Effect of neem extract on the cotton aphid

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Abstract – The aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), a harmful pest on cotton, causes direct damage, reducing plant vigor, and indirect damage by honeydew secretion and transmission of several viruses. Due to the problem of pesticide resistance, alternative techniques for chemical control, such as the use of natural insecticides, have been object of research. The effects of aqueous extracts of neem (*Azadirachta indica* A. Juss) seed powder on the development, survival and fecundity of *A. gossypii* were evaluated. Treatments consisted of neem seed powder in the concentrations of 23.8, 122.0, 410.0 and 1,410.0 mg/100 mL of distilled water. Mortality rate during the nymphal development for aphids maintained on cotton leaf discs treated with the two highest concentrations were, respectively, 60.0% and 100.0%. With the exception of the highest concentration (1,410.0 mg/100 mL), neem concentrations did not extend the aphids' development period. The net reproductive rate (R_0) was of 35.0 nymphs/female for control aphids and of 0.0 nymph/female when the group of females was exposed to neem seed powder at 1,410.0 mg/100 mL since birth. The aqueous extract of neem seeds is efficient against the aphid *A. gossypii*, causing nymph mortality and reducing their survival period and fecundity.

Index terms: Insecta, *Aphis gossypii*, *Azadirachta indica*, *Gossypium hirsutum*, aqueous extract.

Efeito de extrato de nim sobre o pulgão-do-algodoeiro

Resumo – O pulgão *Aphis gossypii* Glover (Hemiptera: Aphididae), praga prejudicial à cultura do algodoeiro, causa danos diretos, reduzindo o vigor da planta, e indiretos, ao secretar substância doce e transmitir viroses. Em virtude da resistência desse afídeo aos inseticidas, técnicas alternativas ao controle químico, como o uso de inseticidas naturais, têm sido pesquisadas. O objetivo deste trabalho foi avaliar os efeitos de extratos aquosos de pó de amêndoas de nim (*Azadirachta indica* A. Juss) sobre o desenvolvimento, sobrevivência e fecundidade de *A. gossypii*. Os tratamentos consistiram de pó de amêndoas de nim nas concentrações de 23,8, 122,0, 410,0 e 1.410,0 mg/100 mL de água. Os porcentuais de mortalidade durante o período ninfal para os pulgões mantidos sobre discos foliares de algodoeiro imersos nas duas maiores concentrações, foram, respectivamente, 60,0% e 100,0%. Com exceção da maior concentração (1.410,0 mg/100 mL), as demais não prolongaram as fases de desenvolvimento dos pulgões. A taxa líquida de reprodução foi de 35,0 ninfas/fêmea na testemunha e de 0,0 ninfa/fêmea no grupo dos pulgões expostos à concentração de 1.400 mg/100 mL, desde o nascimento. O extrato aquoso das sementes de nim é uma alternativa eficiente para o controle de *A. gossypii*, causando mortalidade de ninfas e reduzindo os períodos de sobrevivência e fecundidade.

Termos para indexação: Insecta, *Aphis gossypii*, *Azadirachta indica*, *Gossypium hirsutum*, extrato aquoso.

Introduction

The aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is one of the most common species in cotton (Heneberry & Jech, 2001), and is usually found on the abaxial surface of leaves, feeding on the phloem (Freeman & Smith, 2002). As a result of feeding, plant yield is reduced. The secretion of honeydew contaminates plants, allowing associated fungi to grow and causing, in addition, more than 50 types of diseases in plants, due to the transmission of viruses (Heneberry & Jech, 2001).

One of the first approaches utilized by growers to protect cotton plants from the aphid *A. gossypii* and from other pests is the use of insecticides. As such applications are quite frequent, most natural enemies are eliminated when the aphids are still capable of developing resistance to those products (Godfrey et al., 2000), making subsequent treatments inefficient and leading to an increase in aphid population levels (Godfrey & Fuson, 2001). Alternative techniques for chemically controlling of cotton aphids are the biological control and the use of natural insecticides and resistant plants (Weathersbee

III et al., 1995; Kabissa et al., 1996; Chitra et al., 1997; Mann et al., 2001).

