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Effects of botanical insecticides on the instantaneous population growth rate of *Aphis gossypii* Glover (Hemiptera: Aphididae) in cotton

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ABSTRACT. Botanical insecticides have been studied aiming the alternative pest control. The present study investigated the effects of these insecticides on the instantaneous population growth rate (ri) of *Aphis gossypii*. Botanical insecticides were tested in the following concentrations: Compostonat®, Rotenat-CE® and Neempro (0, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75%); Natuneem® and Neemseto® (0, 0.25, 0.50, 0.75 and 1.00%) and essential oils of *Foeniculum vulgare* Mill., *Cymbopogom winterianus* (L.), *Chenopodium ambrosioides* L. and *Piper aduncum* L (0, 0.0125, 0.025, 0.0375 and 0.05%). Cotton leaf discs, CNPA 8H cultivar with 5 cm in diameter were immersed for 30 seconds in products broth and dried for 30 minutes. Eight replicates per concentration were used and each disc was infested with five apterous adult females of *A. gossypii* uniform in size and confined for 10 days. Compostonat®, Rotenat® and Neempro provided negative r_i decreasing *A. gossypii* population. Natuneem® and Neemseto® and the essential oil of *F. vulgare* showed positive r_i increasing the population. The coefficients of determination (R^2) of regression lines ranged from 0.46 to 0.85. The essential oils of *C. winterianus*, *C. ambrosioides* and *P. aduncum* were not statistically significant precluding the establishment of regression lines.

Keywords: cotton aphid, bioactivity, *Gossypium hirsutum*, natural insecticides.

Efeitos de inseticidas botânicos na taxa instantânea de crescimento populacional de *Aphis gossypii* Glover (Hemiptera: Aphididae) em algodão

RESUMO. Inseticidas botânicos têm sido estudados, visando o controle alternativo de pragas. O presente trabalho objetivou estudar os efeitos desses inseticidas sobre a taxa instantânea de crescimento populacional (r_i) de *Aphis gossypii*. Os inseticidas botânicos foram testados nas seguintes concentrações: Compostonat®, Rotenat-CE® e Neempro (0; 0,50; 0,75; 1,00; 1,25; 1,50 e 1,75%); Nautneem® e Neemseto® (0; 0,25; 0,50; 0,75 e 1,00%); e os óleos essenciais de *Foeniculum vulgare* Mill., *Cymbopogom winterianus* (L.), *Chenopodium ambrosioides* L. e *Piper aduncum* L. (0; 0,0125; 0,025; 0,0375 e 0,05%). Discos de folhas de algodoeiro, cultivar CNPA 8H, com 5 cm de diâmetro foram imersos por 30 segundos nas caldas dos produtos e secos por 30 minutos. Utilizaram-se oito repetições por concentração, e cada disco foi infestado com cinco fêmeas adultas ápteras de *A. gossypii* de tamanho uniforme, confinadas durante 10 dias. Compostonat®, Rotenat® e Neempro proporcionaram r_i negativas, declinando a população de *A. gossypii*. Natuneem®, Neemseto® e o óleo essencial de *F. vulgare* apresentaram r_i positivas, aumentando a população. Os coeficientes de determinação (R^2) das retas de regressão variaram entre 0,46 a 0,85. Os óleos essenciais de *C. winterianus*, *C. ambrosioides* e *P. aduncum* não apresentaram significância estatística, impossibilitando o estabelecimento das retas de regressão.

Palavras-chave: pulgão-do-algodoeiro, bioatividade, *Gossypium hirsutum,* inseticidas naturais.

Introduction

Aphis gossypii Glover (Hemiptera: Aphididae) is a pest of great economic importance to cotton crop. Adults and nymphs suck sap from the phloem, inoculate toxins, excrete sugary substances (honeydew), favoring the sooty mold development, and are vectors of virus in cotton, such as the vermilion and vein mosaic form Ribeirão Bonito (MICHELOTTO; BUSOLI, 2003). The rapid multiplication of *A. gossypii* requires the producer to maintain short intervals of insecticide applications, which can lead to selection of resistant insect populations (BARROS et al., 2006).

Due to the concern of researchers, farmers and society as a whole, about the side effects of pesticides overuse, researched on new alternative methods of pest control has a major boost in recent decades, citing as example studies with botanical insecticides (AHMAD

et al., 2003; CLOYD et al., 2009; ESTRELA et al., 2006; ISMAN, 2006; MARTINEZ; VAN EMDEN, 2001). These products, derived from secondary metabolism of plants, are composed of complex mixtures of chemical substances such as monoterpenes, sesquiterpenes and flavonoids playing important roles in the processes of tritrophic interactions (plant-insect-natural enemy) and in the control of insects, mites, fungi and nematodes (FAZOLIN et al., 2005; SCHMUTTERER, 1990; TAVARES; VENDRAMIM, 2005). They act on insects by ingestion, contact and fumigation, and can be used as powders, extracts, essential oils and oil emulsion (ABRAMSON et al., 2006; RAJENDRAN; SRIRANJINI, 2008). They are generally biodegradable, low toxicity to vertebrates and may have selectivity for natural enemies (COSME et al., 2007; SILVA; MARTINEZ, 2004). Its effects on insects include mortality, feeding deterrence and oviposition, reductions in fecundity, fertility and growth process (BOEKE et al., 2004; MARTINEZ, 2002; ROEL et al., 2000).

