Toxicity of neem oil to *Bemisia tabaci* biotype B nymphs reared on dry bean

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Abstract – The objective of this work was to determine the most susceptible nymphal stage of *Bemisia tabaci* biotype B to neem (*Azadirachta indica* A. Juss.) oil applied to dry bean (*Phaseolus vulgaris* L.) in a screenhouse. A solution of commercial oil (Dalneem) extracted from neem seeds was sprayed directly on each nymphal instar at 0, 0.1, 0.25, 0.5, 1 and 2% concentrations for lethal concentration (LC) determination, and at 0, 0.5 and 1% concentrations for lethal time (LT) determination. The number of living and dead nymphs was recorded five days after spraying for LC determination, and daily during six days for LT determination. The LC_{50} estimated for fourth instar nymphs occurred at 0.56% concentration. For all instars, LC_{50} and LC_{95} were estimated at 0.32 and 2.78% concentrations, respectively. The estimated values of LT_{50} at 1% concentration were 2.46, 4.45, 3.02 and 6.98 days for the first to fourth instars, respectively. The LT_{50} occurred at five days for 0.5% and at four days for 1% concentration in all instars. A mortality rate of over 80% was observed on the 6th day for the first to third instars at 1% concentration. The first three nymphal stages were more susceptible to neem oil when compared to the fourth nymphal stage.

Index terms: *Azadirachta indica*, *Phaseolus vulgaris*, immature stages, lethal concentration, whitefly.

Toxicidade de óleo de nim para ninfas de *Bemisia tabaci* biótipo B criadas em feijoeiro

Resumo – O objetivo deste trabalho foi determinar o estágio ninfal de *Bemisia tabaci* biótipo B mais suscetível ao óleo de nim (*Azadirachta indica* A. Juss.) aplicado em feijoeiro (*Phaseolus vulgaris* L.), em casa telada. Foram avaliados o tempo letal (TL) e concentração letal (CL) do óleo comercial de sementes de nim Dalneem. Para CL, concentrações de 0, 0,1, 0,25, 0,5, 1 e 2% do produto foram pulverizadas diretamente sobre as ninfas em cada ínstar. Para TL, o produto foi avaliado a 0, 0,5 e 1% de óleo de nim em cada ínstar. Ninfas vivas e mortas foram contadas cinco dias após a pulverização para CL e diariamente para TL durante seis dias. Para o quarto ínstar, a CL_{50} foi de 0,56% de óleo de nim. Considerando todos os ínstares, CL_{50} e CL_{95} foram estimadas em 0,32 e 2,78% de óleo de nim, respectivamente. Os TL_{50} para 1% de nim foram estimados em 2,46, 4,45, 3,02 e 6,98 dias para o primeiro, segundo, terceiro e quarto ínstares, respectivamente. Os TL_{50} estimados para 0,5 e 1% de óleo de nim foram de cinco e quatro dias, respectivamente, considerando todos os ínstares. No sexto dia, foi observada mortalidade superior a 80% para o primeiro, segundo e terceiro ínstares a 1% de óleo de nim. Os três primeiros ínstares foram mais suscetíveis ao óleo de nim que o quarto ínstar.

Termos para indexação: *Azadirachta indica*, *Phaseolus vulgaris*, estágios imaturos, concentração letal, mosca-branca.

Introduction

Bemisia tabaci (Genn.) is a polyphagous and multivoltine insect pest responsible for high economic losses in many crops, due, in part, to the transmission of plant-pathogenic viruses. Twenty different whitefly (*B. tabaci*) biotypes have currently been reported worldwide, named according to their native occurrence (Lima et al., 2000; De Barro et al., 2006). Biotype B is considered the most aggressive because it was distributed over various regions of the world (De Barro et al., 2000). In Brazil, large agricultural areas are successively single-cropped with whitefly host plants, such as beans, soybeans and cotton. In cropping systems like these, the intensive usage of agrochemicals to control whitefly eliminated the natural enemies of this insect as well, favoring the selection of resistant whitefly individuals (Morales & Anderson, 2001).

