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Assessing potential plants extracts to reduce *Leucoptera coffeella* (lepidoptera: Lyonetiidae) attack in coffee

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ABSTRACT

Leucoptera coffeella (Guérin-Méneville) (Lepidoptera: Lyonetiidae) is one of the major pests of coffee in South America, causing severe defoliation in coffee plants. Chemical control has been widely used for the management of this insect. However, this practice is becoming gradually less efficient due to the selection of coffee leaf miner populations resistant to synthetic insecticides. Plants extracts can be a valuable tool for the management of *L. coffeella*, due to the potential of plants insecticidal properties of them being compatible with the integrated pest management. This study evaluated the effect of nine botanic aqueous extracts on the oviposition and biology of *L. coffeella*, under laboratory conditions. The extracts of *Toona ciliata*, *Trichilia casaretti* and *Trichilia pallida* decreased the oviposition rate of *L. coffeella* on coffee leaves. Along with *Trichilia catigua*, *Chenopodium ambrosioides* and *Melia azedarach*, these extracts were classified as deterrent to oviposition by a preference index and the *C. ambrosioides*, *T. casaretti* and *T. ciliata* extracts caused high egg mortality of *L. coffeella*. Extracts of seeds of *A. indica* and *T. pallida* negatively affected the development and survival of *L. coffeella*, and reduced the mined area by larvae. In conclusion, the extracts of *A. indica* (S), *T. pallida*, *C. ambrosioides*, *T. casaretti* and *T. ciliata* exhibited high insecticidal activity and might be useful in integrated management programs for *L. coffeella*.

Key words: Coffee leaf miner; Pest management; Plant extracts.

1 INTRODUCTION

Brazil stands out for being the largest producer and exporter of coffee worldwide. In the crop season 2019, more than 49 million bags were produced with 34.40 million bags for arabica coffee and 15.01 for conilon (Conab, 2019). In addition, more than 36 million bags were exported, resulting in revenues of approximately US$ 4.7 billion (Cecafé, 2019). However, the productivity of coffee plants is severely affected by the attack of several insects, with emphasis to the coffee leaf miner, *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) (David-Rueda et al., 2016).

The larvae of coffee leaf miner penetrate coffee leaves and feed on the leaf parenchyma tissues, resulting in superficial injuries on coffee plants leaves, and posteriorly, necrotic lesions, which decrease photosynthetic capacity of plants and affect their productivity and longevity. Overall, plants suffering intensive attack of *L. coffeella* show the upper third completely defoliated and might require up to two years to recover, especially when defoliation occurs in crop years of high productivity. The losses by coffee leaf miner can range from 30 to 80% of production (Souza; Reis; Regitano, 1998; Custódio et al., 2009). Although chemical control is still the most used method of management of *L. coffeella*, this tactic is considered disadvantageous due to high cost, risks to the environment and human health, as well as the selection of resistant insect populations (Guerreiro Filho, 2006; Sharma, 2008).

A promising tool of *L. coffeella* management might be the utilization of plants extracts with insecticidal properties, which could be compatible for use in integrated pest management (IPM) (Torres et al., 2006). Some species of plants synthesize secondary metabolites with characteristics of repellence, feeding or oviposition deterrence, growth inhibition, sterilants or other toxic proprieties, which constitute a chemical defense against pests (Saxena, 1989). These substances might be grouped in five chemical majority types: nitrogen compounds (mainly alkaloid), terpenoids, phenolics, proteinase inhibitors, and growth inhibitors (Maia; Moore, 2011).

