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ORIGINAL ARTICLE

Failure control of *Plutella xylostella* (Lepidoptera: Plutellidae) and selectivity of their natural enemies to different insecticides

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Abstract

Control failure of pests and selectivity of insecticides to beneficial arthropods are key data for the implementation of Integrated Pest Management (IPM) programs. Therefore, the aim of this study was to assess the control failure likelihood of *Plutella xylostella* and the physiological selectivity active ingredients to parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) and to predators *Polybia scutellaris* (Hymenoptera: Vespidae) and *Lasiochilus* sp. (Hemiptera: Anthocoridae). In bioassays, *P. xylostella* larvae and *O. sokolowskii*, *P. scutellaris* and *Lasiochilus* sp. adults were used. Concentration-mortality curves of six insecticides for *P. xylostella* were established. These curves were used to estimate the mortality of *P. xylostella* at the recommended concentration, in order to check a control failure of insecticides to this pest. Furthermore, the lethal concentration for 90% of populations (LC_{90}) and the half of LC_{90} were used in bioassays with the natural enemies to determine the selectivity of these insects to insecticides. All tested insecticides showed control failure to *P. xylostella*, indicated by high LC_{90} and low estimated mortalities (less than 80%). The cartap insecticide was selective in half of LC_{90} to *Lasiochilus* sp. and moderately selective in LC_{90} and the half of LC_{90}, to *Lasiochilus* sp. and *P. scutellaris*, respectively. Deltamethrin was moderately selective in the half of LC_{90} to predator *Lasiochilus* sp. Cartap, carbaryl, and deltamethrin reduced the mortality of *Lasiochilus* sp. in the half LC_{90}. The results also showed that the insecticides methamidophos, carbaryl, parathion methyl and permethrin were not selective to any of the tested natural enemies. The role of insecticides in IPM systems of *Brassica* crops is discussed based on their control failures to *P. xylostella* and selectivity to their natural enemies.

Key words: *Brassica* crops, diamondback moth, insecticide selectivity, natural enemies, biological control

Introduction

The diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a cosmopolitan pest which causes severe damage to *Brassica* crops, e.g. in Brazil (França and Medeiros 1998; Castelo Branco and Amaral 2002), and in other countries (Talekar and Shelton 1993; Godin and Boivin 1998). The total annual expenses of worldwide management of *P. xylostella* has been estimated at approximately $4 billion (Zalucki *et al.* 2012). This pest can decrease the quality of cabbage by 58 to 100% (Castelo Branco and Guimarães 1990) and therefore, hinders the commercialization of this crop. Larvae of *P. xylostella* feed on cabbage leaves, reducing their commercial value and if no control methods are used, the damages are irreversible (Barros *et al.* 1993). This has prompted an increased interest in the development of integrated pest management programs for the species (Furlong *et al.* 2013).

Insecticide control is the major method of suppression of *P. xylostella* damage (Furlong *et al.* 2013). However, the indiscriminate use of insecticides may pose risks to human health and the environment (Pimentel 2005). Furthermore, pesticide applications prove counterproductive because they kill beneficial organisms such as natural enemies of pests (Rahmani and Bandani 2016; Stanley *et al.* 2016) and increase the chances of developing pest resistance to pesticides (Guedes 1999).

The evolution of insecticide resistance in pests has been focused upon for Integrated Pest Management programs (IPM) on several crops. This problem is particularly serious for *P. xylostella* because several groups of insecticides have often shown control failures to this species, indicating their great ability to develop resistance (Vestrad *et al.* 2004; Santos *et al.* 2011; Troczka *et al.* 2017). For this reason, monitoring *P. xylostella* populations for insecticide control failures is extremely important for the application of effective pest and resistance management strategies.

