e-ISSN: 2177-5133
www.comunicatascientiae.com.br

Comunicata Scientiae 6(3): 359-364, 2015

DOI: 10.14295/CS.v6i3.808

Original Article

Toxicity and application of neem in fall armyworm

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Abstract

Aqueous extracts of neem, *Azadirachta indica* A. Juss., leaf and seed cake were tested for toxicity in *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) utilizing different methods of application (foliar and systemic). Probit analysis was used to determine the LC_{50} and regression analysis for mortality at different concentrations of the extracts (0.5%, 1.0%, 1.5%, 2.0% and control treatment). Two caterpillar morphometric variables (larval length and cephalic capsule width) and the scale of damage of attacked plants were measured and, analyzed using the Kruskal-Wallis test (H=16.93; P=0.0304). The LC_{50} values for neem seed cake and leaves were 0.13% and 0.25%, respectively. For larval length and cephalic capsule width, the larvae were more affected to the seed cake extract than leaf extract, however there was no significant difference between the methods of application for these variables. There was no difference in the scale of damage by the extracts and the methods of application analyzed. Both methods of application provided similar results and, the main differences were associated with more efficient of the seed cake extract.

Keywords: caterpillar, control, residue, *Zea mays*

Toxicidade e aplicação de nim em lagarta-do-cartucho do milho

Resumo

Extratos aquosos de folha e torta da semente de nim, *Azadirachta indica* A. Juss., foram analisados para avaliar a toxicidade em lagartas de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) em diferentes métodos de aplicação (foliar e sistêmico). Para cada método de aplicação, foram analisadas diferentes concentrações dos extratos de nim (0,5%; 1,0%; 1,5%; 2,0% e tratamento controle). Foi analisada a variável mortalidade das lagartas, para determinar a CL_{50} utilizando uma análise de Probit e análise de regressão para avaliar o efeito das diferentes concentrações. . As variáveis morfométricas de lagartas (comprimento larval e largura da cápsula cefálica) e escala de danos de plantas atacadas foram mensuradas e analisadas pelo teste de Kruskal-Wallis (H=16.93; P=0.0304). Os valores de CL_{50} para extrato de torta e de folha de nim foram de 0,13% e 0,25%, respectivamente. Para comprimento larval e largura da cápsula cefálica, as lagartas foram mais suscetíveis ao extrato de torta da semente do que o extrato de folhas, entretanto não houve diferença significativa entre os métodos de aplicação para estas variáveis. Também não houve diferença para escala de danos nos extratos e nos métodos de aplicação analisados. Ambos os métodos de aplicação possuem resultados semelhantes e as principais diferenças estão associadas com maior eficiência do extrato de torta.

Palavras-chave: controle, lagarta, resíduo, *Zea mays*

Received: 12 Juny 2014
Accepted: 05 May 2015

Introduction

Maize, *Zea mays* L. (Poaceae), is one of the most important grains in the worldwide (Olawuyi et al., 2014) and its production is affected by various biotic and abiotic factors such as mineral nutrition (Gunes et al., 2007) and attack by defoliating insects like the fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), which is considered a severe maize pest in America (Tavares et al., 2010; Dalvi et al., 2011).

Neem, *Azadirachta indica* A. Juss. (Meliaceae), has been used as fertilizer and in the control of pests in maize crops, and emerging as a viable alternative for small farmers [Mordue (Luntz) & Blackwell, 1993; Kabeh & Jalingo, 2007]. Indeed, this fertilizer in many regions is residue from the pressing of the neem seed for the extraction of the oil (Abbasi et al., 2005; Schmutterer, 2009), which is used as botanical insecticide and provide toxicity in maize caterpillars (Viana & Prates, 2003; Akhtar et al., 2008; Lima et al., 2010).

Most studies with pest control utilizing neem are reported using oil from the seed, which have been a commercial product (Dabrowski & Seredynska, 2007; Carvalho et al., 2008; Lokanadhan et al., 2012; Ikeura et al., 2013; Stanley et al., 2014), or leaf extracts (aqueous or organic) as the crop protector (Broglio et al., 2014). However, the application of extracts of neem seed cake and azadirachtin (compound toxic to caterpillars) utilizing root system (in the soil), caused a systemic effect in the control of sucking pests (Buss & Park-Brown, 2006; Gonçalves & Bleicher, 2006). On the other hand, the insecticides with contact-effect, most commonly used to control *S. frugiperda*, often fail to reach the insect, particularly the late-larvae instars, that are located between the young leaves inside the stalk of the plant as described by Palumbo & Kerns (1994), in which the chemical insecticide activity depends on the plant architecture. For this and other reasons, the application of products with systemic action in crops has a large advantage due to translocation of the active compound to all parts of the plant, besides being selective to natural enemies.

