

BIOLOGICAL CONTROL

Effect of Neem Seed Oil Aqueous Solutions on Survival and Development of the Predator *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae)

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Efeito de Soluções Aquosas de Óleo de Sementes de Nim, *Azadirachta indica*, na Sobrevivência e Desenvolvimento do Predador *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae)

ABSTRACT - The effects of the neem seed oil aqueous solutions on survival and performance of egg, larva and adult stage of the Coccinellid predator *Cycloneda sanguinea* (L.) were evaluated under laboratory conditions. In a first trial, eggs and 2nd-instar larvae were sprayed with the neem solutions at 0; 0.5 and 2.25 ml/l and kept at 25 ± 1°C, 70% RH and 14h photoperiod. Egg viability and duration and larval and pupae survival were evaluated as well as the predatory capacity of the larvae, adult longevity and reproduction. Egg spraying did not affect egg hatch or larvae survival and development. When the larvae were sprayed, significant mortality was observed only at the higher concentration ($P \leq 0.05$) and larval development and predatory capacity were not affected. Also, adults originated from treated larvae showed no alterations on sex rate, fecundity, fertility and longevity, thus indicating that at the tested concentrations the neem oil do not reduce the reproductive potential of the species. In a second experiment, adults collected in the field were sprayed with neem oil or kept on *Parthenium* sp. twigs sprayed with the neem oil aqueous solution at 5 ml/l, to evaluate survival. The neem oil at this concentration caused no mortality to the adults, since all of them, sprayed or kept on sprayed twigs, presented a longevity curve similar to untreated ones. The results indicate that the neem oil presents potential to be used in association with biological control of pests, when *C. sanguinea* is present.

PALAVRAS-CHAVE: Meliaceae, biological control, botanical insecticide, predator, ladybird, *Parthenium*

RESUMO - Os efeitos de soluções aquosas do óleo de sementes de nim sobre o predador *Cycloneda sanguinea* (L.), nos estágios de ovo, larva e adulto, foram avaliados em laboratório. Em um primeiro experimento, ovos e larvas de 2^o ínstar foram pulverizados com as soluções de nim nas concentrações de 0; 0,5 e 2,25 ml/l de água apresentaram mortalidade significativa ($P \leq 0,05$) somente na maior concentração e o desenvolvimento larval e a capacidade predatória não foram afetados. Os adultos originados de larvas tratadas não apresentaram alterações da razão sexual, fecundidade, fertilidade e longevidade, indicando que nas concentrações testadas o óleo de nim não reduz o potencial reprodutivo da espécie. Em um segundo experimento, adultos da joaninha coletados no campo foram pulverizados com a solução aquosa de óleo de nim ou mantidos sobre ramos de losna-branca pulverizados na concentração de 5 ml/l de água, para avaliação da sobrevivência. A pulverização do óleo de nim nesta concentração não causou mortalidade nos adultos de *C. sanguinea*, uma vez que os insetos de ambos tratamentos apresentaram curva de longevidade semelhante à da testemunha. Os resultados demonstram que o nim apresenta potencial para ser utilizado em associação com o controle biológico de pragas, quando *C. sanguinea* for empregada.

PALAVRAS-CHAVE: Insecta, Meliaceae, controle biológico, inseticida botânico, joaninha, *Parthenium*, losna-branca

The neem tree, *Azadirachta indica* A. Juss. (Meliaceae), has been widely studied because it presents a great number of compounds with insecticidal properties and is effective on reducing population of several pest species (Jacobson 1989, Schmutterer 1990, Saxena 1997, Mordue (Luntz) & Nisbet 2000). Its main active compound, azadirachtin, is toxic to over 500 insect species and acts mainly as food deterrent and growth disruptor. Martinez & van Emden (2001) obtained longer period of the larva and reduced larval activity of *Spodoptera littoralis* (Boisduval) treated with sub-lethal doses of azadirachtin. This insectistatic effect, also observed in other species (Rembold 1995) favors the association of the neem products with biological control, because it extends the exposure time of the pests to natural enemies (Martinez 2002). Another factor favorable to this association is the fact that azadirachtin presents higher toxicity by ingestion than by contact (Lowery & Isman 1995, Martinez-Carvalho 1996). This makes this compound potentially less toxic to natural enemies in the field, since these insects would not feed directly on the plants. Besides, 90% of the azadirachtin is eliminated from the insect body until 7h after ingestion (Rembold *et al.* 1984), thus reducing the concentration ingested by the natural enemies that feed on preys that could have fed on treated plants.

