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Growth regulator insecticides for the control of the lesser mealworm beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

Insecticidas reguladores del crecimiento para el control del escarabajo de la cama *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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Abstract: The purpose of this study was to evaluate the efficiency of the insecticides methoxyphenozide, teflubenzuron, pyriproxyfen, chlorantraniliprole and also *Azadirachta indica* in the control of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). The study was carried out in the Microbial Control laboratory of Universidade Estadual Londrina (Brazil) and consisted of two bioassays. In the first, *A. diaperinus* larvae were fed with corn feed treated with the insecticides in seven dosages. In the second bioassay, the different treatments were sprayed on the larvae only at the three highest dosages. Evaluations were performed daily, quantifying larval mortality, and on the 30th day, the number of pupae and adults were evaluated. Teflubenzuron, chlorantraniliprole and *A. indica* were responsible for the greatest percentage of mortalities in larvae fed with treated feed. The treatments with methoxyphenozide and pyriproxyfen caused the lowest mortalities; however, with pyriproxyfen the larvae were not transformed into pupae and adults up to the 30th day of assessment. The treatment with teflubenzuron showed the greatest toxicity, with the lowest survival time (ST_{50}) When the products were applied directly on to the larvae, the most efficient treatments were teflubenzuron, chlorantraniliprole and *A. indica*; however, there was a reduction in the mortality percentages with teflubenzuron and chlorantraniliprole in relation to the feed bioassay. For *A. indica*, the application method did not affect the mortality levels. Pyriproxyfen, which had not led to mortality through feed ingestion, obtained an increase of up to 30% after being applied directly on to the larvae.

Key words: Growth regulator insecticides. Chlorantraniliprole. *Azadirachta indica.* Toxicity. Survival analysis.

Resumen: El objetivo del trabajo fue evaluar la eficiencia de los insecticidas metoxifenozide, teflubenzuron, piriproxifeno, clorantraniliprole y *Azadirachta indica* en el control del escarabajo de la cama *Alphitobius diaperinus*. Se realizaron dos bioensayos en el laboratorio del control microbiano de la Universidade Estadual Londrina (Brasil). En el primero, las larvas de *A. diaperinus* fueron alimentadas con pienso de maíz tratado con los insecticidas en siete dosis (i.e. dosis recomendada - DR y porcentajes del 50; 25; 12,5; 6,25; 3,12 y 1,65 de la misma). En el segundo bioensayo, lós diferentes tratamientos fueron aplicados en las larvas pero sólo en las tres más altas dosis. La cuantificación de la mortalidad de larvas se hizo diariamente hasta el día 30, cuando se evaluó el número de pupas y adultos. Las mayores mortalidades de larvas se presentaron en larvas alimentadas con raciones con teflubenzuron, clorantraniliprole y *A. indica*. Los tratamientos con metoxifenozide y piriproxifeno causaron menor mortalidad, sin embargo, con este último, las larvas no alcanzaron a formar pupas ni adultos. Teflubenzuron presentó la mayor toxicidad con menor TL_{50}. Cuando los productos fueron aplicados directamente en las larvas, los tratamientos más eficientes fueron teflubenzuron, clorantraniliprole y *A. indica*, sin embargo, hubo reducción en los porcentajes de la mortalidad con teflubenzuron y clorantraniliprole. Para *A. indica*, el método de aplicación no interferió con los niveles de mortalidad. Piriproxifeno, que no había causado mortalidad en el consumo de ración, aumentó hasta el 30% cuando fue aplicado a la larva.

Palabras clave: Insecticidas reguladores del crecimiento. Clorantraniliprole. *Azadirachta indica*. Toxicidad. Análisis de supervivencia.

Introduction

The lesser mealworm *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae) is considered the main pest in poultry houses around the world, found in large quantities in poultry litter and manure (Pfeiffer and Axtell 1980; Lambkin *et al.* 2007). Its presence is also observed in the compacted soil floor of broiler houses and it may reach a depth of 0.8 m (Chernaki-Leffer *et al.* 2001). Moreover, it can be found under feeders, feeding on chicken feed and also consuming dead or moribund chicks (Axtell and Arends 1990).

This pest can be a source and vector of several pathogens like bacteria, viruses, fungi, protozoa and platyhelminthes parasites that cause harmful diseases to poultry and humans (Despins and Axtell 1995; Mcallister *et al.* 1995; Goodwin and Waltman 1996; Chernaki-Leffer *et al.* 2002; Vittori *et al.* 2007). Also, *A. diaperinus* can be harmful to poultry, since the poultry can consider it to be an alternative source of food, but eating it may result in lesser weight gain compared to those poultry which feed on the nutrient-balanced feed (Axtell and Arends 1990; Matias 2000).