In the last years, several studies have shown the efficiency of neem (from *Azadiractha indica*) to pest control (Isman, 2020). Besides the experimental data, many formulations deriving from *A. indica* showed insecticidal activity against a great number of different insects (Benelli et al., 2016; Isman, 2020). Other botanical species also have potential to be used in pest control. For example, extracts of *Toona ciliata*, *Trichilia casaretti*, *Trichilia pallida*, *Chenopodium ambrosioides* and *Ruta graveolens* showed insecticidal activity against *Bemisa tabaci* biotype B (Baldin et al., 2007; Baldin et al., 2015). *Melia azedarach* can be used to control *B. tabaci* biotype B and *Spodoptera frugiperda* (Baldin et al., 2007; Scapinello et al., 2014). Moreover, the insecticide activity of *Trichilia catigua* extracts has been reported for *S. frugiperda* and *Zabrotes subfasciatus* (Matos et al., 2009; Silva; Baldin; Pannuti, 2016).

Thus, this study was conducted in order to evaluate the effect of nine botanic aqueous extracts of these species on the oviposition and biology of *L. coffeella*, aiming to identify vegetable species with potential to be utilized in the management of this insect.

2 MATERIAL AND METHODS

The study was conducted at the Laboratório de Resistência de Plantas e Plantas Inseticidas (LARESPI), Department of Crop Protection, at the College of Agronomic Science, Botucatu, São Paulo, between 2008 and 2009.

2.1 *Leucoptera coffeella* stock rearing

A stock rearing of *L. coffeella* was maintained under controlled conditions (25 ± 2 ºC, 70 ± 10% RH, and 14:10 L:D). Mined coffee leaves were collected from *Coffea arabica* cv. Tupi trees under field conditions, in Botucatu, São Paulo (22°50'01''S; 48°25'38''W; 796 m of altitude) in order to obtain an initial population of the insects. Under laboratory conditions, these leaves had the petioles inserted in a sponge, fixed into Gerbox containers (0.11 x 0.11 x 0.035 m), embedded in aqueous solution, containing benzyladenine plant growth regulator (Sigma-Aldrich, product number B3408, Saint Louis, USA) (0.001 mol/ m^3) (Reis Júnior et al., 2000). At the end of the larval stage, the leaves were transferred to plastic tubes (0.08 x 0.03 m) covered with plastic film until the emergence of adults (Venzon et al., 2005). The adults were collected using an aspirator and utilized to the maintenance of the stock rearing and conduction of the bioassays.

2.2 Plant extracts

Plants were collected in experimental areas belonging to the University of São Paulo (ESALQ), Piracicaba-SP, São Paulo State University (UNESP/FCA), Botucatu-SP and in commercial production area in 2008, and further and identified (Table 1). Next, plants structures were dried in an incubator during 48h (40 °C) and crushed in electric mills until a fine powder was obtained. In order to extract the chemical compounds from the plant samples, 0.005 kg of each powder were mixed to 0.0001 m^3 of distilled water and kept under agitation during 24h (Baldin et al., 2007). Suspension of the solutions were filtered with organdy tissue, and the aqueous extracts at 5% (m/v) were obtained.

2.3 Bioassays

In order to evaluate the effect of plants extracts on the oviposition of *L. coffeella*, the coffee plants (*Coffea arabica* cv. Tupi) were cultivated in 0.0028 m^3 seedling tubes with autoclaved substrate. The substrate was composed of soil, sand and corral manure in a 1:1:1 ratio. The substrate was fertilized according to the crop recommendations (Vieira, 2017).

The vegetable extracts were sprayed on seedlings (approximately 0.15 m high and 16 true leaves) using a manual sprayer (0.00002 m^3/plant). After 15 minutes, the seedlings were singly packaged inside cages (0.5 x 0.5 x 0.7 m) covered with glass, where 100 coffee leaf miner adults (sex ratio 1:1) with two days after adult emergence were released. For the control treatment, it was utilized distilled water in the same volume (0.00002 m^3). After 48 hours, the number of eggs laid on plants was evaluated with aid of a stereomicroscope (Nikon - Stereo Zoom Microscope SMZ 645, Tokyo, Japan, 40x magnification). The oviposition preference index (OPI) was estimated by the following equation: OPI = [(T–S)/(T+S)] x 100, where T is the number of eggs in the treatments and S is the number of eggs of the standard treatment (distilled water). The index varies from +100 (stimulant) to -100 (deterrent), while the value 0 indicates neutrality (Schlick-Souza; Baldin; Lourenção, 2011). This experiment was conducted in a completely randomized design and each cage represented one

Table 1: Plant species utilized in the extracts development for testing against coffee leaf miner.

