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Deltamethrin residues applied in different formulations in staked cucumber and the actions of insecticides on the pickleworm control

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ABSTRACT

Pesticide residues, especially in vegetables and fruits, are a reason for concern with respect to the health of consumers and workers that need to return to areas that have been treated with these agrochemicals. The objectives of this experiment were to (a) study the behavior of deltamethrin residues from different formulations on cucumber fruits; and (b) correlate insecticide contents in the fruits with pickleworm control. Treatments were as follows: (a) control; (b) three 30 mL applications of deltamethrin 25 EC 100 L^{-1} water (emulsifiable concentrate formulation); (c) three 7.5 mL applications of deltamethrin 100 EC 100 L^{-1} water (emulsifiable concentrate); and (d) three 3.75 mL applications of deltamethrin 200 CS 100 L^{-1} water (concentrate suspension). Samples were taken at (-1), zero, 1; 3; 5; 7, and 14 days after the last application. Residues were determined by the gas chromatography technique, using an electron capture detector. In order to evaluate insecticide effectiveness, six infestation surveys were conducted during the same sampling times. Although at low levels, the deltamethrin residues in the fruits were above the maximum residue level (MRL = 0.03 mg kg^{-1}), even one day after the end of the safety interval (2 days), and were higher for the CS formulation. The insecticide was effective to control the pest during the entire evaluation period.

Keywords: *Cucumis sativus*, *Diaphania nitidalis*, pesticide residues.

RESUMO

Resíduos de deltametrina, aplicada em diferentes formulações em pepino tutorado e ação do inseticida no controle da broca-das-cucurbitáceas

Resíduos de agrotóxicos, principalmente em hortaliças e frutas, são motivo de preocupação para a saúde dos consumidores e operários que necessitam retornar às áreas tratadas com esses agrotóxicos. Os objetivos deste estudo foram: (a) estudar o comportamento dos resíduos de deltametrina resultantes de aplicações de diferentes formulações em frutos de pepino; e (b) correlacionar os teores do inseticida nos frutos com o controle da broca-das-cucurbitáceas. Os tratamentos foram: (a) testemunha; (b) três aplicações de 30 mL de deltametrina 25 CE 100 L^{-1} de água (formulação concentrado emulsionável); (c) três aplicações de 7,5 mL de deltametrina 100 CE 100 L^{-1} de água (concentrado emulsionável); e (d) três aplicações de 3,75 mL de deltametrina 200 SC 100 L^{-1} de água (suspensão concentrada). As amostras foram tomadas a (-1), zero, 1; 3; 5; 7 e 14 dias após a última aplicação. Os resíduos foram determinados por técnica de cromatografia gasosa, com o uso de detector de captura de elétrons. Para a avaliação da eficiência do inseticida foram feitos seis levantamentos de infestação nas mesmas épocas. Os resíduos de deltametrina nos frutos, embora em baixos níveis, encontravam-se acima do limite máximo de resíduo (LMR = 0,03 mg kg^{-1}, mesmo um dia após o término do intervalo de segurança (2 dias), sendo maiores quando decorrentes da formulação SC. O inseticida nas formulações em que foi aplicado foi eficiente no controle da praga durante todo o período de avaliação.

Palavras-chave: *Cucumis sativus*, *Diaphania nitidalis*, resíduos de agrotóxicos.

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The development of techniques to ensure agricultural productivity, both in quality and quantity, has caused an increase in the use of pesticides, which control harmful organisms in a relevant manner. Although pesticides are used to control pests, diseases, and weeds, they may contaminate foods, other products, and the environment due to their application at a given stage of the crop's production cycle, storage, transport, or due to soil contamination from previous treatments, crop rotation, or to irrigation water contamination (Eom, 1994).

The importance of using pesticides, the conducting of research, and regular and frequent monitoring programs are actions needed to provide direction to governments and rural extension agencies as to the use of these agricultural chemicals, as well as to provide accurate information to the population about their adverse effects (Lucas, 1998).

