Impact of Aqueous Plant Extracts on *Trigona spinipes* (Hymenoptera: Apidae)

by

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ABSTRACT

The stingless bees are an important component of the insect biomass in many tropical areas, due to their collection of nectar and pollen. *Trigona spinipes* is a widely distributed species in South America, and described as a pollinator of many crops that can be used in a commercial pollinating system. The effects of plant extracts on insects are studied because of the demand for organic food and their selectivity to natural enemies. Plant insecticides are reported as a potential agent for the control of insect pests, however little is known about their impact on beneficial insects. This study investigated the survival of *Trigona spinipes* (Hymenoptera: Apidae, Meliponini) Fabricius, after exposure to the leaf extracts of *Azadiracha indica* (Meliaceae), *Lippia sidoides* (Verbenaceae), *Sapindus saponaria* (Sapindaceae), *Anonna squamosa* (Anonnaceae) *Cymbopogon winterianum* (Poaceae), *Corimbia citriodora* (Myrtaceae), *Jatropha curcas* (Euphorbiaceae) and *Ricinus communis* (Euphorbiaceae) and of seeds of *Azadiracha indica*, *Ricinus communis* Nordestina and AL Guarany varieties and *Jatropha curcas*. The extracts that had the greatest influence on the survival of the bees were *A. indica* at 3% and 7% of concentration, *A. squamosa* at a concentration of 10% with 68.89% survival and green leaf of *R. communis* at a concentration of 7%. The results show that

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although the extracts were effective in controlling pests, they may also affect the pollinator *Trigona spinipes*.

Key words: stingless bees, plant insecticides, trigonineos.

INTRODUCTION

One third of human food comes from plants pollinated by bees, making the study on bees important to ensure food safety and security. This shows the need for production models integrating forest ecosystems and agroforestry to improve the performance of these insects (Ashman *et al.* 2004, Biesmeijer *et al.* 2006, Williams 1994).

Stingless bees are an important component of the insect biomass in many tropical areas, due to their collection of nectar and pollen (Johnson and Hubbell 1974). The genus *Trigona* includes one of the most populous groups of stingless bees (Wille 1983). This genus has highly efficient communication mechanisms for the location of food sources (Kerr 1963). The *Trigona* genus is described as pollinator of many crops and can be used in a commercial pollinating system (Sanchez *et al.* 2001).

Trigona spinipes is a widespread species in South America (Schorkopf *et al.* 2007). It has exposed nests, partially protected by a strong layer of bitumen, which maintains temperature regulation inside the nest, protecting the offspring (Wille 1983). These bees usually leave the nest to pollinate after other pollinators have already made their visits to the flowers. Typically, two individuals work together in the same flower; however, up to four bees may be simultaneously working in the same flower (Koschnitzke 2011).

In the last two decades, focus has been placed on the search for new environmentally friendly pesticides aiming to reduce the use of harmful synthetic pesticides (Lobitz *et al.* 1997, Pereira *et al.* 2002; Isman 2006).

The effects of plant extracts on insects are studied because of the demand for organic food and their selectivity to natural enemies (Junior Jones *et al.* 1979; Matos Neto *et al.* 2004). One research line with plant extracts deals with the deleterious effect on organisms (Wheeler *et al.* 2001; Brahmachari 2004; González-Coloma *et al.* 2005; Defagó *et al.* 2006).

Plant insecticides cause less impact and are relatively harmless to natural enemies, pollinators and other non-target organisms. In addition, they are rapidly degraded in the environment and present a low risk to users and consumers (Isman 2006; Schmutterer 1990, Tsuzuki *et al.* 2000).

Plant extracts from species such as *Lippia sidoides*, *Sapindus saponaria* and *Azadirachta indica* have bactericidal, fungicidal, molluscicidal, larvicidal insecticidal and acaricidal properties (Choudhury 1994; Girão *et al.* 2003; Isman 2006; Kunle *et al.* 2003; Murgu *et al.* 2006, Ross *et al.* 1995). However, the effects of plant extracts on pollinators are not well known.

