Potential of Microbial Control of Diamondback Moth, *Plutella Xylostella* (Linnaeus), (Lepidoptera: Plutellidae) on Two Cabbage Cultivars under Different Fertilization Treatments

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Abstract: The susceptibility of the Egyptian field populations of the diamondback moth, *Plutella xylostella* (L.), on two cabbage cultivars (the green cultivar (Globe Master), *Brassica oleracea* var. *capitata* and the red cultivar (Ruby Perfection), *Brassica oleracea* var. *capitata*) to *Bacillus thuringiensis* subsp. *aizawai* and *Beauveria bassiana* has been evaluated. Both pathogens significantly reduced *P. xylostella* population densities, increased control measures and reduced infestations of the cabbage plants. The cabbage cultivar, Globe Master, recorded with high population density than the Ruby Perfection cultivar. The control of diamondback moth on Globe Master was more obvious, with a single spray application of the bacterial and fungal pathogens. The effect of *B. thuringiensis* subsp. *aizawai* at the highest dose was more significant than the effects of *B. bassiana*. The effects of the different fertilizations on the population density showed that the green cabbage cultivar (Globe Master) fortified with ammonium sulphate recorded with high population density. This is followed by the plants fortified with ammonium nitrate and urea. In the red cabbage cultivar, Ruby Perfection, there was nearly no significance in population densities for plants fortified with the different fertilization treatments. In *B. bassiana* treatments the use of urea, recorded higher *P. xylostella* population density followed by ammonium sulphate and lastly ammonium nitrate, on Globe Master cultivar. While with the Ruby Perfection cultivar this come in the following order: ammonium sulphate, ammonium nitrate and urea.

Key words: Brassicaceae, *Plutella xylostella*, *Bacillus thuringiensis*, *Beauveria bassiana*, cabbage cultivars, ammonium sulphate, ammonium nitrate, urea

INTRODUCTION

In Egypt, crucifers are attacked by several insect pests such as the imported cabbage worm, *Pieris rapae* (L.), diamondback moth, *Plutella xylostella* (L.), black cutworm, *Agrotis ipsilon* (Hufn.) . Among these insect pests, diamondback moth is the most destructive[8]. It occurs throughout the world on cruciferous plants. The level of diamondback moth infestation varies according to locality, type of cabbage plants, the outlining plants and the level of natural enemies (Abdel-Razek, unpublished data). If no control measures are undertaken, feeding injury caused by this caterpillar may reduce production to zero. It's exceptional pest status is due to the diversity and abundance of host plants, the lack or disruption of it's natural enemies, it's reproductive potential (over 20 generations per year in the tropics) and it's genetic elasticity facilitating rapid development of resistance to chemical insecticides[10,15,23]. It's control on cruciferous crops worldwide costs about $1 billion annually[17,20], in addition to the crop losses it causes.

Indiscriminate use of chemical insecticides have contributed to the development of resistance to almost every synthetic insecticide applied in the field including relatively new chemicals such as avermectins, neonicotinoids, oxadiazines, pyrazoles and insect growth regulators[16,10,12]. This has promoted evaluation of alternative pest management strategies mainly biological and cultural control. In addition, some insect population resistant to chemical insecticides have been controlled with *B. thuringiensis* products[11]. The recently introduced products based on *B. thuringiensis* subsp. *aizawai* appear to be providing effective control of diamondback moth. This is consistent with reports describing resistance to *B. thuringiensis* subsp. *kurstaki*[9,19,17,5,4].

The development of fungal entomopathogens as biological control agents has been the subject of considerable research, for example *B. bassiana* applied to seedling grown in a nursery was effective at controlling diamondback moth before they were transplanted into the field[18]. In open field trials of using *B. bassiana* significantly reduced the numbers of diamondback moth.
larvae when used alone[21] and when integrated with B. thuringiensis could control three lepidopteran pests on brassicas[22]. These approaches reduce the number of B. thuringiensis application and therefore contribute to resistance management. So, the developments of microbial control strategies for incorporation into IPM programs against this pest are considered to be a great need.

