Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2023; 11(3): 129-131

Received: 11-03-2023
Accepted: 19-04-2023

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Enhancing efficacy of *Bacillus thuringiensis kurstaki* against larvae of cowpea pod worm, *Etiella zinckenella* Treitschka (Lep., Pyralidae) utilizing a feeding stimulant

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DOI: https://doi.org/10.22271/j.ento.2023.v11.i3b.9202

Abstract
The commercial microbial preparation Dipel 2X (*Bacillus thuringiensis kurstaki*) was tested against newly hatching larvae of the cowpea pod worm, *Etiella zinckenella* in 4 concentrations (1, 0.5, 0.25 and 0.125%) once alone and by adding sugar as feeding stimulant in two concentrations (0.25 and 0.125%). The larvae were reared on a modified semi-artificial diet based on cowpea seeds with other ingredients. Young green cowpea pods were sprayed with tested treatments and received eggs of the pest just prior to hatching. The newly hatching larvae showed an interesting behavior on the pods treated with Bt and sugar through lapping the sweet sprayed material on the pods for a period much longer than on pods treated with Bt alone prior to penetration by feeding with the chowing mouth parts. Thus, ingesting higher doses of the Bt spores and crystals leading to death of the larvae in high rates (98.3, 90, 86.6 and 71.6%) in case of adding 0.125& sugar. Meanwhile, these rates recorded 100% for the Bt concentrations of 1 and 0.5% mixed with 0.25% sugar on the fourth day post treatment.

Keywords: *Etiella zinckenella,* Cowpea pod worm, Bt, feeding stimulant, biological control

Introduction

Like most boring lepidopteran larvalpests, females of the cowpea pod worm, *Etiella zinckenella* lays eggs on targeted plant parts (bean pods) and after hatching the newly young larvae crawl on pod surface to locate suitable points for penetration using their chewing mouth parts (Rahouma, 2018) [11]. Thus, the larvae become available for a very short time exposed on plant surface leading to the use of chemical insecticides as an effective control method used by most farmers even with their known negative side effects of insecicides to environment and human health (El Husseini, 1990 and 2006) [4]. Environmentally, safe control methods like use of the entomopathogenic bacterium *Bacillus thuringiensis* (Bt), was not effective against such pests because of the very low doses consumed by the newly hatching larvae on the points of penetration (El Husseini *et al.*, 2012) [6]. Adding sugar or molasses to the sprayed Bt suspension changed the behavior of the newly hatching larvae of the apple leaf roller, *Pandemis heparana* leading them to lap the sweetened sprayed material for a longer period prior to chewing for penetrating the fruits. Thus, they consumed enough dose of the sprayed Bt suspension causing death of the larvae before they bore or during boring in the treated fruits (El Husseini and Sermann, 1977) [2]. The same phenomenon was obtained in case of microbial control of the spiny bollworm, *Earias insulana* (El Husseini and Afifi, 1981) [3] and also for controlling *Spoladea recurvalis* (El Husseini, 2019) [7].

In the present study, sugar was used as feeding stimulant in low concentrations mixed with different concentrations of the commercial entomopathogeneic bacterium Dipel 2X (*B. thuringiensis kurstaki*) for controlling newly hatched larvae of the cowpea pod worm, *E. zinckenella*on cowpea pods under laboratory conditions.

Materials and Methods

Rearing *Etiela zinckenella*

The laboratory colony started with adult moths of *E. zinckenella* obtained alive from a light trap in the Experimental Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt and transported to the Biological Control Laboratory. Moths were confined in groups each of about 6 individuals in 2L glass jars provided with a piece of cotton wool soaked in 10% glucose solution as nutrient for the moths and 10 clean young green pods of cowpea as oviposition site. Laid eggs were separated from the pods using moistened fine brush and placed on small pieces of parchment paper placed on surface of the semi-synthetic diet described by Alfazeiry *et al.* (2012) [1] and modified in the present investigation by replacing the yellow lentils with dried cowpea seeds and agar. The cooked dietprovided a firm suitable physical property as successful insect rearing semi-artificial diet. In Japan, Matsui (1981) [10] used another components to rear *E.zinckenella* on different semi-synthetic diets. The hatched larvae were left to feed on the dietuntil pupation. Pupae were washed with distilled water and placed on filter paper in 2L glass jars for moth emergence. Eggs were obtained as described above for the experimental study.

Treatments with Bt and feeding stimulant

Four successive concentrations of *B.t. kurstaki* were preparedin three sets in distilled water as suspensions of 1, 0.5, 0.25 and 0,125%. To every set of concentrations, 0.125 gr sugar was added as feeding stimulant and another set was enriched with 0.25 g of sugar. The third set served as treatments without sugar as feeding stimulant. To each concentration set, a treatment with spraying only distilled water served as untreated control. Accordingly, 15 treatments were carried out including 3 untreated controls each was replicated 3 times. Young green cowpea pods were hanged by clips in 42 groups each of 10 pods to a stretched rob and each was sprayed by one of the prepared concentrations. Meanwhile, 3 groups were sprayed with distilled water as control. The sprayed pods were left to air dry and pods of each treatment were transferred to a petri-dish of 15cm in diameter. Each single pod received 2 eggs of *E. zinckenella* just prior to hatching and the dishes were covered by perforated polyethylene sheet fixed in place with a rubber band and kept under laboratory temperature of 25 ± 1 °C. Thus, the total number of tested eggs were 20 to find out the mortality of 20 larvae. The bioassay treatments were inspected for 4 successive days post treatment to record mortality among each replicate.

