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The control and protection of cotton plants using natural insecticides against the colonization by *Aphis gossypii* Glover (Hemiptera: Aphididae)

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ABSTRACT. The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a key pest of cotton, irrespective of the use of conventional or organic management. In organic systems, however, the use of synthetic insecticides is not allowed, increasing the difficulty of controlling this pest. This work evaluated aphid control and the ability of products to prevent aphid infestation using natural insecticides compared to a standard synthetic insecticide. The control trial was conducted with four products [*Beauveria bassiana* (Boveril®), neem oil (Neemseto®), and cotton seed oil compared to thiamethoxam (Actara®)], and untreated plants served as the control group. The trial testing the efficacy of these products in preventing aphid infestation was conducted using the same products, excluding Boveril®. The evaluations were conducted 72 and 120h post-treatment for the efficacy and the protection against colonization trials, respectively. The aphid control by cotton seed oil, Neemseto®, and thiamethoxam was similar, with 100% control being achieved on the thiamethoxam-treated plants. Regarding the plant protection against aphid colonization, the insecticide thiamethoxam exhibited a better performance compared to the other tested products with steady results over the evaluation period. The natural products exhibited variable results with low protection against plant colonization throughout the evaluation period.

Keywords: Insecta, organic cotton, cotton aphid, alternative control, population growth rate.

Controle e proteção de plantas de algodão com inseticidas naturais contra a colonização de *Aphis gossypii* Glover (Hemiptera: Aphididae)

RESUMO. O pulgão *Aphis gossypii* Glover (Hemiptera: Aphididae) é uma importante praga do algodoeiro, independente do sistema de cultivo convencional ou orgânico. Entretanto, no cultivo orgânico não é permitido a utilização de inseticidas sintéticos, o que dificulta o controle desta praga. Assim, avaliou-se o controle e a proteção da planta à infestação pelo pulgão com inseticidas permitidos em cultivo orgânico. O experimento de controle empregou três inseticidas naturais [*Beauveria bassiana* (Boveril®), óleo de nim (Neemseto®) e óleo de algodão comparado ao inseticida sintético tiametoxam (Actara®)]. O experimento visando a proteção da planta à infestação foi conduzido com os mesmos inseticidas exceto o Boveril®. As avaliações foram realizadas após 72 e 120h para os experimentos de controle e de proteção da planta, respectivamente. O controle do pulgão 72h após a aplicação foi similar entre óleo de algodão, Neemseto® e Actara® e atingiu 100% de controle com o Actara®. Em relação à proteção da planta contra a colonização, o Actara® destacou-se com maior proteção das plantas quando comparado aos demais inseticidas, sendo a eficiência mantida em todos os intervalos de avaliação. Já os demais tratamentos apresentaram variabilidade com relação à proteção ao longo dos intervalos de avaliação.

Palavras-chave: Insecta, algodão orgânico, pulgão do algodoeiro, controle alternativo, taxa de crescimento populacional.

Introduction

The cotton crop has made a considerable contribution to Brazilian agribusiness, with cotton production and the textile industry accounting for more than 16.4 million of direct and indirect employment in Brazil (VALDEZ, 2011). The cultivated area is expected to increase for the upcoming seasons, due to the worldwide demand for natural fibers (CONAB, 2011). In Brazil, cotton cultivation occurs primarily in the Cerrado and semiarid areas of the western and northeastern states. In the Cerrado, cotton is produced in large fields, adopting all available technologies, whereas it is produced by small growers in the semiarid areas (BARROS;

TORRES, 2010; FONTES et al., 2006). The cotton fields in the semiarid region consist of small areas, ranging from 0.3 to 8 hectares, using family labor and under a low input of technologies, such as chemical fertilization, mechanization, and pest control practices, and most of these fields can be classified as organic cotton because of the use of organic fertilizer and lack of pesticide utilization (BARROS; TORRES, 2010). Cotton plants, however, host a variety of herbivorous pests, and the lack of the proper adoption of pest control practices in organic cotton fields makes it difficult to produce a profitable crop.