This study was conducted to compare the effects of the two microbial pathogens, B. thuringiensis subsp. aizawai and B. bassiana, for controlling the diamondback moth on two cabbage cultivars fertilized with different fertilization compounds. The effects of different fertilization compounds on abundance of P. xylostella on the two cabbage cultivars treated with different pathogens have also been studied.

**MATERIALS AND METHODS**

**Microbial biopesticides studies:** Two cabbage cultivars were used, the green cultivar (Globe Master), *Brassica oleracea* var. *capitata* and the red cultivar (Ruby Perfection), *Brassica oleracea* var. *capitata*. These cultivars were pulled from seed beds and transplanted on November 15th, 2005 at the National Research Centre farm in El-Emam Malek village, Nobariadistrict (130 Km North West Cairo). An area of 60 m$^2$ was divided into two equal blocks of three plots each. The middle plots of each block were used as control experiment for the two microbial pathogens, B. thuringiensis subsp. aizawai and B. bassiana, obtained from the fungal entomopathogens collection at the Ministry of Agriculture, Cairo, Egypt.[21] The same methods of plantation and microbial application on the two cabbage cultivars (Starting 22 Jan., 2006).

All the treatments were assigned to the plots in a randomized complete block design with a three replications. Sampling of *P. xylostella* all stages (except the eggs) were recorded at weekly intervals after spraying with both pathogens and for one month period (Starting from 22 Jan., 2006). Three randomly selected plants were sampled in each sample row to provide fifteen plant samples for each plot on each sample date.

**Fertilization studies:** The same methods of plantation and microbial application on the two cabbage cultivars was applied as in the previous experiment to test the effects of three different nitrogen fertilizations [ammoniumsulphate (21.5 % Azotes), ammoniumnitrate (33.5 % Azotes) and urea (46.5 % Azotes)] on the population densities of *P. xylostella* on the two cabbage cultivars. Potassium fertilization was applied at the rate of 150 Kg/f. All fertilizations were applied at six doses with equal intervals ended one month before harvest.

**Statistical analysis:** All data were subjected to analysis of variance (ANOVA). Means were separated by Duncan's multiple range tests. Percentage data were arcsine transformed.

**RESULTS AND DISCUSSIONS**

**Microbial biopesticides studies:** In cabbage cultivar , Globe Master ,the population density of *P. xylostella* showed a high significant reduction from 5.00±0.18 at control experiment to 0.40±0.11, 0.33±0.18 and 0.80±0.08 at cabbage plants treated with 500gm/f. and 250 gm/f. and 125 gm/ f. of B. thuringiensis subsp. aizawai, respectively. While, between the different concentrations tested, only a significant reduction in population density of *P. xylostella* on plants treated with 500 and 250 gm/f. as compared to those treated with 125 gm/f. (*P < 0.05*) with a percentage reduction in population to 92, 92 and 76 % for these concentrations, respectively compared to control treatment (Table 1). The data in Table 2 showed also, that these first two treatments (500 and 250 gm/f.) with B. thuringiensis subsp. aizawai on Globe Master cultivar was higher in their effects regarding the infestation, control and trend percentages, but generally those and the last treatment were significantly reducing the percentages of infestation from 87% in control plants to 20, 20 and 53 % at 500, 250, 125 gm/f. treated plants, respectively. On the other hand, they increase control percentages of *P. xylostella* on plants to 92, 92, and 76%, respectively.