These practices have contributed to the inefficacy of whitefly chemical control (Barbosa et al., 2004), raised production costs, and reduced crop yields, including dry beans (*Phaseolus vulgaris* L.).

According to biological control practices, pest populations should be reduced, but not exterminated, because their absence could result in agroecosystem imbalance. Based on that principle and aiming for coexistence with insect pests, agricultural practices with low environmental impact have been stimulated by market demands, especially by the increasing number of organic food consumers worldwide (Zehnder et al., 2007). Natural products, like neem (*Azadirachta indica* A. Juss.) oil, tree essential oils, mineral oils and algae extracts have insecticidal, repellent and antifeedant effects and may also act as growth regulators and fertility suppressors of *B. tabaci* (Acosta et al., 2006; Cavalcante et al., 2006; Vasconcelos et al., 2006).

Neem is the most studied natural insecticide and has demonstrated high efficiency in the control of *B. tabaci* nymphs (Price & Schuster, 1991; Liu & Stansly, 1995; Souza & Vendramim, 2000, 2005; Kumar & Poehling, 2007). The insecticidal effect of neem oil on nymphs is due to its antiecdysteroid attributes (Coudried et al., 1985). Most previous studies have been carried out using first instar nymphs and a few studies showed results on other stages (Liu & Stansly, 1995; Souza & Vendramim, 2000, 2005); however, few publications compare neem oil efficacy among nymphal instars. The insecticidal effect of neem in specific nymphal stages of *B. tabaci* was recently reported. However, mean comparisons between these stages were not performed (Kumar et al., 2005; Kumar & Poehling, 2006, 2007). These authors also observed high susceptibility of the first three nymphal stages to neem foliar application, inferring that the first instar was more susceptible than the fourth one. Most researches on neem efficiency against *B. tabaci* nymphs used tomato as the host plant (Kumar et al., 2005; Souza & Vendramim, 2005). There is little information about the effect of neem on insect pests of dry bean. Information about the time of action and concentrations of neem oil required to control this pest is fundamental and necessary when applying botanical insecticides in cropping system management aimed at restoring equilibrium in insect populations.

The objective of this work was to determine the nymphal stage of *B. tabaci* biotype B most susceptible to neem oil.

Materials and Methods

The *Bemisia tabaci* biotype B was obtained from a colony maintained on dry bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) plants in a screenhouse at Embrapa Arroz e Feijão in Santo Antônio de Goiás, GO, Brazil (16°28'0"S, 49°17'0"W). The insect biotype used to initiate the colony was identified through PCR-RAPD of genomic DNA at Instituto Agronômico do Paraná, Londrina, PR, Brazil. The commercial product Dalneem (Dalquim Ltd., Itajaí, Brazil), an emulsion processed from neem seeds, was used in the bioassays.

The lethal concentration (LC_{50} and LC_{95}) of Dalneem to *B. tabaci* nymphs was evaluated in the first experiment. Seedlings of dry bean cultivar Pérola (with two cotyledonary leaves) in soil-filled 200 mL cups were randomly distributed in the rearing screenhouse and submitted to whitefly oviposition for three hours to simulate field conditions. After that, the seedlings were randomly distributed in blocks in another whitefly-free screenhouse to reach a specific nymphal stage. Independent blocks of seedlings containing nymphs of each instar (first to fourth) were sprayed on the abaxial face of the leaves with 500 µL of neem oil per leaf with a micro-sprayer connected to a vacuum pump (Paasche airbrush H-set Type) at 0.1, 0.25, 0.5, 1 and 2% neem oil concentrations, which were determined in preliminary tests.

The experiment was carried out as a completely randomized design in a factorial arrangement (nymphal instar and neem concentration) with four replicates of one seedling with its two cotyledons. Controls were sprayed with distilled water. Living and dead nymphs were counted and recorded five days after neem or water application using a stereoscopic microscope at 40 × magnification. Nymphs that were dark-colored and appeared dried were considered dead.

