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Running title: Effects of botanical insecticides on the parasitoid

**Toxicity and sublethal effects botanical and extracts and insecticides on the parasitoid *Diachasmimorpha longicaudata***

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**Novelty statement**

It is a work of great interest for the integrated management of fruit flies, particularly on the species *Ceratitis capitata*, the Mediterranean fly, considered the main pest of world fruit culture. In the present work, insecticides and botanical extracts with toxic action on fruit flies were studied, regarding the toxic and sublethal effect on parasitoids, with interesting results that may support the selection of products to control these flies, indicating that botanical products may present toxicity and determine sublethal effects (reduction of parasitism) to parasitoid *D. longicaudata*.

**Abstract**

The knowledge about the action of chemical and botanical insecticides on natural enemies is of great importance for the improvement in integrated management programs of fruit flies. The objective of this study was to evaluate the toxic and sublethal effects of extracts and insecticides botanical on the parasitoid *Diachasmimorpha longicaudata*. The toxicity was evaluated by topical application in the parasitoids, with mortality observation. The treatments consisted applying of the ethanolic extracts of *Metrodorea maracasana* and of *Conchocarpus mastigophorus* (70 mg mL-1), of the insecticides Azamax® (5 mL L-1) and Natuneem® (10 mL L-1) and two control treatments (solvent application and without application), with assessment of mortalility 24, 48, 72 and 96 hours after application. The sublethal effects of the insecticides Azamax® and Natuneem® and ethanolic extracts of *M. maracasana* were evaluated by applying them to adults, “parasitism units” (larvae) or both. The reduction in parasitism was calculated. The Natuneem® and the ethanolic extracts of *C. mastigophorus* and *M. maracasana* are harmless to *D. longicaudata* adults. The Azamax® is considered slightly harmful to the parasitoid *D. longicaudata*. Azamax® behaved as slightly harmful in the three application situations, while Natuneem® ranged from slightly (adult) to moderately (in the larvae and double application) harmful. The ethanolic extract of *M. maracasana* was harmless when applied to the adult or larvae and moderately harmful in double application (adult and larvae). The ethanolic extracts of *M. maracasana* extract has potential for bioprospecting, aiming at the management of tefritids.

**Keywords**:Bio-prospection; *Metrodorea maracasana*; Mortality; Selectivity

**Introduction**

Brazil has the largest plant biodiversity in the world, with more than 46,000 species cataloged and, like many species not yet identified (BFG, 2018). These species are distributed in the Amazon, Atlantic Forest, Caatinga, Cerrado, Pampas and Pantanal biomes (BFG, 2015). The Caatinga biome, with a semi-arid climate, has a very diversified biological patrimony, the only one located entirely within the Brazilian territory and corresponding to approximately 10% of the country (MMA, 2011). Endemic plants in the Brazilian semiarid region have shown promise for the discovery of new biologically active substances (Reis *et al*., 2010).

In the management of fruit flies (Diptera: Tephritidae) of economic importance for Brazil and other countries that produce or import “in natura” fruits, chemical control is the most used method for population suppression. Although effective, the use of insecticides causes environmental and toxicological problems, as well as biological imbalance by eliminating beneficial organisms present in the agro-ecosystem (Degrande *et al*., 2002).

The use of biorational products as plant extracts is of great importance, as they are compounds that generally affect the pest, but have low toxicity to non-target organisms and natural enemies (Horowitz and Ishaaya, 2004; Teixeira, 2016). Combined to screening of biorational products, it's bioprospecting, in the sense of making formulations and form of using these natural products with potential in the management of agricultural and forest pests (Pinto *et al*., 2002), enabling aggregate of value to this plant biodiversity.

Plant extracts have great potential for the development of strategies for population suppression of fruit flies, they present several advantages in relation to synthetic insecticides, among which highlights the low persistence in the environment, contributing to food security; few possibilities for selecting resistant insect populations, reducing contamination of the environment; in addition to being compatible with Integrated Pest Management programs - IMP (Barbosa *et al*., 2006).