The expansion of the poultry industry and the current breeding systems contributed to the development of an ideal habitat for lesser mealworms. These systems reuse the chicken litter with each lot exchange, providing the environment with suitable temperature and moisture for rapid population growth and the spread of new areas of infestation from one lot to another (Salin 2000).

The difficulty in controlling this pest leads to significant economic losses and sanitary problems in poultry production.

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Hence studies are encouraged to obtain solutions for the management of its populations (Bates *et al.* 2004).

The control of *A. diaperinus* populations is done through environment management, such as frequent cleaning of the poultry house and removal of the litter after each lot; however, it is an expensive and laborious activity (Axtell and Arends 1990). Another method is the use of chemical products at the end of the poultry cycle such as: pyrethroids (Hamm *et al.* 2006); organophosphorus compounds, chlorates and carbamates (Morales 1991); macrocyclic lactones (Miller 1990); and boric acid (Dufor *et al.* 1992). One negative result is the resistance factors directly multiply with numbers of insecticide applications (Lambkin and Rice 2006).

In addition to these insecticides, other methodologies are being studied for the control of the lesser mealworm, such as bioinsecticides and products with a differentiated mode of action. In this context, insect growth regulator (IGR) insecticides are an alternative because they act in a more specific way and they are less toxic to mammals (Silva and Mendes 2002). In the IGR group, the benzoylphenyl ureas (e.g., teflubenzuron), the diacyl-hydrazines (e.g., methoxyfenozide) and pyridyloxypropyl ether (pyriproxyfen) stand out.

The benzoylphenylureas (chitin synthesis inhibitors) are active during larvae ecdysis, specifically affecting chitin deposition, preventing it from secreting a new cuticle and freeing it from the exocuticle (Silva *et al.* 2003). These products have already been cited as efficient in lesser mealworm control (Weaver 1996). The diacyl hydrazines act as ecdysone antagonists, promoting acceleration in the ecdysis process (Dhadialla *et al*. 1998; Omoto 2000). The juvenoids (pyridyloxypropyl ether) are juvenile hormone analogues and cause development disorders in the insects (Ferreira 1999).

Azadirachtin, a botanical compound extracted from the neem plant, *Azadirachta indica* A. Juss. (Meliaceae), is also considered to be a growth regulator because it is active in ecdysis, probably competing with ecdysteroids at the larvae's receptor site (Sharma 1992; Dev and Koul 1997; Adel and Sehnal 2000).

The anthranilic diamides (e.g., chlorantraniliprole), even though they are not included in the IGR group, exhibit a different mode of action against the insects. They are ryanodine receptor activators and cause insects to lose control of muscular activity (Cordova *et al.* 2006).

This new generation of insecticides may be considered as an alternative in pest control because they have more specific action and cause less toxicity to warm-blooded animals. Edwards and Abraham (1985) observed low toxicity to vertebrates of some IGR in their study, such as methoprene and fenoxycarb, and suggested that these compounds could be administered via feed in poultry raising, remaining biologically active even after passing through the poultry digestive tract.

The purpose of this study was to evaluate the effect of growth regulator insecticides (methoxyphenozide, teflubenzuron, pyriproxyfen), chlorantraniliprole and *A. indica* in the control of *A. diaperinus* larvae.

Materials and methods

Insects. Chicken litter containing *A. diaperinus* larvae was collected in a commercial poultry house in Londrina, Paraná. This material was taken to the laboratory where the larvae were separated from the litter and from the adult insects for later use in the bioassays.

Insecticide. The active ingredients evaluated were methoxyfenozide (INTREPID®), teflubenzuron (NOMOLT®), pyriproxyfen (TIGER®), chlorantraniliprole (PREMIO®) and *A. indica* (NIMAZAL®). Since there was no reference to their use for *A. diaperinus* control, the dosages used were based on the highest recommended dosage (RD) for other pests, obtained from the instructions of the commercial products (Agrofit 2012). The commercial products were diluted in distilled water to obtain seven different concentrations of each active ingredient (recommended dosage-RD, and variables percentages of this RD - i.e. 50; 25; 12.5; 6.25; 3.12 and 1.56).

Bioassays. The bioassays were run in the Microbial Control laboratory of Universidade Estadual Londrina (Brazil) with 4th instar (± 0.5 cm) *A. diaperinus* larvae (Silva *et al.* 2005). Two bioassays were performed, evaluating indirect and direct contact with the products. For the first assay, called "Insecticides Applied on the Feed", 8 mL of each concentration were sprayed on 12 g of sterilized corn feed with the airbrush sprayer connected to a Fanen-Diapump vacuum pump-compressor at a pressure of 0.8 kgf.cm^{-}1. The dosage in ppm of active ingredient for grams of feed was 1000 ppm for methoxyfenozide, 350 ppm for teflubenzuron, 233 ppm for chlorantraniliprole, 133 ppm for pyriproxyfen and 120 ppm for *A. indica.* After its complete homogenization, it was distributed in six acrylic boxes (2.5 cm diameter x 1.5 cm height) composed of 20 PET bottle caps glued side-by-side. Into these boxes, the larvae were subsequently individualized to avoid cannibalism.