The use of neem cake as a fertilizer and protector of plants have been been proposed, especially for sucking insects (Schmutterer, 1990) and diseases (Abbasi et al., 2005). However, studies that use the neem cake as a feeding inhibitor for defoliating caterpillars is no explored. The aim of this study was to evaluate the different applications and toxicity of aqueous extracts of neem for controlling *S. frugiperda*.

Material and Methods

The *S. frugiperda* caterpillars originated from oviposition collected in fields and grown in the Entomology Laboratory, Agrarian Sciences Center, Federal University of Alagoas at temperature 26.6±1 °C and 75% relative humidity, located in Rio Largo, AL, Brazil (9° 29` S, 35° 49` W and 165 m asl). The larvae fed on maize leaves (variety BR106), free of insecticides. In the pupal stage the insects were placed in tubular recipient with 20 cm diameter and 30 cm length, lined internally with paper, until emergence and mating. The adults were fed with sugar (10% in distilled water) and only insects originating from the second generation were the ones used in the experiment.

Neem leaves were collected from plants with two years of age, which the voucher was deposited in the herbarium of the Environmental Institute of Alagoas, registered as MAC 34904. The neem leaves collected were dehydrated at 65 °C for 48 hours and later triturated until a powder of low granulometry was obtained. The powder of the neem seed cake was provided by Cruangi Agroindustry, located in Timbaúba, PE, Brazil. Thereafter, the ground materials were stored in closed glass vials. The aqueous extracts were prepared of the mixture of ground materials with distilled water, at a ratio of 100 g of powder to 900 mL of water. After mixing, the concentrated solution was kept at rest for 24 hours followed by filtration of the solution.

Extract toxicity bioassay

The two neem based treatments used were: i) aqueous extract of neem leaves; and ii) aqueous extract of neem seed cake. Each treatment was analyzed at four concentrations (0.5%, 1.0%, 1.5%, 2.0%) and control treatment with 48 replications for each concentration. The experimental units consisted of a newly hatched

caterpillar, placed on a Petri dish (5 cm diameter) with filter paper moistened with distilled water and a portion of maize leaf (2 cm x 4 cm) that was immersed for two seconds in the solution corresponding to each concentration of extract, and dried on paper towels for ten minutes. These portions were the diet of each caterpillar, with a replacement of the portions every 48 hours.

A control treatment using maize leaf portions without the treatments was also examined. Dimethyl sulfoxide (DMSO, 1%) was added to all solutions to facilitate solubilization. Caterpillar mortality in each treatment was corrected using the Abbott (1925) formula and the data submitted to Probit analysis (Proc Probit) and linear regression with the SAS program and the graphics done in Sigma Plot program.

Extract application methods bioassay

The maize was grown in a greenhouse, in 700 mL polyethylene terephthalate (PET) disposable containers with sugarcane filter cake and crushed coconut bagasse substrate at a ratio of 2:1. The variety BR106 was grown with two seeds per container. At 18 days after sowing, an infestation with first instar caterpillars (24 hours after hatching) was implemented on the maize plants, distributing two caterpillars per plant. The plants were watered daily with 100 mL of water per container. The treatments were: i) aqueous extract of leaves and ii) aqueous extract of seed cake, as well as the control. The treatments were applied at a concentration of 2% only and two methods: i) foliar and ii) systemic. The experimental design was completely randomized with 25 replications per treatment.

The extracts were administered 24 hours after infection with *S. frugiperda* caterpillars. For the foliar method a homemade atomizer was used at a volume of 100 mL, without allowing contact with the substrate in the containers of the growing plants. For the systemic method, 60 mL of extract was applied on the substrate in each container. Evaluations were made for scale of damage (zero to five) five days after treatment applications, considering the value of a zero score the absence of damage to the plant, and grade five the complete destruction of the stalk, as well as morphometric characters such as larval length (cm) and cephalic capsule width (mm) of the 25 caterpillars for each treatment. The data was submitted to the Shapiro-Wilk ($P>0.05$) normality test, followed by the Kruskal-Wallis ($P<0.05$) test using the R package software.

