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Research

Bioactivity of Six Plant Extracts on Adults of *Demotispa neivai* (Coleoptera: Chrysomelidae)

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ABSTRACT. *Demotispa neivai* Bondar (Coleoptera: Chrysomelidae) damage oil palm fruits, which makes it necessary to develop products to control this insect. The mortality, repellency, and antifeeding effects on adults of *D. neivai* of six plant extracts of *Azadirachta indica* A. Juss. (Sapindales: Meliaceae), *Ricinus communis* (L.) (Malpighiaes: Euphorbiaceae), *Citrus sinensis* Oesbek (Sapindales: Rutaceae), *Nicotiana tabacum* (L.) (Slanales: Solanaceae), *Capsicum annuum* (L.) (Solanales: Solanaceae), and *Artemisia absinthium* (L.) (Asterales: Asteraceae) were determined: 1) the lethal concentration $LC_{50\text{-}90}$, lethal time of *D. neivai* was evaluated after spraying the fruits of oil palm; 2) repellent effects of each ingredient were evaluated by calculating the index of repellency; 3) antifeeding effects with the rate of inhibition calculated between doses of 20 and 24 g/liter. The mortality of *D. neivai* was higher with the extracts *Ci. sinensis*, *R. communis*, *N. tabacum,* and *Ca. annuum.* The mortality of *D. neivai* increased in the first 72 hr in all treatments. The extracts of *N. tabacum*, *Ca. annuum,* and *A. indica* were more repellent to *D. neivai* that those of *Ci. sinensis*, *Ar. Absinthium,* and *R. communis.* Antifeeding effect was higher with *Ci. sinensis* and *R. communis.* The increased mortality of *D. neivai* by *Ci. sinensis* can be explained by the effect of this compound on the respiratory system of insects. Extracts of *Ci. sinensis*, *R. communis*, *N. tabacum,* and *Ca. annuum* repelled and caused mortality of *D. neivai* and, thus, can be used in integrate pest management programs of this pest in oil palm plantations.

Key Words: antifeeding effect, Coleoptera, insect pest management, mortality, repellency

Among the factors that can limit production of oil palm *Elaeis guineensis* (Jacquin) (Arecales: Arecaceae), there is *Demotispa neivai* (Bondar) (Coleoptera: Chrysomelidae), a pest in Brazil, Colombia, Ecuador, Panama, Suriname, and Venezuela (Genty et al. 1978, Martínez et al. 2013). This insect damages the exocarp of oil palm fruits that become tenement and with ash color drying and tissue from the first week of formation (Aldana et al. 2004, Martínez et al. 2013). *D. neivai* was also reported on *Bactris gasipaes* Kunth (Arecales: Arecaceae), *Cocos botryophora* Mart. (Arecales: Arecaceae), *Cocos nucifera* (L.) (Arecales: Arecaceae), and *Desmoncus polyacantha* Mart. (Arecales: Arecaceae) (Staines 1996, 2002). Furthermore, damage by *D. neivai* prevents the maturity of the racim with losses estimated in 7% of extraction oil from *E. guineensis* (Genty et al. 1978, Martínez et al. 2008).

Pesticides such as acephate, methamidophos, and monocrotophos are used in oil palm plantations by the control of *D. neivai* (Genty et al. 1978); however, recent studies demonstrated the presence of pesticide traces in crude palm oil (Yeoh et al. 2006). Conventional insecticides are expensive and can cause collateral effects such as pest resistance, environmental pollution, toxic waste, emergence of new pests, and reducing insect fauna (Simmonds et al. 2002, Haouas et al. 2011).