Species	Family	Structure	Origin
Azadirachta indica A. Juss	Meliaceae	S	ESALQ/USP*
Azadirachta indica A. Juss	Meliaceae	L+B	ESALQ/USP*
Trichilia pallida Swartz	Meliaceae	L	ESALQ/USP*
Trichilia casaretti C. DC.	Meliaceae	L	ESALQ/USP*
Trichilia catigua Juss	Meliaceae	L	ESALQ/USP*
Melia azedarach	Meliaceae	L	ESALQ/USP*
Toona ciliata M. Roemer	Meliaceae	L	Commercial
Chenopodium ambrosioides L.	Chenopodiaceae	L+B+I	FCA/UNESP†
Ruta graveolens L.	Rutaceae	L	FCA/UNESP†
Distilled water	---	---	---

S= seed; L= leaf; B= branch; I= inflorescence; * = identified by Dr. Paulo César Bogorni, ESALQ/USP; † = identified from the Horticulture Department, FCA/UNESP.

replicate per treatment, and a total of five replicates were used in the bioassay.

For the biology bioassays with *L. coffeella*, two independent experiments were carried out. In the first, treatments were applied on leaves containing eggs, while in the second they were applied onto leaves containing intact mines with larvae. To obtain leaves with eggs it was utilized a methodology similar to that of the oviposition bioassay, in which the insects were removed from the cages and the leaves were examined, leaving only two eggs per leaf. These leaves were divided in two groups: one in order to evaluate the use of vegetable extracts in eggs and the other to evaluate the possible mortality effects on mines of *L. coffeella*. The treatments utilized on leaves with mines were applied just when it was detected the initial formation of them.

The extracts were sprayed on the leaves as described in the oviposition bioassay. Next, the leaves were stored inside Gerbox containers (0.11 x 0.11 x 0.035 m), placed in vertical position and their petioles soaked in aqueous solution with benzyladenine (0.001 mol/ m^3) (Reis Júnior et al., 2000). It was evaluated the following parameters from coffee leaf miner: mortality of eggs, larvae, and pupae, and the mined area per larva. In order to evaluate the ovicidal effect of the plants extracts, it was considered the number of initial mines developed after a period of seven days. The larvicidal effect was observed 28 days after spraying of the extracts on the mined leaves.

Both bioassays were conducted in a completely randomized design, with 10 treatments and 10 replicates to evaluate the ovicidal effect and eight replicates for the larvicidal effect. Each replicate comprised one leaf containing two eggs or two mines of *L. coffeella*. In order to avoid emerging adults from escaping the treatments, it was connected Gerbox containers, covered on top with organdy tissue.

The data obtained from the tests were submitted to analysis of variance, with normality determined using the Shapiro-Wilk test and homoscedasticity determined by the Levene's test (Winer et al., 1991). When necessary the original data were transformed in $(x+0.5)^{1/2}$. Tukey tests were used (α= 0.05) to compare the means when significant effects were found by *F-test*. Statisticaly analysis was performed using SAS 8.2 software (SAS institute 2001).

3 RESULTS AND DISCUSSION

The aqueous extracts of *T. ciliata* was the most efficient, decreasing the number of eggs of *L. coffeella* on coffee leaves. The extracts *T. casaretti* and *T. pallida* also reduced the rate of ovipostion. On the other hand, the two treatments involving *A. indica* were the most oviposited by moths, along with the control. Regarding the OPI, none treatment was considered stimulant. *Azadirachta indica* (S), *A. indica* (F+R) and *R. graveolens* were classified as neutral, while *T. ciliata*, *T. casaretti*, *T. pallida*, *T. catigua*, *C. ambrosioides* and *M. azedarach* were deterrent to oviposition (Table 2).