Azadirachtin, a chemical complex found in seeds of neem, *Azadirachta indica* A. Juss, is the main component responsible for the toxic, repellent, anti-feedant, growth-inhibiting, oviposition-inhibiting and sterilizing effects in insects (Ahmed & Grainge, 1986; Schmutterer, 1990; Mordue & Nisbet, 2000; Martinez, 2002). More than 400 insect species belonging to the orders Diptera, Hymenoptera, Coleoptera, Lepidoptera, Orthoptera and Hemiptera are susceptible to neem effects (Martinez, 2002). The extract of this plant reduces the population of several aphid species in many crops, causing high mortality and decreasing fecundity, as well as inhibiting population growth (Lowery et al., 1993; Stark & Rangus, 1994; Partridge & Borden, 1997; Ulrichs et al., 2001; Tang et al., 2002).

Neem extracts are usually safe for beneficial organisms, such as bees, predators and parasitoids, mammals, and for the environment (Ahmed & Grainge, 1986; Tang et al., 2002). This plant is an interesting option for integrated pest management programs, since it is selective, presents a less negative impact on the ecosystems and works in association with biological control organisms (Stark & Rangus, 1994; Immaraju, 1998; Tang et al., 2002).

The objective of this work was to evaluate the effects of aqueous extracts of neem seeds on the development, survival and fecundity of *A. gossypii* on cotton.

Material and Methods

This work was carried out from August to October 2002. *A. gossypii* rearing was started with aphids collected from a cotton field located at Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Campus Jaboticabal, SP, Brazil. The aphids were then transferred to cotton plants of cultivar IAC 22 maintained in 500 mL plastic cups, containing a mixture of three parts soil, one part sand and one part manure and 0.5 part vermiculite as substrate. Every 15 days the aphids were transferred to new plants, at a plant age of 20 days after emergence. The rearing was maintained in a greenhouse lined with anti-aphid netting, to avoid infestation by other aphid species and by natural enemies.

The wingless *A. gossypii* females were selected at random, transferred to cotton leaf discs of 5.0 cm in diameter and maintained on 1% agar in Petri dishes. Neonate nymphs were obtained after 24 hours and then these nymphs were used in the experiments.

In order to evaluate the effect of azadirachtin on *A. gossypii*, the extracts were prepared beforehand, 16 hours prior to the experiment start. The treatments consisted of neem seed powder at concentrations of 23.8, 122.0, 410.0 and 1,410.0 mg/100 mL water and control consisted of distilled water. Discs measuring 5 cm in diameter were cut out from the center of cotton leaves, cultivar Coodetec, from field-grown plants. Each leaf disc was dipped into aqueous extract of neem seed powder for 30 seconds and then air dried.

Leaf discs corresponding to each treatment were individualized and maintained with their abaxial surface facing up on a 1.0 cm layer of agar 1% in a Petri dish with 12 cm in diameter. Neonate nymphs were individualized on the surface of each leaf disc with the use of a number zero soft hair brush. Each Petri dish was sealed with *voile* fabric, kept in place by rubber bands and maintained in incubator at 25±1ºC, with a 12-hour photophase and relative humidity of 70±10%. Every four days the dishes containing leaf discs and agar were changed and the new leaf discs were not submitted to the neem treatments. During the reproductive period, the neonate nymphs were daily counted and then removed. A completely randomized design was utilized, with 60 replicates per treatment. Each experimental unit consisted of a Petri dish containing a layer of agar and the aphid. Data were submitted to analysis of variance and means were compared by Tukey test at 5% of probability.

Daily observations were made on the number of molts undergone by the aphids, including those individuals that died before reaching adult stage and the survival period was also evaluated. Determinations included the duration of each instar, nymphal period of surviving aphids, mortality during that period, reproductive period, daily and total offspring produced, longevity and duration of the biological cycle of *A. gossypii* aphids. The effect of neem concentrations on *A. gossypii* was also determined by means of fertility life tables, with parameters calculated according to Silveira Neto et al. (1976):

$R_0 = \sum (m_x l_x)$,

where R_0 is the net reproductive rate; m_x is the number of females produced per female at age x; and l_x is the survival rate at age x; and

$r_m = (\log_e R_0) / T$,

where $\log_e R_0$ is anti-logarithmic of R_0 and T is mean generation time.

Results and Discussion

Effects of neem on nymph development and survival

A. gossypii nymphs showed a decrease in the number of molts as the neem concentrations increased (Table 1). Aphids reared on cotton leaf discs submitted to a concentration of 1,410.0 mg/100 mL showed on average less than one molt, while control nymphs showed 3.5 molts on average.

