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Sublethal Effects of Strains and Commercial Bt-Based Products against Different Populations of *Plutella xylostella*

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***Abstract* – The aim of this study was to determine the sublethal effects of isolate and *Bacillus thuringiensis*(*Bt*) based bioinsecticides against four different populations of *Plutellaxylostella* from brassicas production sites in Brazil (PX, PA, PC and SBT). We tested ten isolates and the products Btt090®, Dipel®, Xentari® and Agree® by spraying them on kale leaf discs and offering the discs to second instar larvae of each population. We assessed the larval and pupal viability and period, pupal weight, and sex ratio. Dipel®, Xentari®, Agree®, 49.19A, E47 and HD1 caused 100% mortality for all the populations. Isolate 20.7L caused the lowest larval viability in the SBT population. Treatment with isolates 20.7L and T08.024 produced the longest larval period in the PA and SBT populations, whereas treatment with isolate E7 produced the longest larval period in the PA population. Isolate 20.7L affected the pupal viability of the PX population, and isolate T08.024 affected the pupal viability of the PC population. The pupal period was higher in the PA population that was treated with*Bttenebrionis*. The pupal weight of the PX and PC populations was negatively affected by the 20.7L and T08.024 isolate, respectively. Isolate T08.024 affected the pupal weights of the PA and PC populations, whereas isolate E28 affected the pupal weights of the SBT population. Isolates 20.7L and T08.024 had the strongest effect on the sex ratios of the SBT and PA populations, respectively. The T08.024 and E28 isolates had the greatest negative effect on the biological characteristics of *P. xylostella*. Because the populations were affected differently by the different products/isolates, the population and area of occurrence of the pest must be considered prior to implementing control measures.**

***Keywords* – Entomopathogenic Bacteria, Biological Control, Diamondback Moth.**

I. INTRODUCTION

The bacterium *Bacillus thuringiensis* Berliner (Bt) is the entomopathogen that is most widely studied and used in integrated pest management [5], and it is present in different regions of the world and in several substrates, including soil, water, dead insects and the surfaces of some plants [2][19].

During its sporulation and/or stationary phase, this microorganism produces inclusions of crystalline proteins (protein crystals) called δ-endotoxins or Cry proteins that have entomopathogenic activity toward various species of Lepidoptera, Coleoptera and Diptera[4][40][42].

The protein crystals act at the larval stage and, upon being ingested, are solubilized in the midgut of the insects due the action of the alkaline pH and proteases, which activate the pro-toxins (Cry proteins). The toxins bind to specific receptors located in the gut epithelium and form pores that favor the leakage of the intestinal contents into the hemocoel. As consequence, the larvae stop feeding, become immobile and die by starvation or septicemia [4][22][32].

Because of their high specificity and lack of environmental risks, the Cry proteins are important alternatives to chemical insecticides for agricultural pest control [33]. In the bio-pesticide market, the Bt-based products are the most used [3][18], and they represent 53% of the bio-pesticide trade worldwide [9].

According to the Ministry of Agriculture, Livestock and Supply of Brazil, the registered and authorized Bt-based pesticides in the Brazilian market are Able®, Agree®, Bac-Control WP®, Bactur WP®, Dipel®, Dipel WP®, Dipel WG®, Thuricide® and Xentari® [24]. Most of these Bt-based pesticides are recommended to control *Plutellaxylostella*(Linnaeus, 1758) (Lepidoptera: Plutellidae) in horticulture, but despite their effective control, cases of selection of pest populations resistant to *B. thuringiensis* have been reported [30][43].

Another method to control *P. xylostella*that has been studied is the use of Bt-Brassicas under laboratory [34] and field conditions [45]. *Cry1* genes were introduced into many Brassica species, including *Brassica oleracea* subsp. *Itálica*, so that Cry1Ac, Cry1C or Cry1A and Cry1C are expressed in the same plant [26][6] [7], and all of its plant materials exhibit resistance to *P. xylostella*[14][29].