This study investigated the survival of *Trigona spinipes* (Hymenoptera: Apidae, Meliponini) Fabricius exposed to aqueous solutions prepared from leaves and seeds of *Azadirachta indica* A. Juss (Meliaceae), *Lippia sidoides* Cham. (Verbenaceae), *Sapindus saponaria* Linnaeus (Sapindaceae), *Anonna squamosa* Linnaeus (Annonaceae), *Corymbia citriodora* Hill & Johnson (Myrtaceae), *Cymbopogon winterianum* Jowitt. (Poaceae), *Ricinus communis* Linnaeus (Euphorbiaceae) and *Jatropha curcas* Linnaeus (Euphorbiaceae).

MATERIALS AND METHODS

The test bioassays were conducted at the Laboratory of Agricultural and Forestry Pests of the Department of Forest Engineering/Federal University of Sergipe – Brazil – at 25ºC ± 2°C, relative humidity (RH) 60% ± 10% and photophase of 12 hours.

We used leaf extracts of *Azadiracha indica*, *Lippia sidoides*, *Sapindus saponaria*, *Anonna squamosa*, *Cymbopogon winterianum*, *Corimbia citriodora*, and *Ricinus communis* and seeds of *Ricinus communis* varieties Nordestina and AL Guarany, *Jatropha curcas* and *Azadiracha indica.*

The leaves and seeds were dried in an oven at 40°C for two days, then, milled to obtain powder. The extract was prepared with 10g of the plant powder and 100mL of water. The mixture was stored in a hermetically sealed container for 48 hours. The suspensions were filtered through cotton fabric, thereby obtaining an aqueous extract at 10% that was preserved in amber flasks. The concentrations used in the bioassays were prepared by diluting 10% of the concentrated solution in distilled water to obtain concentrations of 3% and 7%.

Insects (10 individuals for each concentration and plant species tested) were inoculated in the chest with 1μL of the aqueous extract at different concentrations (3, 7 and 10%) and control (distilled water). The bees were assessed at 24, 48 and 72 hours after inoculation, kept in Petri dishes and fed with sugar solution.

We used a completely randomized 4x3 factorial design, consisting of 12 treatments, and the factors were analyzed at three different concentration levels and the control, which were evaluated at three different times. The analyses were carried out in the ANOVA through the family of optimal transformation Box-Cox (1964) and by applying the Hartley test (1950) to check the homogeneity of variances.

After using the ANOVA, we applied the F test ($p < 0.05$) to verify possible differences between the factors and the interaction, which, when present, were submitted to the Tukey test ($p < 0.05$).

RESULTS

The plant extracts made from leaves of *L. sidoides*, *C. winterianum*, *J. curcas* and seeds of the Nordestina variety *R. communis* did not affect the survival of *T. spinipes* bees during the evaluation period. Other extracts had a greater influence on the survival of the bees. The extracts of *A. indica* at concentrations of 3% and 7% produced 62.2 and 68.89% survival, respectively, *A. squamosa* at concentration of 10% produced 68.89% survival and green leaf of *R. communis* at concentration of 7% produced 63% survival (Table 1).

When the time factor was isolated for the extracts used in this experiment, we observed that time had a negative influence on the survival of *T. spinipes*. The mortality of the bees increased after 48 hours for the extracts of leaves of *A. Indica* and *R. communis* and seeds of *J. curcas* and after 72 hours for the leaf extracts of *C. citriodora*, *C. winterianum* and *S. saponaria* (Table 2).

The combination of factors for concentration and time showed a negative effect on the survival of the bees for the leaf extracts of *S. saponaria* (Table 3) and seeds of *J. curcas* (Table 4). These results show that the factors influenced most negatively when analyzed together rather than separately.