To estimate the lethal time (LT_{50} and LT_{95}) after Dalneem application, four experiments per instar were independently carried out in a completely randomized design in factorial arrangement, with four replicates of one seedling with two cotyledonary leaves. Seedlings containing nymphs in each instar were sprayed with Dalneem at 0, 0.5 and 1% concentrations, based on the results obtained from the first experiment. The numbers of living and dead nymphs were recorded two hours after spraying (day zero), and every day for the following six days, always at the same time

of day. Two leaves of each replicate were removed from the seedlings to record dead and living nymphs each day.

In the first experiment, regression analyses were performed on proportional data to test the relationship of nymphal mortality with neem oil concentrations. Tests of normality and homogeneity of variance were performed using the HOVTEST WELCH option of the GLM procedure of SAS (SAS Institute, 2002). Non-homogeneous percentage mortality values were transformed using arc sin $(x/100)^{0.5}$ and submitted to factorial analysis of variance to determine the single-factor and interaction effects for instars, neem oil concentrations, and days after spraying. Whenever a significant interaction was observed between factors, the level of one factor was compared to each of the other levels by Tukey's multiple range test, at 5% probability. Neem oil concentrations were transformed to $\log_{10}(x)$, and proportional mortality data were submitted to Probit Analysis. Comparisons were performed for each two independent probit regressions based on fiducial limits as described by Payton et al. (2003). All statistical analyses were carried out using GLM and PROBIT procedures of SAS (SAS Institute, 2002).

Results and Discussion

In the first experiment nymphs mortality for all nymphal stages of *B. tabaci* biotype B increased as neem oil concentrations increased (Figure 1), indicating a contact dosage-dependent toxic effect on *B. tabaci* nymphs. Similar results were obtained by Kumar et al. (2005) and Souza & Vendramim (2005). A phytotoxic effect was observed on dry bean leaves treated with neem oil at concentrations higher than 2%.

Many studies corroborated the efficiency of neem oil as a larvicide for *B. tabaci*. Puri et al. (1994) reported that some neem oil commercial formulations at 0.5 and 1% reduced the number of *B. tabaci* nymphs by 97 to 99%. Souza & Vendramim (2000) reported that neem seed extract was more efficient in reducing the number of first instar nymphs than the extracts of fresh fruits of *Melia azedarach* L. and twigs of *Trichilia pallida* Swartz. High mortality (up to 73%) of the first and third instar nymphs of *B. tabaci* was also recorded by Souza & Vendramim (2005) when they evaluated translaminar, systemic and topical effects of neem seed extract at 0.5% concentration. In some cases, treatments with neem oil formulations were equally or more efficient in reducing the number of *B. tabaci* nymphs than were synthetic agrochemicals (Salas & Mendoza, 2001).

