

Toxicity of neem oil to the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae)

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Neem (*Azadirachta indica* A. Juss.)-derived pesticides have been used against a wide range of agricultural pests including tetranychid mites. Approaches combining lethal and sublethal toxicity studies of neem pesticides towards tetranychid mites are necessary to a comprehensive evaluation of such products. Here, we evaluated the lethal and sublethal toxicity of the neem oil Bioneem to the cassava green mite *Mononychellus tanajoa* (Bondar) by integrating lethal concentration (LC) with population growth and biological parameter studies. According to Probit analyses the concentration of neem oil Bioneem which kills 50% of the population (LC₅₀) of *M. tanajoa* was 3.28 $\mu\text{L cm}^{-2}$, which is roughly twice the field concentration recommended of this biopesticide to control pest mites (1.7 $\mu\text{L cm}^{-2}$). The growth rate of the cassava green mite steadily decreased with dosages of neem oil. Furthermore, sublethal concentrations of the neem oil corresponding to the LC₅₀ reduced the periods of the immature stages of *M. tanajoa* resulting in a shorter developmental time. Similarly, the number of eggs per day and the number of eggs per female per day, a proxy for fecundity, were drastically reduced in *M. tanajoa* females exposed to the LC₅₀ of neem oil. Based on our comprehensive approach we conclude that the neem oil showed lethal as well as sublethal toxicity on growth rate and biological parameters such as duration of immature stages and fecundity of the cassava green mite *M. tanajoa* and it could be used as an ecological alternative for the management of this pest.

Key words: *Azadirachta indica*, biological studies, botanical pesticides, growth rate, sublethal effects.

INTRODUCTION

Cassava *Manihot esculenta* Crantz (Euphorbiaceae) is considered a key cash crop and staple food source for smallholders in many tropical and subtropical regions (Suja et al., 2010). The cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) is regarded as a serious pest of cassava fields in Northeastern Brazil and in other tropical countries (Moraes and Flechtmann, 2008; Rêgo et al., 2012). Cassava green mite damage may include reduction of photosynthetic rate, deformation and death of shoots, leaf dropping, yield losses and plant death (Moraes and Flechtmann, 2008). Although there are predatory mites associated with the cassava green mite in the field, outbreaks of this pest usually occur annually during the dry season of the year (Onzo et al., 2005;

Moraes and Flechtmann, 2008; Rêgo et al., 2012; 2013).

Botanical pesticides based on neem (*Azadirachta indica* A. Juss.) efficiently control a wide range of agricultural pests including tetranychid mites (Gonçalves et al., 2001; Mordue (Luntz) et al., 2005; Venzon et al., 2005; Gauraha and Singh, 2011). Although azadirachtin is the most biologically active compound, neem seed extract also contains deacetylnimbin, deacetylsalannin, nimbin, and salanin (Stark and Walter, 1995; Caboni et al., 2006). In addition to being toxic to pests, these active chemical neem compounds have also been emphasized by their low toxicity to mammals, short field persistence and relative selectivity to natural enemies (Caboni et al., 2006; Isman, 2006; Khater, 2012).

The mean lethal concentration (LC₅₀) is broadly used as a parameter for evaluating and comparing toxicity of a pesticide to arthropods (Desneux et al., 2007; Ullah et al., 2011); however, it does not consider sublethal effects of this concentration. Non-lethal concentrations of pesticides affects physiology and behavior of arthropods causing, for instance, reduction of fertility, foraging interference, repellence, feeding deterrence, changes in biological parameters, morphological abnormalities, interferences in foraging behavior, and reduced growth rate (Teodoro et al., 2005; Kim et al., 2006; Marcic, 2007; Desneux et al., 2007; Teodoro et al., 2009; Cordeiro et al., 2010).

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Population growth toxicity studies provide valuable insights on the effects of pesticides on a given species (Stark and Banks, 2003; Martínez-Villar et al., 2005). The instantaneous rate of increase (r_i), which is closely correlated with the intrinsic growth rate (r_m), is a measure of population increase over a given period of time and it has been successfully used to estimate population growth of arthropods (Stark and Rangus, 1994; Teodoro et al., 2005). In the specific case of sublethal effects of pesticides on the biology of pest mites, changes in longevity, fertility, survival, duration of developmental phases have been reported (Gonçalves et al., 2001; Marcic, 2007; Alzoubi and Cobanoglu, 2008). Studies focusing on the sublethal toxicity of botanical pesticides like neem oil on growth rate and biological parameters of phytophagous mites are needed to fully assess the effects of these pesticides to such pests.

