 101 after treatments of the plant in the green house to last insects, respectively. The cabbage pests could be control by the fungi *B. bassiana* and *M. anisopilae* under laboratory conditions, in the green house and in the field. The percentage of infestation reached to 20, 15 and 21% of the *P. xylostella*, *P. rapae* and *S. exigua*, respectively after 90 days of treatment with *M. anisopilae* and 21, 20 and 21% after treatment with *B. bassiana* to the same last insects after 90 days.

Key words: *Beauveria bassiana*, *Metarhizium anisopilae*, *Bacillus thuringiensis*, *Piers rapae*, *Plutella xylostella*, *Spodoptera exigua*, microbial control

INTRODUCTION

Cabbage is one of the important vegetable crop in Egypt. Egypt cabbage is grown during winter. Cabbage has a short growing duration, Egypt cabbage is an efficient food producer from which farmers can easily gain cash income and it is a valuable source of calcium, crude fiber and vitamin C. It is subjected to infestations by different pests mainly whiteflies, aphids, the cabbageworm *Piers rapae*, the diamondback moth *Plutella xylostella*, fall armyworm *Spodoptera exigua* and the cotton leafworm, *S. littoralis*.

Bacillus thuringiensis, is a soil dwelling bacterium, produces an insecticidal protein crystals within the bacterial cells during sporulations. The crystals proteins known as endotoxin, δ- crystal, is the primary active ingredients of *B. thuringiensis*, formulations. Ingestion of δ- crystal by susceptible insects results in gut paralysis and feeding inhibitions of midgut epithelial cells and eventually, death^[1]. In the last decade, tense of commercial formulations of *B. thuringiensis* have been produced worldwide for controlling a wide range of lepidopterous, coleopterous and dipterous insect pests.

The ubiquitous fungi *Beauveria bassiana* (Balsamo) and *Metarhizium anisopilae* (Metschnikoff) Sorokin are common disease agents associated with dead and

morbunt insects in nature^[2]. These fungi have been scrutinized worldwide as microbial control agents of soil inhabiting insects in particular, Quintela and McCoy^[3], reported that at fungal concentrations of 10⁶ and 10⁷ conidia mL⁻¹ of *B. bassiana* and *M. anisopilae* were causing the larvae, developments its movements and mobility and imidaclopride of doses 100 ppm or greater, larval mortality reached to 90-100%.

To ally the fear of the hazardous effect of chemical residues to human and animal's health, several studies were conducted to determine the most effective control method without using insecticides. One of the discovered method is the use of the natural enemies of the insects such as bacteria, fungi and viruses^[3-8].

Damage caused by cabbageworm *p. rapae* by eating the plant foliage rapidly and can strip infested plants in a short time. Larvae bore into heads contaminating them with body parts and a greenish brown excrement. The diamondback *P. xylostella* moth feed outer leaves causes a damage to the plant. The fall armyworm *S. exigua* larvae begin feeding on its eggs shell immediately after hatching then will move to the plant tissue near the soil surface. Larvae grow rapidly and can frequency do considerable damage. They will move in groups to other field after devouring plants in the hatching area. Infestations of these insects can occur throughout the growing season

and the control of them should be applied as necessary based on field monitoring. When these pests found and not good controlled it causes a losses of the yield^[4,9,10].

This study addresses the effect of the bacteria *B. thuringiensis*, the fungi, *B. bassiana* and *M. anisopilae* on the cabbage insect pests, the fall armyworm *S. exigua*, the cabbageworm *P. rapae* and diamondback moth *P. xylostella*.

MATERIALS AND METHODS

Rearing colonies of target insects: Samples of the target insects *Piers rapae*, *Plutella xylostella* and *Spodoptera exigua*, infested the cabbage plant crop collected and reared (in NRC) under laboratory conditions 26±2°C and 60-70% RH. The emerged moths were collected sexed and kept a liter cylinder glass (one male and one female). The inner surfaces of the jars were lined with waxy paper for oviposition. The emerged moths were provided small cotton pieces soaked in 10% honey solution for feeding. Strips of waxy paper carrying egg masses, which transferred to a fresh cabbage leaf.