The instantaneous population growth rate (r_i) has been used in the assessment of lethal and sublethal effects of insecticides on pests and natural enemies by having more consistent results, compared with other techniques used in toxicology. This rate is a direct measure of population growth, and also integrates survival and fecundity, as increase intrinsic rate of (r_m), and both provide similar results (WALTHALL; STARK, 1997).

Several authors evaluated the performance of insecticides and acaricides on pests and natural enemies, using the instantaneous population growth rate (ri), among which are the aphid *Acyrthosiphon pisum* (KRAMARZ et al., 2007), *Myzus persicae* and the ladybug *Eriopis connexa* (VENZON et al., 2007), white mite *Polyphagotarsonemus latus* (VENZON et al., 2006), spider mite *Tetranychus urticae* (KIM et al., 2006), and phytoseiid predator *Phytoseiulus persimilis* (TSOLAKIS; RAGUSA, 2008), parasitoids *Ttrychogramma pretiosum* and *Telenomus remus* (TAVARES et al., 2009), and the coffee red mite (*Olygonicus ilicis*) (MOURÃO et al., 2004).

To test a promising alternative tactics that can be used in the integrated management of cotton pests, especially organic and family crops, this study propose to evaluate the r_i of *A. gossypii* under different concentrations of botanical insecticides.

Material and methods

This work was performed at the Agricultural Entomology Laboratory, Department of Agronomy (DEPA), Rural Federal University of Pernambuco (UFRPE), using acclimatized chambers with temperature and relative humidity being monitored, and 12h photoperiod.

***Aphis gossypii* rearing**. Insects were reared according to the technique adapted from the Biology and Rearing Insects Laboratory (LBCI), Department of Fitossanidade, Faculty of Agricultural and Veterinary Sciences of Jaboticabal (FCAVJ), University Estadual Paulista (UNESP), being kept in acclimatized room at 27 ± 1°C, 70 ± 5% RH and 12h photophase.

Cotton seeds (*Gossypium hirsutum* L. race *latifolium* Hutch), cultivar CNPA 8H, were sown in styrofoam cell trays (272 x 280 mm, 64 cells) containing Base Plant® substrate, consisting of pine barks, vermiculite, peat, lime acid correctives and additives, with moisture content between 50 and 55%. Subsequently, styrofoam cell trays were placed inside plastic trays with water maintaining the appropriate level for plant roots absorption, and also contributing to the moisture maintenance in the cultivation environment.

The rearing was initiated with aphids collected on cotton plants seeded in areas of the DEPA. Plants were kept inside cages of germination and infestation, with dimensions of 1.0 x 1.20 x 0.60 m covered with 'voile' fabric. Inside the cages were installed fluorescent 'daylight' and 'Grolux' lamps to stimulate the photosynthetic process. Trays were placed on PVC pipe supports at approximately 60 cm from lamps. Pots with water and detergent were kept at the cages bottom to prevent ants' infestation. Plants remained in germination cages for approximately 20 days and then were transferred to infestation cages by placing leaves with aphids on them. Colonies were periodically observed to prevent the presence of parasitoids, predators and other undesirable insects. The rearing process was established to ensure the adequate supply of plants and aphids for performing experiments.

Botanical insecticides used. The botanical insecticides Compostonat® (essencial oils of Neem, Karanja and Castor beans; Natural Rural Ltda.), Rotenat-CE® (*Derris* sp.; Natural Rural Ltda.) and Neempro (*Azadirachta indica* A. Juss.; Quinabra e Trifolio-M GmbH., Química Natural Brasileira Ltda.) were tested at 0, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75% concentrations; Natuneem® (*A. indica*; Natural Rural Ltda.) and Neemseto® (*A. indica*; Cruangi Neem do Brasil Ltda.) at 0, 0.25, 0.50, 0.75 and 1.00% concentrations and the essential oils of *Foeniculum*

vulgare Mill., *Cymbopogom winterianus* (L.), *Chenopodium ambrosioides* L. and *Piper aduncum* L. at 0, 0.0125, 0.0250, 0.0375 and 0.0500% concentrations.