Here, we aimed to evaluate both lethal and sublethal toxicity of a neem-based pesticide to the cassava green mite *M. tanajoa* by integrating lethal concentration estimates with demographic and biological studies.

MATERIALS AND METHODS

Mite rearing

Mononychellus tanajoa colonies were established from mites collected from a cassava plot maintained without pesticide applications in São Luís (02°31'48" S, 44°18'10" W), Maranhão state, Brazil. Potted cassava plants cv. Amarela BR 1667 containing mite colonies were maintained in a greenhouse at uncontrolled conditions. Overexploited plants were replaced by new ones and no pesticides were applied. All bioassays were conducted at 27 ± 10 °C, $60 \pm 20\%$ relative humidity, and a 10:14 h photoperiod.

Concentration-mortality bioassays

Concentration-mortality bioassays were carried out to estimate the lethal concentrations of the neem oil Bioneem (Bioneem, Araçuaí, Minas Gerais, Brazil) to adult females of *M. tanajoa* at the beginning of their reproductive period (ca. 10 d old). A preliminary test was conducted in order to determine a wide concentration range where the highest concentration unable to kill *M. tanajoa* and the smallest concentration able to kill 100% of *M. tanajoa* were determined. Afterwards, six concentrations within this range were used to estimate the lethal concentration values (LCs). The neem oil was sprayed through a Potter tower (Burkard, Rickmansworth, UK) in cassava leaf discs (3.5 cm diameter) at 0.34 bar (34 kPa) pressure with a 2.3 mL spray aliquot. Control leaf discs were sprayed with distilled water.

Sprayed cassava leaf discs were allowed to dry for 1 h before being placed in a Petri dish (10 cm diameter \times 1.5 cm depth) filled with distilled water. Each leaf disc was centrally perforated by a pin attached to the bottom of the

Petri dish. Subsequently 10 adult females of *M. tanajoa* were placed on each leaf disc. Six replicates (leaf discs) for each concentration were used. Mite mortality was assessed after 72 h exposure and concentration-mortality curves were estimated by Probit analysis using PROC PROBIT procedure (SAS Institute, 2002). The likelihood ratio chi-square (χ^2) goodness-of-fit was applied to evaluate whether the data adequately conformed to PROBIT model (Robertson et al., 2007).

Growth rate bioassays

The instantaneous rate of increase (r_i) was used to evaluate the sublethal effects of the neem oil to *M. tanajoa* based on reproduction and mortality data. This index is calculated using the equation $r_i = [\ln(N_f/N_0)]/t$, where N_f is the final number of live mites (including eggs and immatures), N_0 is the initial number of mites, and t is the time interval (7 d) elapsed between onset and end of the bioassay (Walthall and Stark, 1997). Positive r_i values indicate population growth, $r_i = 0$ indicate a stable population, and negative r_i values represent population decline and heading towards extinction (Walthall and Stark, 1997).

Five adult females of *M. tanajoa* as previously described were placed on arenas sprayed with different lethal concentrations (LC₅, LC₁₀, LC₂₅, LC₅₀, LC₉₅, LC₉₉) of neem oil, which were based on the concentration-mortality curves previously obtained. This allowed us to evaluate the effects of increasing LCs on the growth rate of *M. tanajoa*. Control arenas (LC₀) were sprayed only with distilled water. Five replicates for each concentration were used. A male was added to each arena and replaced whenever it died. Regression analysis was conducted to assess the effects of increasing LC of neem oil on the r_i of *M. tanajoa* using Sigma Plot (Systat Software Inc., 2008).