Schmutterer (1990) stated that neem-based products, in general, are safe to several species of natural enemies, although some species show higher susceptibility (Hough-Goldstein & Keil 1991, Matter *et al.* 1993, Roger *et al.* 1995, Simmonds *et al.* 2000). Therefore studies are necessary to better understand the neem action on natural enemies, to support recommendations of neem use in pest control. The objective of this research was to evaluate the action of neem seed oil on survival and development of *Cycloneda sanguinea* (L.), an important and polyphagous predator, present on different crops and possessing great potential for utilization in biological control programs.

Material e Métodos

The tests were carried out in the Laboratório de Entomologia, of the Instituto Agronômico do Paraná - IAPAR, Londrina, PR, at $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH and 14h photoperiod. Aqueous solutions the neem seed oil ACE-Nim EC[®], Dominican Republic, were used at the recommended dose, 5 ml/l of water (0.5% azadirachtin).

Adults of *C. sanguinea* were collected in the experimental field of IAPAR and kept in acrylic cages (83 x 50 x 60 cm) and fed on aphids, to be used in the experiments.

Egg Spraying. *C. sanguinea* eggs, obtained from the laboratory rearing, were placed on petri dishes, laid with filter paper, and sprayed by means of a Potter Tower with the neem seed oil aqueous solution (NSOAS) at 0.5; 2.25 and 5 ml/l of water. In each treatment, 30 eggs were used, with three replications of 10 eggs each. The egg hatch was daily evaluated and the newborn larvae were single placed in glass vials (4.7 cm high x 2.3 cm diameter), and fed on aphids. Larvae survival and duration were evaluated. The experimental design was at random, and the Tukey test at

5% significance was used to compare the means whenever a one-way ANOVA indicated significant differences between them.

Larva Spraying. Thirty 2nd-instar *C. sanguinea* larvae, originated from the laboratory rearing, were sprayed with the NSOAS at the concentrations: 5; 2.25 e 0.5 ml/l, and only distilled water on control treatment. Three replications with ten larvae were used in each treatment. After spraying, single larvae were placed in glass vials (4.7 cm high x 2.3 cm diameter), closed with cotton, and fed on aphids, *ad libitum*. Ten aphids were daily offered until the 3rd instar, when the number was increased to 30, always providing more aphids than the daily consumption, as indicated by earlier observations. The number of aphids eaten was registered daily as well as larvae survival, development stage and pupae survival.

After emergence, adults from the same day were coupled, placed in cages (50 cm high x 15 cm de diameter) and fed on aphids, *ad libitum*. The number of eggs was daily counted and collected with a thin wet brush. The eggs were transferred to petri dishes, laid with filter paper wet with distilled water, and kept at $25 \pm 1^\circ\text{C}$. The following biological parameters were evaluated: sex rate, length of preoviposition and oviposition period, egg number per batch and per female, hatch percentage and longevity.

The experimental design was at random, and the Tukey test at 5% significance was used to compare means whenever a one-way ANOVA indicated significant differences between them. In the evaluation of aphid consumption, only the larvae that had a whole stage were considered for analysis.

Adult Spraying. *C. sanguinea* adults, collected at the experimental field of IAPAR were sprayed or placed on *Parthenium* sp. twigs during 24h, earlier sprayed with the NSOAS at 5 ml/l. Both insects and twigs in the control groups were sprayed with distilled water. The insects were kept in cages (50 cm high x 15 cm diameter), and after 24h they were fed on aphids, *ad libitum*. Thirty adults were used in each treatment, with three replications of 10 adults each. Survival was registered daily during 10 days.