Insecticides were tested in randomized experimental design with eight replications. It was used leaf discs of 5 cm in diameter, obtained from cotton plants, cultivar CNPA-8H with approximately 20 days old, grown in green-house. The disks were immersed in insecticide broods for 30 seconds and dried for 30 minutes. Afterwards they were placed into Petri dishes containing 1% agar-water medium, being each disc infested with five apterous adult females of *A. gossypii* with uniform size. Plates were sealed with PVC film and placed in environmental chambers for 10 days at 25 ± 1°C, 67 ± 5% RH and 12h photophase.

To evaluate insecticides effects on the population growth of *A. gossypii*, the r_i was estimated according to the equation:

$r_i = \ln(N_f / N_0) / \Delta t$, where N_f is the final number of aphids (nymphs and adults); N_0 is in the initial number of aphids transferred and Δt is the change in time, in the case of ten days (WALTHALL; STARK, 1997). Positive value of r_i indicates population growth increase; $r_i = 0$ means that the population is stable; and negative value of r_i indicates population decline to extinction, when $N_f = 0$ (STARK; BANKS, 2003).

Regression analysis were performed to correlate the concentrations of insecticides tested with the r_i values by the statistical program SAS (SAS, 2001). The regression lines were plotted using the graphics program Sigma Plot (SYSTAT SOFTWARE INC., 2006).

Results and discussion

The regression equations relating to the r_i were significant for the insecticides Compostonat® ($F = 313.87$, $p < 0.001$, $R^2 = 0.85$), Rotenat® ($F = 157.43$, $p < 0.0001$, $R^2 = 0, 74$), Neempro ($F = 235.97$, $p < 0.001$, $R^2 = 0.81$), Natuneem® ($F = 167,74,08$, $p < 0.0001$, $R^2 = 0.82$), Neemseto® ($F = 31.79$, $p < 0.0001$, $R^2 = 0.46$) and the *F. vulgare* essential oil ($F = 41.50$, $p < 0.0001$, $R^2 = 0.52$) (Figures 1, 2, 3).

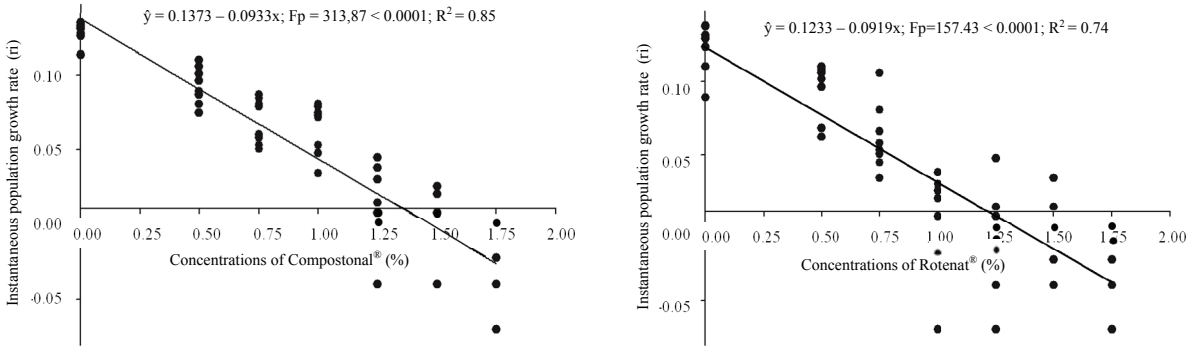

Figure 1. Instantaneous growth rate (ri) observed (●) and estimated (-) to *A. gossypii* in cotton leaf discs treated with Compostonat® and Rotenat®.

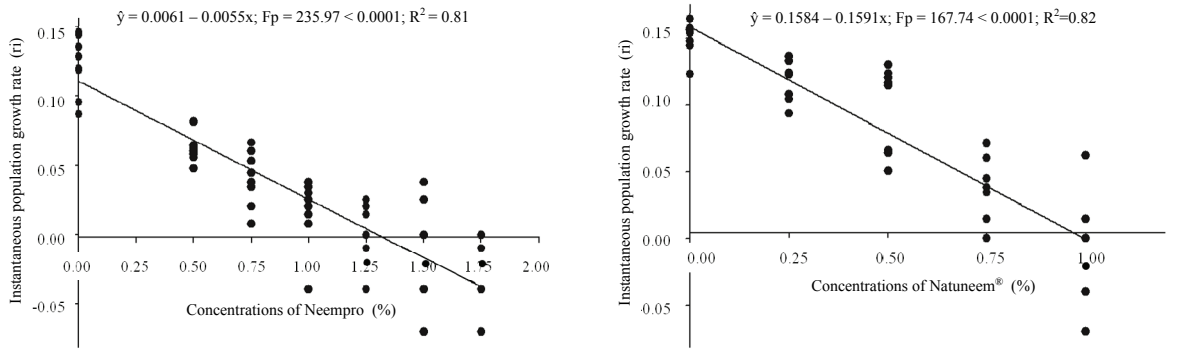

Figure 2. Instantaneous growth rate (ri) observed (●) and estimated (-) to *A. gossypi* Glover in cotton leaf discs treated with Neempro and Natuneem®.