Results and Discussion

The angular coefficient values of the mortality curve of the extracts, obted by Probit analysis, showed significant differences ($x^2=12.83$; $P=0.0003$) between extracts from neem, indicating that *S. frugiperda* caterpillars respond differently. In addition, to evaluate the toxicity of the neem seed cake, the LC_{50} (lethal concentration) values were determined, where the seed cake extract had the lower value (0.13%), being more lethal than the leaf extract (0.25%) (Table 1).

Table 1. Angular coefficients (slope ± standard error) and LC_{50} values of *Azadirachta indica* aqueous extracts in mortality of the *Spodoptera frugiperda* caterpillars

Aqueous Extract	Slope ± SE	LC_{50} ($CI_{95\%}$) (g L^{-1})	x^2	P
Leaf	2.23 ± 0.57	0.25 (0.141 – 0.471)	0.17	0.8395
Seed Cake	1.91 ± 0.53	0.13 (0.016 – 0.321)	0.18	0.8293

SE=Standard Error, LC=Lethal Concentration, CI=Confidence Interval, x^2=Pearson's Chi-square test, P=probability for Chi-square test.

The concentrations used in the treatments were adjusted to the model because the observed mortality frequencies did not differ from expected frequencies, being confirmed by the Pearson's Chi-square test ($P>0.05$).

In addition to determining the LC_{50} values, the behavior of larval mortality against the concentrations in the extracts needed to be verified. For this, a regression analysis was performed with mortality percentages (Figure 1), where the linear model was statistically significant for both the leaf ($F=110.46$; $P=0.0018$) as well as for the seed cake ($F=69.63$; $P=0.0036$).

Despite having the same model, the seed cake extract showed higher mortality percentages at all concentrations, confirming its lethality compared to the leaf extract. It is important to observe the absence of significant

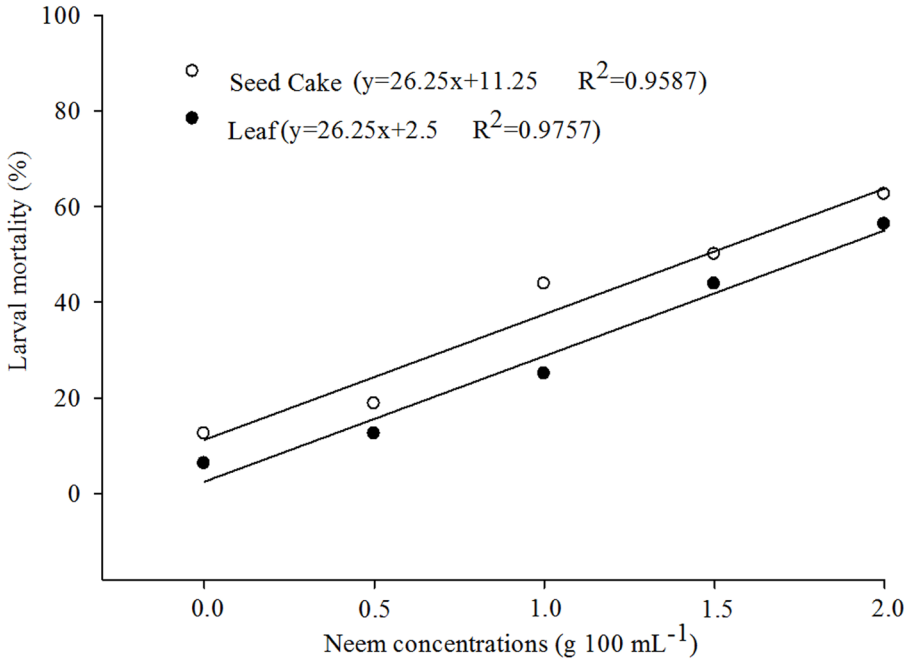

Figure 1. Larval mortality of *Spodoptera frugiperda* after *Azadirachta indica* aqueous extracts application

interaction between extract and concentration.