These results are similar to those obtained by Stark & Rangus (1994) for *Acyrthosiphon pisum* (Harris) nymphs exposed to bean plants treated with Margosan-O, a commercial neem formulation. According to these authors, the molting process for this species was totally interrupted at the two highest concentrations (80 and 100 mg azadirachtin/L) with a mean of 0.7 molts. For the aphid *Toxoptera citricida* (Kirkaldy), nymphs reared on citrus seedlings sprayed with Neemix at concentrations from 2.2 to 18 mg of azadirachtin/100 mL showed a similar behavior, undergoing 0.4 molts on average (Tang et al., 2002). Azadirachtin, the active principle of neem, causes an interruption in the concentrations of ecdisone and juvenile hormone in the hemolymph, affecting molting, metamorphosis and reproduction (Mordue & Nisbet, 2000).

In the present study, *A. gossypii* nymphs exposed to higher concentrations of neem frequently died during the molting process, and parts of the old integument remained attached to the body of the insect. Another observed symptom was a change in nymph coloration from yellow to brownish black. An identical symptom was reported by Stark & Rangus (1994) for nymphs and adults of *A. pisum* maintained on bean seedlings treated with high concentrations of Margosan-O. Azadirachtin causes a number of effects on pest insects, including growth regulation, which brings about a disruption of development and molting (Schmutterer, 1990; Mordue et al., 1998). The active principle found in neem causes severe damage in insects by interfering with molting. Larvae and nymphs are affected since they depend on this process for their development and growth. Molting could be interrupted, causing the death of the insect either during the larval or nymphal stage or during the pupal period (Martinez, 2002).

A. gossypii aphids exposed to the higher concentration of neem (1,410.0 mg/100 mL) showed 85.6% reduction in survival period in relation to aphids submitted to the control (Table 1). Aphids submitted to a concentration of 1,410.0 mg/100 mL survived only until the first instar and after this stage 100% mortality was observed. For surviving nymphs, the duration of instars and of the nymphal period were not significantly different between aphids submitted to neem treatments and control. The first, second, third and fourth instars lasted on average 1.6, 1.3, 1.3 and 1.4 days, respectively. These results are similar to those found by Xia et al. (1999) and Michelloto (2002) for this same aphid species maintained on cotton plants. The nymphal period lasted 5.5 days, a value that is close to those obtained by Akey & Butler Junior (1989) and Kersting et al. (1999) for *A. gossypii*, reared on cotton cultivars Stoneville 825 and Çukurova 1518 at 25ºC, respectively.

With the exception of the highest concentration utilized, the concentrations did not extend the development stages of surviving aphids (Table 1). Neem can cause a delay in insect development (Martinez, 2002), however, its potential is affected by the rate and frequency of application (Partridge & Borden, 1997), life stage during which the aphid is exposed, as well as by the type of exposure (Stark & Rangus, 1994).

Table 1. Development (days) of *Aphis gossypii* on cotton treated with aqueous extracts of neem (*Azadirachta indica*) seed powder. Temperature of 25±1ºC, relative humidity of 70±10% and photophase of 12 hours[(1)].

Treatment (mg/100 mL of water)	No. of molts	Mean survival period	Instar duration				Nymphal period
			1st	2nd	3rd	4th	
Control	3.5±0.40a	17.4±2.69a	1.5±0.26a	1.2±0.12a	1.3±0.14a	1.4±0.29a	5.4±0.32a
23.8	3.1±0.37ab	14.7±3.19ab	1.6±0.18a	1.3±0.12a	1.2±0.12a	1.4±0.28a	5.2±0.28a
122.0	2.8±0.44bc	13.0±3.57b	1.6±0.26a	1.2±0.18a	1.3±0.28a	1.4±0.14a	5.4±0.53a
410.0	2.2±0.46c	7.2±1.48c	1.7±0.39a	1.5±0.16a	1.4±0.29a	1.5±0.26a	5.9±0.73a
1,410.0[(2)]	0.6±0.25d	2.5±0.40d	1.4±0.31a	-	-	-	-
Mean	2.4	11.0	1.6	1.3	1.3	1.4	5.5
CV (%)	16.1	23.2	18.5	11.4	17.3	17.7	9.1

[(1)]Means followed by a common letter in the column did not differ significantly at 5% probability level by Tukey test; parameters calculated considering only aphids that reached the adult stage; data are means±standard error. [(2)]Treatment showing 100% mortality in the nymphal stage.