The major barrier to the adoption of Bt-plants is the ability of *P. xylostella* to develop resistance to Cry1Aa and Cry1C [44][27][38]. One solution to this problem would be the adoption of Bt-Brassica expressing dual *cry*genes; however, *P. xylostella*can develop resistance to Cry toxins even in field situations [38]. For this reason, researchers agreed on the adoption of Bt-Brassicas expressing both Cry1C and Cry1B because cross-resistance to these two proteins has not been detected. Sales of foliar spray of Bt-strains or Bt-based products for the control of *P. xylostella* can generate subsidies for studies of genes that have been proved effective and that can be used together to manage this pest.

P. xylostella is a major pest of brassica plants, causing losses that frequently reach 90% of cauliflower and cabbage production when insecticides are not used [8]. In tropical areas, where pest pressure is high, it is common to spray insecticides daily, causing populations of *P. xylostella* to become resistant to most major chemical and biological insecticides [13][21][38]. Thus, the aim of this study was to evaluate the susceptibility and sublethal effects of bio-insecticides and *B. thuringiensis*strains on four populations of *P. xylostella*from different Brazilian productions.

II. Material and Methods

The insects were reared and the bioassays were conducted in the Laboratory of Biology and Rearing Insects (LBRI), FCAV/Unesp, Jaboticabal, São Paulo.

Populations of Plutellaxylostella

We used four *P. xylostella* populations for the bioassays,

Population PX was collected from cabbage plants in Recife-PE on January 15, 2007 (initial population: 143 adults; 74 generations reared under laboratory conditions).

Population PA was collected from cabbage plants in Alegre-ES on July 22, 2008 (initial population: 160 adults; 41 generations reared under laboratory conditions).

Population PC was collected from cabbage plants in Recife-PE in Recife-PE on May 19, 2008 (initial population: 150 adults; 44 generations reared under laboratory conditions).

Population SBT was collected from an organic kale crop in Itobi-SP on November 19, 2010 (initial population: 7 caterpillars, 12 eggs and 3 pupae; 8 generations reared under laboratory conditions).

The populations were kept reproductively isolated by not introducing new individuals; the rearing methodology followed [12].

Bioassays

We used 10 strains of *B. thuringiensis* from the Germoplasm Bank of entomopathogenic*Bacillus*, located in the Laboratory of Genetics of Bacteria and Applied Biotechnology (LGBAB), FCAV/Unesp. The standard strain (Lepidoptera-specific) that was used as the positive control was *B. thuringiensis*var. *kurstaki* HD-1, and *B. thuringiensis* var. *tenebrionis* (Coleoptera-specific) was used as the negative control.

Most proteins of the Cry1 family are toxic to lepidopterous insects [28], and for this reason, strains with and without the *cry1* gene were tested [41] (Table 1). *B. thuringiensis* var. *tenebrionis* expresses the Cry3Aa protein [10] and Cry8 [25].

The isolates were placed on Petri dishes containing Nutrient Agar 'Na' (meat extract 1.5 g/L, yeast extract 1.5 g/L, sodium chloride 5.0 g/L, bacteriological peptone 5.0 g/L and agar 15.0 g/L) and incubated at 28°C for 5 days to allow the bacteria to grow and sporulate. After this period, the bacteria were transferred to Falcon tubes containing 10 mL of deionized, autoclaved water and 0.05% Tween 20®. The suspensions were homogenized, and the mixture containing spores, crystals and vegetative cells was centrifuged three times (3,600 rpm for 20 minutes); the supernatants were discarded to eliminate extracellular toxins, such as β-exotoxins, and water was then added to the pellets. After the final centrifugation, the pellets were resuspended, and two serial dilutions were prepared for counting the spores using a Neubauer chamber [1]. The concentrations of the suspensions were adjusted to 3×10^8 spores / mL.

