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Effect of aqueous neem seed extract via irrigation on larvae of *Liriomyza sativae* in melon crop

Ewerton M Costa¹; Francisco EL Silva²; Elton L Araujo²

¹Universidade Federal de Campina Grande (UFCG), Pombal-PB, Brazil; ewertonmarinho10@hotmail.com; ²Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró-RN, Brazil; edivino_ufersa@hotmail.com; elton@ufersa.edu.br

ABSTRACT

The leafminer [*Liriomyza sativae* (Diptera: Agromyzidae)] is one of the main pests of melon crop (*Cucumis melo*) in the states of Rio Grande do Norte and Ceará. Thus, adopting strategies to control this destructive insect is essential. The aim of this study was to evaluate the effect of aqueous neem seed extract, applied to soil via irrigation, on leafminer larvae, in the melon crop. An experiment was conducted in greenhouse, in completely randomized design, consisting of five treatments (four doses of aqueous neem seed extract: 1; 3; 5 and 10 g/100 mL) and the control consisting only of distilled water and 10 replicates (melon plants). The treatments were applied in a single dose, via soil irrigation. In the first stage of the evaluation, 24 hours after application, the number of dead larvae per plant was counted. In the second step, pupal mortality was recorded. The total mortality was also calculated considering the number of larvae and the number of emerged adults. We verified that, increasing the concentration of aqueous neem seed extract, an increase in the larval and pupal mortality of the leafminer in melon crop was noticed. The concentration 10 g/100 mL presented the best results, resulting in 36.4% of larval mortality, 96.5% of pupal mortality and 96.8% of total mortality.

Keywords: *Cucumis melo*, *Azadirachta indica*, leafminer, integrated pest management.

RESUMO

Efeito de extrato aquoso de sementes de nim, via irrigação, sobre larvas de *Liriomyza sativae* em meloeiro

A mosca minadora [*Liriomyza sativae* (Diptera: Agromyzidae)] é uma das principais pragas do meloeiro (*Cucumis melo*) nos estados do Rio Grande do Norte e Ceará, o que torna imprescindível a adoção de estratégias de controle dessa praga. Nesse trabalho foi avaliado o efeito do extrato aquoso de sementes de nim, aplicado via irrigação no solo, sobre larvas da mosca minadora, em meloeiro. O experimento foi conduzido em casa de vegetação em delineamento inteiramente casualizado, composto por cinco tratamentos (quatro doses do extrato aquoso de sementes de nim: 1; 3; 5 e 10 g/100 mL além do tratamento testemunha consistindo de água destilada) e 10 repetições (plantas de meloeiro). Os tratamentos foram aplicados em dose única, via irrigação no solo. Na primeira etapa da avaliação, realizada 24 horas após a aplicação dos tratamentos, contabilizou-se o número de larvas mortas por planta. Na segunda etapa foi registrada a mortalidade pupal e calculada a mortalidade total, considerando o número de larvas e número de adultos emergidos. O aumento da dose do extrato aquoso de sementes de nim proporcionou aumento da mortalidade larval e pupal da mosca minadora em meloeiro. A dose de 10 g/100 mL apresentou os melhores resultados, ocasionando 36,4% de mortalidade larval, 96,5% de mortalidade pupal e 96,8% de mortalidade total.

Palavras-chave: *Cucumis melo*, *Azadirachta indica*, mosca minadora, manejo integrado de pragas.

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The leafminer *Liriomyza sativae* (Diptera: Agromyzidae) is one of the most important pests of melon crop (*Cucumis melo*) in the Brazilian semiarid region in states of Rio Grande do Norte (RN) and Ceará (CE) (Araujo *et al.*, 2013), affecting the main producers and exporters of Brazilian melon (IBGE, 2017). Among damages caused by the leafminer are the reduction of photosynthetic capacity and total soluble solid content of fruits (°Brix), which compromise commercialization

of the production (Costa-Lima *et al.*, 2010; Araujo *et al.*, 2013; Costa *et al.*, 2017).

Thus, integrated management strategies to reduce this pest population are essential. Despite the use of different control strategies, such as covering plants with TNT (non woven fabric) during the first 28 days of the crop in the field, and the destruction of crop remains, the leafminer control in the production areas of RN and CE depends on synthetic insecticide application

(Guimarães *et al.*, 2009; Lima *et al.*, 2012).

