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Toxicity and residual control of *Plutella xylostella* L. (Lepidoptera: *Plutellidae*) with *Bacillus thuringiensis* Berliner and insecticides

Toxicidade e controle residual de *Plutella xylostella* L. (Lepidoptera: *Plutellidae*) com *Bacillus thuringiensis* Berliner e inseticidas

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ABSTRACT

Plutella xylostella *L. is the most important worldwide pest of cruciferous plants and indiscriminate use of insecticides has led to the resistance of the species to different groups. This research was conducted to compare the toxicity and persistence of two strains of* ***Bacillus thuringiensis*** *to* ***P. xylostella*** *larvae. Concentrations between 125 and 500g 100L^{-1} of water of the commercial products were evaluated and compared to the insect growth inhibitor diflubenzuron and to the neurotoxic pyrethroid deltamethrin. The efficacy of the insecticides was compared between treated plants kept indoor greenhouse and outdoor. Third instar larvae were more susceptible to* ***B. thuringiensis*** *than first instar ones. Agree and Dipel showed similar control rates of third instars until 10 days after treatment, but on the 15th day, Agree was significantly more efficient than Dipel. Both* ***B. thuringiensis*** *formulations showed a reduction in mortality after 10 days when the treated plants were exposed to natural weather conditions in comparison to the same treatments kept inside greenhouse. Dimilin (100g 100L^{-1} of water) and deltamethrin (30ml of commercial product 100L^{-1} of water) were not efficient to control third instar larvae of* ***P. xylostella****. This inefficiency cannot be attributed to a resistant population of* ***P. xylostella*** *since the larval population used in the experiments was not subjected to insecticide pressure, once the crop was organically cultivated all year round. The results showed that both formulations of* ***B. thuringiensis*** *are sound alternatives for the control of the diamondback moth in organically conducted cruciferous crops, considering the high residual control provided under subtropical weather conditions.*

Key words: *diamondback moth, bioinsecticide, entomopathogens, residual persistance.*

RESUMO

Larvas de ***Plutella xylostella*** *L. são as principais pragas de crucíferas cultivadas e o uso excessivo e indiscriminado de inseticidas tem levado a resistência da espécie para diferentes grupos de inseticidas. Este trabalho foi conduzido para comparar a toxicidade de duas formulações de* ***Bacillus thuringiensis*** *para larvas de primeiro e terceiro ínstar de* ***P. xylostella****. Concentrações entre 125 e 500g 100L^{-1} de água do produto comercial foram avaliadas e comparadas com o inibidor do crescimento diflubenzuron e com o piretroide deltametrina. A eficiência dos inseticidas foi comparada em plantas tratadas e mantidas dentro e fora da casa de vegetação. Larvas de terceiro ínstar foram mais suscetíveis a* ***B. thuringiensis*** *do que larvas de primeiro ínstar. Agree e Dipel apresentaram taxas de controle semelhantes até 10 dias após a aplicação, porém após 15 dias, Agree foi significativamente mais eficiente do que Dipel. Ambas as formulações apresentaram uma redução na mortalidade larval quando as plantas foram expostas às condições climáticas em comparação aos mesmos tratamentos mantidos em casa de vegetação. Dimilin (100g 100L^{-1} de água) e deltametrina (30ml de produto comercial 100L^{-1} de água) não foram eficientes para o controle de lagartas de terceiro ínstar de* ***P. xylostella****. Tal ineficiência não pode ser atribuída a um possível efeito de resistência da população de* ***P. xylostella*** *a esses produtos, uma vez que a colônia da espécie utilizada nos experimentos não estava sujeita à pressão de seleção, por se tratar de uma população coletada em área conduzida organicamente durante todo o transcorrer do ano. Os resultados mostram que as duas formulações de* ***B. thuringiensis*** *são alternativas viáveis para o controle de* ***P. xylostella*** *em cultivo orgânico de crucíferas, tendo em vista o expressivo controle residual observado para as condições climáticas dos subtrópicos.*

Palavras-chave: *traça-das-crucíferas, bioinseticida, entomopatógenos, persistência residual.*

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INTRODUCTION

The diamondback moth, ***Plutella xylostella*** (L.) (Lepidoptera: ***Plutellidae***) is a cosmopolitan pest which causes heavy damage to cruciferous plants, especially in cabbage, both in Brazil (CASTELO BRANCO et al., 1996; FRANÇA & MEDEIROS, 1998), and elsewhere (TALEKAR & SHELTON, 1993; GODIN & BOIVIN, 1998). After the second instar, the larvae bore into leaf tissues of cabbage heads thus reducing the commercial value of the product. Damage can reach 100% of the heads, which are then classified as inadequate for marketing (OOI & KELDERMAN, 1979). According to BARROS et al. (1993), there is a straight correlation between the phenological development of the plants and damage level. Due to the irreversible condition of the damage, control measures are adopted before the beginning of head development.