In addition to the strains, we tested the commercial products Btt090® (*B. thuringiensis* var. *tolworthi*), Dipel® (*B. thuringiensis* var. *kurstaki*), Agree® (*B. thuringiensis* var. *aizawai* + *kurstaki*) and Xentari® (*B. thuringiensis* var. *aizawai*) using the doses recommended by the manufacturers [24].

Each treatment used ten 8-cm diameter leaf-discs of kale (*Brassica oleracea* var. *acephala* cv. Manteiga) that had been sprayed on each side with 1.0 mL of a suspension of the products/strains; the control leaf-discs were sprayed with autoclaved deionized water containing 0.05% Tween 20. The sprays were applied using an airbrush coupled to a compressor (Schulz MS Model 2.3, Joinville, SC, Brazil) at a pressure of 25 lbs/in^2. After air drying, the discs were placed individually in Petri dishes (9.5 cm diameter × 2.0 cm height) on filter paper that had been moistened with water. We placed 10 *P. xylostella* second-instar larvae on the leaf discs, repeating this procedure for all of the tested populations. The discs were replaced every three days to measure foliar consumption.

The Petri dishes were wrapped with polyvinylchloride (PVC) plastic film and maintained in a room at 25 ± 1°C with a relative humidity of 70 ± 10% and a photoperiod of 12 hours for the assessment of the viability, larval period and leaf consumption. The leaf area consumed was measured every three days using a laser meter (IC 203 - CID Bio-Science®, Camas, WA, USA). The pupae were isolated and maintained on Elisa plates until adult emergence, allowing the pupal weight, pupal duration and viability and adult sex ratio to be determined.

Statistical analyses

To evaluate the combined effect of the products/strains and the different populations, the experimental design was completely randomized. The main effects of the strains/products, the populations and their interactions were analyzed using a General Linear Model (GLM). All of the data were subjected to Bartlett and Kolmogorov tests to evaluate the distribution of the variances and the homoscedasticity of the residuals. The data were subjected to an ANOVA, and when the results were significant ($p < 0.05$), the means were compared using the Tukey test. All of the analyses were performed using the statistical program SAS [35].

III. Results and Discussion

The larval viability values were significantly different among the *P. xylostella* populations and for each individual population treated with a different strain or commercial product. The isolate that uniquely caused significant differences among the populations was 20.7L, causing the lowest viability of the SBT larvae (46.0%) and the least effect on the viability of the PA (78.0%) and PC (86.0%) larvae (Table 2).

The lowest viabilities of the PX larvae were caused by 41.7L (33.0%), E28 (13.0%) and T08.024 (24.0%) (Table 2). T08.024 was the only isolate that affected the viability of the PA and PC larvae (21.0 and 20.0%, respectively), and the lowest SBT larval viability values were obtained by treatment with E28 (38.0%) and T08.024 (35.0%).

Agree®, Dipel®, Xentari®, 49.19A, E47 and HD1 caused 100.0% mortality of all the populations.

The larval period values were significantly different between populations and between their treatments with the

products and isolates. Treatment with isolates 20.7L and T08.024 caused the longest larval period in the PA population (8.80 and 8.73 days, respectively) and the SBT population (8.83 and 9.33 days, respectively), and E7 caused the longest larval period in the PA population (8.76 days) (Table 3). Treatments 153.30A, 20.7L and Btt090® caused the longest larval periods in the PC population (7.91, 7.96 and 8.26 days, respectively), and T08.024 treatment caused the longest larval period in the SBT population (9.33 days).

Pupal viability was reduced in the PX population by treatment with isolates 20.7L and E28 (60.2 and 38.3%, respectively) and in the PC population by treatment with isolate T08.024 (19.4%). The control pupal viability values of the population were significantly different, being lowest in the control PA population (75.5%). The pupal viability in the PX population was reduced by treatment with E28, and the lowest pupal viabilities in PC and SBT populations were obtained by treatment with isolate T08.024 (19.4 and 46.1%, respectively); the differences among the values for the populations and products/isolate were significant (Table 4).