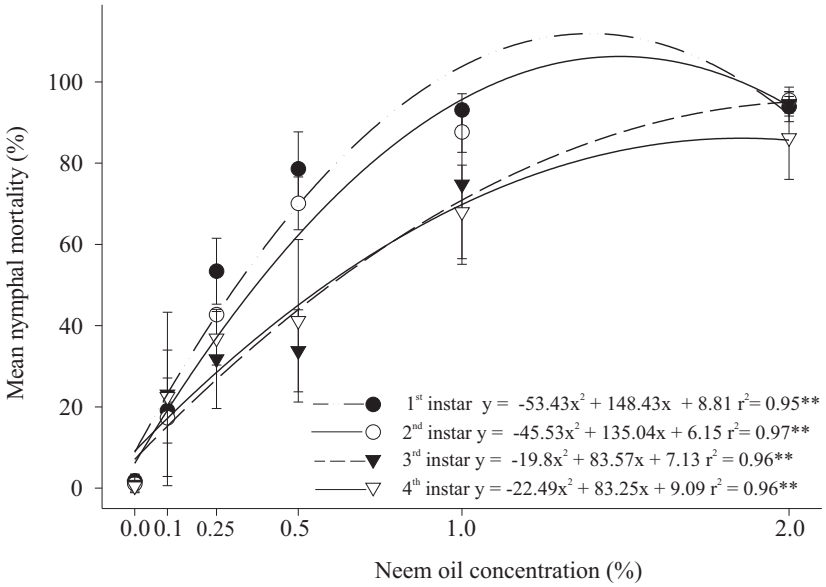

Figure 1. Mortality of *Bemisia tabaci* biotype B nymphs reared on leaves of dry bean seedlings after treatment with neem oil at different concentrations. **Significant at 1% probability.

Differences in nymphal mortality between instars were observed only at concentrations above 0.25%. At this concentration, the first instar mortality was significantly higher (53.4%) only when compared with the third instar (31.8%). Both first and second instars had higher mortality with neem oil at 0.5% than the third and fourth instars (Table 1). These results suggest that applications of neem oil at 0.5% might be more effective if directed against first and second instar nymphs. At concentrations equal or higher than 1%, the only differences that were detected in nymphal mortality were between the first and fourth instars when the oil was tested at 1% concentration. Kumar & Poehling (2006) reported that neem oil was highly toxic to the first instar nymphs after the hatching of viable eggs, corroborating that this stage is very susceptible to the effect of neem. The existence of different degrees of susceptibility among young and fourth instar nymphs was also observed by Kumar et al. (2005), who recorded different rates of nymphal mortality on the four instars of *B. tabaci* after treatment with some formulations of neem oil. Elling et al. (2002) also reported higher susceptibility of young larval instars of *Trialeurodes vaporariorum* after spraying 0.5% of NeemAzal when compared with the fourth instar. According to these authors, treatment of early nymphal stages with this product resulted in a significantly lower number of *T. vaporariorum* fourth instar nymphs in comparison to the number of adults emerged from fourth instar nymphs.

First, second, third and fourth instar nymphs showed no difference ($p<0.05$) in LC_{50} and in LC_{95} (Table 2). Means of LC_{50} and LC_{95} averaged over all nymphal stages were 0.32 and 2.78% concentration, respectively.

In the experiments to estimate lethal time, the mortality of nymphs was significantly affected by nymphal stage ($F = 138.53$; $df = 3, 210$; $p<0.0001$), neem oil concentration ($F = 1041.52$; $df = 2, 210$; $p<0.0001$) and days after spraying ($F = 434.02$; $df = 6, 210$; $p<0.0001$). Interaction was also observed between the three factors ($F = 6.67$; $df = 36, 210$; $p<0.0001$).

On the first day after treatment, significant differences in mortality between treated and control nymphs were observed only for first instar nymphs (Table 3). Neem oil had a significant effect on nymphal mortality on the third and fourth instars after two days, and after four days for the second instar. The highest mortality was observed between three and five days after spraying neem oil on dry bean leaves, for all instars.

Averaged over all nymphal stages, the mean estimated time to death for 50% of the individuals in the population (LT_{50}) was about five and four days for the 0.5 and 1% concentrations, respectively (Table 4). These results

Table 1. Mortality of *Bemisia tabaci* biotype B nymphs of different stages (first to fourth instars), reared on dry bean leaves, after spraying neem oil at different concentrations[(1)].

Concentration (%)	Mortality (%)			
	1st instar	2nd instar	3rd instar	4th instar
0.00	1.8±1.7a (143.5)	1.1±2.2a (57.0)	0.9±1.8a (44.0)	0.5±0.9a (30.7)
0.10	19.1±8.0a (173.7)	17.3±16.7a (51.5)	23.1±20.2a (84.7)	22.5±1.3a (83.0)
0.25	53.4±8.1a (205.2)	42.7±1.3ab (43.0)	31.8±12.2b (100.5)	36.9±6.6ab (98.5)
0.50	78.6±9.1a (116.7)	70.1±6.5a (80.7)	33.8±10.1b (41.0)	41.2±20.0b (114.5)
1.00	93.1±4.0a (124.0)	87.6±5.0ab (59.5)	74.8±19.7ab (48.0)	68.0±11.5b (83.2)
2.00	93.9±3.7a (93.2)	95.5±3.2a (40.0)	94.6±3.0a (76.0)	86.2±10.2a (37.2)

[(1)]Means±SE followed by the same letters do not differ by Tukey's test, at 5% probability; values in parentheses indicate mean sample size; data were converted to arc sin $(x/100)^{0.5}$.