In the second assay, called "Insecticides Applied on *A. diaperinus* Larvae", the insecticide solutions were sprayed directly on the larvae. The products used were the same as the previous assay; however, they were tested only at the three highest doses (RD, and percentages of this RD - i.e. 50; 25). Twenty 4th instar larvae, in a petri dish were sprayed with 0.5 mL. Them they were individualized in the caps and fed with sterilized corn feed.

For each bioassay, six replications with 20 larvae were performed, for a total of 120 larvae per treatment, plus a control group where the insecticides were substituted by distilled water. The insects were kept in an incubator (25 ± 1 ºC, 12-hour photophase and relative humidity of 75 ± 10%) for 30 days. Mortality was assessed daily, and the number of live larvae, pupae and adults was determined only on the 30th day of evaluation.

Statistical analysis. Mortality data were analyzed by probit analysis (Finney 1971) (POLO-PC, Leora Software 1987) to obtain the median lethal concentration (LC_{50}) its 95% confidence intervals and the slopes of dose-mortality curves. The median survival times (ST_{50}) for all products were estimated using the Kaplan-Meier Product-Limit estimator method (JMP, SAS Institute 2008) and were compared using the log-rank test (Kabfleisch and Prentice 1980). The data of larvae mortality, live larvae, pupae and adults, in percentage, did not meet the requirements for a parametric test; thus, they were subjected to the Kruskal-Wallis test and the mean rank values compared by the Dunn test at 5% probability (Ayres *et al.* 2007).

Table 1. Mortality of larvae, live larvae, pupae and adults (%) of *Alphitobius diaperinus* (4th instar) thirty days after ingestion of the feed treated with different insecticides at different dosages (Londrina, Brazi).