An important trait of these pesticides is that there main use has always been targeted at major crops, especially cereals; however, in the case of vegetables, despite their short cycles and the fact that they are grown in relatively small areas, these pesticides become, in a way, adapted to vegetable crops.

Vegetables frequently ensure good financial return per area unit, especially because consumers often prefer products with good aspect as if that would guarantee their health and quality (Baptista & Trevizan, 2007).

Among cultivated vegetables, cucumbers occupy a prominent position because of their importance and acceptance by the population in general, in the form of salads and pickles. Accordingly, about 41,000 tons of this cucurbit were traded at CEAGESP-SP in 2002 (FNP Consultoria & Agroinformativos, 2004).

The cucumber crop has many and serious phytosanitary problems

represented by insect pests and diseases. Among insects, the most important ones are: aphids, *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), the whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), and the cucurbit borers *Diaphania nitidalis* (Cramer) (pickleworm) and *D. hyalinata* (L.) (melonworm) (Lepidoptera: Crambidae), whose control is normally achieved with the use of insecticides (Gallo *et al.*, 2002).

Fenitrothion residues, applied as an emulsifiable concentrate formulation, were evaluated in cucumber rind and pulp at zero, 3, and 8 days after the first application, and at zero, 8, and 14 days after a second application, with the insecticide used at dose rates of 150 and 300 g a.i. 100 L^{-1} water. The residues were concentrated in the rind and underwent rapid degradation (90-96% in 3 days), but did not penetrate the pulp (Ferst, 1991). In a study conducted to evaluate residues of pyrethroid insecticides in vegetables, it was observed that deltamethrin and cypermethrin residues in asparagus decreased to levels below 0.1 mg kg^{-1} on the following day after the last application of the insecticides at the recommended dose; in steam-cooked onion, deltamethrin residues were below the limit of quantitation of the method (LOQ = 0.1 mg kg^{-1}) (Ripley *et al.*, 2001).

Studies performed at the Universidade Estadual de Campinas' Cafeteria in the period from October 1998 to April 2001, involving several vegetables including cucumber, with deltamethrin, cypermethrin, and permethrin residues, demonstrated some situations in which these pyrethroids were inadequately used, with levels above the legal MRL, or their occurrence in vegetables for which they have not been registered, especially cypermethrin and permethrin. However, deltamethrin residues were not found in the samples analyzed (Oviedo *et al., 2003*).

The objectives of this study were to evaluate the behavior of deltamethrin residues applied as different formulations in a staked cucumber crop and its action on pickleworm control.

MATERIAL AND METHODS

The Experiment was conducted under field conditions in the city of Piedade, São Paulo State, Brazil (geographic coordinates: latitude 23° 42' 43'' S; longitude 47° 25' 40'' W), altitude 942 m. Seeding was performed on 08/11/03, in pits spaced at 1 m between rows × 0.5 meter between plants. The 'Caipira' type, 'Safira' hybrid cucumber cultivar was used, conducted in the staked system, without irrigation (water provided by rainfall only). The experimental design consisted of randomized blocks with four treatments and three replications.

Isolation between blocks was provided by borders consisting of two double rows of plants, and plots were separated within each block by ten plants arranged in double rows. Each plot was 7 m in length with double rows of plants spaced 0.5 m from each other and 0.5 m between plants. Fertilizations were made in the planting pits with 100 g of the 4-16-8 NPK formula and two ammonium sulfate sidedressing applications (10 g $plant^{-1}$) at thinning and at the beginning of fruit formation.

The applied pesticide (deltamethrin) is a pyrethroid insecticide, named (*S*)-a-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxilate. Some toxicological data are: acute oral LD_{50} = 330 mg kg^{-1} for dogs; acute dermal LD_{50} for rats and rabbits >2,000 mg kg^{-1}; inhalation LC_{50} (4 hours) for rats 2.2 mg L^{-1} air; acceptable daily intake (ADI) 0.01 mg kg^{-1} body weight; acute oral LD_{50} = 445 mg kg^{-1} for the 25 g L^{-1} EC formulation in rats (Tomlin, 2002).