The pupal period of the PA population was lengthened by treatment with *Bttenebrionis* (4.8 days). The treatments that caused the longest duration of the PX pupal period were 153.30A (4.3 days), E7 (4.4 days) and *Bttenebrionis* (4.7 days). Likewise, the pupal period of the PC population was lengthened by all the treatments, with the exception of the T08.024 isolate, which caused the shortest pupal period (1.4 days) (Table 5).

The pupal weight in PX population was negatively affected by treatment with 20.7L (4.0 mg), and that of the PC population was negatively affected by treatment with T08.024 (1.9 mg). Treatment with isolate T08.024 negatively affected the pupal weight in the PA and PC populations (3.8 and 1.9 mg, respectively), whereas that of the SBT population was hampered by treatment with E28 (3.5 mg) (Table 6).

None of the products/isolates affected the sex ratio of the adult survivors of the PA and SBT populations. However, we observed lower values for the 20.7L-treated PX (0.4) and PC (0.3) populations. The lowest value for the PC population was observed with the T08.024 (0.1) treatment when compared those obtained with the other products/isolates (Table 7).

We observed different responses among the *P. xylostella* populations while evaluating the sublethal effects of the selected bioinsecticides and *B. thuringiensis* isolates. Many studies of the sublethal effects of insecticides on pests are conducted to discover the negative and non-lethal effects on biological aspects that affect the population dynamics of insects [39].

In studies conducted by [12] to investigate the sublethal effects of *B. thuringiensis* on *P. xylostella*, the T08.024 isolate also showed low larval viability; however, the concentration that was used was lower in this investigation, 2.3 × 106 spores/mL.

The variable susceptibility of the populations may be related to factors intrinsic to the population or to the mode of action of the bacteria, such as the optimal condition for the dissolution of the crystals or protoxin activation and, especially, the presence of specific receptors on the epithelial membrane of the insects' midgut that bind the Cry proteins [17][20][31][37].

The receptor binding of some toxins may not be strong enough to kill an insect, such as the less specific toxins that reversibly bind to receptors in an unstable mode [28]. The differences in the efficiency of the products/isolates against the tested populations might be related to decreased receptor binding by the toxins (Ferre and Van Rie, 2002; [36].

Treatment with the T08.024 and 20.7L isolates caused the longest larval period in the PA and SBT populations, and treatment with the E7 isolate caused the longest larval period in the PA population. Treatments with the 153.30A or 20.7L isolates or the Btt090® product caused the longest larval period in the PC population. These results suggest that although these isolates/products did not kill 100% of the insects, they lengthened the larval phase of the pest, which is harmful to the diamondback moth because the prolongation of its life cycle increases its exposure to natural enemies and decreases the number of generations per year, which reduces the insect population in the field [23].

Regarding the reproductive traits of the survivor insects, the sex ratio of the PC population was reduced by treatment with isolate T08.024. The T08.024 isolate also caused the smallest number of eggs laid/female/day in the PX population. These results demonstrate the potential of this isolate, which despite not causing 100% larval mortality, negatively affected the reproductive traits of females, reducing the number of offspring as a result of the greater percentage of males in the population and the lower egg production by the females.

The results of this study demonstrate the efficiency of the isolates or the Bt-based commercial products against the diamondback moth in Brazil. The studied populations responded differently when exposed to the action of the bacteria. Treatments that had lower efficacy for controlling a certain population should not be discarded because they can exhibit high efficacy for other populations.

Given that the climatic conditions were controlled in the laboratory, we assume that the responses to the products/isolates were related to the inherent characteristics of each population and, therefore, the adoption of control tactics that are particular to the management of *P. xylostella* and depends on the region where the pest occurs. However, before these results can be extrapolated to field conditions, it is necessary to conduct further research to verify whether environmental variations influence the efficiency of *B. thuringiensis* in controlling the studied populations.

