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Indirect effect of neem oil on *Podisus nigrispinus* (Hemiptera, Pentatomidae): biology and predatory capacity [1]

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ABSTRACT

This study evaluated the effects on the development and predatory capacity of *Podisus nigrispinus* fed on *Spodoptera frugiperda* that have ingested different concentrations of neem oil. The predatory capacity of *Podisus nigrispinus* was assessed, separating nymphs (fourth instar) and adults (males and females). The treatments consisted of *S. frugiperda* larvae reared in neem oil aqueous solutions (0.077, 0.359 and 0.599%), deltamethrin EC 25 (0.100%) and control arranged in a completely randomized design, with ten replicates. Insects were offered three larval densities (one, three and six), in the third or fourth instars. The predated larvae were examined at 24 and 48 hours after the beginning of the experiment. Biological parameters of *Podisus nigrispinus* were evaluated in groups of ten second-instar nymphs transferred to pots, in five replicates. Insects were offered 2-6 third and/or fourth-instar larvae reared in the same neem oil concentrations in a completely randomized design. The following parameters were evaluated: duration of each nymph stage (days), nymph mortality (%), weight of fifth-instar nymphs (mg), sex ratio, weight of males and females (mg) and longevity of unfed adults (days). The predatory capacity of nymphs and adults of *Podisus nigrispinus* was influenced by the neem oil at the concentrations of 0.359% and 0.599% in the highest density. The concentration of 0.359% lengthened the nymphal stage and the concentration of 0.599% reduced the weight of males.

Key words: spined soldier bug, fall armyworm, biological control, integrated pest management, botanical insecticide.

RESUMO

Efeito indireto do óleo de nim sobre *Podisus nigrispinus* (Hemiptera, Pentatomidae): biologia e capacidade predatória

Este trabalho estudou os efeitos do óleo de nim no desenvolvimento e na capacidade predatória de *Podisus nigrispinus*, alimentados com lagartas de *Spodoptera frugiperda*, submetidas a concentrações de óleo de nim. A capacidade predatória de *P. nigrispinus* foi avaliada, individualizando-se ninfas (quarto ínstar) e adultos (machos e fêmeas), constando de dez repetições, sendo os tratamentos lagartas de *S. frugiperda* criadas nas soluções aquosas de óleo de nim (0,077 , 0,359 e 0,599%), deltametrina 25 CE (0,100%) e testemunha, em delineamento inteiramente casualizado. Foram ofertadas aos insetos três densidades de lagartas (uma, três e seis), de terceiro ou quarto ínstares. Avaliaram-se as lagartas predadas às 24 e às 48 horas do início do ensaio. Na biologia de *P. nigrispinus*, ninfas de segundo ínstar foram transferidas, em grupo de dez, para potes, repetidos cinco vezes. Diariamente, foram

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oferecidas de 2 a 6 lagartas de terceiro e,ou, quarto ínstares, criadas nas mesmas concentrações de teste, em mesmo delineamento estatístico, sendo as avaliações: duração (dias) de cada estádio ninfal, mortalidade ninfal (%), peso (mg) de ninfas no quinto ínstar, razão sexual, peso (mg) de machos e fêmeas e longevidade (dias) dos adultos sem alimento. A capacidade predatória de ninfas e adultos de *P. nigrispinus* foi influenciada pelo óleo de nim, na maior densidade estudada, nas concentrações de 0,359 e 0,599%. A concentração de 0,359% alongou o período ninfal e a concentração de 0,599% ocasionou menores pesos de machos.

Palavras-chave: percevejo predador, lagarta-do-cartucho, controle biológico, manejo integrado de pragas, inseticida botânico.

INTRODUCTION

Maize is a widely distributed crop throughout Brazil, both because of its many uses on the farm, either in human or animal feeding, and the tradition of this cereal cultivation by Brazilian farmers (Magalhães *et al*., 2002). Consequently, the fall armyworm, *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae), a serious pest of the crop, is also widespread in the Brazilian territory (Miranda & Suassuna, 2004), mainly because of the wide range of host plants throughout the year in crop succession, such as, corn or soybeans in the summer and maize or sorghum in the off-season (Barros *et al*., 2010).