Laboratory trails

Effect of *B. thuringiensis* Dipel 2X var. kurstaki (23000 IU) on the diamondback moth *P. xylostella*, the cabbage worm *P. rapae* and the beet army worm *S. exigua*: A pieces of cabbage leaves were sprayed with 6 concentrations (500, 250, 125, 63, 32 and 16 µg mL⁻¹) of *B. thuringiensis* (*Bt*) and leave for drying under laboratory conditions. The treated leaves were kept in petri dishes (one/ dish) lined with moistened filter paper. Tenth larvae of the third larval instars were introduced to each dish to feed for 24 h. The larvae were then transferred to similar petri-dishes and fed on untreated cabbage leaves till death or pupations. The experiments were replicated four times. Control were made by feeding the larvae on untreated cabbage leaves. The percentages of mortality were counted and calculated according to Abbot^[11], while LC₅₀ were calculated through probit analysis^[12]. The experiments were carried under laboratory conditions 26±2°C and 60-70% RH.

Cultivations of the fungi *Beauveria bassiana* and *Metarhizium anisopilae* and recovery of spore: *Beauveria bassiana* (BR3) and *Metarhizium anisopilae* (RM3) were obtained. The spores of *B. bassiana* and *M. anisopilae* were collected from the surface of mycelium growth and spores suspensions with Tween-80 (2 drops), was prepared. The preparations diluted in water and adjusted at concentrations 8x10⁸ conidia mL⁻¹.

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Montpellier. The fungus were received on an agar plate as sporulated culture. Conidiospores from this plate pervel as inocula for all experiments. Conidiospores. Conidiospores of both fungi were harvested from fungal cultures that were produced on potato dextrose agar plus 0.4% yeast extract (PDAY) and incubated for 10-15 days at 25±1°C. Conidial inoculum was taken from pure fungal cultures, with no more than two serial passages from a host insect. Conidial viability was determines by counting germ tubes produced on PDAY after 18 h using light microscope at 400X. Conidial viability was 95-100%. The surface of the cultures was gently brushed in the presence of 20 mL of sterilized water in order to free the spores, the suspension was then filtered through muslin. The concentration of the spore suspension was adjusted using a haemacy tameter to 1x 10⁸ conidia mL⁻³.

In vitro effect of the fungi *B. bassiana* and *M. anisopilae* on the target insects: The fungi *B. bassiana* and *M. anisopilae* were taken in 6th concentrations 16.5, 8.25, 4.125, 2.02, 1.06 and 0.5 conidia mL⁻¹. A piece of cabbage leaves were dipping in the last prepared preparations and leave for drying under laboratory conditions then put in petri dishes (one/dish) for each concentrations (4 replicated/each), tenth third larval instars of each of the target insects were putted in each petri dishes. Control were made by feeding the larvae on untreated cabbage leaves. The percentages of mortality were counted and calculated according to, Abbot^[11], while LC₅₀ were calculated through probit analysis^[12].

The experiments were carried under laboratory conditions 26±2°C and 60-70% RH.

Semifield (green house) trials: Cabbages were planted in the green house in (NRC) 40 plots of the plant one found in each plot. Natural infestation takes place, the plant were sprayed by the bioinsecticides of *Bt*, *B. bassiana* and *M. anisopilae* at the concentrations of 300 µg mL⁻¹ of *Bt* and 8.25x10⁸ conidia mL⁻¹ for each of *B. bassiana* and *M. anisopilae*. Control samples leave without treatments. The cabbage examined each 2 days by transferring the cabbage leaves to the lab, the percentage of infestations were calculated until the end of the experiment. Control were made by feeding the larvae on untreated cabbage leaves.

Each treatment was replicated 4 times. The percentages of mortality were counted and calculated according to Abbot^[11], while LC₅₀ were calculated through probit analysis^[12].

Field trials: A trials was carried out in 2 cabbage populations (300 m² apart from each other) at NRC farm at El- Nobaria during the season 2003-2004, naturally

infested with the *P. xylostella*, *P. rapae* and *S. exigua*. Fifty cabbages, in each population were applied at the rate of 20 g 5 m L⁻¹ 10 m⁻² of the pathogens *B. thuringiensis*, *B. bassiana* and *M. anisopilae*.

Each treatment weekly sprayed figures and replicated 4 times. The infestations of *P. xylostella*, *P. rapae* and *S. exigua* after 20, 50 and 90 days of treatments were estimated. Four plots were treated by water as check.

RESULTS AND DISCUSSION

The data indicates that LC₅₀, 121, 90 and 61 µg mL⁻¹ after treated the *P. rapae*, *P. xylostella* and *S. exigua*, with *B. thuringiensis*, at different concentration under laboratory conditions, respectively (Table 1). At the same time the LC₅₀, were 122, 98 and 101 to the same insects after treated with *B. thuringiensis*, in the green house, respectively.