Among the cotton pests, the cotton aphid *Aphis gossypii* Glover is an important pest that infests at the beginning of the crop season, delaying early plant development; the aphid infestation might extend through the development of the plants if control practices are not adopted. Plants infested with cotton aphids exhibit reduced development and curled leaves, especially the young leaves driving the growth of the main stem and the leaves of the reproductive branches (EBERT, 2008; LECLANT; DEGUINE, 1994). Beyond the damage caused directly to the plant due to the feeding behavior, aphids secrete honeydew on the leaves and the open lint, seriously endangering the cotton yield. In addition, the honeydew favors the development of black sooty mold fungus, which affects plant development and results in stick lint, causing problems during the spinning process at the textile mills (DEGUINE et al., 2000). Large colonies of cotton aphids are commonly produced due to the intrinsic biotic characteristics of the insects, such as rapid development and a parthenogenic mode of reproduction in the tropics, which are enhanced when coupled with high temperatures and plants under water stress (GODFREY et al., 2000; VAN EMDEN; HARRINGTON, 2007), both of which are common environmental conditions in semiarid regions.

The control of cotton aphids in cotton fields is primarily addressed with seed treatment or foliar spraying with systemic or contact broad-spectrum insecticides (ALMEIDA et al., 2008; TORRES; SILVA-TORRES, 2008). Nevertheless, the increased value of the fiber produced under organic systems has stimulated the small growers in the semiarid regions to adopt biorational pest control methods. The cotton cultivated under low environmental-impact production and family agriculture systems fetches a higher price, which compensates for the low yield commonly obtained. In addition, the recent cultivation of colored fiber cottons (i.e., degrees of green and brown colors) under organic systems has a large opportunity to expand in the semiarid region, especially among the small growers. Thus, biorational methods of aphid control will be required where organic production systems are intended. Previous studies have shown that alternative products, such as entomopathogenic fungi (LOUREIRO; MOINO JÚNIOR, 2006; STEINKRAUS et al., 2002), natural oils, and plant product derivates (BAGAVAN et al., 2009; EL SHAFIE; BASEDOW, 2003; LIN et al., 2009; MAREGGIANI et al., 2008; SANTOS et al., 2004), have the potential to control cotton aphids. Based on the requirements for organic production, the utilization of natural insecticides is one way to control cotton aphid. Therefore, this study investigated the control of cotton aphids established on cotton plants and the ability of the treatment to prevent the colonization of treated plants. The tested products were the natural oil from seed cotton, a commercial formulation of neem oil, and a commercial formulation of *Beauveria bassiana* in comparison to thiamethoxam, a synthetic insecticide recommended to control cotton aphid in conventional cotton fields.

Material and methods

The experiments were conducted under greenhouse facilities and under controlled conditions in the Biological Control Laboratory of the Universidade Federal Rural de Pernambuco (UFRPE). Cotton plants of the variety BRS Verde were used for aphid-colony rearing and in the experiments. The plants were grown in plastic pots filled with 500 g of a mixture of soil and humus (3:2 by weight) and fertilized weekly with a 20 g L^{-1} of urea solution at rate of 20 mL per pot, beginning eight days after seedling emergence. Four seeds were planted per pot and were subsequently thinned to two plants per pot after five days from plant emergence. The aphid colony was maintained under greenhouse conditions with potted cotton plants kept inside cages of 1 x 1 x 0.8 m (WxLxH) that were covered with *voile* fabric to avoid other opportunist arthropods.

The study was conducted in the following two goals: (i) to evaluate the effect of the tested products in reducing the aphid population on infested plants and (ii) to verify the ability of the tested products in preventing the colonization of treated cotton plants exposed to aphid colonization.

Control of cotton aphid. To investigate the effect of the products in reducing aphid infestation, at 21 days after emergence, the cotton plants were

subjected to aphid infestation by allowing them to contact other cotton plants inside the aphid-rearing cages for a period of 48h. After this colonization period, the plants were moved to the laboratory and randomly evaluated. The number of aphids was counted in the upper two fully expanded leaves of each plant using a bench 10x magnification lens to obtain the aphid infestation prior to the treatment. The petioles of these leaves were smoothly smeared with entomological glue (Cola Entomológica®, Biocontrole Ltda, São Paulo State, Brazil) to avoid aphid dispersion or hosting aphids dispersing from other parts of the plants. The experiment consisted of a completely randomized design with five treatments (four insecticides and control plants with no insecticide treatment) and five replications each. Each replication consisted of a pot with two plants. Thus, the replication mean was considered as the average of the aphids counted on four leaves (i.e., two leaves per plant x two plants per pot corresponding to 20 leaves counted from 10 plants per treatment).