Results and Discussion

Survival and Duration of Eggs and Larvae - Egg Spraying.

The egg hatch of *C. sanguinea* in all neem treatments was similar to that in the control group. The length of egg stage varied from 1.7 to 2.7 days among the treatments, although the differences were not statistically significant. No changes were observed on survival and duration of the larvae originated from sprayed eggs (Table 1), what coincided with the results reported by Kaethner (1990) apud Schmutterer (1990), where no reduction of egg hatch and larval eclosion was registered for *Coccinella septempunctata* L. and *Crysoperla carnea* Steph., originated from eggs treated with AZT-VR-K (1000 ppm) or a combination of this compound with neem oil (250-30000 ppm).

However, Schmutterer (1990) stated that the ovicide action of neem oil treatments is common, and the product can

Table 1. Mean percentage (\pm EP) of egg hatch, larvae survival, and duration of eggs and larvae of *C. sanguinea*, when the eggs were sprayed with different concentrations of neem seed oil aqueous solution. Temp.: $25 \pm 1^\circ\text{C}$; RH: $60 \pm 10\%$, photoperiod: 14h (n = 30)

Concentration (ml/l)	Egg hatch (%)	Larva survival (%)	Mean duration (days)	
			Egg	Larva
5	50.0 \pm 20.00	90.0 \pm 10.00	2.7 \pm 0.36	9.2 \pm 1.19
2.25	57.0 \pm 15.28	97.0 \pm 5.77a	2.7 \pm 0.78	9.7 \pm 0.44
0.5	60.0 \pm 10.00	100.0 \pm 0.00	1.6 \pm 0.23	9.5 \pm 0.21
Water	53.0 \pm 15.28	97.0 \pm 5.77	1.7 \pm 0.30	9.9 \pm 0.25

The means were not significantly different according to ANOVA.

obstruct the egg membrane, thus impeding the respiratory changes of the embryo, in a dose-dependent manner. The author has not attributed this fact to the action of the growth regulators present in the neem oil, but to the higher adherence of the product to the egg membrane, as observed with other vegetable oils. The ovicide effect of neem oil based products on phytophagous insects was also reported by Souza & Vendramim (2000).

Reports on the action of neem products on embryo development are rare, but studies have demonstrated that larvae originated from treated eggs can present high mortality due mainly to the contact of the insect with the chorion, at the larva eclosion (Prabhaker *et al.* 1999) or as a consequence of consumption of neem residues present on the chorion (Tanzubil & McCaffery 1990). In our research, it is not possible to state that the newborn larvae have eaten the chorion treated with the neem oil, but even if this happened, survival and larvae development were not affected, at any concentration.

Survival and Food Consumption of Larvae and Duration of Larva and Pupae - Larva Spraying. Larvae survival when NSOAS was sprayed on 2nd-instar larvae at 5ml/l was 60%, significantly different of the control group, where 94% survival was observed. However, at 2.25 ml/l, survival was statistically similar to that observed in the control group. The duration of larva period, six days, showed no variation among treatments (Table 2). Food consumption during the

whole larvae period was not reduced in any treatment. At 5 ml/l, the mean consumption was 90 aphids, in comparison with 84 aphids eaten by the larvae of the control group. This result indicates that the voracity of the treated larvae was not affected. Survival and duration of the pupae were not affected and varied from 98.4% to 99.7% and 5.0 to 5.3 days, respectively (Table 2).

The significant mortality of the larvae sprayed with the NSOAS at 0.5% indicates that *C. sanguinea* may be susceptible to neem products, what contradicts the results obtained by Srivastava & Parmar (1985) with Coccinellid predators, and the general tendency observed with natural enemies (Schmutterer 1990). At 0.225% the larvae demonstrated lower susceptibility to the neem oil, and this result is very relevant, if we consider that in most studies, neem oil has caused insect pest mortality at doses between 0.1 and 5% (Schmutterer 1990).