Two emulsifiable concentrate formulations: deltamethrin 25 EC (25 g a.i. deltamethrin L^{-1}) and deltamethrin 100 EC (100 g a.i. deltamethrin L^{-1}) and one concentrate suspension formulation: deltamethrin 200 CS (200 g a.i. deltamethrin L^{-1}) were used. Treatments were: (a) control; (b) three applications of 30 mL deltamethrin 25 EC.100 L^{-1} water; (c) three applications of 7.5 mL deltamethrin 100 EC.100 L^{-1}; (d) three applications of 3.75 mL deltamethrin 200 SC.100 L^{-1}. Thus, all deltamethrin treatments had 0.75 g a.i. deltamethrin.100 L^{-1} water. Applications were made by means of a backpack sprayer, kept at 60 psi CO_2 constant pressure, equipped with D2-23 spray nozzles; a 800 L ha^{-1} spray volume was applied each time, as recommended, sufficient to completely wet the plants. The applications were made at 7-day intervals from one another.

Samples were taken one day before the last application (-1) and at zero, 1; 3; 5, and 7 days after the last spray, and were obtained at random, respecting their spatial distribution within the plants, so as to make them as representative as possible. Seventy-two samples were analyzed, as follows: 4 treatments × 3 replicates × 6 harvests.

Samples consisted of 12 fruits each, stored in plastic bags and transported in a Styrofoam box containing dry ice. In the laboratory, the samples were processed on the same day they were harvested, and were ground/homogenized; subsamples weighing about 400 g each were taken; the subsamples were sealed in plastic vials and maintained at -20°C until analysis time.

During the experiment, daily temperature and precipitation data were obtained on-site for the period, to allow correlations to be made with insecticide effectiveness on pickleworm control.

The analytical method was adapted from Andersson & Palsheden (1998), and consisted of residue extraction with ethyl acetate in the presence of Na_2SO_4. Then, an aliquot of the extract was concentrated by evaporation and the residues were resuspended in an ethyl acetate/cyclohexane mixture; next, the extractives were cleaned-up by the gel permeation chromatography (GPC) technique, and elution was performed using the same solvent mixture. The quantitative determinations were done by gas chromatography using an electron capture detector.

For the analyses, 10 g of the homogenized samples were transferred to a 100 mL Duran-Schott flask, to which 50 mL ethyl acetate and 10 g Na_2SO_4 were added. The preparations were then homogenized in an Ultra-turrax device for two minutes at 132,243

g and centrifuged for five minutes at 1,223 g. Ten mL supernatant aliquot samples were transferred to 50 mL polypropylene tubes and evaporated to dryness in a water-bath at a constant temperature of 40º C under a slight airflow previously dried through a silica gel filter.

The extracts were cleaned up through gel permeation chromatography (GPC), using two PLGel columns; to accomplish that, the residues were resuspended in 2 mL of an ethyl acetate/cyclohexane (1/1, v/v) mixture; the tube walls were thoroughly rinsed and the extracts were collected in 15 mL centrifuge tubes, which were placed in a mini-shaker apparatus for 1 minute and under ultrasound for another minute, after which the extracts were filtered through a 0.20 µm Millipore membrane using 5 mL hypodermic syringes, and transferred to GPC vials. Next, 500 µL of the extracts were injected into the GPC which was operated at a flow of 1 mL min^{-1} with the same organic solvent mixture; the eluates were discarded during the first 14 minutes and the cleaned extracts were collected during the subsequent 2-minute period in 15 mL centrifuge tubes. These were taken to a Turbo Vap LV evaporator until the extracts were completely dried under a slight N_2 flow at 35ºC.