Chemical control is still the most used control method for *S. frugiperda* (Cruz *et al*., 1982; Costa *et al*., 2005), but the indiscriminate use and improper handling of pesticides causes many serious problems to the environment and human health. Thus, less impactful methods of pest control, or even associations of methods such as biological control and botanical insecticides, meet the integrated pest management requirements and show good applicability (Lara, 1991; Carvalho, 2009; Espindula *et al*., 2010).

The use of natural enemies, such as spined soldier bugs, is a viable and economic alternative management control of lepidopteran pests. *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae), a promising biological control agent, stands out for preying on defoliating insects of various crops (Lisboa *et al*., 2004; Lins Júnior *et al*., 2007; Espindula *et al*., 2010), but little is known of the action of botanical insecticides on these natural enemies.

Some studies reported in the literature sought to investigate the possible effects of natural products on predators and parasitoids. Silva *et al*. (2009) investigated the influence of different plant products on oviposition and embryonic development of *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae). The authors observed that the aqueous extract (leaf and flower) of oleander (*Nerium oleander* L.) was the most selective for the insect, the aqueous extract (leaf and seed) of neem (*Azadirachta indica* A. Juss) increased the oviposition capacity and the essential oil of fennel (*Foeniculum vulgare* Miller) reduced oviposition and also influenced the embryonic development.

Costa *et al*. (2007) evaluated the effect of neem seed oil on the development, survival and fecundity of *E. annulipes*. They found an increase in the nymphal period with increasing neem oil concentrations and that the frequency of neem applications affected the oviposition period and reduced oviposition, while maintaining egg laying.

Cosme *et al*. (2007) studied the effects of azadirachtin, the active ingredient of neem on eggs and first and fourth-instar larvae of the predator *Cycloneda sanguinea* (Linnaeus) (Coleoptera: Chrysopidae). Azadirachtin at the concentration of 10 mg/L was favorable to the integrated pest management because of the positive association between the botanical insecticide and the natural enemy. However, azadirachtin at 50 and 100 mg/L has moderate to high toxicity, which is toxic to *Cycloneda sanguinea*, and all treatments reduced egg viability.

Carvalho (2009) compared the effect of neem and cinnamon (*Melia azedarach* L.) extracts on *Podisus nigrispinus* and found that the treatments with neem had lower percentage of survival than the treatments which cinnamon.

Therefore, the objective of this study was to evaluate the development and predatory capacity of *Podisus nigrispinus* fed on *Spodoptera frugiperda* that have ingested different concentrations of neem oil.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Plant Resistance to Insects, Department of Plant Protection, FCAV/UNESP, Jaboticabal, SP, under controlled temperature (25 ± 2 ^{o}C), relative humidity (70 ± 10%) and photoperiod of 12 hours. Larvae of *S. frugiperda* were obtained from mass rearing colonies fed on artificial diet, according to Kasten Junior *et al*. (1978). Spined soldier bugs were also obtained from mass rearing colonies fed on larvae of *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), according to Zanuncio *et al*. (1994).

Predatory capacity of Podisus nigrispinus

The predatory capacity of *P. nigrispinus* was assessed by separating nymphs (fourth instar) and adults (males and females) in plastic containers (height 4.5 cm; diameter7.5 cm), both with less than 24 hours of development.

To supply distilled water to predators and maintain humidity inside the container, a tube (1.3 mL dental anesthetic cartridge) was inserted through a 1 cm hole in the lid, with the end inward and sealed with a cotton swab.

In both assays (nymphs and adults), the treatments consisted of the predators feeding on larvae of *S. frugiperda* kept in maize leaves (variety AL 34). Leaves were immersed in a neem oil solution (obtained by cold pressing of neem kernels, containing 352.8 ppm azadirachtin) at concentrations of 0.077, 0.359 and 0.599%, 25 EC deltamethrin (0.100%) and water (control) for 3 minutes, dried at room temperature and fed to larvae. The experiment was arranged in a completely randomized design with ten repetitions.

Nymphs and adults were fed three prey densities: one, three and six larvae, all in the third or fourth instars (approximately 15 mm in length), and remained in feeding for 24 hours before the assay starts to stimulate predation, as described by Oliveira *et al*. (2008).

Twenty-four hours after the assay has started, the larvae were removed and those that showed integument injuries, lack of mobility and body content total or partially sucked were considered as predated. The predated larvae were replaced and, after 24 hours, a second evaluation has been carried out.