In Ruby Perfection cabbage, the population density of *P. xylostella* was significantly reduced due to B. thuringiensis subsp. aizawai treatment with a mean
reduction after the four week counts of 0.15±0.03, 0.33±0.02, and 0.50±0.03 for the three treatments, respectively as compared with 1.50±0.09 for the control. The percentage reduction in population was significantly increased to 90, 78, and 67% for the treatments with 500, 250, and 125 gm/f, respectively (Table 1). The single application of *B. thuringiensis* subsp. *aizawai* on Ruby Perfection cabbage reduced the percentages of infestation from 53% in control plants to 6, 21 and 33% in 500, 250 and 125 gm/f treated plants, while the control percentages increased to 95, 88, and 82% for the same treatments, respectively (Table 2).

One spray application of *B. bassiana* at the rate of 200 gm/f. (64 × 10⁸ spores/ml), 100gm/f. (32×10⁸ spores/ml) and 50 gm/f. (16×10⁶ spores/ml) significantly reduced *P. xylostella* population density on the green cultivar (Globe Master), *Brassica oleracea* var. *capitata* to 0.32±0.02, 0.53±0.05 and 0.88±0.05, respectively as compared to 5.50±0.13 on control plants. The different treatments led to 93, 89, and 82% reduction in population at 64 × 10⁸ spores/ml, 32×10⁸ spores/ml and 16 × 10⁸ spores/ml, respectively. The other cabbage cultivar, Ruby Perfection, showed a mean population density of 1.50±0.09 for the control. This was significantly reduced to 0.37±0.04, 0.44±0.03 and 0.63±0.06 for the treatments 64 × 10⁸, 32×10⁸ and 16 × 10⁸ spores/ml, respectively (Table 3).

### Table 1: Relative *P. xylostella* population densities and percentage reduction in population at weekly intervals in the control and *B. thuringiensis* treated two cabbage cultivars in Nobaria District, 2006.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>B. oleracea var. <em>capitata</em> (Globe Master)</th>
<th>B. oleracea var. <em>capitata</em> (Ruby Perfection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.00±0.20</td>
<td>5.00±0.20</td>
</tr>
<tr>
<td>500 gm/F</td>
<td>0.33±0.02</td>
<td>0.50±0.13</td>
</tr>
<tr>
<td>250 gm/F</td>
<td>0.40±0.03</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>125 gm/F</td>
<td>0.40±0.04</td>
<td>0.40±0.05</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letter are not significantly different at the level of (P<0.05). Number in parentheses is the percentage reduction in population.

### Table 2: Infestation, control and trend percentages of *P. xylostella* after application of *B. thuringiensis* at different concentrations in diamondback moth-infested two cabbage cultivars in Nobaria District, 2006.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>B. oleracea var. <em>capitata</em> (Globe Master)</th>
<th>B. oleracea var. <em>capitata</em> (Ruby Perfection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.00±0.20</td>
<td>5.00±0.20</td>
</tr>
<tr>
<td>500 gm/F</td>
<td>0.33±0.02</td>
<td>0.50±0.13</td>
</tr>
<tr>
<td>250 gm/F</td>
<td>0.40±0.03</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>125 gm/F</td>
<td>0.40±0.04</td>
<td>0.40±0.05</td>
</tr>
</tbody>
</table>

### Table 3: Relative *P. xylostella* population densities and percentage reduction in population at weekly intervals in the control and *B. bassiana* treated two cabbage cultivars in Nobaria District, 2006.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>B. oleracea var. <em>capitata</em> (Globe Master)</th>
<th>B. oleracea var. <em>capitata</em> (Ruby Perfection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.00±0.20</td>
<td>5.00±0.20</td>
</tr>
<tr>
<td>500 gm/F</td>
<td>0.33±0.02</td>
<td>0.50±0.13</td>
</tr>
<tr>
<td>250 gm/F</td>
<td>0.40±0.03</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>125 gm/F</td>
<td>0.40±0.04</td>
<td>0.40±0.05</td>
</tr>
</tbody>
</table>

Means in the same column followed by the same letter are not significantly different at the level of (P<0.05). Number in parentheses is the percentage reduction in population.
The field application of *B. bassiana* significantly reduced percent infestation of plants of Globe Master cultivar from 87% to 27, 53, 73% at the treatments of 64 × 10³ spores/ml, 32 × 10³ spores/ml and 16 × 10³ spores/ml, respectively. The control of *P. xylostella* on Globe Master cultivars reached to 94 and 84% at 64 × 10³ and 16 × 10³ spores/ml, respectively. The other cabbage cultivar, Ruby Perfection, showed significant reduction in percent of infested plants and significant control of *P. xylostella* on treated plants (Table 4).