The residues were resuspended in exactly 0.5 mL of the same ethyl acetate/cyclohexane mixture containing the internal standard (bifenthrin pyrethroid

insecticide), and the extracts were transferred to gas chromatograph autosampler vials using Pasteur-pipettes. The extract aliquot samples were injected into the gas chromatograph in the splitless mode, programmed with a linear temperature gradient program. The analytical instrument used was a Hewlett Packard gas chromatograph, model 6890, equipped with an electron capture detector (µ-ECD, Ni^{63}), HP-608 megabore column, 30 m in length, 0.53 internal diameter and 0.5 µm film thickness, auto sampler device, and an HP GC ChemStation rev.A.06.03 software. The equipment operation conditions were: injector temperature = 250ºC; column temperatures: 100ºC (start), for 1 minute; ramp of 25ºC min^{-1} up to 280ºC (12 minutes); detector temperature = 320ºC. Gas flows were: H_2 (carrier) = 6 mL min^{-1}, N_2 (make up) = 30 mL min^{-1}. Under these conditions, the retention times were: deltamethrin 10 minutes 40 seconds; bifenthrin internal standard 8 minutes 45 seconds.

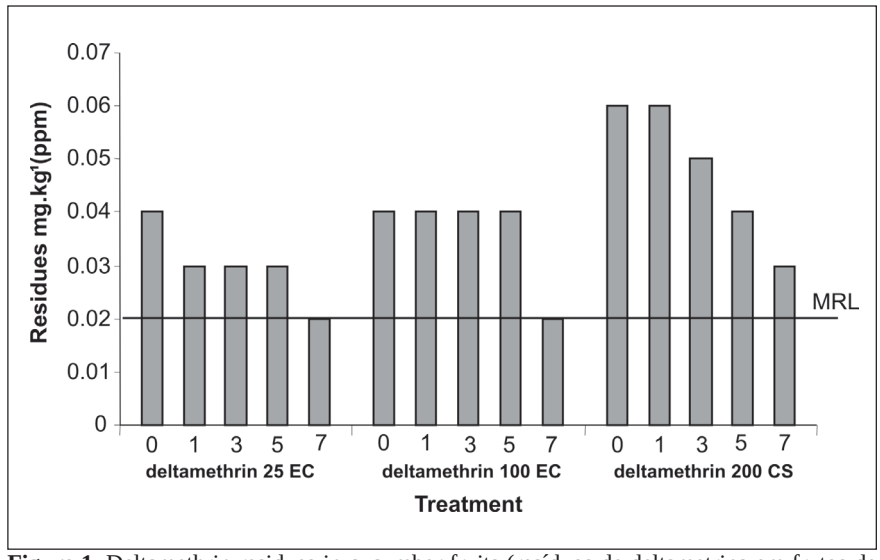

Figure 1. Deltamethrin residues in cucumber fruits (resíduos de deltametrina em frutos de pepino). Piracicaba, USP-ESALQ, 2006.

The residues were calculated directly from the correspondent calibration curve (r^2 = 0.9984), previously plotted by comparison with the standards, obtained from injections of 10; 20; 40; 100; 200; 400, and 800 pg of deltamethrin, diluted in the ethyl acetate/cyclohexane mixture (1/1, v/v) containing the internal standard, into the chromatographic system.

In order to determine the limit of quantitation (LOQ) and the percentage of fruit residue recoveries, 10 g of homogenized, deltamethrin contamination-free cucumber subsamples were spiked with the insecticide so as to obtain concentrations of 0.02 mg kg^{-1} and 0.2 mg kg^{-1} with three replications each, and were processed in the same way as the experimental field samples.

The results obtained were handled by obtaining statistical parameters such as the mean, standard deviation of the mean, and coefficient of variation. In order to validate the analytical method, recoveries ranging from 70 to 120% were considered acceptable (Catta-Preta, 2002).

In order to evaluate the occurrence of the pest and effectiveness of treatments, infestation was initially evaluated in the plots when the fruits were still very small, before beginning the applications. After two applications, (-1 day) samples were taken and, after the third and last application, another five infestation surveys were subsequently carried out, corresponding to the dates when samples were obtained. In each of these surveys, before grinding for residue determination, the 12 fruits sampled were sliced and examined for the presence or absence of the caterpillar or damages that might have been done to the fruits. These data were recorded and transformed to be expressed as percentages of infested fruits, which were submitted to analysis of variance and then to Tukey test for comparisons between treatments. The SANEST statistical program - *Sistema de Análise Estatística para o Cálculo das Tabelas de Variância* (Statistical Analysis System for the Calculation of Variance Tables) - was used in the analyses.