However, few insecticides to control the leafminer in melon crop are registered (Agrofit, 2017), which makes the active principle rotation difficult favoring the selection of resistant populations. Moreover, the consumers look for products cultivated under minimum insecticide sprays and with no residues above the maximum limits (Guimarães *et al.*, 2009). Thus, develop products different from the conventional

insecticides which can be used in integrated management systems in the melon crop is extremely important.

Among promising alternatives to control the leafminer, insecticides based on vegetable extracts can be relevant, especially neem-based insecticides [*Azadirachta indica* (Meliaceae)]. Products based on neem, mainly oil from seeds, have been efficient, for different crops and regions in the world, in order to control leafminer larvae species such as *Liriomyza huidobrensis* (Weintraub & Horowitz, 1997; Dequech *et al.*, 2010), *Liriomyza trifolii* (Dimetry *et al.*, 1995; Yildirim & Baspinar, 2012; Rai *et al.*, 2013) and *L. sativae* (Hossain & Poehling, 2006; Hossain *et al.*, 2008; Silva *et al.*, 2015; Silva *et al.*, 2016; Costa *et al.*, 2016).

The main insecticide application method is spraying it directly on leaves. However, this way of application presents the great disadvantage of rapid photodegradation of neem derivatives, which reduces its efficiency, especially when applied in the field (Hossain *et al.*, 2008). Thus, the application of neem-based insecticides via irrigation in the soil can be an efficient alternative to control the leafminer fly. Hossain *et al.* (2008) and Silva *et al.* (2015) observed significant larval and pupal mortality of *L. sativae* after application, via soil irrigation, of synthetic insecticides based on neem on tomato and melon crops, respectively.

In relation to neem seed aqueous extract, only information of its effect when sprayed directly on larvae of leafminer on melon leaves can be found in literature (Costa *et al.*, 2016). Therefore, the aim of this study was to evaluate the effect of neem seed aqueous extract, applied via soil irrigation, on leafminer larvae of *L. sativae* on melon plants.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse in Centro de Ciências Agrárias at Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte, Brazil. The authors used adults of *L. sativae*, at the

age 24 to 72 hours, from Laboratório de Entomologia Aplicada of UFERSA, which follows the methodology recommended by Araujo *et al.* (2007).

Neem seeds from ripe fruits were used to prepare the aqueous extract, collected in the municipality of Mossoró (5°12'48''S; 37°18'44''W, altitude 37 m). Seeds were dried over newspaper, in shade and under 25±2°C temperature in a closed room. After, seeds were crushed to powder in a blender. Doses of extract were evaluated at 1; 3; 5 and 10 g of neem seed powder, added to 100 mL of distilled water. The mixture was kept at rest for 24 hours in the dark, in order to extract bioactive substances. All mixtures were filtered through void fabric (sterilized) prior to application.

To evaluate the effect of the doses of neem extract on larvae of leafminer, melon plants, cultivar Iracema (yellow type), were cultivated in 500 mL soil capacity pots, in a greenhouse. When plants presented two developed leaves were taken to the laboratory and submitted to leafminer infestation for 20 minutes in cages (50x50x50 cm) coated with anti-aphid screen, each cage with an average of 200 couples of leafminer fly (adults at 24 to 72 hours age). Afterwards, plants were transported back to the greenhouse. After 72 hours, the number of larvae per plant was counted and then the treatments via soil irrigation were applied. The extracts were applied manually in a single dose, 50 mL per plant, with the aid of a millimeter plastic container.

Treatments consisted of distilled water (control) and four doses of neem seed aqueous extract (1; 3; 5 and 10 g/100 mL). The experimental design was completely randomized with five treatments (extract with neem seed and control) and 10 replicates (melon plants) per treatment.

Evaluations were divided in two steps. The first was carried out 24 hours after applying the treatments, evaluating the number of dead larvae (larvae without movement) per plant. Larval mortality rate (ML) was calculated using the formula $\%ML = (\text{number of dead larvae} / \text{total number of larvae}) \times 100$. In the second step, the pupal mortality was obtained evaluating the larvae which

have survived the treatments, registering the number of viable pupae and emerged adults. Pupal mortality rate (MP) was calculated using the formula: $\%MP = (\text{number of emerged adults} / \text{number of pupae}) \times 100$. The pupae were obtained according to the methodology described by Araujo *et al.* (2007). The total leafminer mortality rate was evaluated based on the initial number of larvae and number of emerged adults.