Chemical control is the main method to reduce the damage caused by the diamondback moth larvae; usually the number of insecticide applications can reach as much as 15 or 20, independently of the presence of larvae in the field (LIM, 1990; CARBALLO, 1992; SAMPSON, 1992). As a result of excessive use of insecticides, diamondback moth is among the lepidopteran species with largest number of records of resistance to active ingredients and there are reports of more than 50 insecticides (VASQUEZ, 1995), including ***Bacillus thuringiensis*** (FERRÉ et al., 1991; TABASHNIK et al., 1994a, b).

Biological methods based on the use of predators, parasitoids and entomopathogenic microorganisms are sound alternatives to overcome the inefficiency of chemical control (MONNERAT & BORDAT, 1998; SILVA-TORRES et al., 2010a,b; 2011). Bioinsecticides based on ***B. thuringiensis*** are commercially produced since 1970 and at present represent over 90% of the market for biological products for pest control (DIAS et al., 2004).

In this research the efficiency and persistence of two formulations of ***B. thuringiensis*** were evaluated and compared to an insect growth inhibitor and a neurotoxic pyrethroid insecticide on first and third instar larvae of ***P. xylostella*** up to 15 days after application. The persistence of the insecticides was compared in plants kept indoor and outdoor a greenhouse.

MATERIAL AND METHODS

The larvae used in the experiments were obtained from a colony kept in the laboratory at 20±1ºC and photo phase of 12:12 hours (D:L). Larvae were collected in a cauliflower crop in Colombo, Paraná and were reared in laboratory for ten generations before being used in the experiments. Field collected larvae were continuously introduced in the colony to renew the genetic pool of the colony. The larvae were collected in an organic crop, and thus not subjected to insecticide pressure. The treatments were conducted on Fuyutoyo cabbage plants grown in 20L polyethylene pots maintained in a greenhouse. The insecticides were sprayed at pre-heading stage, 45 days after the plants were transplanted to the pots.

The following treatments in g or ml of commercial product $100L^{-1}$ of water were applied with a 2L manually-pressurized sprayer to the leaves of cabbage plants until the leaves were wet: Dipel (***B. thuringiensis*** var. kurstaki) (500g); Agree (***B. thuringiensis*** var. aizawai GC 91, transconjugated (hybrid) with toxins of ***B. thuringiensis*** var. kurstaki) (125g, 250g and 500g); diflubenzuron (Dimilin 25PM) (100g); deltamethrin (Decis 25CE) (30ml) and a control treatment sprayed only with water. A preliminary experiment showed that the inclusion of the adjuvant Agral to the solutions did not improve the efficiency of the insecticides. Based on the results obtained in the first experiment, another one was conducted to evaluate a lower concentration of Agree (125g $100L^{-1}$ of water) and the inclusion of a neurotoxic pyrethroid, deltamethrin (30ml $100L^{-1}$ of water). Each treatment consisted of 10 replications (plants) and after the leaves had dried, two hours after spraying, one leaf was removed from each plant and placed in a 15cm diameter Petri dish lined with a paper towel to offer the larvae. Mortality was assessed on first and third instar larvae. Ten larvae were placed on each treated leaf which together with an untreated control were kept in a climatic chamber at 20±1°C, 12:12 hours D:L photophase and relative humidity of 60±10%. After three, six, 10 and 15 days after application (DAA) a new set of leaves were collected from the previously treated plants and exposed to another set of larvae of similar ages to assess the residual effect of the insecticides. Due to the slower action of entomopathogenous insecticides, evaluation of mortality was assessed during four days after exposure of the larvae to the treated leaves.

In order to compare the residual effect of the insecticides between plants grown outdoor under the natural weather conditions (precipitation, wind and solar radiation) and plants kept indoor another experiment employing a similar procedure was carried out and larval mortality was compared between plants maintained under the two environmental conditions.

The experiments were conducted in a completely randomized design, in a factorial arrangement with four (insecticides) x five (residual times) treatments. Analysis of variance (ANOVA) was used to compare treatment and time effects within each experiment. Means classification were performed using Tukey's least significant difference test (P<0.05). Treatment efficiencies were compared by correcting mortalities through Abbott's formula (ABBOTT, 1925). Normality and homogeneity of variance of the data was checked using Shapiro-Wilk's test and Levene's test, respectively.