In this sense, plant extracts represent an alternative for pest control as repellents, deterrents of oviposition and feeding, growth regulators, and toxicity to larvae and adults with low pollution and quick degradation in the environment (Bourguet et al. 2000, Tavares et al. 2009, Chermenskaya et al. 2010). Plant secondary metabolites such as lignans, neolignans, alkaloids, chalcones, kawapirones, flavones, essential oils, and amides are important in plant–insect relationships (Parmar et al. 1997, Abou-Fakhr et al. 2001, Martín et al. 2011). Repellent from plants are obtained from compounds with unpleasant odors or irritants (Parmar et al. 1997, Bourguet et al. 2000, Abou-Fakhr et al. 2001) and with phagoinhibiting is and biocide activity (Akhtar and Isman 2004, Abbassy et al. 2007). Preliminary studies showed lethal and sublethal effects of aqueous plant extracts on oil palm pests, especially on *Rhynchophorus palmarum* (L.) larvae (Coleoptera: Curculionidae) and *D. neivai* adults (Pérez and Iannacone 2006, Martínez et al. 2008).

There are a variety of plants that have insecticidal properties, deterrents, and repellents used in agriculture for pest control; however, these plants could be an alternative in the control of oil palm pest. This study evaluated the lethal concentration $LC_{50\text{-}90}$, lethal time, and sublethal effects of six plants extracts on *D. neivai* adults in the laboratory and semi-controlled field experiments, in order to contribute for the development of new strategies for controlling this insect pest affecting an important source of food.

Materials and Methods

Insects. Adults of *D. neivai* were collected in commercial plantations of oil palm with 10 years old in the Municipality of Puerto Wilches, Santander, Colombia (07°20′ N, 73°54′ W) with 28.46°C mean temperature, 76–92% relative humidity, 145–225 h of sunshine per year, and 2,168 mm annual rainfall. Insects were daily collected by hand and transferred to the laboratory of the Entomology of the Research Center in Oil Palm (Cenipalma) in Barrancabermeja, Santander, Colombia, in plastic containers (25 by 40 cm), with perforated lid to allow air flow and fed on palm exocarp. Healthy males and females without amputations or apparent malformations were used in the bioassays.

Plant Extracts. Six species of plants were used as sources of natural products in this study (Table 1). *Azadirachta indica* A Juss (Sapindales: Meliaceae) is a herb native to Asia and introduced in America, *Ricinus communis* (L.) (Malpighiales: Euphorbiaceae) also from India, *Citrus sinensis* Oesbek (Sapindales: Rutaceae) of Central Asia and distributed throughout the Americas, *Nicotiana tabacum* (L.) (Solanales: Solanaceae) native to tropical America, *Capsicum annuum* (L.)

Table 1. Plant material used to prepare extracts for studies of their bioactivity against adults of *D. neivai* (Coleoptera: Chrysomelidae)

Family name	Scientific name	Tissue used	Date of collection
Asteraceae	*Ar. absinthium*	Leaves	Jan./2010
Euphorbiaceae	*R. communis*	Leaves	Jan./2010
Meliaceae	*Az. indica*	Seeds	Jan./2010
Rutaceae	*C. sinensis*	Fruits	Jan./2010
Solanaceae	*C. annuum*	Fruits	Feb./2010
Solanaceae	*N. tabacum*	Leaves	Feb./2010

(Solanales: Solanaceae) native to America, and *Artemisia absinthium* (L.) (Asterales: Asteraceae) distributed in Europe and introduced in America. Tissues of these plants were collected from experimental field agrobiology Safer-Agrisave (Medellin, Colombia) and dried in an oven at 35°C for 3 wk in darkness. Subsequently, tissues were ground and stored in glass jars (1,000 ml) at 18 ± 2°C in the dark until extraction. Preparations of extracts were made with 10 g of a sample from each plant in an Erlenmeyer flask of 100 ml with 50 ml of methanol. Flasks were covered with aluminum foil and placed in agitation at 100 oscillations per minute during 24 h (OS-300 Allsheng, China). Suspensions were obtained, filtered through meshes of tissue and transferred to a flask of 250 ml for evaporation in a rotary evaporator (Büchi R-114 AG, Switzeland) at 30 ± 2°C. The resulting residue was weighed and dissolved in acetone to produce the primary solution of 400 g per plant. The primary solution per plant extract was diluted with distilled water to obtain concentrations of six series adjusted to 4, 8, 12, 16, 20, and 24 g/liter.