Fertilization studies: Plants growth and insect population for the two cabbage cultivars as a result of different fertilizers and pathogen treatments were recorded at Tables (5 and 6). The Globe Master cultivar was the most affected by the different fertilizers as compared with the other cultivar (Ruby Perfection). Those plants fertilized with ammonium sulphate was first to grow followed by those fertilized with ammonium nitrate and urea, the later was retarded in growth in both cultivars. Results also, showed that Globe Master cultivar was higher in weight, length, diameter at all fertilization treatments with the ammonium sulphate was the better followed by ammonium nitrate and urea (Abdel-Razek, personal communication).

Table 5 showed that control plants fertilized with ammonium sulphate recorded significantly highest *P. xylostella* population density (5.00±0.20) as compared with the reduction occurred at *B. thuringiensis* subsp. *aizawai* treated plants (0.43±0.07, 0.36±0.06, 0.33±0.08) with 500, 250 and 125 g/f., respectively. However, the different treatments of bacterial pathogen did not show any significance in population density on plants fertilized with ammonium nitrate and urea for the Globe Master cultivar except with the reduction recorded compared to the control. In Ruby Perfection cultivar plants the population density of *P. xylostella* was significantly lower in the control and 500 g/f (B. *thuringiensis* subsp. *aizawai*) as compared with the Globe Master cultivar and with no significant in the other treatments, although they appeared lower (Table 5).
Table 6 showed the same trend in population density for the control of the two cabbage cultivars and between the different fertilization treatments. Data regarding treatments of Globe Master cultivar with different *B. bassiana* concentrations showed that plants fertilized with urea recorded higher *P. xylostella* population density, this is followed by plants fertilized with ammonium sulphate and later plants fertilized with ammonium nitrate.

On the other hand, in Ruby Perfection cultivars significantly higher population density on control plants were recorded for the different fertilization treatment (1.2±0.26, 0.40±0.26 and 0.60±0.27 at ammonium sulphate, ammonium nitrate and urea, respectively. This is compared with the lower population density of (0.36±0.10, 0.27±0.10, 0.10±0.07), (0.44±0.18, 0.33±0.10, 0.20±0.09) and (0.63±0.14, 0.53±0.14, 0.44±0.05) for the treatments with 64×10⁸, 32×10⁸ and 16×10⁸ spores/ml and the same fertilizations, respectively.

However, in the treatments with fungal pathogens, the fertilization with ammonium sulphate led to higher population density at the different concentrations tested followed by plants treated with ammonium nitrate and urea, respectively but with no significant differences (Table 6).

The diamondback moth has a short development period relative to the time it takes for cabbage to develop. Population may increase in the field as a result of reproduction by a population in a single field within a season, without necessary being influenced by the outside population immigration[2].

The results of this study show that activity of diamondback moth in cabbage after a single application of either the bacterial and fungal biopesticide can vary according to the kind and concentration of the biopesticide. If these effects on the population density, percent infestation, control and amount of damage can be interpreted as characteristics to the biopesticide and rate, then this information could prove to be useful in deciding which of the biopesticides and rate to use to achieve a desired result in the diamondback moth management program.