The evaluation of the action of insecticide compounds on food consumption is a very important parameter, mainly with predators. The results described herein have shown that *C. sanguinea* larvae had no significant change on their predatory capacity, and do not agree with the results reported by Banken e Stark (1998), who obtained significant reduction of the predatory capacity of larvae and adults of *C. septempunctata* sprayed with neem oil at 600 ppm, nor they agree with Roger *et al.* (1995) who observed reduction on aphid consumption by *Coleomegilla maculata lengi* Timb. adults, sprayed with neem oil at 50%.

Table 2. Mean survival (\pm EP), duration and total food consumption of larvae and survival and duration of pupae of *C. sanguinea*, when 2nd-instar larvae were sprayed with different concentrations of neem seed oil aqueous solution. Temp.: $25 \pm 1^\circ\text{C}$; RH: $60 \pm 10\%$, photoperiod: 14h (n = 30)

Concentration (ml/l)	Larva			Pupa	
	Survival (%)	Duration (days) ¹	Total consumption ²	Survival (%) ²	Duration (dias) ²
5	60.0 \pm 10.00 b	6.0	90.3 \pm 3.54	99.7 \pm 0.58	5.3 \pm 1.53
2.25	86.7 \pm 11.55 a	6.0	89.5 \pm 2.10	98.7 \pm 1.53	5.0 \pm 0.00
0.5	93.3 \pm 11.55 a	6.0	86.4 \pm 4.31	99.7 \pm 0.58	5.3 \pm 0.58
Water	93.3 \pm 5.77 a	6.0	84.0 \pm 6.10	98.4 \pm 1.53	5.3 \pm 0.58

Means followed by the same letter, in the columns, do not statistically differ, by the Tukey test, at 5% significance.

¹Data not statistically analysed (no variance).

²The means were not significantly different according to ANOVA.

Besides aphid consumption, the *C. sanguinea* larvae showed no changes on their development, and 100% emergence was registered at the higher concentration of neem solution (Table 2). This result corroborates the results obtained by Lowery & Ismam (1995) with *Coccinella undecimpunctata* L. sprayed with neem oil at concentrations higher than 2%.

Sex Rate, Reproduction and Longevity - Larva Spraying.

The sprays of NSOAS on the *C. sanguinea* larvae at different concentrations have not affected sex rate, which was near 0.5 in all treatments. Also, no concentration affected mean longevity of males and females that varied from 26.4 to 32.8 days, and from 49.3 to 50.3 days, respectively. In all treatments mean longevity was higher for females than for males (Table 3). The length of preoviposition and oviposition was not changed (Table 3). Larvae spraying with NSOAS did not affect the number of eggs per batch, egg hatch or the number of eggs per female. The total number of eggs per females was higher at the higher neem concentration (5ml/l) than in the group control, although statistically non significant (Table 4), thus indicating that the neem oil solutions sprayed on *C. sanguinea* larvae did not affect reproductive maturation of the adults at the concentrations tested. Therefore, the pattern of the fertility curve of the females originated from untreated larvae was similar to the pattern of females treated with different concentrations of the neem oil solution (Fig.

1). Similar results were observed in the field by Eisenlohr *et al.* (1992) apud Schmutterer (1995), who obtained no reduction of egg laying by Coccinellid and Sirphid predators already settled in peach orchards, when Neem Azal-F was sprayed to control aphids. Although growth disruption and mortality are more often reported as neem action, the effects on reproductive parameters are very important because they drastically interfere on next generations of several insect species.

Adult Spraying. *C. sanguinea* adults that were sprayed with NSOAS at 5 ml/l or that were placed on twigs sprayed with the same solution presented longevity curve similar to the control group (Fig. 2), thus indicating no contact action of neem solutions to adults. This result may be related to the impermeability of their tegument mainly in the dorsal region, that is protected by hard and thick elitra. Similar results were obtained by Banken & Stark (1998), who demonstrated that *C. septempunctata* adults sprayed with Neemix, presented no mortality; according to Mani (1996), adults of predators the Coccinellid *Cryptolaymus monrouizieri* Mulsant e *Chilocorus nigritus* (Fabr.) also presented no mortality when sprayed with neem oil at 2%. The contact of the adult with the treated twigs also caused no harm to the insects, as also reported by Fernandez *et al.* (1992) with adults of the predator *Cyrtorhinus lividipennis* (Reuter), that presented no mortality when placed on rice plants sprayed with neem oil at 3% and aqueous extract of