IV. Conclusion

The Agree®, Dipel® and Xentari® products and the 49.19A, E47 and HD1 isolates have activity against *P. xylostella*.

Among the isolates that did not cause 100% mortality, the T08.024 and E28 isolates negatively affected the biological characteristics of the pest to a greater extent compared with the other isolates.

The different *P. xylostella* populations were affected differently by the products/isolates, and therefore, the population and the area of occurrence of the pest should be considered when choosing a bioinsecticide.

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Table 1: Isolates of *Bacillus thuringiensis* used in the bioassays.

Isolates/Strains	Frequency of *cry1* gene
153.30A	*Fa*
49.19A	*Aa; Fa; Ab; Ae; Bd; Ea*
E7	*Aa; Ca; Ea; Ab; Ae; Ab; Fa; Bd; Ac*
E28	*Aa; Ca; Da; Fa; Bd*
E47	*Aa; Ca; Da; Bd*
41.7L	*Aa; Da; Fa; Bd*
20.7L	*Aa; Ca; Fa; Ab; Ae; Bd; Ac*
T08.024	*Aa; Ca; Fa; Ab; Ae; Bd; Ac*
HD-1	*Aa; Ab; Ae*
Bttenebrionis	-

Table 2: Effect of products and *Bacillus thuringiensis* isolates on the larvalviability (%) of different *Plutellaxylostella* populations (means ± SE).

Products/isolates	Populations			
	PX	PA	PC	SBT
153.30A	77.0 ± 4.49Aa	70.0 ± 4.94Aabc	75.0 ± 5.82Aa	70.0 ± 4.71Aabc
20.7L	64.0 ± 11.47ABa	78.0 ± 6.29Aab	86.0 ± 6.86Aa	46.0 ± 5.62Bcd
41.7L	33.0 ± 8.70Ab	53.0 ± 7.00Abc	62.0 ± 10.83Aab	61.0 ± 8.88Abcd
Btt090®	68.0 ± 6.63Aa	59.0 ± 7.67Aabc	63.0 ± 8.17Aab	72.0 ± 4.16Aabc
E28	13.0 ± 3.35Ab	41.0 ± 10.69Acd	40.0 ± 9.55Abc	38.0 ± 6.63Ad
E7	66.0 ± 5.21Aa	71.0 ± 8.62Aabc	88.0 ± 3.89Aa	66.0 ± 6.36Aabc
T08.024	24.0 ± 5.42Ab	21.0 ± 3.48Ad	20.0 ± 9.89Ac	35.0 ± 5.82Ad
Bttenebrionis	74.0 ± 6.70Aa	68.0 ± 5.74Aabc	75.0 ± 4.28Aa	76.0 ± 5.42Aab
Control	88.0 ± 2.49Aa	89.0 ± 2.77Aa	87.0 ± 2.60Aa	90.0 ± 2.11Aa

Means ± standard errors with the same uppercase letter (rows) or lowercase letter (columns) are not different by comparison using the Tukey test (P > 0.05).

Table 3: Effect of products and *Bacillus thuringiensis* isolates on the larval duration (days) of different *Plutellaxylostella* populations (means ± SE).

Products/Isolates	Populations			
	PX	PA	PC	SBT
153.30A	8.11 ± 0.30Aa	8.86 ± 0.37Aa	7.91 ± 0.33Aa	8.20 ± 0.24Aab
20.7L	6.17 ± 1.03Ba	8.80 ± 0.11Aa	7.96 ± 0.33Aba	8.83 ± 0.24Aab
41.7L	8.03 ± 0.98Aa	7.97 ± 0.21Aa	6.41 ± 1.10Aab	6.95 ± 0.79Ab
Btt090®	8.29 ± 0.17Aa	8.45 ± 0.16Aa	8.26 ± 0.27Aa	8.56 ± 0.20Aab
E28	6.43 ± 1.45Aa	8.12 ± 1.36Aa	7.81 ± 0.88Aab	7.27 ± 1.22Aab
E7	8.09 ± 0.14Ba	8.76 ± 0.22Aa	7.42 ± 0.15Cab	8.04 ± 0.16BCab

