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Mortality and behavior of *Neopamera bilobata* (Hemiptera: Rhyparochromidae) subjected to an insecticide based on *Sophora flavescens*

Mortalidade e comportamento de *Neopamera bilobata* (Hemiptera: Rhyparochromidae) submetido ao inseticida à base de *Sophora flavescens*

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Highlights

Control of the strawberry seed bug with environmental friendly products.
Thiamethoxam results in an 18.62% of probability of survival for the seed bug.
Sophora flavescens can reduce the probability of survival of the seed bug to 0 %.

Abstract

Aiming at the production of a safe food and new alternatives to chemical pest control, this study investigated the mortality of adults of the strawberry seed bug, *Neopamera bilobata* (Hemiptera: Rhyparochromidae), subjected to different products, most of which have low toxicity and low environmental impact. The insects were collected in cultivated areas of Fazenda Escola farm, at the State University of Londrina. A stock culture was established in the Entomology Laboratory (T: 25 ± 1 ºC; 12-h photophase) and maintained on sunflower seeds and distilled water for the development of bioassays and experiment in a greenhouse. The insects were preliminarily subjected to the following treatments: water; a formulation with orange peel essential oil (1 mL L^{-1}); a product based on *Sophora flavescens* (3 mL L^{-1}); a formulation with orange peel essential oil (1 mL L^{-1}) + *S. flavescens* (3 mL L^{-1}); *S. flavescens* (3 mL L^{-1}) + neutral detergent (1 mL L^{-1}); silicone adjuvant (1 mL L^{-1}); sucrose ester (1 mL L^{-1}); and thiamethoxam (0.1 g L^{-1}) as a positive control.

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Due to the efficiency of *S. flavescens*, with mortality greater than 90%, different concentrations (1, 2, 3, 4, and 5 mL L^{-1}) were used on the adults. Higher doses of *S. flavescens* led to lower survival rates. After the dose of 4 mL L^{-1} was determined, another step was carried out in a greenhouse, using 18 pots with a capacity of 4 L of substrate containing plants of cultivar Albion surrounded by a voile fabric cage. Ten adult bugs were added to each pot; nine pots were sprayed with the *S. flavescens*-based product and the other nine only with distilled water. *Sophora flavescens* reduced the survival rate of *N. bilobata* in the laboratory and in the greenhouse. In conclusion, the *S. flavescens*-based product has the potential to control the bug *N. bilobata* at the tested rates, thus warranting further studies.
Key words: Alternative control. Pests of strawberry. Strawberry seed bug.

Resumo

Buscando a produção de alimento seguro e novas alternativas ao controle químico de pragas, objetivou-se avaliar a mortalidade de adultos do percevejo-dos-frutos do morangueiro, *Neopamera bilobata* (Hemiptera: Rhyparochromidae) submetidos a diferentes produtos, sendo a maioria de baixa toxidez e baixo impacto ao ambiente. Os insetos foram coletados em áreas cultivadas da Fazenda Escola da Universidade Estadual de Londrina. Foi estabelecida uma criação estoque no Laboratório de Entomologia (T 25 ± 1ºC, com fotofase de 12 horas) e mantidos com sementes de girassol e água destilada, para realização dos bioensaios e experimento em casa de vegetação. Preliminarmente, os insetos foram submetidos aos seguintes tratamentos: água; produto formulado com óleo essencial de casca de laranja 1 mL L^{-1}; produto à base de *Sophora flavescens* 3 mL L^{-1}; produto formulado com óleo essencial de casca de laranja 1 mL L^{-1} + *S. flavescens* 3 mL L^{-1}; *S. flavescens* 3 mL L^{-1} + detergente neutro 1mL L^{-1}; adjuvante siliconado 1 mL L^{-1}; éster de sacarose 1 mL L^{-1} e tiametoxan 0,1g L^{-1} como controle positivo. Em função da eficiência de S. flavescens com mortalidades superiores a 90%, os adultos foram submetidos a diferentes concentrações (1, 2, 3, 4 e 5 mL L^{-1}). Quanto maior a dose de S flavescens menor foi a proporção de sobrevivência. Determinada a dose de 4 mL L^{-1} realizou-se outra etapa em Casa de Vegetação, dispondo-se 18 vasos, com capacidade de 4 litros de substrato, com plantas da Cultivar Albion, envoltas por gaiola de voil. Em cada vaso foram adicionados 10 adultos do percevejo, sendo que 9 vasos receberam a pulverização com o produto à base de S. flavescens e outros 9 somente água destilada. Observou-se que *S. flavescens* reduziu a proporção de sobrevivência de *N. bilobata* em laboratório e em casa de vegetação. Conclui-se que o produto à base de *S. flavescens* tem potencial para o controle do percevejo *N. bilobata* nas doses testadas, merecendo estudos complementares.
Palavras-chave: Controle alternativo. Pragas do morango. Percevejo-dos-frutos.