Furthermore, when monitoring insecticide control failures of crop pests, the conservation of natural enemies is fundamental to IPM programs in agricultural systems. Natural enemies are key components of an IPM system because they counteract insecticide-resistant pests and minimize the use of pesticides. However, the use of broad-spectrum insecticides has negatively affected the biological control in several agricultural systems with significant effects on the survival, longe-Bopapevity, fecundity, reproduction, and behavior of natural enemies (Naranjo 2001; Bopape *et al.* 2014; Rahmani and Bandani 2016). Therefore, the selection of insecticides should be based on their effectiveness against the target pest and on the safety of non-target organisms, including natural enemies. Understanding the effectiveness against pests and the potential impact of insecticides on natural enemies is important for successful integration of chemical and biological control measures.

Among the biological control agents of *P. xylostella*, parasitoids (Labou *et al.* 2016), predator wasps (Silva-Torres *et al.* 2010) and predator bugs (Eigenbrode *et al.* 1996) are important for the biological control of this pest. *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) is a parasitoid of larvae and pupae on several *Brassica* crops around the world (Wang *et al.* 1999; Cordero *et al.* 2007). This parasitoid has great potential for use in biological control programs of *P. xylostella*, due to its great parasitism rate which can be 100% in some cases (Zu-Hua *et al.* 2003). In Brazil, the wasp *Polybia scutellaris* (White) (Hymenoptera: Vespidae) and the bug *Lasiochilus* sp. (Hemiptera: Anthocoridae) have often been found on *Brassica* crops infested by *P. xylostella*, indicating the function of these natural enemies in the regulation of population dynamics of this pest.

Thus, to develop the IPM of *Brassica* crops it is necessary to monitor insecticide control failure to *P. xylostella* and choose insecticides which are selective to natural enemies of this pest. Thus, we aimed in this study to: 1) obtain concentration-mortality curves for six insecticides used to control *P. xylostella*, 2) determine the toxicity of six insecticides to *P. xylostella*, 3) study the physiological selectivity of insecticides to natural enemies: *O. sokolowskii*, *P. scutellaris*, and *Lasiochilus* sp.

Materials and Methods

Insects

Plutella xylostella larvae were obtained from mass rearing colonies maintained in the laboratory of Integrated Pest Management of the Federal University of Viçosa, Brazil. Adults of the parasitoid *O. sokolowskii* and adults of the predators *P. scutellaris* and *Lasiochilus* sp. were collected on *Brassica* crops in Viçosa, Minas Gerais state, Brazil. The insect colonies in the laboratory were placed under conditions of 25 ± 2°C, relativity humidity (RH) 75 ± 5% and a photoperiod of 12 : 12 h (L : D).

Bioassays

Insecticides used in bioassays are usually recommended for the control of *P. xylostella* in Brazil, and are listed in Table 1. Three concentrations of each insecticide were used in order to verify mortality rates greater than zero and less than 100%.

Kale leaves (*Brassica oleracea* L. var. *acephala*) were immersed for 5 s in an insecticide solution and shade-dried for 2 h. The leaves were subsequently placed in Petri dishes (9 cm diameter × 2 cm height), covering the entire bottom of the dish. Ten 3rd instar larvae were placed in each Petri dish, which was put in a rearing chamber at 25 ± 0.5°C, 75 ± 5% RH and 12 h photoperiod. Insect mortality was assessed after 24 h of exposure and the insects were considered dead if unable to walk when prodded with a fine paintbrush. Three concentrations of each insecticide were used to verify mortality rates greater than zero and less than 100%. After defining the concentration-mortality range, six more intermediate concentrations were used. Four replicates were used for each concentration.

Table 1. Insecticides used in bioassays of toxicity with third instar larvae of *Plutella xylostella* adults of *Oomyzus sokolowskii*, *Polybia scutellaris* and *Lasiochilus* sp.