Biological parameters studies

The sublethal toxicity of the neem oil was further evaluated by comparing some biological parameters of the cassava green mite *M. tanajoa* exposed to either the LC₅₀ or the LC₀ (control) of the neem oil. LC₅₀ was chosen for being commonly used as a parameter for evaluating and comparing the toxicity of a pesticide to arthropods. Ten mated females of *M. tanajoa* were confined to cassava leaf discs for 4 h to obtain eggs of similar age. Leaf discs (3.5 cm diameter) were sprayed through a Potter tower with either the LC₅₀ of neem oil (3.28 μ L neem oil cm⁻²) or distilled water (LC₀, control) at the same conditions described above. Eggs were individually transferred to leaf discs and observed twice a day (08:00 and 16:00 h) until reaching adulthood. Newly molted females and males were observed once a day (08:00 h) until their death. One male of the stock culture was added to each leaf disc containing a newly molted female and the periods of preoviposition, oviposition, postoviposition, number of eggs per female, and number of eggs per female per day were

recorded. The biological parameters periods of immature development, egg-to-adult, preoviposition, oviposition, postoviposition, eggs per female, and eggs per female per day of the cassava green mite *M. tanajoa* exposed to the LC₅₀ of neem oil and the control (LC₀) were compared by *t* tests using the software Statistica 7.0 (StatSoft Inc., 1984-2004). Periods of egg, larva, protochrysalid, protonymph, deutochrysalid, deutonymph, teliochrysalid, preoviposition, and postoviposition were log-transformed prior analyses as they violated assumptions of a normal distribution. Untransformed means \pm standard deviation are shown.

RESULTS

Table 1 shows the lethal concentrations (LC₅, LC₁₀, LC₂₅, LC₅₀, LC₉₅, LC₉₉) of neem oil that were estimated by concentration-mortality bioassays. The concentration recommended for field applications of the neem oil Bioneem according to the biopesticide label (15 mL neem oil L⁻¹ distilled water or 1.7 μ L neem oil cm⁻²) matched the LC₃₀ according to Probit analysis (data not shown).

The instantaneous rate of increase (*r*_i) of *M. tanajoa* linearly decreased with increasing lethal concentrations of neem oil (Figure 1). Positive values of *r*_i indicate population growth of *M. tanajoa* exposed up to the LC₅₀, i.e., 3.28 μ L neem oil cm⁻² (*r*_i = 0.40 \pm 0.05 d⁻¹). Mite populations exposed to LC₉₅ (25.27 μ L cm⁻²) and LC₉₉ (58.87 μ L cm⁻²) of neem oil were

Table 1. Toxicity of the neem oil Bioneem to the cassava green mite *Mononychellus tanajoa* (n = 360, $\chi^2 = 12.78$, degrees of freedom = 4, *p* = 0.81). LC = lethal concentration; CI = confidence interval.

Slope \pm SE	LC ₅ (95% CI)	LC ₁₀ (95% CI)	LC ₂₅ (95% CI)	LC ₅₀ (95% CI)	LC ₉₅ (95% CI)	LC ₉₉ (95% CI)
1.46 \pm 0.16	0.4 ¹ (0.2-0.6)	0.7 (0.4-0.9)	1.4 (1.0-1.8)	3.28 (2.6-4.0)	25.3 (17.3-43.3)	58.9 (35.6-123.1)

¹ μ L neem oil cm⁻².

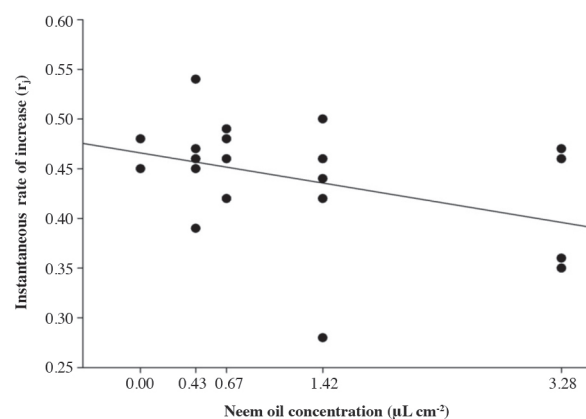


Figure 1. Instantaneous rate of increase (*r*_i) of the cassava green mite *Mononychellus tanajoa* exposed to increasing lethal concentrations of the neem oil Bioneem ($y = 0.4666 - 0.0213x$; $F_{1,23} = 5.84$; $p = 0.024$, $R^2 = 0.20$). LC₅₀: 3.28 μ L neem oil cm⁻².

extinct within 7 d (*N*_f = 0).