For the scale of damage to leaves variable, there were no significant differences for both extracts (H=3.53; P=0.1710) as well as for application methods (H=0.7717; P=0.6799). For the cephalic capsule width variable, there were differences between the means of the extracts (H=12.95; P=0.0115), where the control treatment showed the highest means followed by the leaf and seed cake treatments; while for application methods no differences were verified (H=4.81; P=0.0899). The caterpillar length was significant only for means of the extracts (H=7.69; P=0.0212), where the control treatment showed the highest mean, followed by leaf and seed cake treatments. For application routes there were no significant differences (H=2.28; P=0.3186).

The estimated LC_{50} values showed that *S. frugiperda* caterpillars were more susceptible to seed cake extract. This can be attributed to the higher content of azadirachtin, considered the most potent of the limonoids, or the tetranortriterpenoids with toxic activity to arthropods, because 90% of azadirachtin is concentrated in the neem cake after pressing the seeds (Brechelt & Fernandez, 1995), which may contribute to the control of defoliators. As such, lower LC_{50} values means greater toxicity and, consequently, smaller amounts of the extract to kill 50% of the population that was exposed.

In addition to this, the bioassay showed a static effect (reduced growth) on the development of *S. frugiperda* caterpillars, as many of them showed their exuviae in the terminal part of the body, without being able to release them completely, and this was observed in both neem based treatments. This effect of neem on insects was described by Mordue & Nisbet (2000) as a deterrent to feeding, interfering mainly in the physiology of the ecdysis and in cellular processes, potentially resulting in the death of the insect. According to Martinez & Emden (2001), this process will require some time to be triggered and act on the insect, resulting in low mortality at the final larval stage and high mortality in the pupal stage.

Although the maize leaf had been immersed in a solution with the extracts for two seconds, it is believed that the insects were still able to find neem free space on the leaf due to morphological characteristics (for example, trichomes), allowing small variations in mortality during the first instars, such that the caterpillar still managed to feed in these spaces, thus conferring reduced susceptibility. On the other hand, there was an increasing positive response

as the concentration of the extracts was increased, despite low mortality during the first days. However, Viana & Prates (2003), using an aqueous extract from neem leaves at 1%, found that the mortality of *S. frugiperda* caterpillars was low during the first three days after initial feeding and high by ten days, indicating that neem extracts need a determined time period to exhibit effects on the caterpillars.

Although the insecticidal effect of neem is established, the behavior of *S. frugiperda* caterpillars after ingestion or contact with this insecticide is not known. Viana & Prates (2005) questioned whether caterpillars, fed for a period of time on parts of the plants treated with neem extract and then on untreated parts, due to a fault in spraying or natural growth of the plant, could restore the normal development of the caterpillar and result in damage to the plant. This shows that the efficiency of neem extracts in laboratory studies is very positive, but when we moved to conditions that require more care or that mimic field conditions like a greenhouse, for example, the natural efficiency of neem can be questioned, despite several studies [Mordue (Luntz) & Blackwell, 1993] that can counteract these questions, because extracts used in this bioassay did not show a difference when applied to young maize plants with regard to evaluation of the scale of damage. This similarity between treatments could be explained as a function of natural growth of the leaf area of the plant or the movement of the caterpillars between the leaves.

In comparing the results of the morphometric analysis of the caterpillars that were infested on the plants, one can observe differences between the extracts (H=16.93; P=0.0304). In general, it can be said that the extract from neem seed cake was the one that obtained the best results, showing lower means. *S. frugiperda* being a polyphagous insect shows more sensitivity to neem extracts [Mordue (Luntz) & Blackwell, 1993], but for this sensitivity to be manifested, a marked amount of neem limonoid compounds flowing into the phloem is needed, since studies on sap-sucking insects such as aphids use concentrations greater than 100 ppm (100 mg L^{-1}) (Nisbet et al., 1993). However, studies assessing the economic viability of the amount of seed cake that small farmers can use in a way that does not cause toxicity to the maize plants and that will be effective in controlling *S. frugiperda* is yet to be explored.

Conclusions

The aqueous extract of neem seed cake is more toxic than the leaf extract which is usually used by farmers to control *S. frugiperda*. The seed cake could be used as plant protector. There was no difference between the methods of application.

Acknowledgements

To CAPES/FAPEAL for funding this research and IMA for botanical identification.

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