Dosages		Products											
		Methoxyphenozide		Teflubenzuron		Chlorantraniliprole		Pyriproxyfen		Azadirachta indica		Control	
1.56RD	Mortality	14.24 ± 7.65	b*	98.96 ± 1.04	a*	28.33 ± 6.55	ab*	4.31 ± 1.56	b*	23.34 ± 6.50	ab*	2.56 ± 2.56	b
	Pupae	6.23 ± 3.32	abc	0.00 ± 0.00	c	9.39 ± 1.54	abc	0.00 ± 0.00	c	11.38 ± 2.40	ab	15.44 ± 3.33	a
	Adults	31.09 ± 5.95	ab	1.04 ± 1.04	c	22.06 ± 4.38	abc	0.83 ± 0.83	bc	34.04 ± 5.65	ab	43.80 ± 7.26	a
	Live larvae	48.44 ± 8.49	ab	0.00 ± 0.00	c	40.22 ± 4.45	abc	94.85 ± 1.83	a	31.25 ± 4.91	bc	38.19 ± 6.74	abc
3.12RD	Mortality	6.37 ± 1.63	b	96.21 ± 2.02	a	34.88 ± 5.95	ab	4.65 ± 2.29	b	32.14 ± 2.74	ab	2.56 ± 2.56	b
	Pupae	4.66 ± 1.68	ab	0.83 ± 0.83	b	2.50 ± 1.71	ab	0.00 ± 0.00	b	10.54 ± 3.62	ab	15.44 ± 3.33	a
	Adults	26.67 ± 9.23	ab	0.00 ± 0.00	b	5.13 ± 2.69	ab	0.83 ± 0.83	b	27.27 ± 6.46	a	43.80 ± 7.26	a
	Live larvae	62.31 ± 8.93	ab	2.96 ± 2.09	c	57.49 ± 3.64	ab	94.52 ± 2.10	a	30.05 ± 3.62	bc	38.19 ± 6.74	bc
6.25RD	Mortality	8.08 ± 3.76	bc	96.90 ± 2.22	a	69.29 ± 4.99	ab	2.59 ± 1.16	c	42.82 ± 5.36	abc	2.56 ± 2.56	c
	Pupae	2.97 ± 1.34	ab	0.88 ± 0.88	b	0.83 ± 0.83	b	0.00 ± 0.00	b	6.31 ± 1.55	ab	15.44 ± 3.33	a
	Adults	14.41 ± 8.32	ab	2.22 ± 2.22	b	0.00 ± 0.00	b	0.00 ± 0.00	b	11.32 ± 2.67	ab	43.80 ± 7.26	a
	Live larvae	74.54 ± 9.32	ab	0.00 ± 0.00	c	29.88 ± 4.91	bc	97.41 ± 1.16	a	39.55 ± 6.38	abc	38.19 ± 6.74	abc
12.5RD	Mortality	27.97 ± 6.36	bc	100.00 ± 0.00	a	54.80 ± 7.42	abc	5.85 ± 2.65	c	71.04 ± 6.88	ab	2.56 ± 2.56	c
	Pupae	4.46 ± 2.19	ab	0.00 ± 0.00	b	2.59 ± 1.79	ab	0.00 ± 0.00	b	0.98 ± 0.98	ab	15.44 ± 3.33	a
	Adults	12.55 ± 7.19	ab	0.00 ± 0.00	b	4.21 ± 1.54	ab	0.00 ± 0.00	b	2.84 ± 1.98	ab	43.80 ± 7.26	a
	Live larvae	55.02 ± 4.12	ab	0.00 ± 0.00	c	38.41 ± 5.35	abc	94.15 ± 2.65	a	25.14 ± 8.00	bc	38.19 ± 6.74	abc
25RD	Mortality	8.13 ± 2.62	bc	99.17 ± 0.83	a	84.69 ± 3.63	ab	7.54 ± 5.59	c	57.18 ± 8.06	abc	2.56 ± 2.56	c
	Pupae	15.28 ± 5.96	ab	0.00 ± 0.00	c	0.88 ± 0.88	bc	0.00 ± 0.00	c	3.94 ± 1.25	abc	15.44 ± 3.33	a
	Adults	20.64 ± 6.19	ab	0.83 ± 0.83	b	4.17 ± 2.71	b	0.00 ± 0.00	b	14.42 ± 4.57	ab	43.80 ± 7.26	a
	Live larvae	55.95 ± 11.61	ab	0.00 ± 0.00	c	10.26 ± 1.88	bc	92.46 ± 5.59	a	24.47 ± 5.58	abc	38.19 ± 6.74	abc
50RD	Mortality	7.21 ± 2.70	bc	100.00 ± 0.00	a	89.64 ± 2.92	ab	5.44 ± 2.71	c	60.65 ± 3.99	abc	2.56 ± 2.56	c
	Pupae	14.28 ± 4.09	ab	0.00 ± 0.00	b	0.83 ± 0.83	ab	0.00 ± 0.00	b	3.84 ± 2.03	ab	15.44 ± 3.33	a
	Adults	37.30 ± 8.15	ab	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	8.75 ± 6.57	ab	43.80 ± 7.26	a
	Live larvae	41.21 ± 5.69	ab	0.00 ± 0.00	c	9.53 ± 2.46	bc	94.56 ± 2.71	a	26.76 ± 6.89	abc	38.19 ± 6.74	abc
RD	Mortality	14.44 ± 1.86	b	100.00 ± 0.00	a	97.09 ± 1.32	a	36.98 ± 3.92	ab	52.80 ± 4.86	ab	2.56 ± 2.56	b
	Pupae	6.39 ± 2.99	ab	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	6.11 ± 1.69	ab	15.44 ± 3.33	a
	Adults	15.00 ± 7.28	ab	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	5.28 ± 1.98	ab	43.80 ± 7.26	a
	Live larvae	64.17 ± 9.33	a	0.00 ± 0.00	b	2.91 ± 1.32	b	63.02 ± 3.92	a	35.82 ± 6.47	ab	38.19 ± 6.74	ab

* Means (± standard error) within a line followed by the same letter are not significantly different ($P < 0.05$; Kruskal-Wallis and Dunn).

Results and discussion

Insecticides applied on the feed. The highest mortalities of *A. diaperinus* larvae were observed for teflubenzuron, which led to a mean mortality rate from 96 to 100%. Chlorantraniliprole also caused high mortality which increased with higher dosages. *A. indica* base product also led to increased mortality with an increase in dosages; however, the highest percentage was obtained at 12.5 RD, whereas in RD, the mortality was reduced. This fact may have occurred due to the inhibiting action caused by the main compound of this plant, azadirachtin, which, in addition to being toxic to insects, has a feeding deterrent and repellent activity (Mordue (Luntz) and Nisbet 2000). In higher concentration in the feed, it may have inhibited insect feeding and, consequently, a smaller quantity of the product was ingested. In spite of the high mortalities caused by teflubenzuron, there was no significant difference between chlorantraniliprole and *A. indica*, but it differed from the methoxyphenozide and pyriproxyfen treatments, as these

Table 2. Median survival time (ST_{50}) for *Alphitobius diaperinus* 4th instar larvae after 30 days in contact with feed treated by different insecticides at different dosages (Londrina, Brazil).