The insecticides and concentrations tested were as follows: Boveril PM (*Beauveria bassiana* isolates ESALQ-PL63 and ESALQ-447 at $5x10^8$ conidia g^{-1}) (Itaforte Bioprodutos, São Paulo State, Brazil); Neemseto® (azadirachtin) (Cruangi Neem do Brasil Ltda., Timbaúba, Pernambuco State, Brazil) at 1% concentration; cotton seed oil at 1%; and Actara 250 WG (thiamethoxam at 0.1 g of a.i. 200 mL^{-1}) (Syngenta S.A., São Paulo State, Brazil). Tween 20 at 0.02% was added to each dilution. For Boveril PM, the number of conidia counted in a 200 µL aliquot. Furthermore, the viability of the conidia in BDA and the pathongenicity against 3rd-instar larvae of *Diatraea saccharalis* (Fabr.) were tested. The results of these tests fit the standard values set by the manufacturer.

The plants were sprayed using an electric power Airbush set (Paasche Airsbush Co., Harwood Heights, IL, USA) under 15 lb pol^{-2} pressure. The volume of the insecticide dilution applied per plant was regulated to 1 mL to obtain a homogeneous plant covering according to a previous test. As aphids are also affected by water droplets, the infested plants comprising the control treatments were also sprayed with a dilution of water and Tween 20 at 0.02%. The treated plants were allowed to stand for 2h for the spray to dry the spray, and the plants were transferred to a climatic chamber at 27°C and a 12-h photophase. After 72h, the plants were evaluated by counting the number of live aphids per leaf.

The data of aphid per leaf pre- and 72h post-treatment were compared. In addition, the insectistatic effect of some of the tested products also relates to reproduction (DIMETRY; EL-HAWARY, 1995; ISMAN, 2006; NISBET, 1994); thus, the instantaneous population growth rate (r_i) was calculated based on Stark and Banks (2003) using the following formula: $r_i = \ln (N_f / N_0) / \Delta T$, where N_f indicates for the final number of individuals in the populations, N_0 indicates the initial number of individuals in the population, and Δt indicates the time (days) of observation post-treatment. In this case, Δt was three days. Positive values of r_i indicate a population increase during the observation period, and negative values of r_i indicate population decrease; values of $r_i = 0$ indicate no numerical change in the population.

The number of aphids per leaf and the values of r_i were subjected to normality and homogeneity tests with regard to the assumptions for the analysis of variance (SAS, 2001). The results were subsequently subjected to analysis of variance, and the means were compared using the Tukey HSD test at a 0.05 significance level (SAS, 2001).

Protection of cotton plants against aphid infestation. The products were also investigated with regard to their efficacy in protecting treated plants against aphid colonization; the product formulated with *B. bassiana* was excluded in this experiment, due to a low control efficacy. Cotton plants cultivated without aphid infestation and of the same age (~21 days after emergence) were sprayed using a procedure similar to that described above, and the treated and untreated (control treatment) plants were exposed to aphid colonization at two hours after spraying. To test the aphid colonization on the treated plants, the cotton plants were randomly placed between rows of potted cotton plants heavily infested with aphids, with the leaves touching to allow aphid colonization. A completely randomized design was established, with four treatments (three insecticides and control) and five replications each. Each replication mean was originated from two plants (two plants per pot). The evaluations consisted of whole-plant inspections for the presence of aphids at 24, 48, 72, and 120h after the exposure of the treated plants to the aphid-infested plants.

The average number of aphids per two plants was tested for normality and homogeneity of variance, and square root (x + 0.5) transformation was required to fit the assumptions for the analysis of variance. The data were then subjected to analysis of variance through a repeated measure procedure because the evaluations were conducted over time on the same plants using the SAS statistical package (SAS, 2001). To separate the means among the treatment, the Tukey HSD test was performed at 0.05 level of significance for each evaluation interval.

Results and discussion

Control of cotton aphid. The evaluation of aphid infestation on cotton plants prior to insecticide application resulted in a statistically similar average of aphids per plant ($p > 0.05$) (Figure 1), indicating that we could disregard the effect of the initial population on the final results across the treatments. Thus, we can conclude that the tested insecticides exhibited different control performances on cotton aphid ($F_{4, 20} = 26.89$, $p < 0.0001$). The aphid population on the untreated and plants treated with Boveril increased by 2.12- and 1.89-fold, respectively, during the 72h post-treatment period (Figure 1). The cotton plants treated with 1% cotton seed oil exhibited a slightly decrease of aphid population at 72h post-treatment that was statistically similar to the infestation prior to the treatment ($p > 0.05$). Among the natural products tested, the formulation of neem (Neemseto®) produced the greatest reduction in the aphid population with an average of 19.8 aphids per plant at 72h post-treatment, which was 12.9 times lower than the infestation prior to the treatment; the standard synthetic insecticide thiamethoxam exhibited 100% control of the aphids (Figure 1).