The choice of host plant by the insect to oviposition is related to a complex of stimuli and responses (Städler; Reifenrath, 2009) that might be assessed by its sensorial system, composed by receptors that might recognize the metabolites synthetized by plants, resulting in the increment or reduction of oviposition ratio (Schoonhoven; Jermy; Van Loon, 2005; Navarro-Silva, 2009). In this context, the reduced number of eggs of coffee leaf miner might be related to the direct contact between the adults with the plant molecules, occasioned when the insects moved on the treated adaxial surface of leaves.

Table 2: Mean number (± SE) of eggs of L. coffeella in coffee leaves after 48 hours of exposure to the plants extracts and oviposition preference index (OPI).

Extracts	Number of eggs[1]	OPI[2]	Classification[1]
A. indica (S)	10.60 ± 3.89 a	-9.23 ± 15.25	neutral
A. indica (L+B)	8.65 ± 3.92 ab	-10.93 ± 15.25	neutral
Distilled water	7.98 ± 2.26 ab	0.00 ± 15.25	standard
R. graveolens (L)	7.18 ± 2.04 abc	-10.75 ± 15.25	neutral
M. azedarach (L)	6.29 ± 2.00 abcd	-23.77 ± 15.25	deterrent
C. ambrosioides (L+B+I)	5.62 ± 2.52 abcd	-37.05 ± 15.25	deterrent
T. catigua (L)	3.73 ± 1.42 abcd	-41.56 ± 15.25	deterrent
T. pallida (L)	3.25 ± 1.33 bcd	-53.76 ± 15.25	deterrent
T. casaretti (L)	2.12 ± 0.75 cd	-63.64 ± 15.25	deterrent
T. ciliata (L)	1.76 ± 0.78 d	-70.04 ± 15.25	deterrent
F	26.60*	-	-
CV (%)	24.84	-	-

[1/] Means followed by the same letter per column do not differ by Tukey test (P > 0.05).

T. ciliata is well known as source of cedrelone and toonacilin, substances that exhibit insecticidal and non-feeding-stimulant property (Liao et al., 2007). The species involving the gender *Trichilia* exhibit numerous reports of biological properties and their insecticidal activity has been attributed to the presence of limonoids, like trichilin, hirtin, cedrelone, and other substances (Simmonds et al., 2001; Matos et al., 2009; Vieira et al., 2014). Limonoids contained in *M. azedarach*, like triterpenoid meliartenin, also exhibit bioactivity against insects (Carpinella et al., 2003; Ntalli et al., 2010). Other plant species that act on the behavior of the insects is *C. ambrosioides* (Costa; Tavares, 2006) and it has been wide utilized due to the presence of high levels of ascaridoles in seeds, leaves, and stem (Santos; Corrêa, 2006).

The activity of some of these plants was verified against other insect pests. Aqueous extracts of *T. casaretti*, *T. pallida*, and with emphasis to *T. ciliata*, at 3% (p/v), exhibited high efficiency against *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae), contributing to decreasing the number of adults and eggs per tomato leaf (Baldin et al., 2015). In a study with *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), aqueous extracts of *M. azedarach* 10% (w/v) decreased the ovipositon in cabbage (Dequech et al., 2009). In another experiment with *P. xylostella*, extracts of *C. ambrosioides* and *T. catigua*, 10% (w/v), provoked a deterrent effect on the oviposition preference of the insect (Medeiros; Boiça Junior; Torres, 2005).

Regarding the effects of extracts on the larval stage and consequence mined provoked by them, coffee leaves treated with *A. indica* (S) showed lowest percentage of mined area/larva. On the opposite, *A. indica* (F+R) provided the highest mined area in coffee leaves (Table 3).