Dosages	Products	ST_{50} (d.p.i.)*	95% Confidence interval		SE	d*f*	χ^2	P
			CI low	CI high				
1.56RD	Methoxyphenozide	–	–	–	0.5656	4	350.46	0.0001
	Teflubenzuron	10	–	–	0.6964			
	Chlorantraniliprole	–	–	–	0.7525			
	Pyriproxyfen	–	–	–	0.1441			
	Azadirachta indica	–	–	–	0.6915			
3.12RD	Methoxyphenozide	–	–	–	0.3596	4	435.73	0.0001
	Teflubenzuron	10	–	–	0.3998			
	Chlorantraniliprole	–	–	–	0.7944			
	Pyriproxyfen	–	–	–	0.3501			
	Azadirachta indica	–	–	–	0.6364			
6.25RD	Methoxyphenozide	–	–	–	0.3190	4	420.68	0.0001
	Teflubenzuron	10a	–	–	0.4692			
	Chlorantraniliprole	20b	15	25	0.9917			
	Pyriproxyfen	–	–	–	0.0000			
	Azadirachta indica	–	30	–	0.7869			
12.5RD	Methoxyphenozide	–	–	–	0.4631	4	306.43	0.0001
	Teflubenzuron	10a	–	–	0.5722			
	Chlorantraniliprole	25b	20	–	1.0165			
	Pyriproxyfen	–	–	–	0.3609			
	Azadirachta indica	20ab	10	30	0.9704			
25RD	Methoxyphenozide	–	–	–	0.4325	4	458.67	0.0001
	Teflubenzuron	10a	–	–	0.3296			
	Chlorantraniliprole	15a	10	15	0.8228			
	Pyriproxyfen	–	–	–	0.4920			
	Azadirachta indica	30b	30	–	0.7636			
50RD	Methoxyphenozide	–	–	–	0.4263	4	439.29	0.0001
	Teflubenzuron	10a	–	–	0.2967			
	Chlorantraniliprole	10a	–	–	0.7698			
	Pyriproxyfen	–	–	–	0.2879			
	Azadirachta indica	30b	30	–	0.8361			
RD	Methoxyphenozide	–	–	–	0.5142	4	426.86	0.0001
	Teflubenzuron	15a	–	–	0.8977			
	Chlorantraniliprole	–	30	–	0.5356			
	Pyriproxyfen	–	–	–	0.1028			
	Azadirachta indica	10a	5	15	1.0595			

Median survival times were determined using the Kaplan-Meier Product-Limit estimator (Collett, 1994); d.p.i. = days post inoculation. Different letters after ST_{50} values for each dose are significantly different with a = 0.05. Chi-square results are from test of the null hypothesis that times to death were not significantly different among products treatments at each dose (test of equality over products log-rank).

Table 3. Lethal concentration (LC_{50}) for *Alphitobius diaperinus* 4th instar larvae after 30 days in contact with feed treated by different insecticides at different dosages (Londrina, Brazil).

Products	LC_{50}	Regression equation	χ^2 (p-value)
Methoxyphenozide	–	Y = 3.514 + 0.001. log X	5.682 (0.338)*
Teflubenzuron	–	Y = 6.319 + 0.638. log X	4.95 (0.421)*
Chlorantraniliprole	12.27	Y = 3.577 + 1.308. log X	24.61 (0.000)
Pyriproxyfen	–	Y = 0.175 + 2.039. log X	13.394 (0.019)
Azadirachta indica	–	Y = 4.321 + 0.502. log X	28.57 (0.000)

* Not significant by the chi square test at 5% probability.

latter two led to the lowest levels of mortality, no different from the control (Table 1).

Due to the high mortality caused to the larvae fed on teflubenzuron treated feed, the percentage of live pupae, adults and larvae was practically null. After 30th day of evaluation, the highest number of live larvae, pupae and adults was lower than the control. For chlorantraniliprole and *A. indica*, increasing the dosages led to a low number of larvae transforming into pupae and adults (Table 1).

The benzoylphenyl ureas (teflubenzuron) inhibit the formation of chitin synthetase (Retnakaran *et al.* 1985). Hence, larvae treated with these insecticides cannot shed their exocuticle. This might be what led to the mortality of larvae fed with teflubenzuron treated feed in the present study.

These results contrast with those of Chernaki-Leffer *et al.* (2006), which, in spite of having used products from the benzoylphenyl urea group (triflumuron and diflubenzuron at 10 ppm), obtained a reduced percentage of mortality of *A. diaperinus* larvae. Nevertheless, it is important to highlight that in the study cited, the larvae were exposed to the treated food for three days only, afterwards being fed with untreated feed. In the present study, the larvae remained with the treated food throughout the entire period of evaluation (30 days), which allowed the constant ingestion of the active ingredient. The *A. indica* insecticide action on *A. diaperinus* adults and larvae in laboratory was previously shown in other studies (Szczepanik 2001; Marcomini *et al.* 2009). This effect on larvae may be caused by the azadirachtin, which acts as a chitin synthesis inhibitor, interfering in cuticle formation and resulting in deformation and death of insects (Casida and Quistad 1998).