Data were subjected to analysis of variance (F test) and the means were compared by the Tukey test at 5% significance.

Biological parameters of Podisus nigrispinus

Second instar nymphs of *P. nigrispinus* were transferred to plastic containers (1000 mL) in groups of ten. Water supply and maintenance of moisture inside the container were carried out using the same device of the previous assay.

Nymphs were fed 2-6 third- or fourth-instar *S. frugiperda* larvae (approximately 15 mm in length) daily. Larvae were kept in maize leaves (variety AL 34) that were immersed in a neem oil solution at concentrations of 0.077, 0.359 and 0.599%, 25 EC deltamethrin (0.100%) and the control (water) for 3 minutes, dried at room temperature and fed to larvae. The amount of prey was variable, due to the development of the insect.

The treatments, totaling 50 nymphs per neem oil concentration, were arranged in a completely randomized design with 5 replications.

Daily assessments observed the duration (days) of each nymphal instar, mortality (%) and weight (g) of the fifth-instar nymphs. When nymphs reached the adult stage, the parameters sex ratio, weight (g) of males and females and longevity (days) of unfed adults were evaluated.

Data were subjected to analysis of variance (F test) and the means were compared by the Tukey test at 5% significance.

RESULTS AND DISCUSSION

Predatory capacity of Podisus nigripinus

There was no significant difference in the mean number of predated larvae among the treatments, at the density of one larva, for both nymphs and adults of the predator (Figure 1A and 1B; Figure 2A and 2B). In all treatments, the insects consumed the prey at 24 and 48 hours after the start of the assay.

No significant difference was also found in the mean number of predated larvae among the treatments, at the density of three larvae, for nymphs and adults of *P. nigripinus* (Figure 1C and 1D; Figure 2C and 2D). Similarly, in all treatments, the insects consumed the prey, between 24 and 48 hours after the start of the assay.

At the density of six larvae, it was observed influence of the neem oil concentrations and the insecticide on *P. nigripinus* predation at 24 hours after the start of the assay (Figure 1D). The lowest predation occurred at the neem oil concentration of 0.599% (3.10 larvae) compared with the insecticide (5 larvae).

The larvae treated with the insecticide were less mobile and less resistant to predation compared with larvae from the control and neem oil treatments. The neem oil affects the metabolism and physiology of the insect during its development (Martinez, 2002), which help explain the higher predation in the treatment with insecticide.

De Clercq & Degheele (1994) discussed that high prey densities cause predators to abandon a particular prey, even before it is fully consumed, and attack another one. The highest prey density in association with their little mobility can explain the higher predator consumption in the insecticide treatment than in the others.

Oliveira *et al*. (2008) found that nymphs and adults of *P. nigripinus*, at two densities of *Alabama argillacea* (Hubner) (Lepidoptera: Noctuidae) (1 and 3 larvae/ cotton plant), preyed more larvae at the higher density. The authors pointed out that this was likely due to the amount of prey available, facilitating food location.

Using similar data, Oliveira *et al*. (2001) also found that, both in the laboratory and in the field, the increasing density of *A. argillacea* allowed *P. nigripinus* to locate the prey more easily.

In this study, similar results were found for nymphs at the density of six larvae, after 48 hours of predation (Figure 1F). Predation was higher in the control, the neem oil concentration of 0.077% and the insecticide (3.70, 4.10 and 4.90 larvae, respectively) than at the neem oil concentrations of 0.359 and 0.599% (2 and 2.30 larvae, respectively), indicating a possible antifeedant effect of neem oil on the predator. Other studies showed that, after ingestion of azadirachtin, insects stop or decrease feeding and can die after a few days (Ciociola Junior & Martinez, 2002; Martinez, 2002).

At the density of six larvae, after 24 hours, adults showed a higher predation in the treatment with insecticide (5.20 larvae) than in the control and the neem oil concentration of 0.359% (3.70 and 3.80 larvae, respectively), with intermediate values at the concentrations of 0.077 and 0.599% (4.30 and 4.10 larvae, respectively) (Figure 2E).

After 48 hours of the start of the assay, there was no significant difference between the treatments, ie, they did not influence insect predation (Figure 2F).