Consistent differences among these biopesticides could influence decisions concerning augmentations and conservation of the biological agents of control, timing of application, and rate to use for a particular infestation level and growth stage of a crop. Our data showed that the Globe Master cabbage cultivar was significantly more sensitive and attractive to attack by diamondback moth where a significantly higher population densities were recorded at the different week intervals as compared with the other cultivar, Ruby Perfection. This is may be due to the higher waxy materials included in the leaf contents of the later cultivar. The reduction in population density as a result of *B. thuringiensis* subsp. *aizawai* treatment was more obvious on the Globe Master cultivar compared with Ruby Perfection cultivar.

Based on the infestation levels in treated plants relative to nontreated checks, the higher concentrations of both the bacterial and fungal pathogens used had the longest lasting effect, suppressing the infestation of diamondback moth for the period of one month to about (20 and 60%) and (27 and 27%) for the higher concentrations of *B. thuringiensis* subsp. *aizawai* (500 gm/f.) and *B. bassiana* (64×10⁸ spores/ml) for both the Globe Master and Ruby Perfection cultivars, respectively which could have high relevance on marketability of cabbage. These changes in susceptibility of the two cabbage plants to *P. xylostella* could be attributed to changes in plant characteristics with at most the leaf surface waxes which can account for reduced survival and increase in movement rate of *P. xylostella* on *B. thuringiensis* subsp. *aizawai* and *B. bassiana* treated plants. This observation is in consistent with that of[3].

A period of one month has been reported as a critical period of diamondback moth infestation[4]. So, our investigations for four week were critical in investigating the variation in population density between treatments and untreated check. It is also, appeared that one spray of either pathogens at the recommended field levels was quite sufficient to provide protection for cabbage plants if the infestation started after the head formation as recorded in our experiment. This was also, recorded by Salama, *et al*[5] where they reported if the infestation commenced before head formation two or more spray applications of Diple 2X at 200 gm/f. were necessary to achieve reasonable levels of protection.

Our results also, showed that the reduction in population density of *P. xylostella* after a single application of *B. bassiana* to the two cabbage cultivars was significantly reduced at the different concentrations compared with the control plants and the percentage reduction in population was significantly lower with the Ruby Perfection cultivar treatment. This could be due to inability of fungal spores to sustain on leaf surfaces of this cultivar compared with the Globe Master cultivar.

Diamondback moth populations are commonly regulated by two entomophthoralean species Zoophthora radicans and Erynia bluncii, but were also, susceptible to several species of Hyphomycetes which are not usually found in diamondback moth populations. These include *B. bassiana* and *Metarhizium anisopliae*[6]. It is know that fungi causes infection by direct penetration through the host cuticle without requirement for ingestion. This is advantageous as it limits the potential for target insect to avoid consuming a lethal dose, but it also, means that fungi are reliant on appropriate environmental conditions to infect and multiply. The speed fo kill may be delayed as compared with the *B. thuringiensis* subsp. *aizawai* treatment. So, we recorded a higher population density on *B. bassiana* treated plants as well as low percentage reduction in population of diamondback moth as compared with that recorded for *B. thuringiensis* subsp.
aizawai treated plants. Also, the control percentages were lower than in bacterial treatment.

Since the development of fungal pathogens as a microbial control agent however, there are only limited examples of available marketed products\textsuperscript{[14]}. Exploitation of fungi, like other microbial agents, has focused on using them in a similar way to conventional insecticides. The most significant effects of \textit{B. bassiana} were observed in diamondback moth life stages. Effects were even more pronounced in reduction of adults populations. This delayed effects observed in our trials agree with the observations of Lappa\textsuperscript{[8]} who noted that Colorado potato beetle mortality from \textit{B. bassiana} is not rapid, but it adds up in later life stages. \textit{B. bassiana} may therefore have much greater impact in areas where diamondback moth has several generations each season.

This study demonstrated that diamondback moth in Egyptian fields is still susceptible to pathogens and their susceptibility on Globe Master cultivar was more obvious, compared with that on Ruby Perfection cultivar. The toxic constituents produced by both pathogens used justify the need for further research to overcome the environmental constraints.

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REFERENCES