RESULTS AND DISCUSSION

Agree and Dipel were more efficient than diflubenzuron for first instar larvae (F=153.7; P<0.001; df=3) (Table 1). The toxicity of Dipel to third instar larvae decreased from 73.4% on the third day after application to 36.7% after 15 days post treatment (Table 1). For Agree no statistical differences were recorded among the intervals after application, reaching a highest value of 89.0% mortality in the sixth day after application. First instar larvae of ***P. xylostella*** were significantly more susceptible to Agree and Dipel than to diflubenzuron for all evaluation periods after treatment (F=2.2; P=0.071; df=4).

On third instar larvae, the toxicity of both formulations of ***B. thuringiensis*** was higher than on first instar larvae (Table 2). During the first instar, the larvae develop endophytically, a condition of low exposure of the larvae on the treated surface, whereas in the third instar the larvae remain on the leaf surface and ingest a higher amount of insecticide than first instar larvae, as observed by CARVALHO et al. (2010).

There was a significant difference among the insecticides used against third instar larvae (F=2.4; P<0.001; df=3), as well as among the residual periods evaluated (F=467.9; P=0.048; df=4). The toxicity of Dipel to third instar larvae dropped from 85.0% on the sixth day after application to 56.8% after 15 days post treatment (Table 2). For Agree the highest mortality was recorded 10 days after application (90.0%) while the lowest rate of control was recorded on the day of application (69.0%). The effect of diflubenzuron did not differ from the untreated control and the corrected mortality was below 20% in all residual periods evaluated (Table 2).

According to ARONSON et al. (1986), the symptoms of larval intoxication appear soon after the ingestion of the toxic proteins of ***B. thuringiensis*** and thus damage is reduced even before larval death due to the penetration of the spores through the perithrophic membrane lining the mesenteron.

Table 3 shows third instar mortality in plants exposed to natural weather conditions and those kept inside a greenhouse after insecticide application. During the experimental period, plants were exposed to solar radiation and rain (46.8mm). Like diflubenzuron in the previous experiment, deltamethrin did not provide effective control of the larvae, even when plants were kept inside the greenhouse (Table 3). CASTELO BRANCO et al. (2001) recorded a complete failure of deltamethrin to control larvae of ***P. xylostella***, with less than six percent of mortality, indicating a possible occurrence of resistance to this product.

All treatments with Agree were highly efficient up to ten days after application, except the lower concentration after being kept under natural conditions for 10 days (Table 3). After 15 days of insecticide spray, it was found that all treatments had lost their efficiency (F=39.7; P<0.001; df=15). The daily mortality of larvae during four days after residues 0, 10 and 15 days old shows the gradual loss of persistency of the lower dosage of Agree both in relation to the age of the residue and to the effect of exposing the treated plants to solar radiation and rain (Figure 1). The higher concentration of Agree only lost its efficiency 15DAA, with a drastic reduction in comparison to shorter residual periods, even in plants protected from rain and solar radiation. CASTELO BRANCO (1999) obtained 97% mortality of second instar ***P. xylostella*** in laboratory tests with ***B. thuringiensis*** var. kurstaki (500ml ha^{-1}) and aizawai (350ml ha^{-1}). However when the same treatments were applied in the field, they were not efficient against larvae and the failure in the

Table 1 - Mean (±EP) mortality (%) of first instar larvae of ***Plutella xylostella*** after exposure for four days to cabbage leaves containing insecticide residues up to 15 days after application.

	Residual period (days after treatment) [1]				
Treatment	Zero[1]	Three	Six	Ten	Fifteen
Control	7.0±1.53Ac	8.0±1.33Ac	8.0±1.33Ac	8.0±1.33Ac	8.0±1.33Ac
Agree	72.10±7.90Aa	78.9±8.68Aa	89.0±4.06Aa	81.8±6.12Aa	83.9±5.43Aa
Dipel	63.7±6.40Aa	73.4±9.27Aa	65.0±4.43Ab	72.9±5.19Aa	36.7±6.21Bb
Diflubenzuron	33.4±4.81Ab	36.2±7.84Ab	55.8±6.77Ab	42.1±6.52Ab	54.2±4.41Ab

[1] Means followed by the same low case letters in the columns and upper case letters in the rows do not differ according to Tukey's test (P>0.05).

Table 2 - Mean (±EP) mortality (%) of third instar larvae of ***Plutella xylostella*** after exposure for four days to cabbage leaves containing insecticide residues of different ages up to 15 days after application.