Active ingredient [a.i.]	Commercial name	Chemical group	Recommended concentration [mg a.i. · ml^{-1}]*
Carbaryl	Carbaril 850 PM	carbamate	1.275
Cartap	Cartape 500 PM	nereistoxin	0.60
Deltamethrin	Decis 25 CE	pyrethroid	0.60
Methamidophos	Hamidop 600 CE	organophosphate	0.10
Parathion methyl	Folidol 600 CE	organophosphate	0.0075
Permethrin	Ambush 500 CE	pyrethroid	0.60

*recommended dose to control *Plutella xylostella* in Brazil

Concentration-mortality curves of insecticides to *P. xylostella* were constructed. These curves were used to verify the mortality at recommended concentrations. Additionally, LC_{90} and the half of LC_{90} were determined. The lethal concentration (LC_{90}) was chosen because this is a concentration above the minimum efficacy threshold for insecticides in Brazil (i.e., 80% efficacy) and the half of LC_{90}, for simulating decomposition of an insecticide in the field.

In another bioassay, adults of the parasitoid *O. sokolowskii* and adults of the predators *P. scutellaris* and *Lasiochilus* sp. were submitted to LC_{90} and the half of LC_{90} obtained for *P. xylostella* in the previous bioassay. Then, adults of these insects also were transferred to Petri dishes which contained kale leaves treated with insecticides (as described above), and a plastic container (1.5 cm diameter and 1.0 cm high) with a solution of honey + water at proportions of 1 : 1. The dishes were maintained under the same conditions described above and four replicates also were used for each insecticide.

Data analyses

Results of *P. xylostella* mortality were corrected according to Abbott's formula (Abbott 1925). For the first bioassay, concentration-mortality results were subjected to Probit analysis correcting the data for natural mortality (PROC PROBIT) (SAS Institute 2001). Curves were accepted if the null hypothesis was accepted at the 5% of probability, according to the χ^2 test (Young and Young 1998). LC_{90} and the half of LC_{90} were obtained from these curves.

Results of natural enemy mortality were transformed in arcsin $(x/100)^{0.5}$ and submitted to analysis of variance and their averages were compared using the Scott-Knott test at $p < 0.05$. Insecticides which caused more than 70% mortality in natural enemies were considered as non-selective, those which caused 30–70% mortality were considered moderately selective and those which caused less than 30% mortality were considered selective (Bacci *et al.* 2006).

Results and Discussion

Insecticide toxicity to *P. xylostella*

Concentration-mortality curves to *P. xylostella* of insecticides carbaryl and deltamethrin had the highest slope, while the curves of parathion methyl had the lowest (Fig. 1 and Table 2). Alterations in dosages of carbaryl and deltamethrin caused high variations in mortality of *P. xylostella*, compared to parathion methyl. This signifies that a wrong calibration of sprays during applications of parathion methyl would be less effective on the mortality of *P. xylostella*. However, this insecticide used in the recommended concentration would kill this pest less under field conditions according to the estimated mortality found (E.M. = 7.54%) (Table 2).

All insecticides had an estimated mortality (0.0 to 72.15%) lower than the expected mortality of *P. xylostella* (Table 2). This shows the occurrence of control failure of the tested insecticides to *P. xylostella* since for registration of an insecticide in Brazil by the Ministry of Agriculture, the minimum level of efficiency required is 80% (Bacci *et al.* 2007). On these bioassays, *P. xylostella* was submitted to extreme conditions of insecticide exposure. In the field, mortalities may be lower, because the insecticides degrade after the applications. This shows that in the field, the probability of insecticide control failures to *P. xylostella* may be even greater (Talekar and Shelton 1993; Vastrad *et al.* 2004; Bautista *et al.* 2007).

Cartap and methamidophos had the lowest LC_{90} and E.M. (Table 2), showing that these insecticides were the most efficient for the control of *P. xylostella*. However, the efficacy of these insecticides in the field (mortality higher than 80%) depends on increasing the concentration 1.72 and 2.07 times, respectively.

Insecticide selectivity to *O. sokolowskii*, *P. scutellaris*, and *Lasiochilus* sp.