Table 2. Lethal concentration of neem oil to *Bemisia tabaci* biotype B nymphs of different stages (first to fourth instars), reared on dry bean leaves[(1)].

Instar	LC_{50} (%) (FL)	LC_{95} (%) (FL)	Estimate $\log_{10}$ C	Standard error	χ^2	p
1st	0.24 (0.20–0.29)	1.39 (1.02–2.13)	2.18	0.21	106.62	<0.0001
2nd	0.28 (0.23–0.35)	1.81 (1.31–2.93)	1.11	0.12	88.74	<0.0001
3rd	0.38 (0.23–0.60)	3.93 (1.95–18.09)	0.67	0.17	14.91	<0.0001
4th	0.56 (0.36–0.89)	9.49 (3.80–84.34)	0.33	0.13	6.35	<0.0001
Mean	0.32 (0.27–0.37)	2.78 (2.06–4.17)	1.76	0.14	155.57	<0.0001

[(1)]LC_{50} and LC_{95}, lethal concentrations (%) of neem oil to kill 50% and 95% of individuals, respectively; FL, fiducial limits at 95% of probability; C, neem oil concentration; χ^2, chi-square; p, probability.

corroborated earlier findings of Coudriet et al. (1985), who reported that the highest mortality of *B. tabaci* nymphs was observed between two and five days after treatment with neem oil-based products. Kumar & Poehling (2007) observed that a nymphal mortality of 100% was reached with a neem oil formulation four days after application, while abamectin-treated nymphs died 24 hours after application. The slower effect of neem on nymphal mortality probably resulted from its growth regulator effect, mainly due to the abundant presence of azadirachtin, which blocks the synthesis and release of molting hormones (Isman, 2006). Azadirachtin, the main active component of neem oil, was responsible for the extension of *B. tabaci* larvae periods (Coudriet et al., 1985), resulting in a lethal effect. Natarajan & Sundaramurthy (1990) reported that only 14.3 and 13% of *B. tabaci* nymphs reached adult stage after treatment with neem oil at

Table 3. Mortality of *Bemisia tabaci* biotype B nymphs of different stages (first to fourth instars), reared on dry bean leaves, after treatment with neem oil at different concentrations, during six consecutive days[1].