It is also notable a possible antifeedant effect of neem oil on adult *P. nigripinus*, but not as marked as in nymphs, influencing predation, both at 24 and in 48 hours after the start of the assay. These differences in food intake between adults and nymphs of the genus

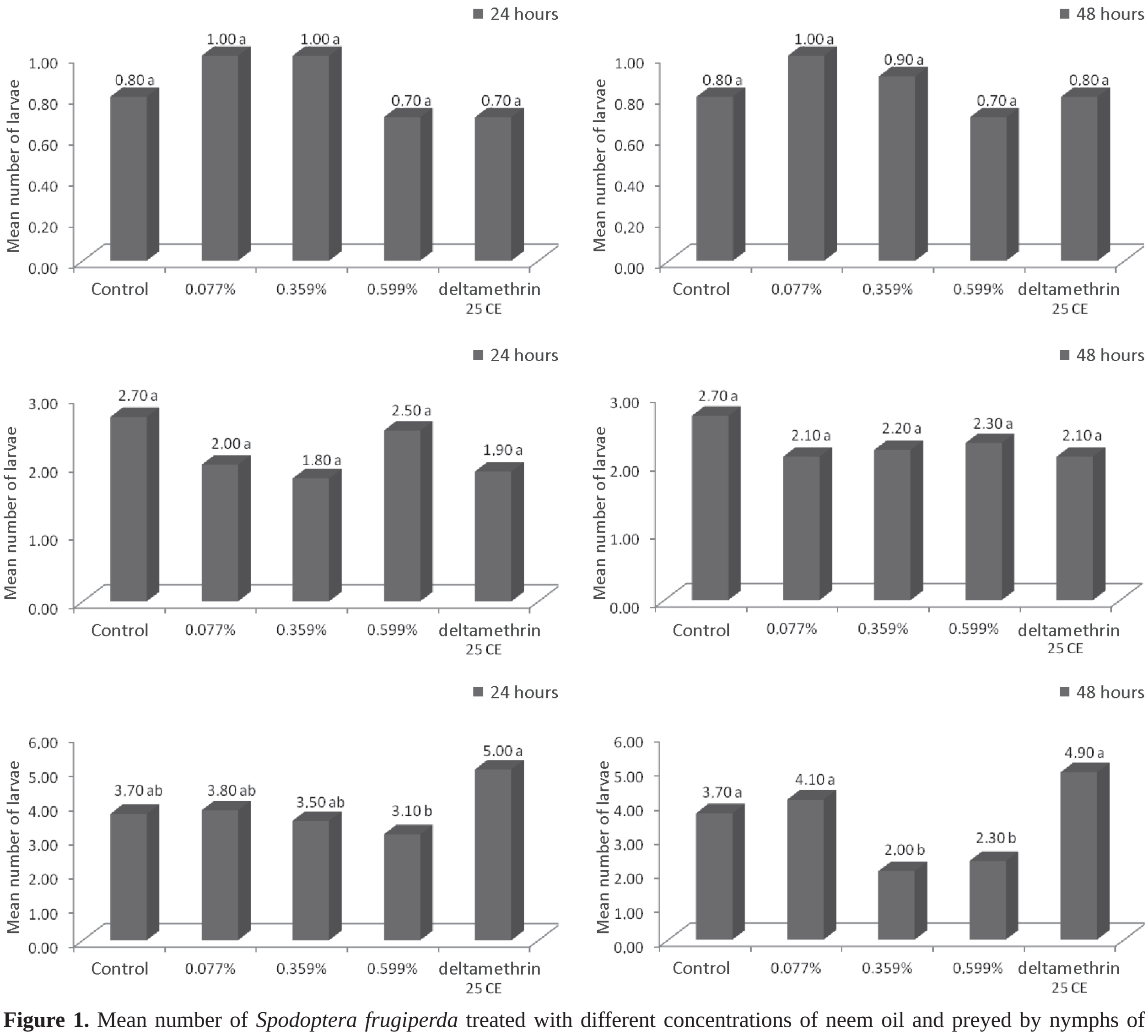

Figure 1. Mean number of *Spodoptera frugiperda* treated with different concentrations of neem oil and preyed by nymphs of *Podisus nigripinus* after 24 and 48 hours of the start of the assay, at the densities of one (A and B), three (C and D) and six (E and F) larvae. Jaboticabal / SP, 2011.

Podisus may be related to the formation of reproductive structures during the sexual maturation of nymphs (Mukerji & Leroux, 1969). It seems that the adult insects consumed the *S. frugiperda* larvae, even though they were contaminated.