Determination of $LC_{50\text{-}90}$ and Semi-Controlled Conditions Test. Six concentrations besides the control (solvent control/liter distilled water) were used per plant extracts: 4, 8, 12, 16, 20, and 24 g/liter of distilled water. Concentrations of the extracts were applied in 5 µl of topical solution in the body of each individual of *D. neivai.* One hundred and twenty insects were used per dose in polystyrene containers (10 by 10 cm) and fed on exocarp palm. Mortality was recorded every 72 h during 15 days. In the field controlled test, 50 insects were caged in bags wrapping a racim of palm oil. Treatments consisted of plant extract with five replications per treatment. The lethal concentration $LC_{50\text{-}90}$ was applied directly on each racim of fruits and the control had distilled water applied with manual pump spray Royal Condor at 40 psi pressure and a volume of 200 cc. Mortality was corrected in the laboratory and semi-controlled field bioassays (Abbott 1925).

Repellency Test. Four Petri dishes were used as an arena, connected to a central board with plastic pipes and diagonally at an angle of 45°. The others dishes were distributed around them in equidistant distances and two plates were put together symmetrically opposed. Fifty adults of *D. neivai* were released into the central board and the control group received exocarp palm. The LC_{90} was applied in the two opposite plates and non-exposed ones represented the control. Four replications per concentration of extract and a control were evaluated by the number of individuals per plate after 24 hr and calculating the repellency index *RI*: $RI = 2\,G/(G + P)$, where G is the percentage of insects in the treatment and P is the percentage of insects in control. The extract was classified as neutral if the index was equal to one (1); attractive, higher than one (1), and repellent; lower than one (1).

Antifeeding Effect. The area of exocarp of oil palm consumed by *D. neivai* was calculated with a millimeter mesh per fruits during 24 hr according to $LC_{50\text{-}90}$ extract. After every 24 hr, the percentage of area consumed (estimated visually with the mesh) was achieved by obtaining food and inhibition index FII: $\text{FII} = [(1 - T/C) \times 100]$, where T is the food consumption per extract and C is the control.

Statistical Analysis. The parameters $LC_{50\text{-}90}$ and its confidence limits were determined by logistic regression based on the concentration probit-mortality with the program XLSTAT-PRO v.7.5 for Windows (XLSTAT 2004). Mortality was evaluated under the semi-controlled conditions and repellency by ANOVA with the test HSD with a significance level ($P \leq 0.05$) (Tukey 1949). The antifeeding effect was evaluated using the paired *t* test or Wilcoxon. Data from the bioassay mortality, repellency, and antifeeding effect on *D. neivai* in semi-controlled conditions were analyzed with SAS User v. 9.0 for Windows (SAS 2002).

Results

Mortality. The homogeneous pattern of response to higher concentrations indicated that the *Ci. sinensis*, *R. communis*, *N. tabacum*, and *Ca. annuum* caused higher mortality of *D. neivai* and lethal effect on this insect with variable values as estimated by the regression model (Table 1). The best results were obtained with concentrations of 20 and 24 g/liter of afore mentioned extracts. The mortality of this insect between concentrations of the each extract showed adjustment by Probit (X^2, $P < 0.001$). This value was lower with *A. indica* ($X^2 = 11.92$, $P < 0.0006$) and *Ar. absinthium* ($X^2 = 81.77$, $P < 0.001$). The mortality of *D. neivai* with the concentrations of *Ci. sinensis*, *R. communis*, *N. tabacum,* and *Ca. annuum* was lower compared with that of *A. indica* and *Ar. absinthium.* The mortality of *D. neivai* was higher and increased up to 3 days between the concentration tested with ~90% from 72 to 144 h of exposure to the plant extracts *Ci. sinensis*, *R. communis*, *N. tabacum,* and *Ca. annuum* and 50% for *Ar. absinthium* and *A. indica* (Fig. 1).