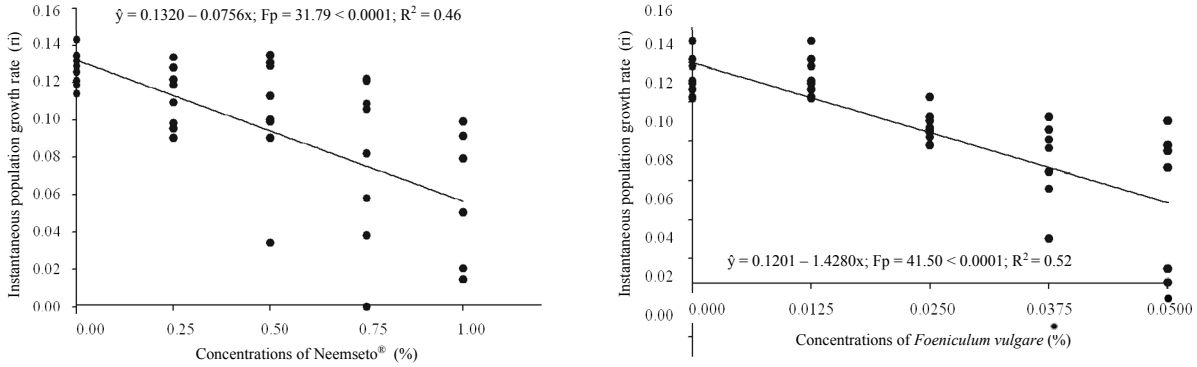

Figure 3. Instantaneous growth rate (ri) observed (●) and estimated (-) to *A. gossypii* Glover in cotton leaf discs treated with Neemseto® and essential oil of *F. vulgare.*

The first three insecticides showed negative r_i, indicating that the *A. gossypii* population has declined towards extinction. Other insecticides and the essential oil obtained positive r_i, confirming that population increased (STARK; BANKS, 2003). Oils of *C. winterianus*, *C. ambrosioides* and *P. aduncum* presented no statistical significance, preventing the establishment of regression lines.

Studies assessing effects of botanical insecticides on *A. gossypii* through the use of r_i are scarce or nonexistent based on the literature searched; but there are similar studies with other pest species and natural enemies. NeemAzal T/S at 0.5 and 1.0% concentrations had reduced population growth of *M. persicae* in laboratory on pepper leaves, with positive r_i. At concentrations of 0.025 and 0.05%, it also had caused lethal and sublethal effects on *Eriopis connexa* (VENZON et al., 2007). Regarding the white mite *Polyphagotarsonemus latus* in Chilli pepper, the r_i values had been negative for lime sulfur brood and 'Calda Viçosa', there had been population decline; a similar result to that found in this study with *A. gossypii* at 1.50 and 1.75% concentrations of Compostonat®, Rotenat® and Neempro. However, positive r_i values had been obtained for mites on plants treated with 'Supermagro' and in the control with water (VENZON et al., 2006). In females of red mites *Oligonychus ilicis*, in coffee trees, the r_i had decreased linearly with increasing concentration of extracts of oil cake, seeds and leaves of neem (MOURÃO et al., 2004).

Considering the good performance of neem-based insecticides and the action mode of these products on pests, it favors its association with biological control. The fact of azadirachtin, the main bioactive compound, to be less effective by contact than by ingestion, favors predators due to the absorption of smaller amounts of active ingredient. Thus, even if preys have been contaminated, the quantity of azadirachtin present would have been quite low, due to its rapid excretion (MARTINEZ, 2002).

According to Venzon et al. (2007), the neem use in the field must be accompanied by regular sampling, being necessary when possible, additional applications in order to reduce the aphid population in case of the remaining population from the first application increase. It is also important to mention the need for research on green-house and field for extension and adjustment of application, validating the use of this alternative tactics as well as assess the effects of botanical insecticides on natural enemies of *A. gossypii*.

Conclusion

Results obtained in this study demonstrate the importance of botanical insecticides in reducing the population of *A. gossypii*, mainly on organic crops, where the synthetic insecticides are not allowed, and family farming due to lack of resources; since in addition to be efficient, they have low toxicity to vertebrates, degrade rapidly, not severely affecting the environment and encourage the population of predators and parasitoids. On the other hand, the use of r_i was very appropriate for its efficiency and speed in obtaining results, eliminating the need for a life table of fertility.

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