Biological parameters of Podisus nigripinus

The mean nymphal mortality was not influenced by the treatments, either consuming *S. frugiperda* larvae treated with the neem oil concentrations or the insecticide (Figure 3). Although there was no significant difference among the treatments, it was observed a lower mortality in the control (34%) than the neem concentration of 0.599% (58%), but none of the treatments showed a high mortality, not even with the insecticide.

Batalha *et al*. (1997) tested the selectivity of several insecticides, including the pyrethroid deltamethrin used in this work, on *P. nigripinus* fed on *S. frugiperda* that was reared on treated leaves. The authors reported that deltamethrin caused prey mortality, with a low LC_{50} compared with the other insecticides tested; however, when it was tested on the predator, the harmful effect was less pronounced, resulting in lower mortality and more selective especially in the nymphal stage. Similar results were reported by Wilkinson *et al*. (1979) and Guedes *et al*. (1992), who found that pyrethroids were less toxic to *P. maculiventris* (Say) and *P. nigripinus* at field doses recommended for the control of the target pest.

Data on duration of each nymphal instar and total nymphal period of *P. nigripinus* fed on preys reared in different neem oil concentrations and insecticide (Table 1) shows influence of the treatments only on the third instar and the total nymphal period. The duration of the third instar was lengthened at the neem oil concentration