Mortality rates were corrected according to the methodology described by Abbott (1925). Obtained data were submitted to analysis of variance, followed by analysis of regression processed by R statistical software (R Development Core team, 2011).

RESULTS AND DISCUSSION

Increasing the dose of aqueous neem seed extract applied via soil irrigation provided an increase in larval and pupal mortality of the leafminer. Doses of 1 g/100 mL and 10 g/100 mL showed the lowest and the highest larval mortality rate, 6.6% and 36.4%, respectively (Figure 1 A). The three highest doses of neem seed extract caused pupal mortality rates higher than 50% providing a considerable reduction in the number of emerged adults. The two highest doses, 5 and 10 g/100 mL, resulted in pupal mortality rate of 78.5% and 96.5%, respectively (Figure 1 B). In relation to total mortality, the three highest doses of aqueous neem seed extract provided mortality rate higher than 60%, with the highest dose, 10 g/100 mL, resulting in mortality rate of 96.8% of the insects (Figure 1 C).

These results permit to verify the systemic action of aqueous neem seed extract on larval and pupal mortality of the leafminer *L. sativae*. Systemic action of aqueous neem seed extract in other pest insects, such as *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Souza & Vendramim, 2005; Gonçalves & Bleicher, 2006; Baldin *et al.*, 2007), *Tuta absoluta* (Lepidoptera: Gelechiidae) (Gonçalves-Gervásio & Vendramim, 2007) *Aphis craccivora* (Hemiptera: Aphididae) (Gonçalves & Bleicher, 2006) had already been reported in

other studies.

There are also reports of systemic action of commercial products (oils), based on neem, on the mortality of *Liriomyza* species. Weintraub & Horowitz (1997) showed mortality rate of 40.3%, 48.6% and 84.4% of *L. huidobrensis* larvae applying Neemix-45® (4.5% azadirachtin), occurring when bean plants (*Phaseolus vulgaris*) were saturated with 5; 10 and 25 ppm of azadirachtin, respectively. Hossain *et al.* (2008) verified that an increase in concentration of NeemAzal-U, via soil irrigation on tomato crop, resulted in an increase of larvae mortality rate and reduction of emerged adults of *L. sativae*. Silva *et al.* (2015), evaluating Azamax neem oil (12 g/L of azadiractina), applied via soil irrigation on larvae of *L. sativae* on melon plants, observed increasing rates of larvae mortality and reduction in number of emerged adults with an increased concentration of the product.

Comparing our results to the ones mentioned above, we concluded that

aqueous neem seed extract, applied via soil irrigation, was as efficient as the commercial oils for larval and pupal mortality of *L. sativae*. Some studies show that leafminer mortality is due to the fact that the compounds present in neem, mainly the azadirachtin show insecticidal action mainly through ingestion and can affect the physiological processes of insects, interfering, for example, in regulatory hormones of metamorphosis, which consequently compromises the normal insect development, and may result in mortality (Schmutterer, 1990; Mordue & Blackwell, 1993; Ascher, 1993; Silva *et al.*, 2009). In relation to insects of order Diptera, such as the leafminer, the effect of neem-based insecticides is variable. Changes occur in larval metamorphosis, larval and pupal mortality of insects after exposed to the products (Schmutterer, 1990; Weintraub & Horowitz, 1997; Hossain & Poehling, 2006; Hossain *et al.*, 2008; Silva *et al.*, 2009; Alvarenga *et al.*, 2012; Rai *et al.*, 2013). The fact that the

compounds present in neem affect insect physiological processes may explain the increase in larval mortality and, mainly, pupal mortality with an increase in the dose of aqueous neem seed extract.