In addition to being efficient in diverse pest control, the complexity and diversity of the mode of action of *A. indica* could avoid the selection of resistant insects (Chaieb *et al.* 2007; Khatter 2011). This characteristic is of great importance in *A. diaperinus* control, in which resistance to cyfluthrin (pyrethroid) and fenitrothion (organophosphate) has already been reported (Lambkin and Rice 2006).

Also chlorantraniliprole may be used as an alternative for *A. diaperinus* control and in resistance management strategies because, besides exhibiting high mortality rates, it has a different mode of action that would reduce the chance of the development of resistant populations. It acts when its molecules bond to the ryanodine receptors in the myofibrils of muscle cells, and causes uncontrolled release of calcium. Consequently, insects lose muscle control, leading to rapid cessation of feeding, regurgitation and failure of the heart muscle (Cordova *et al.* 2006).

Methoxyphenozide is a diacyl hydrazine group product and acts as an agonist of the ecdysteroids, causing acceleration in the ecdysis process (Omoto 2000). However, it doesn't appear to be efficient in *A. diaperinus* larvae control (Chernaki-Leffer *et al.* 2006). These results corroborate with those observed in the present study where methoxyphenozide exhibited the lowest larvae mortality.

Kostyukovsky *et al.* (2000) evaluated the tebufenozide effect on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and also observed low mortality. This product belongs to the same chemical group as methoxyphenozide, and its insecticide action may have occurred due to some characteristic of the product in relation to the coleopteran. According to Sousa (2003) they are more efficient in Lepidoptera control, interfering in the development of caterpillars, with no action on pupae and with sub-lethal action in adults.

Pyriproxyfen, also included in the IGR group, is a juvenoid which acts by prolonging the nymph larval stages (Ferreira 1999). Some studies report that in addition to affecting adult emergence, the compound also affects adult weight, sexual ratio, and insect deformation (Dhadialla *et al.* 1998; Sial and Brunner 2010). This may explain the results obtained in the present study, where on average 90% of the larvae fed with feed treated with this product remained in the early stage (larvae) up to the 30th day of evaluation. Therefore, pyriproxyfen may be seen as a promising agent in the control and management of *A. diaperinus* populations because it will cause a rupture in the insect cycle.

This same effect was obtained by Kostyukovsky *et al.* (2000), who evaluated the mortality and morphological characteristics of *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera, Bostrichidae), *Sitophilus oryzae* (Linné, 1763) (Coleoptera: Curculionidae) and *T. castaneum* larvae after the consumption of food treated with pyriproxyfen by adult parents. The authors observed the hatching of deformed larvae, and soon after a long larval period, they died while still in this stage.

The mortality of larvae by teflubenzuron was already observed on the third day of evaluation; nevertheless, the period of feeding in order to achieve 50% mortality was 10 days in all doses, except in RD, where the ST_{50} was 15 days. This may be due to feeding inhibition caused by the insecticide, which at elevated concentration, made the larvae take more time to begin their feeding and to become intoxicated by the product. The treatment with teflubenzuron showed higher toxicity when compared with other products because it showed the lowest ST_{50}. Nevertheless, in 25RD and 50RD, it did not differ from chlorantraniliprole and in RD

Table 4. Mortality of larvae, live larvae, pupae and adults (%) of Alphitobius diaperinus (4th instar) thirty days after contact with different insecticides at different dosages (Londrina, Brazil).