The LC_{90} and the half of LC_{90} of insecticides methamidophos, carbaryl, parathion methyl and permethrin

Table 2. Regression equations, lethal concentrations (LC_{90}), half of LC_{90}, Chi-square (χ^2) and probability (p) of concentration-mortality curves and estimated mortality (E.M.) [%] of six insecticides for *Plutella xylostella*

Insecticide	Regression equations[1]	Concentrations [mg a.i. · ml^{-1}][2]		χ^2	p	E.M.[3] [%]
		LC_{90}	half of LC_{90}			
Carbaryl	Y′ = 1.66 + 3.78x	16.571	8.286	5.31	0.506	0.17
Cartap	Y′ = 6.06 + 2.30x	1.243	0.622	7.47	0.187	70.92
Deltamethrin	Y′ = 5.85 + 3.62x	1.317	0.659	7.69	0.173	0.00
Methamidophos	Y′ = 6.24 + 2.95x	1.030	0.515	5.89	0.051	72.15
Parathion methyl	Y′ = 3.98 + 1.88x	16.784	8.392	11.17	0.192	7.54
Permethrin	Y′ = 5.47 + 3.07x	1.838	0.919	6.25	0.099	0.47

[1]Y = mortality in probit; x = log of concentrations [mg a.i. · ml^{-1}]
[2]doses used in bioassays with natural enemies
[3]mortality for each insecticide was estimated by replacing the recommended concentration for control of *P. xylostella* (see Table 1) in each concentration-mortality curve

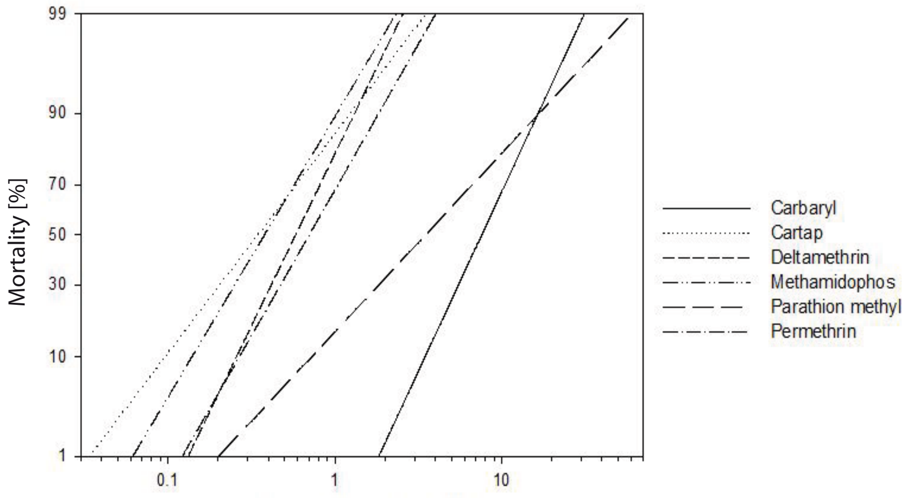

Fig. 1. Concentration-mortality curve of six insecticides used in bioassays with third instar larvae of *Plutella xylostella*

were not selective to parasitoid *O. sokolowskii* and to predators *P. scutellaris* and *Lasiochilus* sp. The insecticides cartap and deltamethrin also were highly toxic to *O. sokolowskii* in both concentrations (Fig. 2). Cartap was not selective in LC_{90}, but was moderately selective in the half of LC_{90} to *P. scutellaris*. This insecticide had a moderate selectivity in LC_{90} and was selective in the half of LC_{90} to *Lasiochilus* sp. Deltamethrin was moderately selective to *Lasiochilus* sp. in LC_{90}, but was not selective to *P. scutellaris* in both concentrations (Fig. 2).