	---Residual period (days after treatment) [1]---				
Treatment	Zero	Three	Six	Ten	Fifteen
Control	31.0±2.33Ab	16.0±2.21Bb	15.0±1.67Bb	10.0±2.58Bb	12.0±2.91Bc
Agree	69.0±2.33Ba	84.0±2.21Aa	85.0±1.67Aa	90.0±2.58Aa	84.5±3.58Aa
Dipel	67.6±2.68ABa	79.0±4.89Aa	85.0±1.67Aa	84.3±4.31Aa	56.8±9.38Bb
Diflubenzuron	17.0±3.00Ac	13.0±2.60Ab	11.0±3.48Ab	12.9±5.96Ab	15.0±4.77Ac

[1] Means followed by the same lower case letters in columns and upper case in rows do not differ by Tukey's test (P>0.05).

control of the diamondback moth was attributed to the fast degradation of ***B. thuringiensis*** in natural weather conditions. HADDAD et al. (2005) evaluated the field persistence of ***B. thuringiensis*** and determined a half-life of 16.5 hours for the recommended dosage. One day after treatment, only 78% of the spores remained on the leaf surface. Our results show that in areas with less solar radiation the persistence of ***B. thuringiensis*** spores is higher than in tropical regions.

The results showed that the formulation of ***B. thuringiensis*** containing aizawai and kurstaki lineages is more efficient than the kurstaki lineage alone. Although high mortality was attained with concentrations as low as 125g of commercial product $100L^{-1}$ of water, the residual effect is shorter than 10 days, while the concentration of 500g of Agree was efficient for up to 15 days after application. The toxicity of deltamethrin to larvae of ***P. xylostella*** was significantly lower than that of ***B. thuringiensis***, even in the lower concentrations of the bioinsecticide evaluated.

The relatively high persistence of the toxic effects of ***B. thuringiensis*** in comparison to data available in the literature (CASTELO BRANCO, 1999; HADDAD et al., 2005) suggests that the performance of this bioinsecticide is less affected by weather conditions in the subtropics than in warmer tropical areas, where solar radiation is described as affecting the persistence of the spores of ***B. thuringiensis*** (POLANCZYK & ALVES, 2003).

The high efficiency of ***B. thuringiensis*** recorded in our experiments show that the lineage of ***P. xylostella*** occurring in Southern Brazil has not acquired resistance to this microbial insecticide, as reported by CASTELO BRANCO & GATEHOUSE (2001) in the Federal District, Brazil.

Considering the need for insecticide options to be employed in organic horticultural crops, the results showed that ***B. thuringiensis*** is a highly useful alternative for the control of ***P. xylostella***. The use of ***B. thuringiensis*** in greenhouses should be based on previous samplings that indicated the need for control measures. In closed environments the selection of resistant populations is

Table 3 - Mean (±EP) mortality of third instar larvae of ***Plutella xylostella*** in plants treated with insecticides and kept either inside a greenhouse or exposed to weather conditions outside the greenhouse for 15 days after insecticide application.

		---Residue age on the leaves (days)[1]---			
Treatment	E*	Zero[1]	Five	Ten	Fifteen
Control	I	10.0±5.48Aa	10.0±3.16Aa	18.0±8.00Aa	0.0±0.00Aa
Control	O	6.0±4.00Aa	12.0±2.00Aa	14.0±6.78Aa	2.0±2.00Aa
Deltamethrin	I	25.3±9.99ABa	48.9±8.86Aa	24.0±6.32ABa	22.0±12.41ABa
Deltamethrin	O	12.9±3.95Ba	26.8±8.86ABb	11.8±0.92Ba	2.0±2.00Bb
Agree 125g	I	90.0±5.48Aa	87.5±5.36Aa	71.2±12.77Aa	34.0±11.66Ba
Agree 125g	O	94.0±4.00Aa	79.1±3.52Aa	36.0±7.02Bb	2.0±2.00Bb
Agree 250g	I	90.0±5.48Aa	90.0±3.16Aa	78.6±9.59ABa	28.0±8.00BCa
Agree 250g	O	94.0±4.00Aa	85.8±2.59Aa	70.6±13.12ABa	12.9±6.53Ca
Means (±EP)	I	53.8±8.97a	59.1±7.93a	47.9±7.78a	21.0±4.99a
Means (±EP)	O	51.7±9.88a	50.9±7.70a	33.1±7.36b	4.7±2.01b

[1] Means followed by the same lower case letters in columns and upper case in rows do not differ by Tukey's test (P>0.05).
* E- Environment; I- Inside greenhouse; O- Outside greenhouse