RESULTS AND DISCUSSION

The Anderson & Palsheden (1998) method proved to be adequate for the analysis of deltamethrin residues in cucumber fruits with LOQ set at 0.02 mg kg^{-1}; recoveries ranged from 72-79%. Below that value, the interfering materials eluted in the chromatograms considerably impaired their resolution.

Table 1. Pickleworm infestation (%) in cucumber (n=12 fruits). Piracicaba, USP-ESALQ, 2006.

Treatment	Sampling (days after last application)					
	-1	zero	1	3	5	7
Control	19 a	25 a	31 a	25 a	36 a	28 a
Decis 25 EC	0 b	6 b	0 b	3 b	0 b	6 b
Decis Ultra 100 EC	0 b	3 b	0 b	3 b	0 b	0 b
Decis 200 CS	0 b	0 b	0 b	0 b	0 b	0 b

Values followed by different letters in columns are significantly different by Tukey test ($p \leq 0.01$)

The deltamethrin residues in the fruits, although at low levels, were above the MRL of 0.03 mg kg^{-1}, even one day after the end of the safety interval (2 days) (Agência Nacional de Vigilância Sanitária, 2007) for all formulations, and were higher in samples of the concentrate suspension formulation (0.05 mg kg^{-1}) (Figure 1). Hascoët & André (1978) reported deltamethrin residue levels lower than 0.01 mg kg^{-1} in cucumber, which the authors considered very low in whole fruits, after two days since the last application. In addition, no residues were found in the pulp, but occurred exclusively in the rind. Ferst (1991) researched the occurrence of chlorpyrifos and fenitrothion residues in cucumber and observed that they were found in the rind only, without penetration into the pulp.

The comparison between residual levels in the treatments that used different formulations showed that CS resulted in higher residues than the ECs over the sampling period, with values on day zero of 0.06 for CS and of 0.04 mg kg^{-1} for the other formulations, and then, at the end of the period (7 days), of 0.03 and 0.02 mg kg^{-1}, respectively. Such trait, according to which higher residues are found in the CS formulation, was also observed for dislodgeable residues in cucumber leaves (Franco *et al*, 2005). The day (-1) sample results (<0.02 mg kg^{-1}) showed that the 7-day period between two applications was sufficient to dissipate the residues, and no overlapping of the applications made until then was observed. Also, it can be seen that residue degradation in the various treatments (formulations) during the sampling period (7 days) was twice as high for all formulation types: from 0.04 to 0.02 mg kg^{-1} for deltamethrin 25 EC and deltamethrin 100 EC, and from 0.06 to 0.03 mg kg^{-1} for deltamethrin 200 CS, which seems to indicate that the occurrence of 21.4 mm of rainfall during the period and the prevalence of average temperatures of 20.5 ± 2.7ºC were not determinative factors for a potential and more accelerated reduction of residues in the fruits.

The results found in the 18 control samples (<LOQ) reveal that the plots in that treatment were very well protected and isolated at application time, and did not present cross contamination, which is sometimes difficult to ensure.

Initial evaluation of pickleworm attack showed no plot infestation. It can be observed that the insecticide, in the three formulations studied deltamethrin 25 EC, deltamethrin 100 EC, and deltamethrin 200 CS), was effective to control the pickleworm during the sampling period (Table 1). It can be seen also that, after 13 days from the beginning of applications the control plots were already infested by the pest, differing from the treatments that had received the insecticide applications, just as they differed during the entire sampling period. Lorini & Foerster (1987) evaluated the effectiveness of several insecticides, including deltamethrin, on pickleworm control; the authors concluded that the insecticide was effective to control the pest and did not differ from the best treatments.

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