Days[2]	C[3] (%)	Mortality (%)			
		1st instar	2nd instar	3rd instar	4th instar
0	0.0	0±0aA (220.5)	0.47±0.55aA (209.7)	0±0aA (130.3)	0.4±0.6aA (164.3)
	0.5	0.21±0.25aA (315.0)	0.81±1.1aA (127.5)	0±0aA (89.3)	0±0aA (161.0)
	1.0	0.08±0.17aA (320.5)	0±0aA (88.5)	0±0aA (89.3)	0.8±0.8aA (221.3)
1	0.0	0±0aA (460.5)	0.6±0.5aA (140.2)	0.1±0.1aA (242.7)	0.3±0.6aA (110.3)
	0.5	2.2±0.65bA (261.5)	0.8±0.6aA (130.7)	1.5±0.8aA (225.3)	2.92±2.1aA (201.7)
	1.0	3.67±1.59bA (207.5)	0.7±0.8aA (87.7)	1.1±1.1aA (178)	4.6±3.7aA (166.0)
2	0.0	0.41±0.5aA (343.3)	1.3±0.9aA (146.0)	0±0aA (202.7)	0±0aA (239.0)
	0.5	9.27±1.8bA (257.8)	1.8±0.6aB (164.0)	11.8±4.7bA (186.6)	7.3±2.8bA (175.3)
	1.0	34.9±10.7cA (233.3)	1.1±1.5aB (46.7)	23±2.1cA (205.0)	6.4±1.6bB (186)
3	0.0	2.2±1.2aA (312.2)	0.3±0.5aA (58.5)	0.5±0.6aA (200.3)	0.9±1.6aA (155.0)
	0.5	62.3±11.1bA (182.0)	5.9±4.2abB (82.0)	44.6±10.2bA (157.0)	11.9±2.9bB (96.3)
	1.0	75.6±4.7bA (160.2)	12.1±12.2aB (140.0)	33.5±4.1bC (155.0)	13.7±6.2bB (204.6)
4	0.0	2.4±1.2aA (296.2)	2.5±2.5aA (109.0)	0±0aB (186.7)	0.7±0.7aAB (285.0)
	0.5	77.7±12.7bA (156.0)	33.9±19.8bB (106.0)	60.1±10.3bAB (92.0)	16.1±1.9bC (146.0)
	1.0	90.5±0.9bA (126.5)	39.7±15.5bB (103.2)	75.1±10.9bA (93.3)	28.8±13.3bB (227.0)
5	0.0	3.0±1.2aA (285.2)	1.6±1.6aA (128.0)	0.7±0.3aA (271.7)	0.8±1.0aA (225.7)
	0.5	70.7±9.5bA (282.0)	71.2±10.8bA (123.0)	74.8±4.9bA (118.3)	10.2±1.3bB (155.3)
	1.0	88.1±3.6cA (204.5)	72.7±7.6bA (79.5)	91.8±5.3cA (149.7)	41.9±9.1cB (86.0)
6	0.0	5.5±1.1aA (334.2)	0.1±0.3aB (141.7)	3.1±3.8aAB (132.0)	0.5±0.8aB (284.0)
	0.5	68.5±9.6bA (209.2)	76.1±13.3bA (75.0)	60.3±23.6bA (118.3)	31.7±11.6bB (102.3)
	1.0	88.6±4.8cA (189.7)	80.6±5.7bA (69.0)	96.2±1.2cA (196.0)	37.8±4.4bB (82.3)

[1]Means±SE followed by the same small letter, in the column, and the same capital letter within a row do not differ by Tukey's test, at 5% probability; data were converted to arc sin $(x/100)^{0.5}$; values in parentheses indicate mean number of insects tested. [2]Days after spraying. [3]Neem oil concentration.

Table 4. Lethal time of neem oil at different concentrations to *Bemisia tabaci* biotype B nymphs of different stages (first to fourth instars), reared on dry bean leaves[1].

Instar	C (%)	LT_{50} (FL)	LT_{95} (FL)	Estimate $\log_{10}$ (days)	Standard error	χ^2	p
1st	0.5	3.48 (3.07–3.95)	9.78 (7.73–14.2)	3.66	0.44	69.34	<0.0001
	1.0	2.46 (2.25–2.66)	6.02 (5.36–6.99)	4.23	0.30	191.03	<0.0001
2nd	0.5	4.49 (4.24–4.75)	7.35 (6.57–8.84)	7.70	0.99	59.59	<0.0001
	1.0	4.45 (4.21–4.72)	7.32 (6.54–8.68)	7.60	0.83	83.32	<0.0001
3rd	0.5	3.43 (2.86–4.09)	11.13 (8.31–18.29)	3.22	0.44	53.34	<0.0001
	1.0	3.02 (2.76–3.26)	6.14 (5.46–7.26)	5.33	0.52	104.85	<0.0001
4th	0.5	21.07 (11.13)[2]	-[2]	1.28	0.30	17.48	<0.0001
	1.0	6.98 (5.49)[2]	26.92 (12.58)[2]	2.80	0.90	9.61	<0.0001
Mean	0.5	4.38 (3.99–4.88)	13.25 (10.5–18.7)	3.42	0.35	93.83	<0.0001
	1.0	3.43 (3.13–3.75)	8.93 (7.47–11.66)	3.96	0.42	87.18	<0.0001

[1]C, neem oil concentration; LT_{50} and LT_{95}, lethal time (days) to kill 50 and 95% of individuals, respectively; FL, fiducial limits at 95% probability; χ^2, chi-square; p, probability. [2]Estimated values exceeded *B. tabaci* life cycle.