The strawberry crop (*Fragaria* × *ananassa* Duchesne) suffers constant economic losses due to damage caused by a number of pests; in particular, the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae); the cyclamen mite, *Phytonemus pallidus* Banks (Acari: Tarsonemidae); and more recently, the seed bug *Neopamera bilobata* Say (Hemiptera: Rhyparochromidae) (Bernardi et al., 2015; Talton et al., 2020; Lahiri et al., 2022).

Neopamera bilobata was first reported in strawberry in studies on the biology and characterization of injuries to the plant, mainly to the berry (deformation) in different stages of development (Kuhn et al., 2014b). Other studies addressing biological parameters have been published (Kuhn et al., 2018), but the challenge of controlling this pest has not yet been overcome.

Although chemical control is still the most used management strategy for pests of strawberry as is the case with thiamethoxam to control the strawberry aphid, *Chaetosiphon fragaefolii* Cockerell (Hemiptera: Aphididae), there has been a constant search for more ecologically sustainable alternative strategies. Moreover, as the strawberry is preferably consumed fresh, greater control is necessary regarding the presence of toxic residues on the berry (Bernardi et al., 2015; Agência Nacional de Vigilância Sanitária [Anvisa], 2020). Because the insect *N. bilobata* has only recently been reported to cause economic damage, no product has been registered for its control, so research with products that are safer for humans and cause less environmental impact is needed.

Plant extracts are important sources of natural insecticides (Isman, 2020). The ovicidal effect of oxymatrine-based product, extracted from the shrubby plant *Sophora flavescens* Aiton (Fabaceae), was observed on *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) on coffee trees (Andrade et al., 2020). In addition, adjuvants, which are products added to the application mixture to enhance the effectiveness of phytosanitary inputs, can have insecticidal effects. In this respect, the use of silicone adjuvant provided a reduction of more than 90% of the populations of *T. urticae* and *Aphis pomi* De Geer (Hemiptera: Aphididae) (Patrzałek et al., 2020).

Therefore, in aiming to contribute with management alternatives for the strawberry seed bug, this study investigated the mortality and behavior of *N. bilobata* adults and nymphs subjected to Actara® (thiamethoxam), Siltac® (silicone adjuvant), Terpex® (formulation with orange peel essential oil), Matrine® (*S. flavescens*-based product), and ESG-G1 (sucrose ester).

A stock colony was maintained in the laboratory from insects collected from strawberry plants cultivated on Fazenda Escola farm at the State University of Londrina (UEL) (Londrina - PR, Brazil). The culture followed the methodology proposed by Kuhn (2014a), with modifications for laying substrate with green felt and feeding on hulled sunflower seeds.

The culture was maintained under controlled conditions (25 ± 1 ºC, with a 12-h photophase). Adults and nymphs from the stock colony were kept in 200-mL transparent plastic containers with perforated plastic lids. Hulled sunflower seeds were provided as food *ad libitum.* For hydration, a 2-mL Eppendorf® tube containing cotton moistened with distilled water was placed in the container.

With the exception of sucrose ester, the treatments were defined based on information provided by strawberry producers in different regions of the country concerning the products that were being tested in the field for the control of the strawberry seed bug (Table 1).