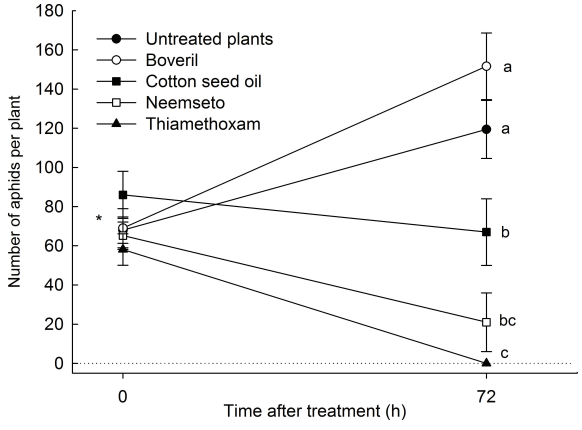

Figure 1. Control of cotton aphid *Aphis gossypii* with natural and synthetic insecticides. Note: *means (± SE) of initial infestation prior to insecticide application do not differ among treatments ($F_{4, 20} = 2.33$, $p = 0.0913$); while mean at 72h post insecticide application followed by different letter differ among treatments by Tukey HSD's test ($p < 0.05$).

The instantaneous population growth rate (r_i) calculated based on the final and initial number of aphids per cotton plant was not determined for the thiamethoxam treatment because of the 100% control at the final evaluation. However, based on r_i, the aphid population exhibited significant changes ($F_{3, 16} = 25.37$, $p < 0.0001$) with regard to untreated plants and those treated with Neemseto, cotton seed oil, and Boveril (Figure 2). The untreated and Boveril-treated plants produced positive r_i values of 0.229 and 0.220, respectively, indicating similar population increases during the observation period. In contrast, the treatments with Neemseto and cotton seed oil resulted in significant reductions in the aphid population, with r_i values of -0.419 and -0.164, respectively. These results were similar between these two treatments, even though Neemseto resulted in 2.55-fold greater reduction (Figure 2).

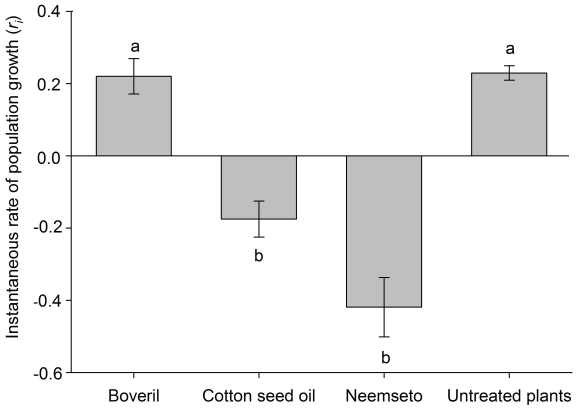

Figure 2. Cotton aphid population increase after application of natural insecticides on cotton plants. Bars holding similar letters do not differ by Tukey HSD's test ($p > 0.05$).

Protection of cotton plants against aphid infestation. The protection of the cotton plants against aphid colonization varied across the treatments and all of the evaluation intervals (Table 1). Among the treatments, the synthetic insecticide thiamethoxam exhibited the best performance in protecting the plants against aphid colonization, irrespective of the evaluation interval: the average population was 4.1 aphids per cotton plant at 120h post-treatment (Table 1). Among the natural products, there were no significant differences at the 120h-evaluation interval (Table 1). Between 24 and 120h for the untreated plants and after treatment with seed oil and Neemseto, the aphid population increased significantly at rates of 6.3-, 7.9-, and 10.9-fold, respectively (Table 1).

We found that the commercial formulation of *B. bassiana* performed differently from previous reports using different isolates of this fungus tested against cotton melon^{-1} aphid. According to Loureiro and Moino Júnior (2006) and Araújo Júnior et al. (2009), *B. bassiana* was pathogenic to *A. gossypii*. In our study, the treatment of aphid-infested cotton plants with Boveril produced similar results as untreated plants: the observed aphid population growth was similar (Figures 1 and 2).