The mortality of *D. neivai* was similar in semi-controlled conditions in the field with the estimated concentration for the LC_{90} ($F_{1,28} = 4.85$, $P < 0.05$) by Tukey test. The *Ci. sinensis* and *R. communis* showed lethal effects on this insect with 95.5 and 89.9% mortality, respectively, followed by *N. tabacum*, *Ca. annuum,* and *A. indica*, 74.4, 64.4, and 62.6%. The mortality rate was lower with *Ar. absinthium*, 46.6% (Fig. 2).

Repellent Effect. The repellency index was higher with *N. tabacum* (RI = 0.22), *Ca. annuum* (RI = 0.37), and *A. indica* (RI = 0.62). *Ci. sinensis* (RI = 0.9), *Ar. absinthium* (RI = 1), and *R. communis* (RI = 1.05) with values varying between treatments ($F_{1,28} = 8.65$, $P < 0.05$) and forming different groups by Tukey test (Fig. 3).

Antifeeding Effect. The plant extracts showed high antifeeding activity for *D. neivai* (Table 2) with the amount of food consumed by adults of this insect differing according to the concentration. The consumption of exocarp of oil palm was lower with the estimated lethal concentration LC_{50} and higher with the LC_{90} with variations up to 95% FII. The FII of *R. communis* and *N. tabacum* was higher than with *Ar. absinthium.* Adults of *D. neivai* had significant response ($F_{1,28} = 8.65$, $P < 0.05$) with *Ca. annuum*, *Ci. Sinensis*, and *A. indica* with moderate ingestion of these compounds (Table 3).

Discussion

Plant extracts have potential for integrated management of phytophagous insects in oil palm (Pérez and Iannacone 2006, 2008). The insecticidal activity of six plant extracts was evaluated against *D. neivai*, the lethal and sublethal effects were observed in the bioassays. The extracts *Ci. sinensis*, *R. communis*, *Ca. annuum,* and *N. tabacum* showed significant effect on adults of *D. neivai* with lethal effects in insects just after the exposure raising 100% mortality at 72 and 144 h. The dose–response bioassays showed increased toxicity of *D. neivai* with increasing concentrations and differing between *Ar. absinthium* and *A. indica.* Similar results were reported for beetles with increased concentration of plant extracts (Kim et al. 2003, Cerna-Chávez et al. 2010). *Ci. sinensis* was toxic to *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *R. communis* to *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae), *Ca. annuum*, and *N. tabacum* to larvae of Lepidoptera (Park et al. 2003, Vandenborre et al. 2010, Ahn et al. 2011). *A. indica* and *Ar. absinthium* extracts were not as active on *D. neivai*, but were lethal to many insect pests (Ahn et al. 1998, Scott et al. 2003, Kessler et al. 2006).