The probable mechanism of the physiological selectivity of the cartap was not elucidated because no biochemical and physiological studies were developed. The physiological selectivity may be associated with a lower penetration into the integument of insects (Guedes 1999), a change in the site of insecticide action or the high rate of activity of the detoxifying enzyme (Yu 1988; Halappa and Patil 2016). The penetration rate of the insecticide into the integument of insects depends on the insecticide affinity and the thickness and chemical composition of the cuticle (Leite *et al.* 1998).

The cartap metabolism by enzymes of the cytochrome P450-dependent monooxygenases system may be associated with the selectivity of this compound to *P. scutellaris* and *Lasiochilus* sp. These enzymes usually detoxify lipophilic compounds, transforming them into polar metabolites which allow their excretion (Brattsten *et al.* 1986). This hypothesis is based on the fact that the cytochrome P450-dependent monooxygenases are the main enzymes involved in metabolic mechanisms that reduce the insecticide toxicity to insects (Guedes 1999). Changes in the acetylcholinesterase enzyme into the bodies of predators *P. scutellaris* and *Lasiochilus* sp. and the fast catalyzing of the hydrolysis of the neurotransmitter acetylcholine by the enzyme may also be responsible for the selectivity of cartap to these insects (Silver *et al.* 1995).

Mortalities of *O. sokolowskii*, *P. scutellaris* and *Lasiochilus* sp. caused by the half of LC_{90} of methamidophos, parathion methyl and permethrin and mortalities of *O. sokolowskii* and *P. scutellaris* caused by the half of LC_{90} of cartap, carbaryl, and deltamethrin were not lower than mortalities caused by their LC_{90} of these insecticides (Fig. 2). This shows that besides the high impact of these insecticides during application, this effect persists even after the decomposition of half of the active ingredients. Moreover, the insecticides cartap, carbaryl, and deltamethrin reduced *Lasiochilus* sp. mortality when the half of LC_{90} was used (Fig. 2). Therefore, we suppose that the mortality of this predator caused by these insecticides is high at the time of application, but it reduces after the break down to half of the initial concentrations of the active ingredients in the environment.