T08.024	8.21 ± 1.00ABa	8.73 ± 1.02Aa	4.57 ± 1.57Bb	9.33 ± 0.24Aa
Bttenebrionis	7.42 ± 0.20Aa	9.17 ± 0.15Aa	7.03 ± 0.23Aab	7.48 ± 0.16Aab
Control	8.61 ± 0.13Aa	7.91 ± 0.33Aa	8.64 ± 0.26Aa	8.80 ± 0.30Aab

Means ± standard errors with the same uppercase letter (rows) or lowercase letter (columns) are not different by comparison via the Tukey test (P > 0.05).

Table 4: Effect of products and *Bacillus thuringiensis* isolates on the pupal viability (%) of different *Plutellaxylostella* populations (means ± SE).

Products/Isolates	Populations			
	PX	PA	PC	SBT
153.30A	77.0 ± 6.10Aab	75.4 ± 5.04Aa	66.9 ± 7.14Aabc	86.2 ± 5.51Aab
20.7L	60.2 ± 10.95Bab	89.2 ± 3.44Aa	78.6 ± 6.15ABab	64.2 ± 7.38ABabc
41.7L	53.0 ± 12.74Aab	77.7 ± 5.40Aa	52.2 ± 9.43Abcd	58.4 ± 10.45Aabc
Btt090®	66.4 ± 7.37Aab	74.2 ± 8.50Aa	71.4 ± 3.51Aabc	86.1 ± 2.64Aab
E28	38.3 ± 14.50Ab	76.0 ± 12.82Aa	43.8 ± 10.23Acd	53.3 ± 10.47Abc
E7	59.0 ± 5.06Aab	66.7 ± 6.02Aa	51.3 ± 9.03Abcd	61.5 ± 7.97Aabc
T08.024	58.3 ± 12.24ABab	71.7 ± 10.84Aa	19.4 ± 10.71Bd	46.1 ± 11.95ABc
Bttenebrionis	82.5 ± 3.48Aa	78.3 ± 6.65Aa	87.0 ± 4.16Aa	69.2 ± 8.05Aabc
Control	83.2 ± 3.65ABa	75.5 ± 1.87Ba	83.4 ± 4.30ABab	89.8 ± 2.02Aa

Means ± standard errors with the same uppercase letter (rows) or lowercase letter (columns) are not different by comparison using the Tukey test (P > 0.05).

Table 5: Effect of products and *Bacillus thuringiensis* isolates on the pupal duration (days) of different *Plutellaxylostella* populations (means ± SE).

Products/Isolates	Populations			
	PX	PA	PC	SBT
153.30A	4.3 ± 0.08Aa	4.3 ± 0.09Aab	4.4 ± 0.10Aa	4.3 ± 0.09Aa
20.7L	3.5 ± 0.60Aab	4.1 ± 0.09Aabc	4.6 ± 0.11Aa	4.2 ± 0.08Aa
41.7L	3.0 ± 0.68Aab	4.5 ± 0.16Aab	3.5 ± 0.59Aa	4.3 ± 0.48Aa
Btt090®	4.3 ± 0.11Aab	4.4 ± 0.12Aab	4.5 ± 0.12Aa	4.5 ± 0.14Aa
E28	2.2 ± 0.74Ab	3.1 ± 0.52Ac	3.4 ± 0.59Aa	3.6 ± 0.61Aa
E7	4.4 ± 0.16Aa	4.4 ± 0.11Aab	4.8 ± 0.09Aa	4.4 ± 0.11Aa
T08.024	3.3 ± 0.74Aab	3.6 ± 0.48Abc	1.4 ± 0.71Ab	3.1 ± 0.69Aa
Bttenebrionis	4.7 ± 0.13ABa	4.8 ± 0.06Aa	4.5 ± 0.10Ba	4.6 ± 0.68ABa
Control	4.5 ± 0.07Aa	4.4 ± 0.08Aab	4.4 ± 0.07Aa	4.2 ± 0.11Aa

Means ± standard errors with the same uppercase letter (rows) or lowercase letter (columns) are not different by comparison using the Tukey test (P > 0.05).