Table 3. Mean sex rate (\pm EP), longevity, duration of preoviposition and oviposition period of *C. sanguinea*, when 2nd-instar where sprayed with different concentrations of neem seed oil aqueous solution. Temp.: $25 \pm 1^\circ\text{C}$; RH: $60 \pm 10\%$, photoperiod: 14h (n = 30)

Concentration (ml/l)	Sex rate	Longevity (days)		Duration (days)	
		Female	Male	Preoviposition	Oviposition
5	0.4 ± 0.21	49.3 ± 29.17	32.8 ± 16.38	7.1 ± 6.94	40.7 ± 16.80
2.25	0.5 ± 0.10	60.3 ± 25.32	47.5 ± 40.38	6.1 ± 3.48	60.0 ± 28.14
0.5	0.5 ± 0.11	53.2 ± 30.96	42.1 ± 28.57	10.0 ± 6.15	57.6 ± 26.63
Water	0.5 ± 0.10	50.3 ± 32.02	26.4 ± 7.04	11.2 ± 7.04	39.2 ± 29.71

The means were not significantly different according to ANOVA.

Table 4. Mean egg laying per day (\pm EP), per female and total, and percentage of egg hatch, after different concentrations of the neem seed oil aqueous solution were sprayed on 2nd-instar larva of *C. sanguinea*. Temp.: $25 \pm 1^\circ\text{C}$; RH: $60 \pm 10\%$, photoperiod: 14h (n = 30)

Concentration (ml/l)	N. eggs/batch	N. eggs/day ¹	Total n. eggs ¹	Egg hatch (%) ¹
5	26.2 ± 3.58 a	8.6 ± 4.71	351.0 ± 180.26	69.5 ± 16.34
2.25	14.9 ± 4.87 b	5.4 ± 1.98	300.6 ± 135.18	65.0 ± 14.66
0.5	15.0 ± 7.00 b	6.0 ± 3.19	342.0 ± 192.25	57.1 ± 25.69
Water	21.1 ± 3.79 ab	9.0 ± 9.51	224.4 ± 260.10	71.9 ± 13.49

Means followed by the same letter, in the columns, do not statistically differ, by the Tukey test, at 5% significance.

¹ The means were not significantly different according to ANOVA.