The mortality of nymphs exposed to neem was dependent upon concentration and a linear relationship occurred between these variables (Figure 1). This observation is similar to the verified by Tang et al. (2002), that the mortality of nymphs and adults of *T. citricida* on citrus treated with Neemix was dependent upon rate, and to results by Lowery et al. (1993), who found a positive correlation between aphid mortality and neem extract rates. In the present study, the percentage mortality during the nymphal period of *A. gossypii* at the two highest concentrations was of 60.0% and 100.0%, respectively.

Effects of neem on longevity and reproduction

The neem extracts negatively influenced *A. gossypii* reproduction, reducing the net reproductive rate (R_0) and the intrinsic rate of population increase (r_m) of aphids exposed to neem treatments since their nymphal stage (Figures 2 and 3). The net reproductive rate (R_0), that is, the number of nymphs laid by each female during one generation, decreased as the extract concentration increased. This rate was of 35.0 nymphs for each female in the control and 0.0 nymph/female for the group of females exposed from birth to a concentration of 1,410 mg neem seed powder/100 mL (Figure 2). The intrinsic rate of population increase (r_m), which represents the population growth index of the aphid, also decreased as concentration increased. The highest r_m was obtained for adults in the control (0.32), while for aphids submitted to the highest neem concentration this rate was zero (Figure 3). These results corroborate those by Stark & Wennergren (1995) on effects of the neem-based insecticide Margosan-O against the aphid *A. pisum*.

The reproductive period of surviving *A. gossypii* aphids, 12.9 days on average, was not significantly influenced by the concentration of neem extracts (Table 2). However, the daily and total offspring produced were lower at the higher concentrations. Adults submitted to the control treatment and to the lowest neem concentration (23.8 mg/100 mL) produced twice as many nymphs in relation to adults maintained on leaf discs treated with 410.0 mg of neem extract/100 mL. Neem extracts at several concentrations reduced the fecundity of the aphids *A. pisum* on faba bean plants (Stark & Rangus, 1994; Stark & Wennergren, 1995), *Myzus persicae* (Sulzer) on sweet pepper, *Nasonovia ribisnigri* (Mosley) on lettuce, and *Chaetosiphon fragaefolii* (Cockerell) on strawberry (Lowery & Isman, 1996), *Elatobium abietinum* (Walker) on conifers (Partridge & Borden, 1997) and *T. citricida* on citrus (Tang et al., 2002).

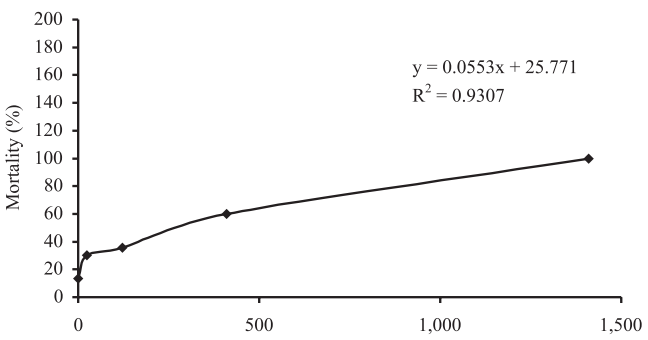

Figure 1. Percentage of mortality during the nymphal period of *Aphis gossypii* on cotton treated with aqueous extracts of neem (*Azadirachta indica*) seed powder. Temperature of 25±1ºC, relative humidity of 70±10% and photophase of 12 hours.

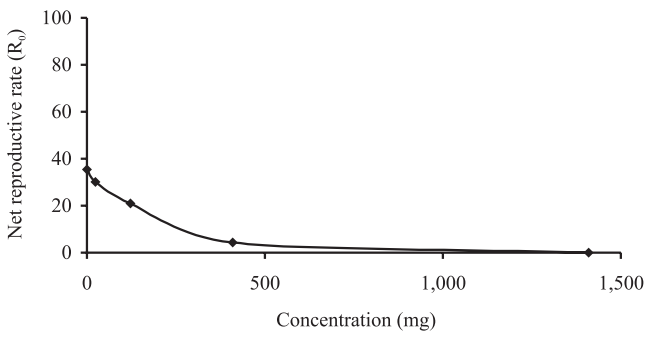

Figure 2. Net reproductive rate (R_0) of *Aphis gossypii* on cotton treated with aqueous extracts of neem (*Azadirachta indica*) seed powder. Temperature of 25±1ºC, relative humidity of 70±10% and photophase of 12 hours.