Results and Discussion

Bioassay results showed that larval death of *E. zenckinella* appeared on the second day post treatment by all tested Bt concentrations. Furthermore, it increased day after day and reached mortality means of 60.00, 51.66, 50.00 and 40.33% for the Bt concentrations 1, 0.5, 0.25 and 0.125% on the fourth day post treatment, respectively as shown in Table (1). Larval mortality decreased as Bt concentration decreased and *vis versa*. This trend agrees with results recorded by different authors on different lepidopteran larval species, *e.g.*, El Husseini and Sermann (1977) [2] on *Pandemis heperana*, El Husseini and Afifi (1981) [3] on *Earias insulana*, and El Husseini (2019) [7] on *Spoladea recurvalis*. Adding sugar as feeding stimulant in a concentration of 0.125% to the same Bt concentrations, highly increased larval mortality on the fourth day post treatment recording total mortalities of 98.34, 90.90, 85.00 and 71.67% descendingly from the highest to the lowest tested Bt concentration (Table 1).

Table 1: Numbers of dead larvae and daily mortality % among newly hatched larvae of *E.zinckenella* fed on cowpea podstreated with different concentrations of *B.t. kurstaki* (Btk) and 0.125% sugar as feeding stimulant (N=20)

Treatment	Mortality % as indicated by days pos-tratment				
	Day 1	Day 2	Day 3	Day 4	Total
Btk 1%	0.00	18.33	20.00	21.67	60.00 d
Btk 1% + 0.125% sugar	0.00	56.67	20.00	21.67	98.34 a
Btk 0.5%	0.00	18.33	18.33	15.00	51.66 e
Btk 0.5%+ 0.125% sugar	0.00	25.00	31.67	33.23	89.90 b
Btk 0.25%	0.00	16.67	15.00	18.33	50.00 e
Btk 0.25% +0.125% sugar	0.00	23.33	31.67	30.00	85.00 b
Btk 0.125%	0.00	11.67	13.33	15.33	40.33 f
Btk 0.125% + 0.125% sugar	0.00	25.00	25.00	21.67	71.67 c
LSD (0.05)					6.842

In a column, means followed by the same letter are not significant at 0.05 probabilities

Increasing the concentration of the feeding stimulant to 0.25% in the sprayed Bt concentrations (Table 2) recorded 100.00 and 100.00, 91.66 66 and 83.43% for the Bt concentrations of 1 and 0.5%. The Bt concentrations 0.5 and 0.125% on the fourth day post treatment, respectively. This induced high mortality rates due to the feeding stimulant could be explained by the high dose of the Bt proteaceous crystal, ingested by the larvaewhere the gut enzymes activate this toxin causing perforations in the mid-gut cell membrane, followed by gut paralysis leading to death (Vachon *et al.*, 2012) [12]. Thus, the more ingested Bt material due to the feeding stimulant, the higher the larval mortality is.

Table 2: Numbers of dead larvae and daily mortality % among newly hatched larvae of *E. zinckenella*fed on cowpea pods treated with different concentrations of *B.t. kurstaki* (Btk) and 0.25% sugar as feeding stimulant (N=20)

Treatment	Mortality % as indicated by days pos-tratment				
	Day 1	Day 2	Day 3	Day 4	Total
Btk 1%	0.00	18.33	20.00	21.67	60.00 c
Btk 1% + 0.25% sugar	0.00	60.67	21.67	18.33	100.00 a
Btk 0.5%	0.00	18.33	18.33	15.00	51.66 cd
Btk 0.5%+ 0.25% sugar	0.00	33.33	31.67	35.00	100.00 a
Btk 0.25%	0.00	16.67	15.00	18.33	50.00 cd
Btk 0.25% +0.25% sugar	0.00	28.33	30.00	33.33	31.66 ab
Btk 0.125%	0.00	16.67	13.33	10.00	40.00 d
Btk 0.125% + 0.25% sugar	0.00	26.67	31.67	25.00	83.34 b
LSD (0.05)					17.924

In a column, means followed by the same letter are not significant at 0.05 probabilities

The present results suggest a recommendation to the farmers specially producer of the organic cowpea to use feeding stimulants like sugar or molasses when applying Bt commercial preparations for controlling the cowpea pod worm, *E. zinckenella.* Such recommendation was also advised by Jousani *et al.* (2017) [8] a successful insecticide with new environmental features and tidings and Kumar *et al.* (2021) [9] in application for sustainable agriculture.

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