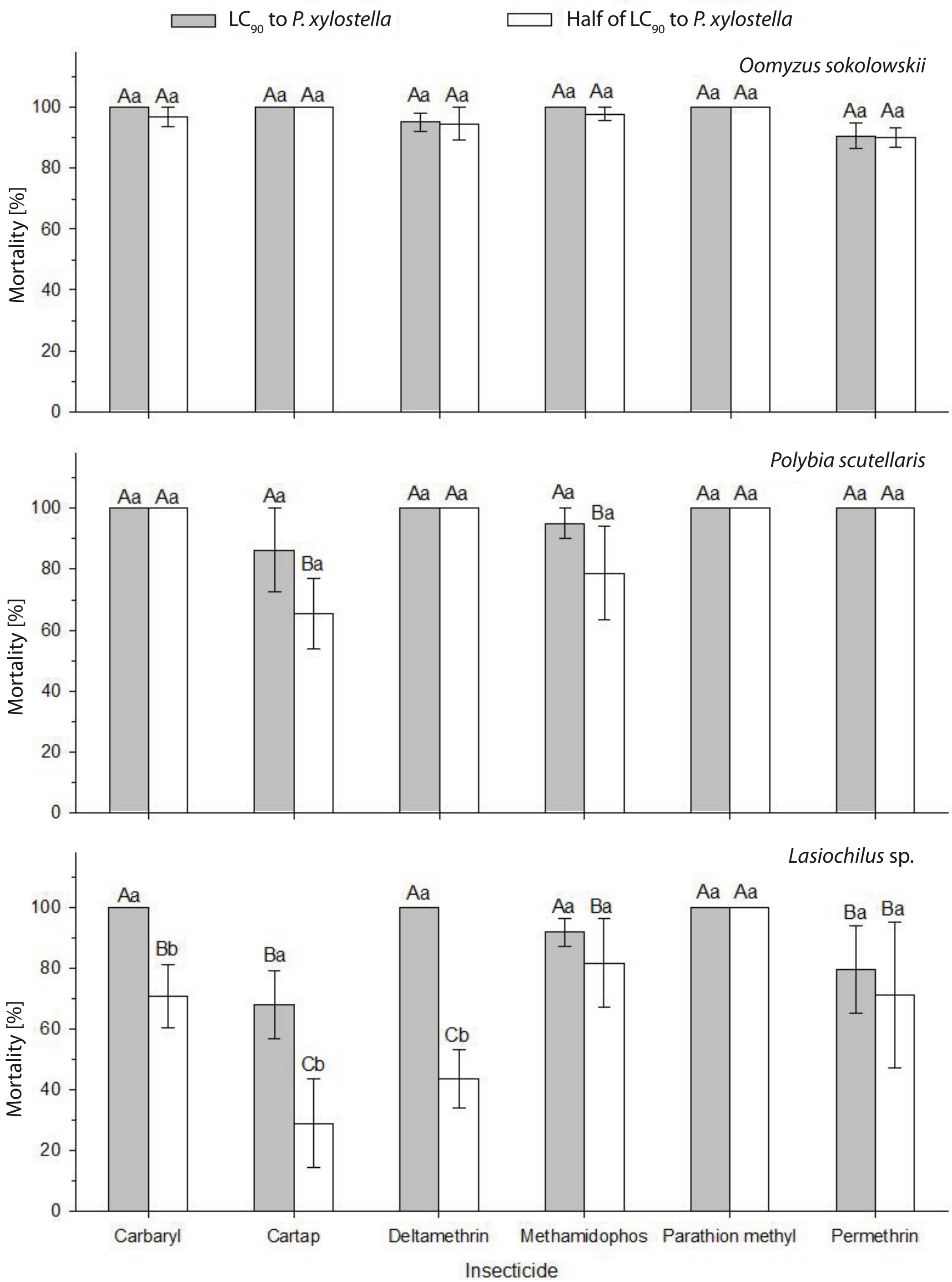

Fig. 2. Mortality of adults of *Oomyzus sokolowskii*, *Polybia scutellaris* and *Lasiochilus* sp. caused by to LC_{90} and the half of LC_{90} of six insecticides used in control of *Plutella xylostella*. Averages with the same letter do not significantly differ by the Scott-Knott test ($p < 0.05$). Capital letters are for comparing the insecticide selectivity in the same concentration and small letters are for comparing the reducing of insecticide in toxicity selective in the half of LC_{90}. Vertical bars = standard error

Both concentrations of cartap and to the half of LC_{90} carbaryl, deltamethrin, and permethrin showed selectivity to predator *Lasiochilus* sp. (Fig. 2). The mechanisms that provide selectivity of these insecticides to *Lasiochilus* sp. may be associated with lower penetration rates into the integument, higher metabolism rates of the compound and/or changes in the site of action of insecticides. The selectivity of pyrethroid deltamethrin and permethrin to *Lasiochilus* sp. may be due to microsomal oxidase enzymes and esterases, as well as changes in their sodium channels (Yu 1988; Leng and Xiao 1995). Changes in the sodium channels affecting the sensitivity of the enzyme (Na–K)-ATPase and Mg_2-ATPase may also be responsible for reducing the neurotoxic action of this insecticide (Zhao *et al.* 1992; Leng and Xiao 1995).

In summary, all tested insecticides (high LC_{90}) showed control failure to *P. xylostella*. The insecticides, methamidophos, carbaryl, parathion methyl and permethrin, were not selective to parasitoid *O. sokolowskii* and to predators *P. scutellaris* and *Lasiochilus* sp. The cartap insecticide was moderately selective to the *Lasiochilus* sp. and to the *P. scutellaris*. Deltamethrin was moderately selective in the half of LC_{90} to predator *Lasiochilus* sp. The role of insecticides in IPM systems of *Brassica* crops is discussed based on the control failure likelihood to *P. xylostella* and selectivity to their natural enemies.

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