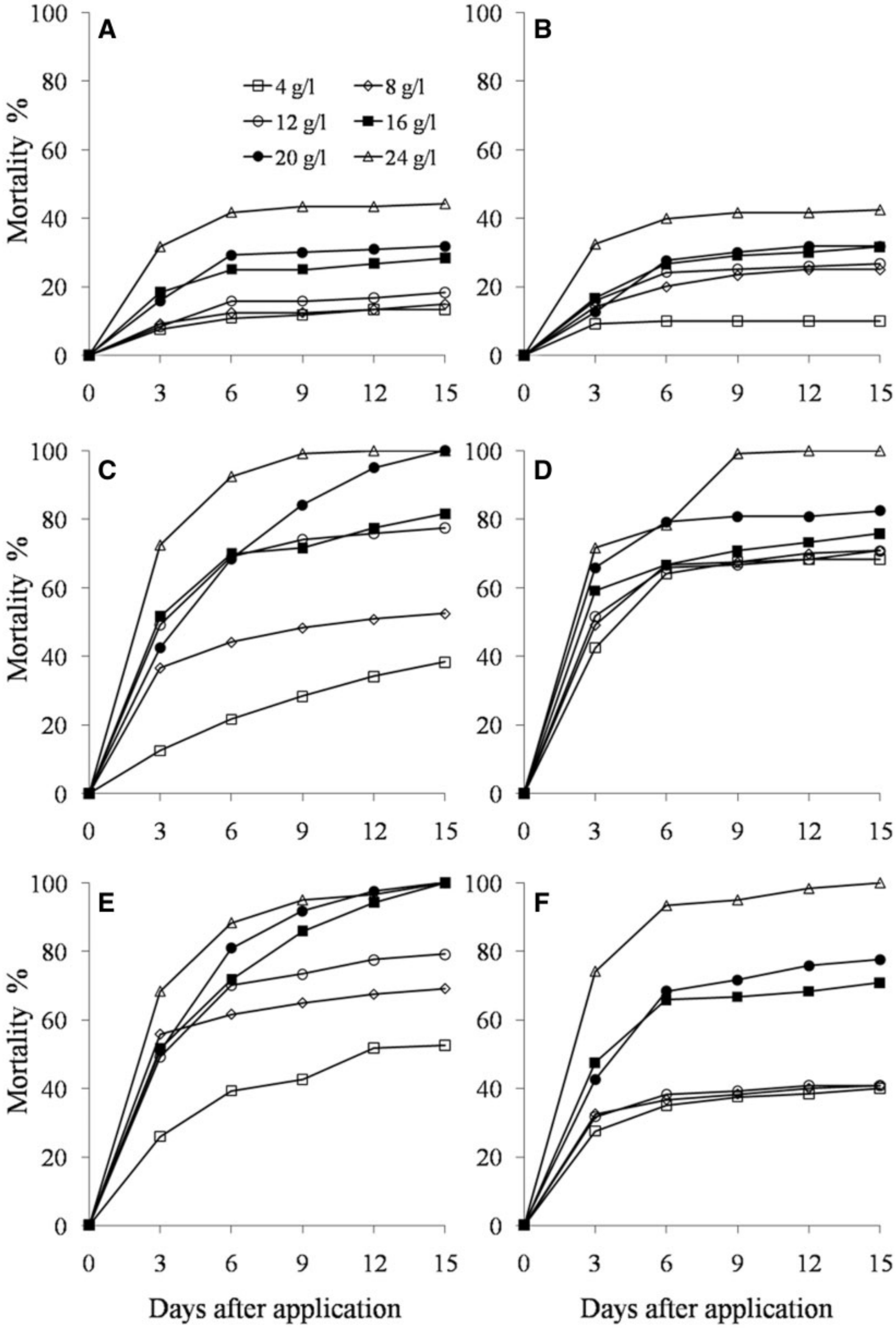

Fig. 1. Mortality of *D. neivai* (Coleoptera: Chrysomelidae) adults by concentration of *Ar. absinthium* (A), *A. indica* (B), *C. annuum* (C), *C. sinensis* (D), *N. tabacum* (E), and *R. communis* (F) during 15 days to calculate the LC_{50-90} ($P < 0.0001$).