We observed that aqueous neem seed extract, applied via soil irrigation, resulted in a significant larval and pupal mortality of the leafminer *L. sativae* on melon, considering that the dose of 10 g/100 mL is the most efficient. These are the first informations about the application of aqueous neem seed extract via soil irrigation, to control leafminer larvae in the melon leaves. The results are promising, because show that this application method allows the product to kills larvae, causing death. Moreover, aqueous neem extract is easy to be prepared and it is also a low-cost product for producers. However, the authors highlight that further studies under field conditions are necessary to confirm the viability of this technology (the use of aqueous neem seed extract) in the integrated management of melon leafminer.

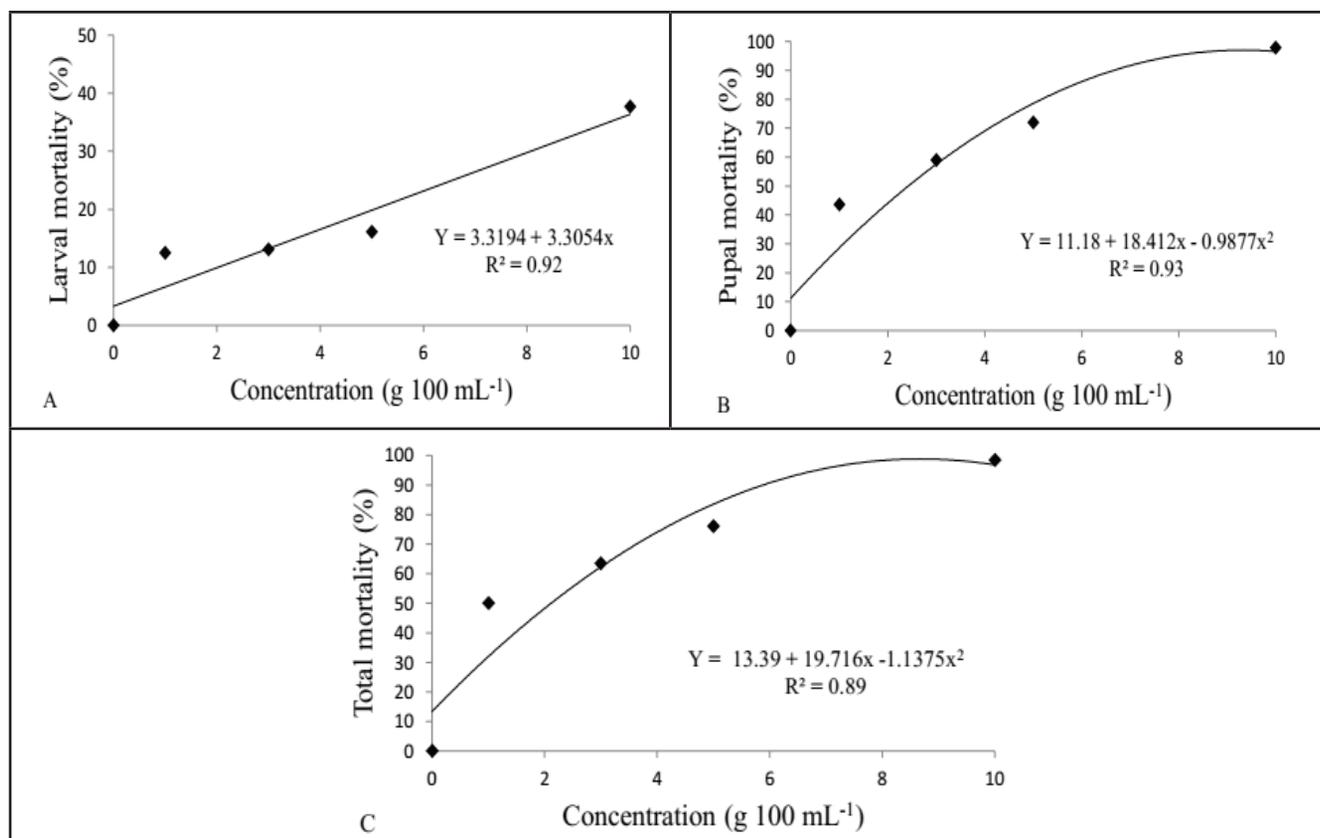


Figure 1. Larval (A), pupal (B) and total (C) mortality rates of *Liriomyza sativae* through application via soil irrigation, at different doses of aqueous neem seed extract (*Azadirachta indica*) in melon plants (*Cucumis melo*). Mossoró, UFERSA, 2017.

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