Table 6: Effect of products and *Bacillus thuringiensis* isolates on the pupal weight (mg) of different *Plutellaxylostella* populations (means ± SE).

Products/Isolates	Populations			
	PX	PA	PC	SBT
153.30A	4.7 ± 0.19Aa	5.3 ± 0.20Aab	4.9 ± 0.16Aa	4.8 ± 0.10Aabc
20.7L	4.0 ± 0.71Ba	5.7 ± 0.16Aa	5.0 ± 0.12Aba	4.8 ± 0.26ABabc
41.7L	4.3 ± 0.52Aa	4.6 ± 0.15Aab	3.7 ± 0.63Aa	3.9 ± 0.46Abc
Btt090®	4.8 ± 0.23Aa	4.6 ± 0.18Aab	5.0 ± 0.12Aa	5.2 ± 0.11Aab
E28	3.3 ± 0.73Aa	4.3 ± 0.75Aab	4.0 ± 0.46Aa	3.5 ± 0.62Ac
E7	5.0 ± 0.08Aa	5.1 ± 0.19Aab	5.2 ± 0.09Aa	5.1 ± 0.16Aab
T08.024	3.7 ± 0.60ABa	3.8 ± 0.45ABb	1.9 ± 0.65Bb	4.6 ± 0.18Aabc
Bttenebrionis	5.1 ± 0.18Aa	5.1 ± 0.11Aab	5.0 ± 0.14Aa	5.1 ± 0.12Aab
Control	5.2 ± 0.13Aa	5.2 ± 0.20Aab	5.1 ± 0.12Aa	5.5 ± 0.15Aa

Means ± standard errors with the same uppercase letter (rows) or lowercase letter (columns) are not different by comparison using the Tukey test (P > 0.05).

Table 7: Effect of products and *Bacillus thuringiensis* isolates on the sex ratio of different *Plutellaxylostella* populations (means ± SE).

Products/Isolates	Populations			
	PX	PA	PC	SBT
153.30A	0.5 ± 0.06Aa	0.4 ± 0.09Aa	0.5 ± 0.08Aab	0.5 ± 0.06Aa
20.7L	0.4 ± 0.07Ba	0.5 ± 0.06ABa	0.3 ± 0.06Bab	0.5 ± 0.05Aa
41.7L	0.5 ± 0.13Aa	0.6 ± 0.09Aa	0.4 ± 0.10Aab	0.4 ± 0.11Aa
Btt090®	0.5 ± 0.10Aa	0.5 ± 0.10Aa	0.4 ± 0.09Aab	0.6 ± 0.07Aa
E28	0.2 ± 0.12Aa	0.4 ± 0.11Aa	0.4 ± 0.13Aab	0.3 ± 0.11Aa
E7	0.5 ± 0.10Aa	0.5 ± 0.07Aa	0.6 ± 0.10Aa	0.5 ± 0.11Aa
T08.024	0.3 ± 0.14ABa	0.6 ± 0.14Aa	0.1 ± 0.07Bb	0.3 ± 0.11Aba
Bttenebrionis	0.5 ± 0.04Aa	0.4 ± 0.08Aa	0.5 ± 0.06Aa	0.5 ± 0.06Aa
Control	0.4 ± 0.07Aa	0.5 ± 0.08Aa	0.4 ± 0.07Aab	0.6 ± 0.07Aa

Means ± standard errors with the same uppercase letter (rows) or lowercase letter (columns) are not different by comparison using the Tukey test (P > 0.05).