Table 3: Mean (± SE) percentage of mined area/larva of *L. coffeella*, 28 days after application of the extracts on coffee leaves. Botucatu-SP, 2008/2009.

Extracts	% mined area [1]
A. indica (L+B)	7.48 ± 1.43 a
T. ciliata (L)	4.43 ± 0.76 abc
T. pallida (L)	3.14 ± 0.52 bc
T. catigua (L)	5.40 ± 1.02 ab
T. casaretti (L)	3.88 ± 0.46 abc
M. azedarach (L)	4.94 ± 0.81 ab
R. graveolens (L)	5.60 ± 1.33 ab
Distilled water	3.62 ± 0.56 abc
C. ambrosioides (L+B+I)	3.64 ± 0.44 abc
A. indica (S)	1.64 ± 0.45 c
F	4.00*
CV (%)	24.35

[1/] Means followed by the same letter per column do not differ by Tukey test ($P > 0.05$).

The contrast involving the potential larvicidal of *A. indica* (S) and *A. indica* (F+R) extracts might be related to the concentration of larvicidal compounds in diferent structures of *A. indica* (Table 3). Although all the part of an *A. indica* plant present azadirachtin, the concentration depends of the structure collected, which in general, is more concentrated in seeds (Silva; Batista; Brito, 2009).

Azadirachta indica is one of the most efficient botanical insecticide worldwide, and its insecticidal activity is mainly linked to azadirachtin A and B, nimbin, salannin and similar compound. (Pavela, 2007). Azadirachtin A is the predominant insecticidal active ingredient derived from neem seed kernel (Tan; Luo, 2011; Lai et al., 2014). This compound has been exploited as an alternative to synthetic insecticides due to its broad spectrum insecticidal action (Boursier et al. 2011; Lai et al., 2014). Studies verified that natural or commercial products based on *A. indica* affect the colonization of adult insect and reduce the oviposition ratio, decrease the number of larvae hatched, and affect the nymphal phase of *B. tabaci* in tomato plants (Kumar; Poehling, 2006; Lynn et al., 2010). The efficacy of azadirachtin is related to its physiological action, as a growth regulator. The molecule is also deterrent for oviposition and able to act as sterilant in some female insects (Morgan, 2009; Martinez, 2011).

Larvae of *L. coffeella*, to present miner habit, were exposed to the extracts after hatched and feed on the treated vegetable tissue. According to literature, *A. indica* present a residual effect, longer enough, to inhibit the first ecdysis of larvae hatched from treated eggs with the botanical ingredient (Schmutterer, 1988). Moreover, *A. indica* extracts possibly present a systemic and translaminar action in plants (Kumar; Poehling, 2006, Baldin et al., 2007), which cause the capacity of these extracts to affect larvae inside mines.

Spraying of plants extracts on *L. coffeella* eggs significantly reduced the egg eclosion, except for *T. catigua*. Regarding the larval and pupal survival, the extracts *A. indica* (S) and *T. pallida* caused elevated mortality in larval and pupal stages, with emphasis to *A. indica* that exhibited 100% of pupal mortality (Figure 1).

Insecticidal activity on the embryonic phase of lepidopterans by botanical extracts is not widely known, especially the ovicidal action (Mazzonetto et al., 2013). The effect ovicidal of the extracts tested in this study is interesting due to ovicidal activity of synthetic and botanic products is not conventional (Martinez; Meneguim, 2003). Eggs of lepidopterans present a lipid or waxy layer inside the chorion that involves the embryonic membrane, which may be responsible for the retention of products with ovicidal action, protecting the embryo (Smith; Salkeld, 1966).

The extracts of *C. ambrosioides*, *T. casaretti* and *T. ciliata* might present secondary compounds with insecticidal activity, which are responsible for the high egg mortality of *L.*

coffeella. Previous studies involving aqueous extract showed the ovicidal activity of *T. ciliata* and *T. casaretti*, and *C. ambrosioides* against *B. tabaci* biotype B (Baldin et al., 2015) and *Zabrotes subfasciatus* Boheman (Girão Filho et al., 2014).