Table 1
Products and doses used in preliminary bioassays to control Neopamera bilobata

Treatment	Active ingredient	Products	Doses
T1	-	Water	-
T2	Thiamethoxam	Actara®	0,1 g L^{-1}
T3	Orange peel essential oil	Terpex®	1 mL L^{-1}
T4	Sophora flavescens	Matrine®	3 mL L^{-1}
T5	*Sophora flavescens* + Orange peel essential oil	Matrine® + Terpex®	3 mL L^{-1} + 1 mL L^{-1}
T6	*Sophora flavescens* + Detergent	Matrine® + Detergent	3 mL L^{-1} + 1 mL L^{-1}
T7	Silicone adjuvant	Siltac®	1 mL L^{-1}
T8	Sucrose ester	ESG-G1 (product under registration)	2 mL L^{-1}

In the first experiment, in the laboratory, five replications were performed for each treatment, in a completely randomized design. Forty polystyrene boxes (11 × 11 × 3.5 cm) were used, each containing 10 male and female adult bugs, without distinction of sex or age. There was a difference between the femurs of males and females: the femur of the male was more robust, with larger spines, with a more tapered abdomen and lighter brown in color, while the femur of females was smaller, with the end of the abdomen more rounded and darker in color. To reduce the dispersive behavior or high mobility, the insects were kept at low temperatures (10 to 12 ºC) for 2 min prior to the application. The products were pre-diluted and placed in manual sprayers that were previously calibrated for a volume of 1 mL per spray. Only one spray was given, at a distance of about 30 cm from the insects. After application, two hulled sunflower seeds and an Eppendorf® tube containing moistened cotton were added to polystyrene box. Mortality was evaluated daily, considering even moribund insects, which did not have control of their locomotor functions, as dead.

The second experiment, also in the laboratory, was conducted with *S. flavescens.* Adults of *N. bilobata* were exposed to five concentrations of *S. flavescens* (1, 2, 3, 4, or 5 mL per liter of water), which corresponded to five treatments, in addition to a control. As a control, water was sprayed. Ten insects were used per polystyrene box (11 × 11 × 3.5 cm), totaling 600 insects in total, without standardized age. The form of application was spraying, as in the first experiment, with the same volume of 1 mL per replication. Treatment dilutions followed the same previous procedures. The bottoms of the polystyrene boxes were painted with odorless black paint so the insects would adjust their behavior to excessive light. Evaluations were performed every 24 h, until significant mortality was observed in the treatments.

The third experiment was carried out in a greenhouse in Londrina - PR, Brazil. Each experimental unit consisted of a pot containing one plant of strawberry cv. Albion surrounded by a voile fabric cage with a zippered opening that was wrapped with a rubber band attached to the upper edge of the pot; and 10 *N. bilobata* adults of undefined age. The plants had been transplanted for eight months and were grown in 4-L pots containing the substrate 9084H (Carolina Soil do Brasil; Santa Cruz do Sul - RS, Brazil). During seedling development, before the experiment, plant size was standardized by pruning. Irrigation was applied in a localized manner, directly into the pot, with a hose, twice daily.

Treatments were as follows: *Sophora flavescens* at a concentration of 4 mL L^{-1}; and water. The experiment was laid out in a completely randomized design with nine replications (pots). Application was carried out using a Brudden manual sprayer, with a capacity of 5 L, under maximum working pressure and application time of 3 s, with a spray solution volume of 20 mL per experimental unit (pot). To avoid the possible presence of residues, the water treatment was applied first.

Evaluations were carried out 48 h and 96 h after establishment by counting mortalities in each pot, which was placed in a larger cage to prevent escapes. Live insects were placed in Falcon tubes and returned to the cages.

To estimate the probability of survival of the insects, after the application of treatments, the cumulative survival values were subjected to analysis of normality of residuals and homogeneity of variances. The data were subjected to non-parametric analysis by applying the Kaplan-Meier survival test, using the Cox regression model with proportional risks, at a 5% significance level. The tests were performed using GraphPad Prism 9 software.

In the test of the effects of products on the survival of *N. bilobata,* the *S. flavescens*-based product in isolation or mixed with other treatments provided the best results (Figure 1A). At the end of the evaluations, 96 h after the treatments were applied, cumulative survival probabilities were 3.21, 0.57, and 1.21% for *S. flavescens, S. flavescens* + formulation with orange peel essential oil, and *S. flavescens* + detergent, respectively. The use of thiamethoxam (commercial standard) resulted in an 18.62% probability of *N. bilobata* surviving at the end of the evaluations. Survival in the water treatment was 60%, which did not differ from the formulation with orange peel essential oil, silicone adjuvant, or sucrose ester.