The same results obtained Fatma *et al.*^[9] who found that the third instar larvae of *P. rapae*, *P. xylostella* and *S. exigua*, showed high susceptible levels *Bacillus thuringiensis* Agerin, the mortality reached to 90%. The results also, agree with, Abdul-Hafez *et al.*^[13] who found that the formulations of dipel and delfin did not affect on eggs of *Pectinophora gossypiella* but had adverse effect on survival and developmental of the hatched larvae. Similarly, Zhang^[14] and Tang^[15] reported that *B.t* applied on eggs of *P. xylostella* did not affect egg hatching but caused substantial mortality for newly hatched larvae. Kares^[16] found that *B.t* formulations, Bactospeine, was virulent to the 2nd, 3rd and 4th larval instars of *P. rapae* in the laboratory and the 2nd instar was the most susceptible one. Other workers came to similar results with different formulations of *B.t.*^[10,16-18], however, *P. xylostella* was reported to develop resistance to *B.t.*^[19-23], found that *B.t* control the diamondback moth caused a high mortality at the different dosages of treatments.

The LC₅₀ of *P. rapae*, *P. xylostella* and *S. littoralis*, were 110, 98 and 66x10⁸ conidia mL⁻¹ after treated with *B. bassiana*. In addition, the LC₅₀ were 113, 87 and 71x10⁸ conidia mL⁻¹, for the same target insects after treatments of *M. anisopilae*, respectively (Table 2). A pathogenic fungus represents one of the main microbial control agents that were applied and are also still under investigations for their utilizations purposes. They are able to cause chronic diseases that kill insect larvae during a short time^[24]. The entomopathogenic *B. bassiana* represented one of these beneficial fungi that is known in the field and semifield of biological control^[24,25].

Hung and Bouicas^[26] injected *B. bassiana* spores in the fifth instar larvae of *S. exigua*; they found that death occurred within 2-3 days post injections. Abd El-Gawad^[27] studied the pathogenicity of *B. bassiana* under laboratory conditions on newly hatched *Sesamia cretica* larvae and the larval mortality increased after the treatments with the fungi. Present results agree with those of Abou-Bakar^[28], who recorded that, the values of LT₅₀ showed prolongation by decrease of tested concentrations of fungi *M. anisopilae* for controlling the cotton leafworm.

The results showed that the LC₅₀ obtained of *P. rapae*, *P. xylostella* and *S. exigua*, were, 130, 87 and 65x10⁸ conidia mL⁻¹ after treated the cabbage plant with the fungi *B. bassiana*. After treatments of the plants with the fungi *M. anisopilae* the LC₅₀ calculated were 121, 81 and 70x10⁸ conidia mL⁻¹ for *P. rapae*, *P. xylostella* and *S. exigua*, respectively, (Table 3). Other workers came to similar results with different concentrations of both the two fungi McCoy^[5] and McCoy *et al.*^[8] reported that the conidia of *B. bassiana* and mycelia of *M. anisopilae* have suppressed root weevil larval populations in grooves when applied at high inoculum rates. Also the found that the entomopathogenic fungi controlled citrus pests.

Table 1: Effect of *B. thuringiensis* against the target insects

Target insects	In the lab				In the green house			
	LC ₅₀	Variance	Slop	confidence limits	LC ₅₀	Variance	Slop	confidence limits
<i>P. rapae</i>	121	0.001	1.02	133-143	122	0.002	2.5	10-143
<i>P. xylostella</i>	90	0.001	1.03	78-111	98	0.001	1.03	88-122
<i>S. exigua</i>	61	0.001	1.03	55-87	101	0.001	1.10	78-132

Table 2: Effect of fungi against the target insects under the laboratory conditions

Target insects	<i>B. bassiana</i>				<i>M. anisopilae</i>			
	LC ₅₀ (spores mL ⁻¹) x10 ⁸	Variance	Slope	95% confidence limits x10 ⁸	LC ₅₀ (spores mL ⁻¹) x10 ⁸	Variance	Slope	95% confidence limits x10 ⁸
<i>P. rapae</i>	110	0.001	1.12	127-88	113	0.001	1.50	132-100
<i>P. xylostella</i>	98	0.002	1.03	112-76	87	0.002	1.27	112-75
<i>S. exigua</i>	66	0.004	1.33	85-55	71	0.002	1.22	81-55

Table 3: Effect of fungi against target insects in the green house (semi field)