Eggs of the cassava green mite *M. tanajoa* exposed to the LC₅₀ (3.88 \pm 0.74 d, n = 129) of neem oil hatched faster than those unexposed (4.76 \pm 0.30 d, n = 407) (*df* = 534, *t* = 20.24, *p* < 0.001) (Figure 2). Likewise, the larval period of *M. tanajoa* exposed to LC₅₀ of neem oil was reduced (0.66 \pm 0.46 d, n = 95) compared with the control (1.03 \pm 0.27 d, n = 101) (*df* = 194, *t* = 9.09, *p* < 0.0001) (Figure 2). LC₅₀-exposed mites had also shorter periods of protochrysalid (LC₅₀: 0.60 \pm 0.27 d, n = 66; LC₀: 0.85 \pm 0.22 d, n = 83; *df* = 147, *t* = 7.51, *p* < 0.0001), protonymph (LC₅₀: 0.75 \pm 0.49 d, n = 63; LC₀: 0.96 \pm 0.24 d, n = 77; *df* = 138, *t* = 5.42, *p* < 0.0001), deutochrysalid (LC₅₀: 0.53 \pm 0.23 d, n = 49; LC₀: 0.75 \pm 0.16 d, n = 73; *df* = 120, *t* = 7.39, *p* < 0.0001), deutonymph (LC₅₀: 0.70 \pm 0.50 days, n = 39; LC₀: 1.00 \pm 0.19 d, n = 70; *df* = 107, *t* = 6.65, *p* < 0.0001), and teliochrysalid (LC₅₀: 0.51 \pm 0.22 d, n = 29; LC₀: 0.99 \pm 0.20 d, n = 67; *df* = 94, *t* = 11.82, *p* < 0.0001) (Figure 2).

Mites exposed to the sublethal concentration of neem oil reached adulthood earlier (LC₅₀: 9.64 \pm 1.01 d, n = 23; LC₀: 10.31 \pm 0.77 d, n = 67; *df* = 88, *t* = 3.29, *p* = 0.001) and had shorter oviposition (LC₅₀: 10.05 \pm 5.84 d, n = 22; LC₀: 16.31 \pm 1.32 d, n = 22; *df* = 42, *t* = 4.90, *p* < 0.0001) and postoviposition (LC₅₀: 1.33 \pm 0.81 d, n = 06; LC₀: 2.59 \pm 0.73 d, n = 22; *df* = 26, *t* = 5.13, *p* < 0.0001) periods than control mites. However, the duration of the period of preoviposition of *M. tanajoa* was not affected by the neem oil (LC₅₀: 1.60 \pm 0.98 d, n = 15; LC₀: 1.28 \pm 0.37 d, n = 56; *df* = 69, *t* = -1.22, *p* = 0.22). Females of *M. tanajoa* exposed to LC₅₀ of neem oil had reduced total (LC₅₀: 15.43 \pm 10.59 eggs per female, n = 16; LC₀: 84.45 \pm 19.79 eggs per female, n = 22; *df* = 36, *t* = 12.65, *p* < 0.0001) and daily (LC₅₀: 1.17 \pm 0.46 eggs per female per day, n = 16; LC₀: 5.17 \pm 1.01 eggs per female per day, n = 22; *df* = 36, *t* = 14.60, *p* < 0.0001) fecundity.

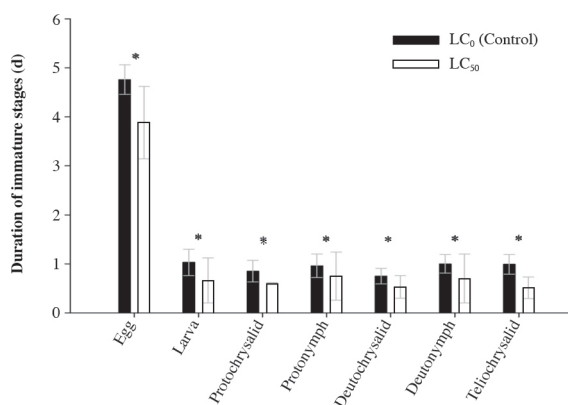


Figure 2. Durations of immature stages of the cassava green mite *Mononychellus tanajoa* exposed to LC₅₀ (3.28 μ L cm⁻²) and LC₀ (control) of the neem oil Bioneem. Means \pm SD are given. Asterisks above bars denote significant differences within each developmental stage (Student's *t* test, *p* < 0.05).