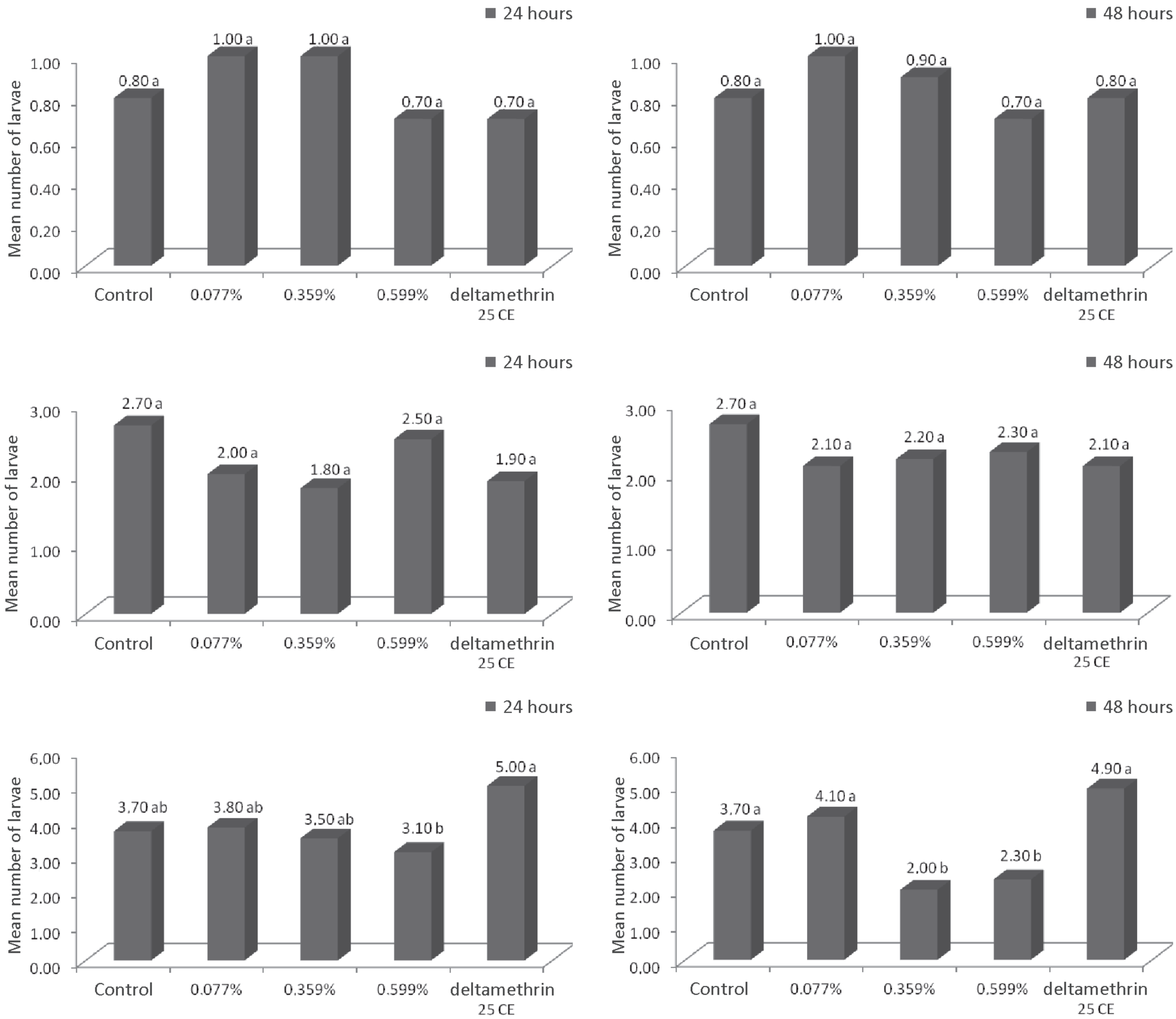

Figure 2. Mean number of *Spodoptera frugiperda* treated with different concentrations of neem oil and preyed by adults of *Podisus nigripinus* after 24 and 48 hours of the start of the assay, at the densities of one (A and B), three (C and D) and six (E and F) larvae. Jaboticabal / SP, 2011.

of 0.359% (4.50 days) (Table 1), which increased the duration of the total period (19.42 days). However, the neem oil concentration of 0.599% caused the shortening of the third instar duration (3.10 days), a difference of almost one day between the concentrations of 0.359% and 0.599%.

The lengthening of the nymphal period caused by the neem oil on beneficial insects, such as pest predators, is considered a good outcome because, as the tested concentrations did not cause mortality, the lengthening of the developmental time to reach adulthood will possibly provide increased predation and favor biological control in the environment (Costa *et al*., 2007).

The insecticide treatment had no influence on the nymphal development of *P. nigripinus*, but reduced the duration of the total nymphal period (16.58 days) compared with the neem concentration of 0.359% (19.42 days). These results confirm that deltamethrin is selective to *P. nigripinus*, in agreement with Battle *et al*. (1997).

The weight of fifth-instar nymphs was not influenced by the treatments and nor was the weight of females (Table 2), which is important because even if the preys were contaminated, the predator fed normally and ensured reserves to reach the adult stage.

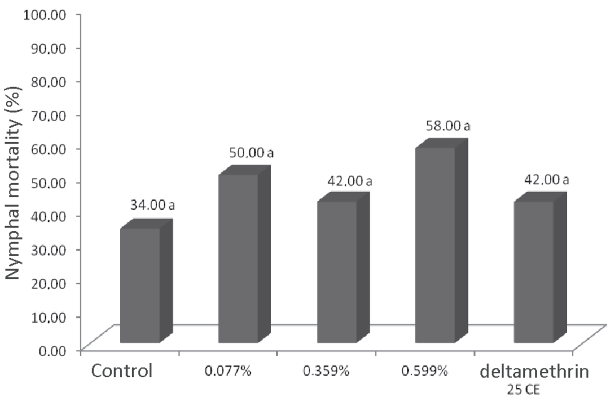

Figure 3. Mean mortality (%) of *Podisus nigripinus* nymphs fed on *Spodoptera frugiperda* treated with different concentrations of neem oil. Jaboticabal / SP, 2011.

The lowest weight of males occurred at the neem oil concentration of 0.599% (28.24 mg) compared with the concentration of 0.077% (46.80 mg) and the weight in the other treatments, which is important as it suggests that smaller males may be less vigorous and less competitive for mating with females (Table 2). Weighing was carried out just after the change to adult stage, with values referring to food intake during the nymphal stage and confirming an antifeedant effect of the prey that ingested azadirachtin (Ciociola Junior & Martinez, 2002; Martinez , 2002).

The treatments had no effect on the sex ratio of *P. nigripinus* (Table 2), keeping the same proportion of males and females. Longevity of unfed adults was also not influenced by the treatments, relying on reserves accumulated during the nymphal stage for a period of approximately 7 days.

Estrela *et al*. (2011) studied the development of *P. nigripinus* fed on *S. frugiperda* larvae and reported similar values for the duration of each nymphal instar of the predator, with means from the second to the fifth instar of 3.75; 3.25; 4.33 and 6.33 days, respectively. Vacari *et al*. (2007) also observed similar values for each nymphal instar of *P. nigripinus* fed on larvae of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae). The authors of both studies suggested a high level of adaptation of this predator to the prey consumed.