Similar to the mined area assay, the effect of aqueous extract of *A. indica* on the biology of *L. coffeella* exhibited distinct results according to the type of vegetable structure utilized. Several insects died during the larval and pupal stage in plants treated with *A. indica* (S), not allowing adult emergence. Some larvae have left their mines while others remain inside the mines, however, without feeding, and as time passes, started to exhibit a lethargic behavior. Similar effect was observed in other studies involving extract of *A. indica* and *T. absoluta* (Trindade et al., 2000).

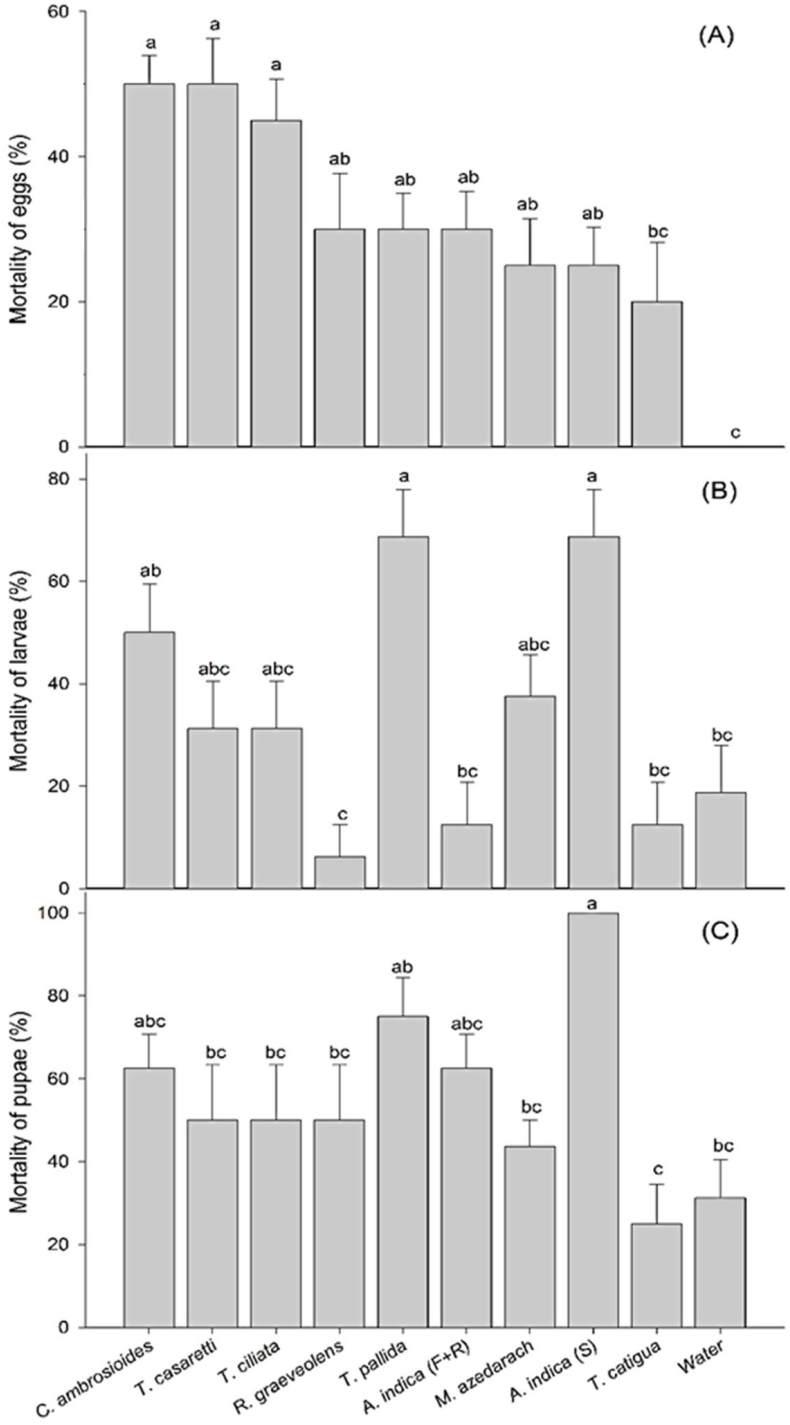

Figure 1: Mortality (%) of eggs (A), larvae (B), and pupae (C) of *L. coffeella* after spraying of plants extracts. Means followed by the same letter per column do not differ by Tukey test ($P > 0.05$).

These deleterious effects in *L. coffeella* appears to be more related to the toxic effect of the *Azadiractina* plant than the fagodeterrent capacity. The insecticidal effects of *A. indica* are found in doses much lower than the necessary to the feeding deterrence (Morgan 2009). Insects, when feed in *A. indica*, or other plant compounds of the plant, do not died immediately, but tend to stop feeding, present a delay in the immature stage development, prolong the juvenile stage, present incomplete ecdise, irregular formation of pupae and adults, and might present a reduction in the viable number of eggs. These effects on the larval development are linked to the reduction in the ecdysone concentration or delay in its release on hemolymph (Mordue; Blackwell, 1993). The effect of the plant might also affect the synthesis of juvenile hormone, and in consequence, the insects do not pass through the farad condition, staying immobile and ceasing the feeding behavior (Govindachari, 1992). The factor might explain the less foliar area consumed by larvae when exposed to the extracts of *A. indica* (S) (Table 3), with high mortality (Figure 1).