Extracts of *J. curcas* and *R. communis*, although belonging to the same family (Euphorbiaceae), have different insecticidal action according to the structure of the plant used. Both extracts reduced the percentage of surviv-

Table 1. Survival of *T. spinipes* after inoculation with the plant extracts used at different concentrations. S. *R. communis* AL = Seeds of *R. communis* variety AL Guarany. S. *R. communis* N = Seeds of *R. communis* variety Nordestina. F.V. *R. communis* = Green leaves of *R. communis.* S. *J. curcas* = Seeds of *J. curcas*. F. *J. curcas* = Leaves of *J. curcas.*

Extract	0%	3%	7%	10%
L. sidoides	93.33[a]	96.66[a]	95.55[a]	94.44[a]
S. saponaria	93.33[a]	83.33[b]	88.75[ab]	90.00[ab]
A. squamosa	93.33[a]	72.22[ab]	90.00[ab]	68.88[b]
C. citriodora	93.33[a]	90.00[a]	75.55[b]	85.55[ab]
A. indica	93.33[a]	62.22[c]	68.89[bc]	87.77[ab]
C. winterianum	93.33[a]	88.89[a]	86.66[a]	84.44[a]
S. *R. communis* AL	93.33[ab]	100.00[a]	98.89[ab]	91.11[b]
S. *R. communis* N	93.33[a]	94.44[a]	100.00[a]	97.78[a]
F.V. *R. communis*	93.33[a]	73.33[b]	63.33[b]	76.66[ab]
S. *J. curcas**	1.97[a]	1.97[a]	1.99[a]	1.84[b]
F. *J. curcas*	93.33[b]	100.00[a]	97.78[ab]	97.78[ab]

Means followed by the same lowercase letter in the rows do not differ ($p<0.05$) by the Tukey test.
*Data transformed by Box-Cox (1964) and subjected to the Hartley test (1950) corresponding to 93.33% (0 and 3%), 98.90 (7%) and 75.56 (10%).

Table 2. Survival of *T. spinipes* in the inoculation time for the extract studied.

Extract	24h	48h	72h
L. sidoides	84.17[a]	76.67[a]	73.33[a]
S. saponaria	92.50[a]	84.17[ab]	81.67[b]
A. squamosa	87.50[a]	79.17[a]	76.67[a]
C. citriodora	93.33[a]	90.00[a]	75.55[b]
A. indica	93.33[a]	62.22[c]	68.89[bc]
C. winterianum	95.83[a]	87.50[ab]	81.67[b]
S. *R. communis* AL	97.50[a]	95.00[a]	95.00[a]
S. *R. communis* N	98.33[a]	95.83[a]	94.54[a]
F.V. *R. communis*	86.67[a]	71.67[b]	71.67[b]
F.*J. curcas*	99.17[a]	96.67[a]	95.83[a]
S.*J. curcas**	1.97[a]	1.94[b]	1.94[b]

Means followed by the same lowercase letter in the rows do not differ ($p<0.05$) by the Tukey test.
*Data transformed by Box-Cox (1964) and subjected to the Hartley test (1950) corresponding to 94.4%, 87.76% and 86.66% respectively, in the times studied.

ing individual bees; however, only the leaf extract of *R. communis* and the seed extract of *J.curcas* showed significant effects on bee survival. This may be related to the presence of secondary compounds at higher concentration in leaves and seeds of the oil plant *Jatropha*.

DISCUSSION

Among the plant families with a potential for use as insecticides, the Meliaceae and Annonaceae stand out as the main sources of active insecticidal principles affecting the development, behavior and reproduction of insects (Schmutterer 1988). This is corroborated by the data obtained in this work, where we observed *A. indica* with the lowest survival rate of a representative of the Meliaceae family.

The effect of these plant insecticides is attributed to terpenoids which are chemicals found in plants that protect them from insect attack (Bernays and Chappman 1994). The impact of tetranortriterpenoids (secondary components) can vary and include behavioral, physiological or repellent manifestations (Tedeschi *et al.* 2001; Qiu *et al.* 1998).