In a study with *P. nigripinus* fed on larvae of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), Espindula *et al*. (2010) found 4.40; 4.40; 4.80 and 5.80 days of duration from the second to the fifth instar, which was close to the findings of this study. Weight values followed the same tendency, with fifth instar nymphs weighing around 20 mg and females (around 50 mg) heavier than males (around 38 mg) (Table 2).

Cosme *et al*. (2007) investigated the effects of synthetic insecticides and azadirachtin and found that the neem-oil has a dose dependent effect on *C. sanguinea*.

Table 1. Mean duration (days) of each nymphal instar and total nymphal period of *Podisus nigripinus* fed on *Spodoptera frugiperda* treated with different concentrations of neem oil. Jaboticabal / SP, 2011

Treatments	Instars[1]				Total[1]
	Second	Third	Fourth	Fifth	
Control	3.50 a	3.32 ab	4.04 a	6.70 a	17.28 ab
0.077%	3.48 a	3.84 ab	4.14 a	6.64 a	17.32 ab
0.359%	3.72 a	4.50 a	4.68 a	7.22 a	19.42 a
0.599%	3.30 a	3.10 b	4.46 a	8.34 a	18.84 ab
deltamethrin 25 CE	3.62 a	3.20 ab	4.28 a	7.24 a	16.58 b
F (treatment)	0.49^{ns}	3.16*	2.12^{ns}	2.25^{ns}	3.60*
C.V. (%)	14.42	20.40	9.10	14.08	7.84

(1) Means followed by the same letter in the column are not significantly different by the Tukey test at 5% probability level.

Table 2. Mean weight (mg) of the fifth nymphal instar of males and females, sex ratio and unfed adult longevity of *Podisus nigripinus* fed on *Spodoptera frugiperda* treated with different concentrations of neem oil. Jaboticabal / SP, 2011

Treatments	Weight of fifth nymphal instar (mg)[1]	Weight (mg) of adults[1]		Sex ratio[1;2]	Longevity of unfed adults[1]
		Male	Female		
control	24.92 a	42.50 ab	62.04 a	0.57 a	7.04 a
0.077%	23.42 a	46.80 a	61.66 a	0.47 a	7.88 a
0.359%	20.26 a	39.98 ab	43.62 a	0.23 a	7.20 a
0.599%	21.54 a	28.24 b	54.96 a	0.60 a	6.80 a
deltamethrin 25 CE	25.20 a	42.08 ab	56.06 a	0.64 a	8.76 a
F (treatment)	2.98^{ns}	3.26*	1.63^{ns}	2.71^{ns}	1.08^{ns}
C.V. (%)	12.02	21.67	24.80	11.72	22.61

[(1)]Means followed by the same letter in the column are not significantly different by the Tukey test at 5% probability level. [2] Data were transformed to $(x + 0.5)^{1/2}$ for analysis.

Azadirachtin at 10 mg/L showed favorable characteristics for use in integrated pest management, but at 50 and 100 mg/L was toxic to fourth instar larvae of *C. sanguinea*.

Costa *et al*. (2007) reported that mortality of *E. annullipes* subjected to different neem oil concentrations was low, allowing its use to control pests. In addition, the nymphal period of the predator increased with the neem concentration, lengthening the period of predation.

The use of neem as an alternative to chemical pesticides, or to be used in association with biological control, is promising for integrated pest management. Once the chain of food stimuli is ceased, even if the predator consumes a contaminated prey, its biological parameters are not influenced by the neem oil. Nevertheless, the neem oil doses to be used should be considered and further tested in field conditions.

CONCLUSIONS

The predatory capacity of nymphs and adults of *Podisus nigripinus* was negatively influenced by neem oil at the density of six larvae: for nymphs, this influence occurred at 24 and 48 hours after the start of the assay, at the concentrations of 0.359 and 0.599%, and for adults, after 24 hours at a concentration of 0.359%;

The neem oil ingested via prey causes no mortality to *Podisus nigripinus*, and no interference with the weight of fifth instar nymphs, female weight, sex ratio and unfed adult longevity;

The neem oil concentration of 0.359% lengthened the nymphal period and the concentration of 0.599% reduced male weight.

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