In the analysis of the different doses of *S. flavescens* (Figure 1B), 120 h after the treatments were applied, the cumulative probabilities of survival were 6.26, 3.87, 1.69, 2.05, and 0% for the concentrations of 1, 2, 3, 4, and 5 mL L^{-1} of mixture, respectively. The water treatment resulted in a survival rate of 44%.

In the greenhouse, after 96 h of application, the *S. flavescens*-based product sprayed on the strawberry plants (Figure 1C) resulted in a cumulative survival probability of 0% for the concentration of 4 mL per liter of mixture, whereas the water treatment led to a survival rate of 42%.

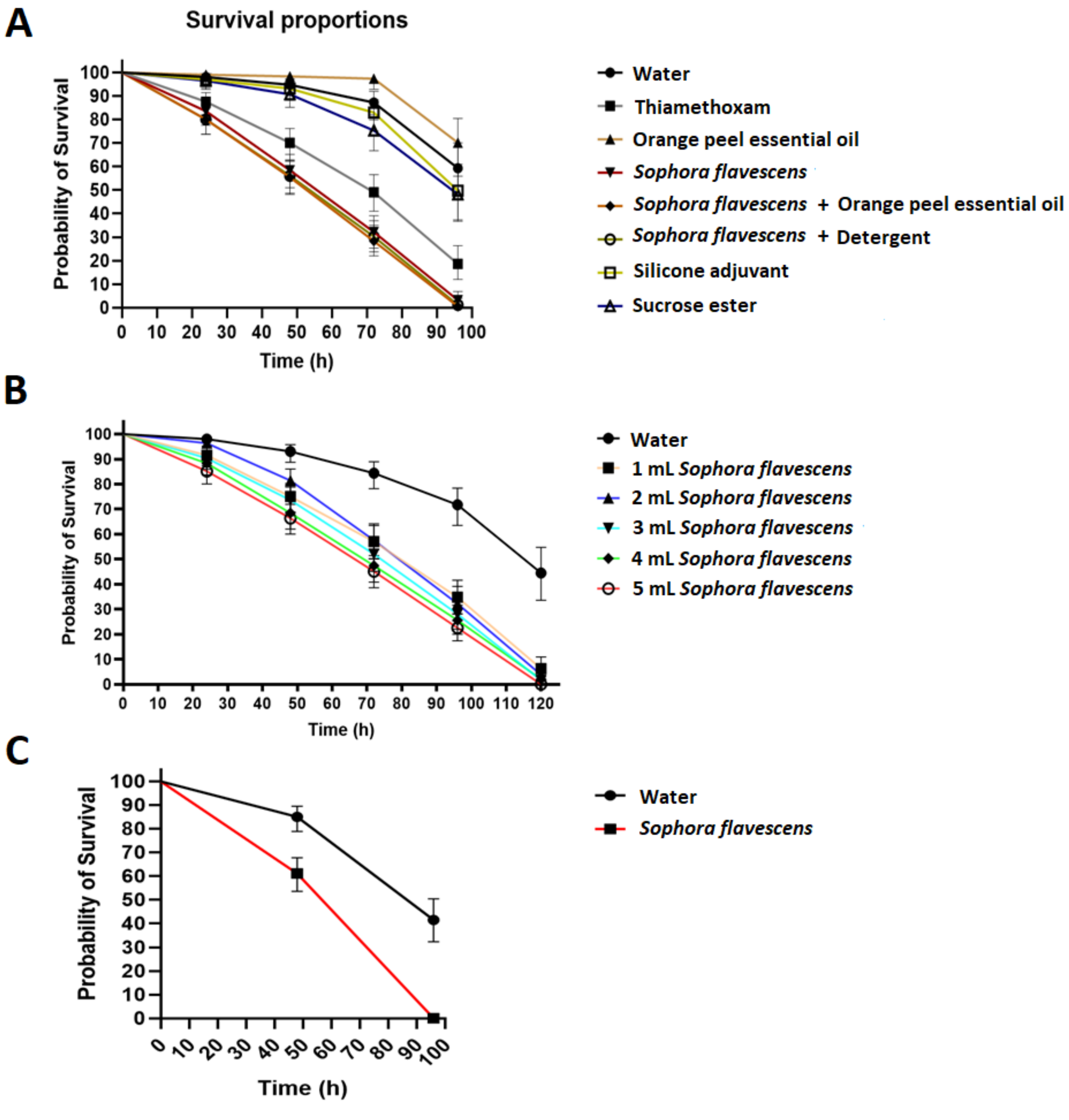

Figure 1. A) Probability of accumulated survival, estimated by the Kaplan-Meier method, of *Neopamera bilobata,* submitted to phytosanitary products and adjuvant after 24, 48, 72 and 96 h after application. B) Accumulated survival probability, estimated by the Kaplan-Meier method, of *Neopamera bilobata* submitted to doses of *Sophora flavescens*-based product after 24, 48, 72, 96 and 120 h after application. C) Accumulated survival probability, estimated by the Kaplan-Meier method, of *Neopamera bilobata* on strawberry submitted to a product based on *Sophora flavescens* (4 mL L^{-1}) in a greenhouse after 48 and 96 h after application. Mean ± 95% confidence interval.

The insecticidal effect of *S. flavescens* on *N. bilobata* was observed both in its isolated use and in combination with the formulation with orange peel essential oil or detergent. The probability of survival of the strawberry seed bug with *S. flavescens,* at the end of 96 h, was lower than that obtained with thiamethoxam, which was used as a positive control (Figure 1A).

The chemical insecticide based on thiamethoxam has the potential to control *N. bilobata.* In the present study, after 96 h of application, its survival probability was 18.62%. Kuhn (2014a) found a mortality rate of 95% in bugs after 120 h of evaluation. With the use of this product, up to 99.3% mortality was detected in *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) on strawberry grown in a greenhouse (Bi et al., 2002). However, despite its effectiveness, it is worth mentioning that thiamethoxam has not yet been registered for the control of *N. bilobata.* Therefore, evaluating new bioinsecticides is important to reduce the negative impacts of phytosanitary products on beneficial insects.