Azadiracha indica contains a diverse group of bioactive substances with high biological effect, among which are azadirachtin, meliantrol, salanina and vilasinina (Lee *et al.* 1991). The effect caused by extracts from the family Anonnaceae may be related to alkaloids, acetogenins and diterpene, chemical compounds found in extracts of leaves, which are derivatives that exhibit toxic effects on a wide range of organisms (Chen *et al.* 2004; Mirela *et al.* 2007; Oliveira *et al.* 2002).

The combined action of all of these substances and their action separately produce different effects on the insects, such as repellency, sterility, egg-laying

Table 3. Results of the interaction between concentration and time of the aqueous extract of the leaf of *S. saponaria* on *T. spinipes.*

	Time (Hours)		
Concentration	24	48	72
0	96.66aA	90.00aA	93.33aA
3	96.66aA	80.00aAB	73.33abB
7	96.66aA	86.66aA	80.00abA
10	96.66aA	90.00aA	70.00bB

Means followed by the same lowercase letter in the columns and uppercase letters in the rows do not differ ($p<0.05$) in the Tukey test.

Table 4. Results of the interaction between concentration and time of the aqueous extract of seeds of *J. curcas* on *T. spinipes.*

	Time (Hours)		
Concentration	24	48	72
0	1.98aA	1.95aA	1.96aA
3	1.98aA	1.96aA	1.96aA
7	2.00aA	2.00aA	1.96aA
10	1.93aA	1.77bB	1.77bB

Means followed by the same lowercase letter in the columns and uppercase letters in the rows do not differ ($p<0.05$) in the Tukey test.

disorientation, lethal effects, and altered activity of growth regulators, among others (Tedeschi *et al.* 2001; Qiu *et al.* 1998). In this work, we can observe an effect on the survival of bees submitted to *A. indica* extract; however, this effect may go beyond the survival reduction, and may include factors such as disorientation, which could interfere with bees locating the colony and food source.

Ricinus communis was another extract in our experiment that caused effects on bees which may have been caused by secondary compounds known for their antimicrobial activity and also for inhibiting mitochondrial respiratory reactions (Ferraz 1999).

Ricinine (secondary compound) is an alkaloid that can be found in all parts of *R. communis*. The ricinine content varies according to the plant part, and 1.3% of ricinine is found in leaf dry matter; 2.5% in etiolated seedlings; 0.03% in the endosperm of the seed and 0.15% in the fruit shell (Holfelder 1998).

In *J. curcas*, the toxic compound curcina is capable of inhibiting the formation of proteins in a fashion similar to ricinine, and can be found at higher concentrations in the seed (Arruda *et al.* 2004; Gandhi *et al.* 1995, Kumar and Sharma 2008; Makkar *et al.* 1998). This fact is corroborated by our data where the extract of green leaves of *R. communis* caused more damage than the seed extracts. This may be related to the higher concentration of the compound in the leaves than in seeds.

It is possible that the smaller damage caused by the seed extracts can be related to stronger action of glutathione S-transferase enzymes, which act more easily given that the ricinine concentration is lower in seeds. These enzymes belong to a group of multifunctional proteins that play an important role in the detoxification insects and may contribute to the development of resistance to insecticides, catalyzing the insecticide degradation (Yu 1996). The induction of the detoxifying enzyme, glutathione S-transferase, usually occurs in the fatty body and midgut. The fatty body is more sensitive to ricinine.

The fact that extracts from the leaves of *L. sidoides*, *J. curcas*, *C. winterianum* and seeds of *A. communis* variety Nordestina did not affect the survival of *T. spinipes* may be attributed to the action of detoxifying enzymes; however, the production of these enzymes has a cost that may not be associated with survival after 72 hours of evaluation. Therefore, further studies should be conducted on the trade-off deriving from the production of these enzymes by bees.

CONCLUSION

This study shows that although the extracts were effective in controlling pests, they may also affect the pollinator *T. spinipes* which warrants the need for further research involving the effect of plant insecticides and their effects on beneficial organisms in order to obtain greater safety, selectivity, economic viability and applicability of integrated programs for insect pest control.

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