Table 1. Mean (± SE) number of cotton aphid per plant after application of natural and synthetic insecticides.

	Time post-treatment (h)				
Treatments[1]	24	48	72	120	Statistics
Untreated plants	33.8 ± 9.32 Ac	50.1 ± 5.04 Ac	112.2 ± 8.88 Ab	213.9 ± 19.59 Aa	$F_{3, 12} = 48.33$, $p < 0.0001$
Cotton seed oil	36.8 ± 9.23 Ab	90.0 ± 28.87 Aab	199.9 ± 50.51 Aa	290.9 ± 88.75 Aa	$F_{3, 12} = 10.44$, $p = 0.0012$
Neemseto®	21.1 ± 7.14 Ab	59.3 ± 10.41 Aab	142.7 ± 22.41 Aa	231.6 ± 42.07 Aa	$F_{3, 12} = 15.31$, $p = 0.0002$
Thiamethoxam	1.4 ± 0.47 Ba	2.2 ± 1.37 Ba	2.2 ± 0.88 Ba	4.1 ± 1.92 Ba	$F_{3, 12} = 0.86$, $p = 0.4872$
Statistics	$F_{3, 12} = 19.36$, $p < 0.0001$	$F_{3, 12} = 40.90$, $p < 0.0001$	$F_{3, 12} = 89.24$, $p < 0.0001$	$F_{3, 12} = 56.41$, $p < 0.0001$	

[1]Means followed by the same capital letters within column and small letters within rows do not differ by Tukey HSD's test ($p > 0.05$).

It is important to highlight that laboratory studies using isolates of *B. bassiana* are conducted in Petri dishes and under conditions that are usually favorable to the fungus, including high humidity. Confining aphids, phloem-sucking insects, on leaf discs can cause the aphids to move and/or stop feeding, resulting in stress to the insect, and the stress caused in the target pest is known to enhance the efficacy of the parasitism by *B. bassiana* (FURLONG; GRODEN, 2003; LORD, 2009). Another explanation that we can consider for the low efficacy of the tested formulation of *B. bassiana* can be the reduced post-treatment evaluation period. According to Tesfaye and Seyoum (2010), *B. bassiana* caused cumulative mortality of *A. gossypii* at 25°C from 73.3 to 93.3% but required ~5 days to produce 50% mortality. Furthermore, Vu et al. (2007) reported that among the fungi tested, *Lecanicillium lecanii*, *Paecilomyces farinosus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Cordyceps scarabaeicola,* and *Nomuraea rileyi*, *L. lecanii* performed as the best in controlling *A. gossypii*.

The aphid population on the plants treated with cotton seed oil demonstrated a slight decrease compared to the untreated plants, suggesting an effect on aphid reproduction. The negative outcome of the instantaneous rate of population growth supports this hypothesis of an effect on reproduction. Future studies using cotton seed oil should consider evaluation intervals that are sufficiently long to allow one aphid generation to ascertain the effect on reproduction.

The aphid control obtained with the commercial neem oil (Neemseto) was comparable to that obtained with the standard synthetic insecticide thiamethoxam (Figure 1). The topical effect of neem seems to predominate in this trial because the treatment consisted of spraying aphid-infested plants. Regarding the standard synthetic insecticide thiamethoxam, the results obtained fit those already reported with an excellent level of control and protection of cotton plants against colonization by aphids (TORRES; RUBERSON, 2004; TORRES; SILVA-TORRES, 2008). None of the natural products tested were able to protect the plants against aphid colonization; the final count varied, on average, from 231.6 to 290.9 aphids per plant at 120h post-treatment.

Although previous results suggest the systemic action of products with azadirachtin (SCHUMUTTERER, 1990; SOUZA; VENDRAMIM, 2005), only a delay in aphid infestation would be expected. Based on our results, although Neemseto showed efficacy in the control trial, the results were similar to the untreated plants when applied prior to infestation, evidencing no protection against aphid colonization (Table 1).

Conclusion

One application of Neemseto and Actara achieved similar control of cotton aphids at 72h after treatment followed by cotton seed oil, whereas the aphid populations on plants treated with Boveril and untreated plants were similar. Regarding protection against aphid infestation, only the insecticide Actara exhibited effectiveness in maintaining the aphid population near zero on cotton plants throughout the evaluation. However, further studies using more than one application and longer evaluation periods for cotton seed oil will be necessary to ascertain the efficacy of this natural product.

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