Minutes after application, the bugs displayed changes in behavior and mortality by contact, with alterations in mobility. The *S. flavescens* plant is used for the extraction of the matrine, oxymatrine, and total matrine compounds, which have several biological activities, including insecticidal and acaricidal (Wu et al., 2021), by contact or ingestion (Huang, Lv, & Xu, 2017; Cheng et al., 2020). Because it is a product obtained from a natural extract, there may be more than one mechanism of action on insects and mites. A study on *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) identified that *S. flavescens* botanical extract has a neurotoxic effect, acting on sodium channels (Du et al., 2004). Depending on the structural modification of the matrine molecule isolated from plants, the insecticidal effect can be enhanced, lipophilicity increased, and negative effects mitigated (Huang et al., 2017), also modifying its mechanism of action on arthropods.

The dose of the *S. flavescens*-based product as recommended in the leaflet for controlling pests of strawberry can also have an effect on *N. bilobata,* as confirmed in this study. Being a commercial product derived from plant extracts, its use recommendation for the strawberry seed bug is satisfactory, since it can be applied in organic agriculture or even in the conventional strawberry crop. This benefit is especially important for the fresh consumption of the berry, avoiding greater risks to the consumer.

The result obtained in the greenhouse demonstrates the potential of using this product to control the pest in field areas, contributing to the sustainability of the ecosystem. Future studies determining its lethal concentration and lethal time, as well as the compatibility of an *S. flavescens*-based product with other biological control methods such as the use of entomopathogens and predatory mites, must be carried out, since these methods are commonly used in the strawberry crop.

In summary, *S. flavescens* causes high mortality in *N. bilobata* in laboratory and greenhouse. Silicone adjuvant, the formulation with orange peel essential oil, and sucrose ester are not effective in controlling *N. bilobata.* The chemical insecticide based on thiamethoxam has the potential to control *N.*

bilobata. Laboratory and greenhouse results indicate that *S. flavescens* is an excellent option for controlling the strawberry seed bug, warranting complementary studies.

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