0.5 and 1% concentrations, respectively. Despite the slower effect of neem oil on *B. tabaci* nymphs when compared to synthetic agrochemicals, it can be the best alternative when considering its fast degradation in the environment, low human toxicity, and low probability of selecting resistant individuals due to the presence of different compounds with insecticide properties. In the present research, values of approximately 13 and 9 days for LT_{95} were estimated for 0.5 and 1.0% concentrations, respectively.

After six days, an increase in neem oil concentration from 0.5 to 1% did not result in higher mortality of the second and fourth instar nymphs (Table 3). Souza & Vendramim (2005) also observed no difference in nymphal mortality of *B. tabaci* third instars treated with 0.5 or 1% of neem oil. However, neem oil at 1% concentration killed significantly more nymphs of the first and third instar when compared to 0.5%. In the present research, mortality higher than 80% was observed for the first three nymphal stages six days after spraying 1% neem oil.

Neem oil at 0.5 and 1% concentrations killed significantly more nymphs of the first, second and third instars when compared with the fourth instar (Table 3). Accordingly, the estimated values of LT_{50} and LT_{95} were higher for the fourth instar when compared with the other instars (Table 4). Kumar et al. (2005) also observed that fourth instar nymphs were the least susceptible to another commercial neem oil product. The lesser susceptibility of the fourth instar nymphs can be explained by considering the two modes of action of neem oil: contact or ingestion. One possible explanation is that fourth instar nymphs have a cuticular layer preventing their contact with the neem oil applied on leaves, as observed by Wang et al. (2003) when evaluating the effect of synthetic agrochemicals on fourth instar nymphs of *T. vaporariorum*. Another explanation was given by Elling et al. (2002) and Kumar et al. (2005) based on the evidence that the fourth instar is divided into three substages, and the nymphs feed in the first substage only (Gill, 1990). These authors affirmed that since fourth instar nymphs feed only in their first substage, they are more capable of avoiding the effects of neem oil by ingestion. Neem compounds can penetrate into the leaves as observed by Souza & Vendramim (2005), who registered translaminar effect of neem seed extract on *B. tabaci* nymphs. According to Isman (2006) there are many plant species with antifeedant properties, but among these, only neem seems to cause toxic effects by ingestion, with deleterious physiological consequences to insects, as observed by Bleicher et al. (2007) and Singha et al. (2007).

The results of the present research suggest that neem oil at a 0.5% concentration may be used in preventive applications to reduce initial infestation of *B. tabaci* on dry bean without possible phytotoxic effects. Moreover, a 0.5% neem oil concentration would be less expensive, considering that one of the major limitations for the use of this product in agriculture is its high refining cost (Isman, 2006). Neem oil at a 1% concentration, however, can kill over 80% of the nymphs from first to third instar and 68% of fourth instar nymphs, so it is an alternative for the reduction of late whitefly infestations. According to Zehnder et al. (2007) arthropods in organic production systems should be managed using multiple strategies, aimed at the reduction of insect pest populations to minimize the need for curative solutions. Based on estimated values of LC_{50} and LT_{50} for neem oil at 0.5% and 1% concentrations, this botanical extract can be one of those organic production strategies.

Conclusions

1. First, second and third instar nymphs are more susceptible to neem oil than fourth instar nymphs.

2. Neem oil at 1% concentration can reduce the population of first to third nymphal instars by over 80%.

3. Based on estimated values of lethal concentration and lethal time, neem oil at 1% concentration can be efficient to reduce populations of *Bemisia tabaci* nymphal stages.

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