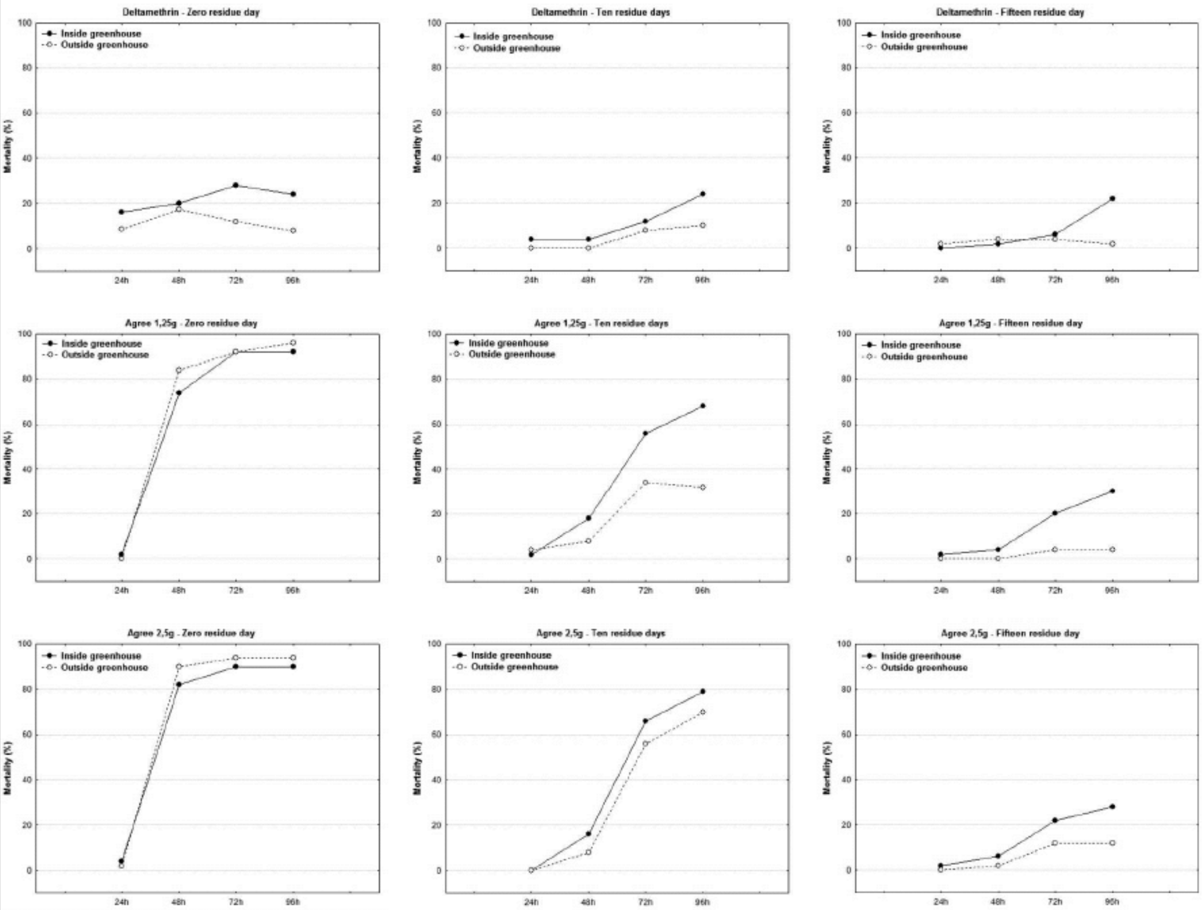

Figure 1 - Mortality of third instar larvae of ***Plutella xylostella*** to the insecticides during the exposition time in each of the three residual ages after spraying.

more frequent because the migration of susceptible individuals is reduced in comparison to open field populations (TABASHNIK et al., 2000). The presence of diamondback moth in brassicaceous plants can be monitored by means of pheromone traps (IMENES et al., 1999) to indicate the proper time for insecticide use. Natural enemies are not affected by ***B. thuringiensis*** (MONNERAT et al., 2000) and adult parasitoids walking on treated surfaces are able to find and parasitize larvae not intoxicated, thus complementing the action of the insecticide. Moreover, ***B. thuringiensis*** can be included in insecticide rotation programs especially in greenhouses, as an alternative to neurotoxic molecules to prevent or delay the appearance of resistant strains of ***P. xylostella*** (CASTELO BRANCO et al., 2003; RAYMOND et al., 2007).

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