Dosages		Products									
		Methoxyphenozide		Teflubenzuron		Chlorantraniliprole		Pyriproxyfen		*Azadirachta indica*		Control	
25RD	Mortality	21.83 ± 4.20	b	67.94 ± 4.00	a	56.89 ± 5.14	ab	50.65 ± 3.04	ab	50.51 ± 4.83	ab	17.70 ± 1.19	b
	Pupae	4.58 ± 3.25	-	3.29 ± 1.35	-	3.53 ± 2.23	-	6.29 ± 3.43	-	3.05 ± 1.25	-	1.00 ± 1.00	-
	Adults	45.52 ± 4.64	a	4.29 ± 1.09	b	15.98 ± 4.55	ab	6.21 ± 3.23	b	21.56 ± 5.14	ab	8.27 ± 3.07	b
	Live larvae	28.08 ± 2.02	ab	24.48 ± 4.99	b	23.59 ± 4.30	b	36.85 ± 5.77	ab	24.88 ± 2.34	b	73.03 ± 2.20	a
50RD	Mortality	37.28 ± 5.41	ab	66.61 ± 6.16	a	51.79 ± 5.29	ab	41.33 ± 3.08	ab	59.59 ± 8.02	a	17.70 ± 1.19	b
	Pupae	6.32 ± 2.76	-	4.22 ± 2.59	-	4.77 ± 2.96	-	2.00 ± 2.00	-	1.67 ± 1.67	-	1.00 ± 1.00	-
	Adults	31.63 ± 2.51	a	7.33 ± 3.09	ab	23.46 ± 1.10	ab	2.18 ± 1.34	b	9.39 ± 4.79	ab	8.27 ± 3.07	ab
	Live larvae	24.77 ± 3.52	ab	21.84 ± 5.43	b	19.98 ± 1.88	b	54.49 ± 5.57	ab	29.02 ± 9.33	b	73.03 ± 2.20	a
RD	Mortality	28.46 ± 7.88	b	83.23 ± 6.37	a	76.22 ± 6.65	a	62.89 ± 6.91	ab	56.69 ± 3.91	ab	17.70 ± 1.19	b
	Pupae	2.00 ± 1.22	-	2.05 ± 1.26	-	2.11 ± 1.30	-	1.00 ± 1.00	-	4.39 ± 3.23	-	1.00 ± 1.00	-
	Adults	26.72 ± 7.58	a	3.16 ± 1.29	ab	9.33 ± 4.14	ab	0.00 ± 0.00	b	16.29 ± 4.32	ab	8.27 ± 3.07	ab
	Live larvae	42.82 ± 6.61	ab	11.55 ± 5.30	b	12.33 ± 4.14	b	36.11 ± 6.51	ab	22.64 ± 4.83	ab	73.03 ± 2.20	a

Means (± standard error) within a line followed by the same letter are not significantly different ($P < 0.05$; Kruskal-Wallis and Dunn).

from *A. indica,* which indicates that at higher concentrations, these products act in a more rapid way (Table 2).

For *A. indica*, the ST_{50} was high for the 25RD and 50RD; however, with the increase of concentration, the lethal time was reduced (Table 2). This slower action was also reported by Szczepanik (2001), where 1st instar larvae fed with poultry feed treated with a neem-based commercial insecticide (Neem-Azal-T^{TM}) at the concentrations of 0.01 and 0.1% died after 25 and 45 days, respectively.

Other studies have shown the great amount of time spent in controlling these insects with the IGR. Weaver (1996) obtained larval control of *A. diaperinus* seven days after applications with hexafluron and triflumuron. These products normally do not show an immediate effect because mortality only occurs after larval ecdysis. Thus, the author recommends its association with pyrethroids, which act in a rapid way and show good results in adult control, while the IGR will act efficiently in larvae control.

The differences obtained in the results did not allow the estimate of the lethal concentration of each product because they did not fit the Probit model. Even for chlorantraniliprole, which obtained LC_{50} of 12.27, it was not possible to check the fit of the values since the p-value for the chi-square test was significant (less than 0.005), indicating that the adjustment to the model is not appropriate. In addition, some products like methoxyphenozide and pyriproxyfen caused mortality below 50% (Table 3).

Insecticides applied on *A. diaperinus* larvae. There was a difference in the results when the insecticides were applied directly on the larvae in relation to the results observed for application on the feed. In spite of teflubenzuron still proving to be the most efficient product, the mortality percentages decreased compared with the product that was applied directly on the feed. For chlorantraniliprole there was also a reduction in the mortality percentages in relation to the first bioassay because, even with reduction in the product efficiency, chlorantraniliprole applied on the insects was one of the most effective treatments, not differing from teflubenzuron. For *A. indica*, this application method did not affect the mortality levels and caused a mean mortality that did not differ from teflubenzuron and chlorantraniliprole. However, methoxyphenozide, in spite of causing the lowest mortality, not differing from the control, obtained an increase of up to 30% in relation to the larvae that ingested the treated feed (Tables 1 and 4).

It was observed that when the product pyriproxyfen was applied directly on *A. diaperinus* larvae, mortality percentages were greater than when the product was applied directly on the feed in all dosages tested (Tables 1 and 4). This mortality percentage increase shows that direct contact between the product and the insect increases control efficiency, which probably occurs due to greater penetration capability of the insecticide through its cuticle. This may explain the results obtained in most of the studies with pyriproxyfen, where the highest mortalities are observed after topical application of the product on adults, nymphs and eggs of *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae) (Chen and Borden 1989; Mojaver and Bandani 2010).

Some compounds may be toxic to insects through contact as well as ingestion, like that which occurred with *A. indica* in this study. This was also observed by Kavillieratos *et al.* (2007), for *T. castaneum* and *S. oryzae* reared in corn and

Table 5. Median survival time (ST_{50}) for *Alphitobius diaperinus* 4th instar larvae 30 days after contact with insecticides at different dosages (Londrina, Brazil).