The plant extracts have repellent and attractive effects on *D. neivai. N. tabacum* and *Ca. annuum* showed repellent effect on *D. neivai*, while *Ci. sinensis*, *Ar. Absinthium*, and *R. communis* altered the behavior of this insect. The repellents compounds obtained of *N. tabacum* and *Ca. annuum* altered mating behavior, oviposition, and food preference of insects (Feeny et al. 1989, Jaenson et al. 2005, Delphia et al. 2007). The response to volatile plants can vary among arthropods. For example, volatile compounds found in orange fruit *Citrus aurantium* (L.) (Sapindales: Rutaceae) were attractive to *Anastrepha ludens* (Loew) (Diptera: Tephritidae) and repellent for *Culex pipiens* (Coquillett) (Diptera: Culicidae) (Won-Sik et al. 2002, Rasgado et al. 2009). In *eucalipts* leaves, the 1,8-cineol was not repellent to most insects (Pavela 2011). On the other hand, 1,8-cineol was attractive to orchid bees of the *Euglossina* genus (Williams and Whitten 1983). In our study, the repellent effect caused by *N. tabacum* and *Ca. annuum* can help disperse the populations of *D. neivai* and reduce damage on oil palm fruits. Different plants have been used as repellent against insect vectors of diseases, the use of *N. tabacum* is a powerful repellent against mosquito that causes malaria in humans while *Ca. annuum* is used to disperse weevil pest on stored products (Lale 1992, Karunamoorthi et al. 2009, Sõukand et al. 2010).

The extracts of *N. tabacum*, *R. communis,* and *Ca. annuum* had high antifeeding effect ($P \leq 0.05$). Lethal concentration LC_{90} caused greater inhibition on *D. neivai.* Secondary metabolites of feeding deterrents are chemicals that inhibit food behavior of insects (Schoonhoven et al. 2005). Compounds such as alkaloids, terpenoids, and phenolic compounds found in nature can inhibit the absorption of food by insects (Wei et al. 2000, Koul 2008). Feeding reduction or inhibition by

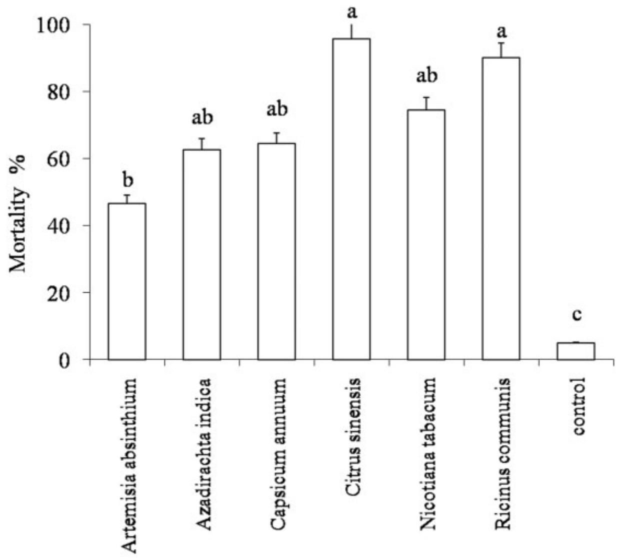

Fig. 2. Mortality in semi-controlled conditions of *D. neivai* (Coleoptera: Chrysomelidae) adults by plant extracts (Tukey $P < 0.05$).

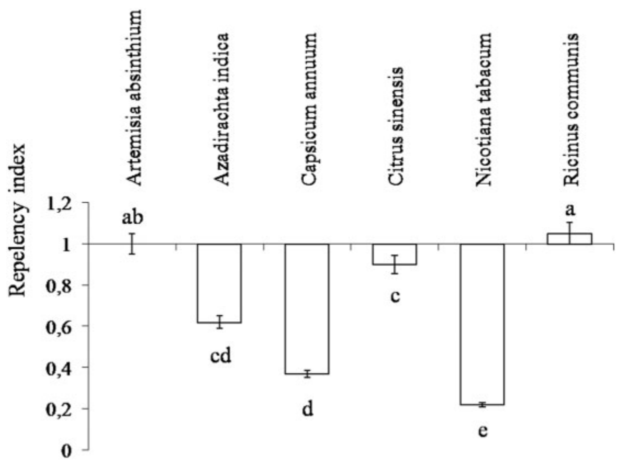

Fig. 3. Repellency of *D. neivai* (Coleoptera: Chrysomelidae) adults by plant extracts (Tukey $P < 0.05$).