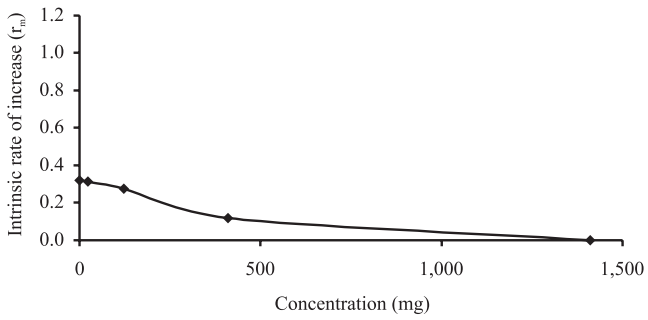

Figure 3. Intrinsic rate of population increase (r_m) of *Aphis gossypii* on cotton treated with aqueous extracts of neem (*Azadirachta indica*) seed powder. Temperature of 25±1ºC, relative humidity of 70±10% and photophase of 12 hours.

Survival *A. gossypii* adults had their longevity and biological cycle duration reduced as a function of the neem extract concentrations (Table 3). The control aphids showed a biological cycle with a mean duration of 20.3 days and a longevity of approximately 15.0 days, while those exposed to cotton leaves treated with a neem concentration of 410.0 mg/100 mL showed a cycle of approximately 15.0 days and a mean longevity of 8.8 days.

Studies on the effect of lethal and sublethal concentrations of natural insecticides on the biology of pest insects are quite important. In spite of the fact that high concentrations of neem can cause high mortality in aphids, these are possibly not economically viable. Intermediate concentrations have been recommended, since they can be utilized in association with other methods, such as biological control (Tang et al., 2002). Since the cotton aphid presents a great diversity of natural enemies, the evaluated concentrations provide basic knowledge for future studies on the association between natural insecticides and biological control.

Neem-based insecticides efficiently control several aphid species in the field (Lowery et al., 1993). In the laboratory, the aqueous extract of neem seeds was efficient against the aphid *A. gossypii*, causing nymph mortality and reducing their survival period and fecundity. Since the potential of this natural insecticide varies according to meteorological conditions (Partridge & Borden, 1997), additional studies are necessary to evaluate its efficiency under field conditions.

Table 2. Reproductive period (days), daily and total offspring in surviving *Aphis gossypii* aphids on cotton treated with aqueous extracts of neem (*Azadirachta indica*) seed powder. Temperature of 25±1ºC, relative humidity of 70±10% and photophase of 12 hours[(1)].

Treatment (mg/100 mL of water)	Reproductive period	No. of offspring/day	Total number of offspring
Control	13.1±1.71a	3.6±0.50a	43.0±3.24ab
23.8	13.2±2.96a	3.6±0.50a	45.6±2.81a
122.0	12.5±3.39a	3.0±0.85a	33.4±3.42b
410.0	12.6±2.28a	1.7±0.60b	20.3±1.43c
1,410.0[(2)]	-	-	-
Mean	12.9	2.9	35.6
CV (%)	20.7	21.4	19.5

[(1)]Means followed by a common letter in the column did not differ significantly at 5% probability level by Tukey test; parameters calculated considering only aphids that reached the adult stage; data are means±standard error. [(2)]Treatment showing 100% mortality in the nymphal stage.

Table 3. Duration (days) of biological cycle and longevity of survival *Aphis gossypii* aphids on cotton treated with aqueous extracts of neem (*Azadirachta indica*) seed powder. Temperature of 25±1ºC, relative humidity of 70±10% and photophase of 12 hours[(1)].

Treatment (mg/100 mL of water)	Biological cycle	Longevity
Control	20.3±1.24a	15.0±1.19a
23.8	20.5±1.70a	15.2±1.70a
122.0	17.0±1.06ab	11.5±1.11ab
410.0	14.8±1.10b	8.8±1.11b
1,410.0[(2)]	-	-
Mean	18.1	12.6
CV (%)	17.5	25.2

[(1)]Means followed by a common letter in the column did not differ significantly at 5% probability level by Tukey test; parameters calculated considering only aphids that reached the adult stage; data are means±standard error. [(2)]Treatment showing 100% mortality in the nymphal stage.

Conclusion

1. The neem extracts negatively influence *A. gossypii* reproduction, reducing the net reproductive rate (R_0) and the intrinsic rate of population increase (r_m) of aphids exposed to neem treatments since their nymphal stage.

2. The aqueous extract of neem seeds is efficient against the aphid *A. gossypii*, causing nymph mortality and reducing their survival period and fecundity.

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