Dosages	Products	ST_{50} (d.p.i.)[1]	95% Confidence interval		SE	d*f*	χ^2	P
			CI low	CI high				
25RD	Methoxyphenozide	–	–	–	0.8779	4	40.89	0.0001
	Teflubenzuron	20a	–	–	0.8754			
	Chlorantraniliprole	20ab	20	25	0.9835			
	Pyriproxyfen	27.5b	25	30	0.9767			
	Azadirachta indica	25ab	20	30	0.9502			
50RD	Methoxyphenozide	–	25	–	0.8427	4	26.37	0.0001
	Teflubenzuron	20a	20	25	0.8591			
	Chlorantraniliprole	20a	20	30	1.1273			
	Pyriproxyfen	30a	25	–	0.8162			
	Azadirachta indica	25a	20	25	0.9205			
RD	Methoxyphenozide	–	–	–	0.6847	4	109.67	0.0001
	Teflubenzuron	15a	10	15	0.7307			
	Chlorantraniliprole	15ab	15	20	0.9605			
	Pyriproxyfen	25b	20	25	1.0343			
	Azadirachta indica	15ab	15	20	1.0087			

Median survival times were determined using the Kaplan–Meier Product-Limit estimator (Collett, 1994); d.p.i. = days post inoculation. Different letters after ST_{50} values for each dose are significantly different with a = 0.05. Chi-square results are from test of the null hypothesis that times to death were not significantly different among products treatments at each dose (test of equality over products log-rank).

wheat. Nevertheless, for *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae), lufenuron and flufenoxuron, that belong to the same chemical group as teflubenzuron (benzoyl ureas), contact resulted in low toxicity (Molina and Carbone 2010). These results agree with the data obtained in this current study, where teflubenzuron was more efficient when ingested than when in direct contact with the larvae. This may be due to the particularities of the insects and how the product is metabolized by them, and also the compounds and mode of action of the insecticide. Some authors cited that the action of these insecticides is mainly through ingestion in the larval stage (Dhadialla *et al.* 1998; Tunaz and Uygun 2004).

Pyriproxyfen, despite causing a higher mortality rate when applied on the larvae, maintained the low number of pupae and adults observed in the ingestion bioassay, confirming its effect as a juvenoid, prolonging the larval stages. Interference in the life cycle of the insects was also reported in other studies, where the emergence of adults of *A. diaperinus* and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) was suppressed when treated with fenoxicarb, an insecticide belonging to the same chemical group as pyriproxyfen (Edwards and Abraham 1985).

After spraying the larvae with the different insecticides, the shortest time leading to death of 50% of the population was with teflubenzuron and chlorantraniliprole, which took 20 days with 25RD and 50RD, and 15 days at RD, proving to be more efficient in control of *A. diaperinus*. Pyriproxyfen was the least toxic product, taking 30 days at the 50RD and 25 days at RD (Table 5).

In conclusion, in all these cases it was observed that the insecticides used in this study need more time to act when compared to conventional products, such as the pyrethroids (Dhadialla *et al.* 1998). However, they are less toxic to poultry and humans (Silva and Mendes 2002). Therefore, a strategy for *A. diaperinus* population control is necessary, taking into account various factors, such as lethal time, the best application period, toxicity for poultry and the environment and resistance management. So the association of these products with contact insecticides, for adult control, mainly in treatment between poultry lots, is a valid alternative.

Table 6. Lethal concentration (LC_{50}) for *Alphitobius diaperinus* 4th instar larvae 30 days after contact with different insecticides at different dosages (Londrina, Brazil).

Products	LC_{50}	Regression equation	χ^2 (p-value)
Methoxyphenozide	–	Y = 2.623 + 0.470. log X	3.765 (0.052)*
Teflubenzuron	–	Y = 3.387 + 0.912. log X	3.223 (0.072)*
Chlorantraniliprole	–	Y = 2.920 + 1.058. log X	5.505 (0.018)
Pyriproxyfen	–	Y = 3.569 + 0.650. log X	5.251 (0.021)
Azadirachta indica	–	Y = 4.244 + 0.377. log X	0.743 (0.388)*

* Not significant by the chi square test at 5% probability.

Hence, larvae might be controlled afterwards by the growth regulator insecticides.

In spite of mortalities above 50% being observed in most of the tested products as well as in the bioassays with insecticides applied on the feed, the heterogeneity of the results did not allow the estimate of the lethal concentration of each product, and the data did not fit the Probit model (Table 6).

The growth regulator insecticides, teflubenzuron, chlorantraniliprole and *A. indica*, affected the survival and development of *A. diaperinus* larvae in the laboratory. Pyriproxyfen did not allow the larvae to reach the adult phase and Methoxyphenozide was not efficient in controlling *A. diaperinus*.

When applied as a contact insecticide, teflubenzuron caused lower mortality rates than when ingested with feed, in contrast to pyriproxyfen, which obtained higher mortality rates when applied directly on the insects.

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