Target insects	Pathogens							
	<i>B. bassiana</i>				<i>M. anisopilae</i>			
	LC ₅₀ (spores mL ⁻¹) x10 ⁸	Variance	Slope	95% confidence limits x10 ⁸	LC ₅₀ (spores mL ⁻¹) x10 ⁸	Variance	Slope	95% confidence limits x10 ⁸
<i>P. rapae</i>	130	0.001	1.02	146-98	121	0.001	1.40	132-90
<i>P. xylostella</i>	87	0.002	1.02	110-66	81	0.002	1.17	100-65
<i>S. exigua</i>	65	0.002	1.30	85-57	70	0.002	1.20	91-50

Hsiao and Javedan^[29] mentioned that conidial suspension of *B. bassiana*, was applied to the surface of kale and cabbage leaves and after exposure to sunlight three days, caused a mortality especially the diamondback moth. In the same time Sabbour and Abd El-Aziz^[30] found that the fungi *B. bassiana* and *M. anisopilae* reduced the LC₅₀ of *Agrotis ipsilon* and *Spodoptera littoralis* during the seedlings and vegetative stages of cotton plants under laboratory and semifield (green house) conditions.

Zhang and Groden^[31] studied the pathogenicity of two strains of the fungus *B. bassiana*, they could to control some *leptinotarsa decemlineata*.

The field trials showed that the treatments with the fungi *B. bassiana* decreased the percentage of infestations by *P. xylostella* to 31, 28 and 21% after 20, 50 and 90 days of treatments, respectively as compared to 52, 53 and 60% in the untreated ones (Table 4). The percentage of infestations by *P. rapae* decreased to 24, 27 and 20% after treated by the fungus *B. bassiana*, after 20, 50 and 90 days, respectively, as compared with 55, 54 and 59% in the control. The beet armyworm *S. exigua*, decreased to 27, 23 and 21% after treated with *B. bassiana* as compared to 58, 55 and 56% in the control, respectively (Table 4).

When the target insects treated with the fungi, *M. anisopilae*, the percentages of infestations of *P. xylostella* were, 26, 21 and 20 after 20, 50 and 90 days, respectively. In addition, the infestations with *P. rapae* decreased to 23, 20 and 15% after 20, 50 and 90 days, respectively. When the beet armyworm *S. exigua*, treated with *M. anisopilae*, the percentage of infestations decreased to 22, 21 and 22% (Table 4). The percentage of infestation decreased to 35, 30 and 33% after treatment to *P. xylostella* with *B. thuringiensis* as compared to 52, 53 and 60% in the control. The percentage of the infestations with *P. rapae* decreased to 32, 44 and 30% as compared to 55, 54 and 59% in the control. In addition, the percentage of infestations with *S. exigua*, decreased to 45, 48 and 39%. Hem *et al.*^[32] control *Helicoverpa armigera* in the field by the fungus of *Beauveria bassiana*.

The same results obtained by Ismail and Sabbour^[33] who found that *Earias insulana Pectinophora gossypiella* and *Heliothis armigera*, can controlled in the field and laboratory by *Bt* and *B. bassiana*^[34], can controlled the *Sesamia cretica*, *Chilo agamemnon* and *Ostrinia*

Table 4: Effect of the different treatments against the target insects in the field

Post Ist application date	Treatments	% of infestations (mean)		
		<i>S. exigua</i>	<i>P. rapae</i>	<i>P. xylostella</i>
20	Control	52	55	58
50		53	54	55
90		60	59	56
20	<i>B. bassiana</i>	31	24	27
50		28	27	23
90		21	20	21
20	<i>M. anisopilae</i>	26	23	22
50		21	20	21
90		20	15	21
20	<i>B. thuringiensis</i>	35	32	45
50		30	44	48
90		33	30	39

nubalis by *Bt.*, *B. bassiana*, *M. anisopilae* and *Verticillium lecanii* in the lab and the field. Tanda and Kaya^[35] reported that over 200 species of insects in nine orders, mainly lepidoptera and coleoptera, have since been recorded as hosts. These insects were susceptible to the bioinsecticides. Gloriana *et al.*^[36], Jayanthi^[37] and El-Khawaas^[38] suggested that the control of the *S. littoralis* by the fungi gives a good results in both field and laboratory. El-Khawaas^[38] recorded that the LC₅₀ of *S. littoralis* was 30.65X10⁵ conidia cm⁻³ at the same time he suggested that the treatments with the fungi caused an elongation to the larval and pupal duration and the mortality reached to 92.59%. El-Sufty^[39] and Hung and Boucias^[40] testing the fungi against the leafworm and beet armyworm, the results showed that the fungus *B. bassiana* causes a higher mortality to bo insect pests. Negasi *et al.*^[41] found that the entomopathogenic fungi could to control the silverleaf whittly *Bemisia argentifolii* successfully and also recorded that the fungi elongate the larval durations.

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