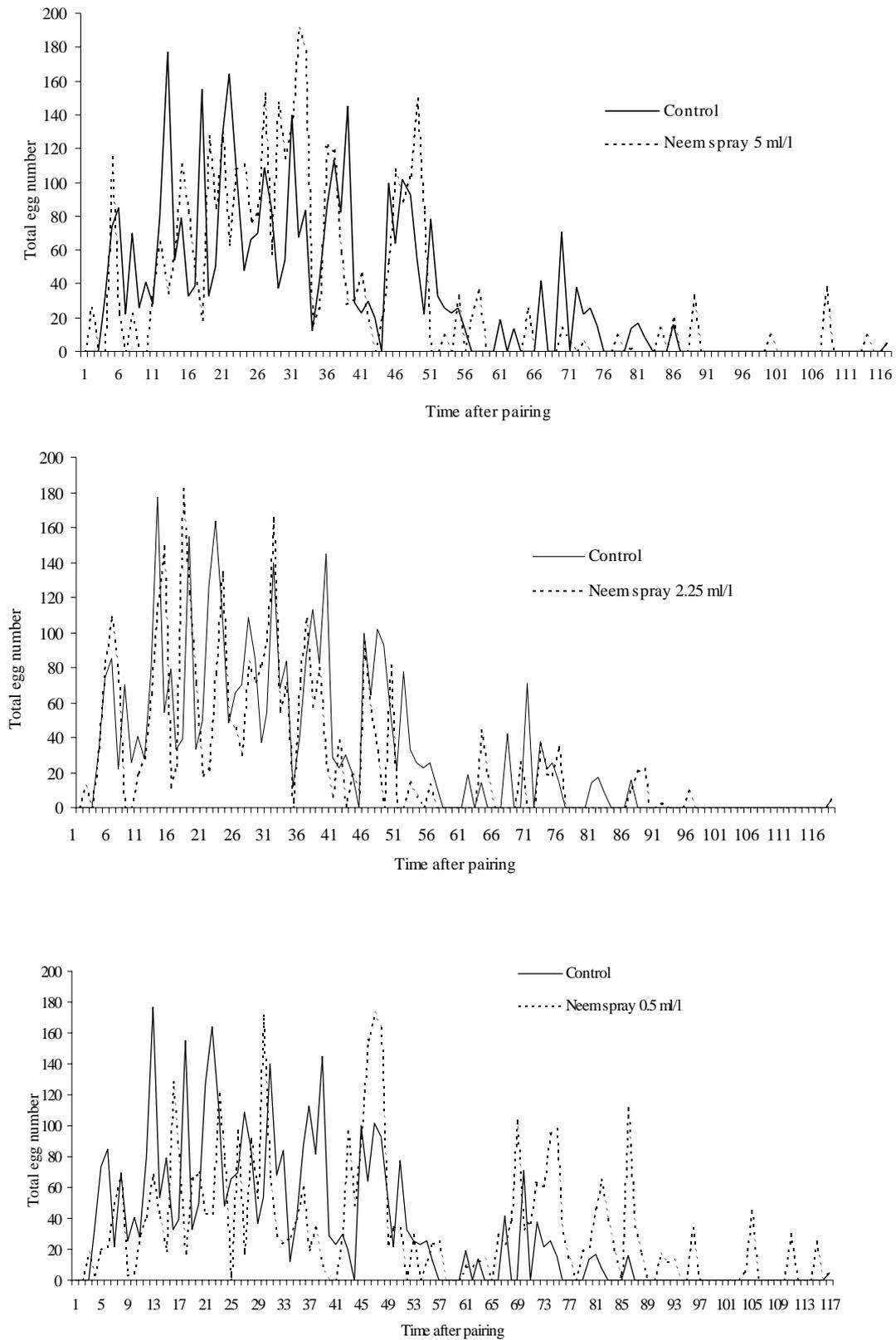


Figure 1. Fertility curves of *C. sanguinea* females after 2nd-instar larvae were sprayed with different concentrations of neem seed oil aqueous solution. Temp.: 25 ± 1°C; Rh: 60 ± 10%, photoperiod: 14h (n = 30)

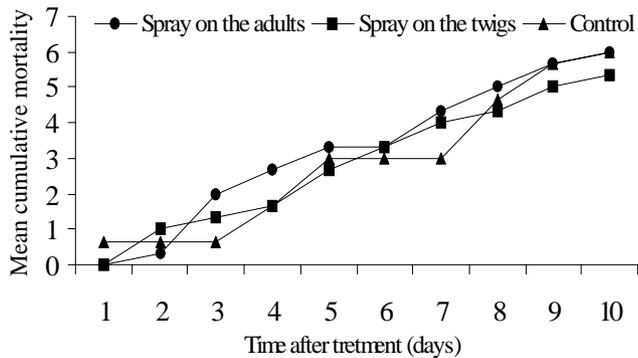


Figure 2. Mean cumulative mortality of *C. sanguinea* adults, treated direct and indirectly with neem seed oil aqueous solution at the concentration of 5 ml/l. Temp.: $25 \pm 1^\circ\text{C}$; RH: $60 \pm 10\%$, photoperiod: 14h (n = 30)

seeds at 5%.

The results showed that treatments with neem seed oil aqueous solutions, except by a medium mortality caused to larvae at 5ml/l, were not harmful to *C. sanguinea*, thus indicating possibility of use of neem products to control aphids when this Coccinellid is present.

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