Table 2. Activity and response of the lethal concentrations of plant extracts on *D. neivai* (Coleoptera: Chrysomelidae)

Ar. absinthium	LC_{50}	1.89	1.55–2.39	81.77	<0.0001
	LC_{90}	3.62	3.05–4.44		
	LC_{99}	6.57	5.54–8.07		
Az. indica	LC_{50}	3.73	2.75–4.18	11.92	0.0006
	LC_{90}	9.69	6.40–11.5		
	LC_{99}	14.5	9.34–20.9		
C. annuum	LC_{50}	0.26	0.24–0.27	221.52	<0.0001
	LC_{90}	0.51	0.47–0.57		
	LC_{99}	1.09	0.93–1.33		
C. sinensis	LC_{50}	0.22	0.48–3.54	12.09	<0.00005
	LC_{90}	0.70	0.41–1.14		
	LC_{99}	1.80	0.75–1.24		
N. tabacum	LC_{50}	0.21	0.20–0.23	216.19	<0.0001
	LC_{90}	0.41	0.37–0.45		
	LC_{99}	0.83	0.71–1.00		
R. communis	LC_{50}	0.32	0.30–0.35	183.64	<0.0001
	LC_{90}	0.84	0.74–0.98		
	LC_{99}	2.35	1.85–3.25		

C, concentrations causing 50, 90, and 99% mortality; E, estimated value; IC, confidence interval; Chi, chi-square value. Significance level at $P < 0.0001$.

Table 3. Index of food inhibition (FII) by plant extracts on *D. neivai* (Coleoptera: Chrysomelidae) adults ($P < 0.05$, compared by Wilcoxon)

Extract	Concentration	$P < 0.05$	FII (%)
Ar. absinthium	LC_{50}	0.001	—
	LC_{90}	<0.0001	11.33
Az. indica	LC_{50}	<0.0001	35.85
	LC_{90}	<0.0001	70.49
Ca. annuum	LC_{50}	<0.0001	61.12
	LC_{90}	<0.0001	95.76
Ci. sinensis	LC_{50}	<0.0001	45.08
	LC_{90}	<0.0001	79.71
N. tabacum	LC_{50}	<0.0001	65.37
	LC_{90}	<0.0001	100.00
R. communis	LC_{50}	<0.0001	63.05
	LC_{90}	<0.0001	100.00

organic extracts or plant allelochemicals have been demonstrated for several insect orders. Aqueous extracts of *R. communis* leaves were active against *C. chinensis* (Upasani et al. 2003) and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Pavela et al. 2010). *R. communis* mixed with fatty acids had an indirect antifeeding effect on *Atta sexdens* (Hymenoptera: Formicidae) in symbiosis with the fungus *Leucoagaricus gongylophorus* (A, Moller) (Agaricales: Agarocaceae) (Bigi et al. 2004). *N. tabacum* did not affect *C. chinensis* and *C. maculatus* (Khalequzzaman and Osman-Goni 2009), while the strongest effects were found on *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Musser et al. 2005). Antifeeding effect was found on *Spodoptera frugiperda* (Smith), *Helicoverpa zea* (Boddie), *Heliothis virescens* (F.), and *Heliothis subflexa* (Guenée) (Lepidoptera: Noctuidae) using *Ca. annuum* extracts (Ahn et al. 2011).

The selectivity of *Ci. sinensis*, *R. communis*, *N. tabacum,* and *Ca. annuum* may allow controlling one or more insect species or plants when applied simultaneously. The insecticide action of these plants can be due to synergism of compounds and its ability to penetrate the insect body through respiratory system. In addition, this too produced an enzymatic phagous-inhibition during digestion and as allelochemicals by interfering in chemical communication. *Ci. sinensis*, *R. communis*, *N. tabacum,* and *Ca. annuum* extracts have lethal and sublethal effects on *D. neivai* and with potential to manage populations of this insect.

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