DISCUSSION

Lethal toxicity, population growth and biological parameter studies were combined to assess the toxicity of the neem oil Bioneem to the cassava green mite *M. tanajoa*, a key cassava pest in Northeastern Brazil and in other tropical countries. Probit analysis confirms the toxicity of neem oil to the cassava green mite; however, the recommended field concentration of this biopesticide to control pest mites only matched the LC₃₀, which indicates that the field rate should be higher in order to efficiently control the cassava green mite.

Organisms exposed to pesticides are known to suffer non-lethal effects (Stark and Banks, 2003; Teodoro et al., 2005; Desneux et al., 2007; Teodoro et al., 2009) that are, therefore, not visible on the mortality bioassays such as lethal toxicity studies. Here, when growth rate and biological parameters were considered as endpoints of non-lethal effects of neem oil the results were corroborative. In our bioassays, mites were exposed to neem oil both by direct contact toxicity and residual activity. The sublethal concentrations of the neem oil reduced the r_1 of the cassava green mite in a concentration-dependent manner. However, r_1 values were positive, indicating population increase of *M. tanajoa* up to exposures to the LC₅₀ of the neem oil. Population extinction ($N_f = 0$) occurred only after mites were exposed to LC₉₅ and LC₉₉. This is in line with a study by Venzon et al. (2005), which found sublethal effects of the neem pesticide NeemAzal on growth rate of coffee red spider mite *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae).

The developmental periods of egg, larva, protonymph, deutonymph, and chrysalid stages of *M. tanajoa* were reduced by LC₅₀ of neem oil resulting in a shorter egg-to-adult period. The reduction in the duration of the phases of development of *M. tanajoa* reduces the exposure time of their sensitive immature stages to toxic compounds of the neem oil. Similarly, Gonçalves et al. (2001) found that the duration of immature stages of *M. tanajoa* were reduced by neem extract. In another study, azadirachtin accelerated instar maturation of the elm leaf beetle *Xanthogaleruca luteola* Müller (Zimmerman et al., 1995). In the present study, duration of oviposition and postoviposition (but not preoviposition) periods of *M. tanajoa* were also reduced by the LC₅₀ of neem oil. The fecundity of *M. tanajoa* was affected by the LC₅₀ of neem oil causing a reduction in the number of eggs laid (daily and total) compared to control. The fecundity of *M. tanajoa* was negatively impacted by an egg reduction of over 80% in comparison to control. The exposure to azadirachtin and neem oil-based pesticides at different concentrations also reduced the fecundity of two-spotted spider mite *Tetranychus urticae* Koch (Martínez-Villar et al., 2005; Gauraha and Singh, 2011).

Although there are phytoseiid predatory mites associated with populations of the cassava green mite,

these natural enemies alone are unlikely to prevent *M. tanajoa* outbreaks, especially in regions like Northeastern Brazil in the dry season, during which population peaks of the pest are at its highest (Onzo et al., 2005; Moraes and Flechtmann, 2008; Rêgo et al., 2012; 2013). In this scenario, the use of relatively selective pesticides like neem may help to achieve an efficient control of the cassava green mite while not suppress natural enemies' populations. In a parallel study, we show that the recommended field concentration of the neem oil Bioneem was unable to kill the generalist predatory mite *Proprioseiopsis neotropicus* (Acari: Phytoseiidae), indicating that this biopesticide is selective to this natural enemy. Several other studies point to a relatively selectivity of neem-derived pesticides towards predatory mites (Cote et al., 2002; Venzon et al., 2005; Khater, 2012).

CONCLUSIONS

Our approach allowed a comprehensive evaluation of neem oil toxicity to the cassava green mite *M. tanajoa* by integrating lethal with non-lethal studies. Neem oil showed lethal as well as sublethal toxicity on growth rate and biological parameters of the cassava green mite and it could be used as an excellent alternative for the management of this pest.

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