Regarding *T. pallida*, there are three tetranortriterpenoids and two compounds, hirtin and diacetylhirtin, with insecticidal activity, extracted in acetone extract of this plant (Simmonds et al., 2001). In other *Trichilia* (*T. hirta*), researchers observed the deleterious effects of limonoids hirtin, causing a delay in the development and an antifeeding behavior in two lepidopterans species (Xie et al., 1994). Another study verified that the application of a pure extract of *T. pallida* on larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae), increased the potential antifeeding effect when compared to the isolated utilization of five tetranortriterpenoids (Simonds et al., 2001). New studies might be conducted in order to check the possible interaction (synergism or addictive) among the compounds studied. This idea might be also explored in this research, because of the probable presence of more than one insecticidal molecule in an aqueous solution.

Trichilia plants have been pointed as valuable in the management of pests, due to the presence of insecticidal compounds, compared with *A. indica* (Cunha et al., 2005). In study involving *T. absoluta* and isolated molecules of *T. pallida*, in extract of dichloromethane, researchers verified that the triterpenoid 24-metilenocicloarta-3E-ol acted similar to azadiractin, reducing the concentration of ecdysone on the hemolymph, which causes the mortality of insects due to their incapacity to release the exuviae (Cunha et al., 2008). Similarly, other research verified the deleterious effects of aqueous extract of *T. pallida* against *T. absoluta* in tomato leaves, causing a prolongation of the larval development and a reduction in eggs viability (Thomazini; Vendramim; Lopes, 2000). Extracts of *T. pallida* in acetate ethyl (2%) reduce the survival and prolong the larval development of *Spodoptera frugiperda* (Roel; Vendramim, 2006).

4 CONCLUSIONS

The aqueous extracts from the species utilized in this study showed different responses regarding the control of *L. coffeella*. Extracts of *A. indica* (S) and *T. pallida* provoked the higher deleterious effect in the biology of the insect, *C. ambrosioides* and *T. casaretti* resulted in the higher ovicide effect and *Toona ciliata* provoked the higher effect in oviposition behavior. This results can be valuable for programs of integrated management of *L. coffeella*.

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