Plant extracts and botanical insecticides can cause, in addition to the lethal toxic effects, several deleterious effects on arthropod pests, such as repellency, feeding inhibition, behavioral changes, adult sterilization, morphogenetic and hormonal system changes and, consequently, negatively affect biological development (Fernandes *et al*., 2017). These effects are considered sublethal, that is, they affect the biology, physiology and behavior of the insect, reducing the development, feeding, fecundity, longevity, sex ratio, mobility, oviposition and prey/host search capability (França *et al*., 2017). In assessing the selectivity of insecticides to natural enemies it is important to consider both lethal and sublethal effects (Paiva *et al*., 2018).

Integrated pest management programs (IPM) in fruit cultivation have encouraged the use of various control methods and tactics, such as cultural, behavioral, varietal resistance, and especially biological control. In Brazil, biological control of fruit flies is mainly carried out by parasitoids of the Braconidae family. The species *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) was introduced in the 1990s by Embrapa Mandioca e Fruticultura, in order to reduce the population density of fruit flies and to increase the population of natural enemies (Carvalho *et al*., 2000). In addition, *D. longicaudata* was chosen for introduction in Brazil due to its specificity for the Tephritidae family and its ease of rearing in the laboratory (Garcia and Ricalde, 2013).

In the search for new insect control agents, it is extremely important to find substances that can have selective action in favor of natural enemies, especially for the success of Integrated Pest Management programs in agroecosystems, for the maintenance of existing natural enemies and/or for the creation and release of predators, pathogens and parasitoids (Torres *et al*., 2007).

Semi-arid plants endemic to the Brazilian flora, such as *Metrodorea maracasana* Kaastra (Rutaceae), found in the Northeast (Bahia) and Southeast (Espírito Santo), have been studied in the control of *C. capitata* and *Anastrepha obliqua* fruit flies (Macquart 1835), with promising results (Gomes, 2014; Costa, 2016). Neem (*Azadirachta indica* A. Juss) (Meliaceae) derivatives have also been used to control fruit flies, especially in relation to oviposition deterrence (Machota Junior *et al*., 2013, Silva *et al*., 2015). Studies on the toxicity of these plants to natural enemies are scarce, although they are important, since natural enemies are more susceptible to insecticide compounds than pests. Thus it is needed to assess how these products affect the effectiveness of insect beneficial (Bueno *et al*., 2017).

Studies have investigated the selectivity of insecticides to the parasitoid *D. longicaudata* (Ruiz *et al*., 2008, França *et al*., 2010, Alvarenga *et al*., 2012, Oliveira, 2014), in general, with the application of products only to parasitism units (groups of larvae), disregarding a probable sublethal effect on adult parasitoids surviving product application. Therefore, the objective of this study was to evaluate the toxic and sublethal effects of insecticides and botanical extracts on *D. longicaudata* adults.

**Material and Methods**

The biological material used in the tests was obtained from a creation kept in the laboratory following the methodology proposed by Carvalho and Nascimento (2002). The adults were kept in an acrylic cage containing water and diet based on honey and agar. Third-instar *C. capitata* larvae were used as parasitoid hosts, and were stored in “parasitism units”. These consisted of groups of 150 larvae wrapped in thin organza-like tissue, fixed to the ceiling of an acrylic cage (30 x 30 cm) containing the parasitoids. The units were periodically exposed to about ten-days-old parasitoids for one hour. After exposure of the parasitism units, the larvae were placed in plastic containers containing vermiculite for pupation and subsequent emergence of adults.

The flies of the species *C. capitata* used in the studies were raised in the same laboratory. Eggs were daily collected and, after asepsis, they were submitted to diet based on oat bran, sugar, beer yeast, soybean meal, distilled water, in addition to preservatives, adapted from Zucoloto (1987), aiming to obtain larvae. About 10 days after larvae hatching, already formed pupae were collected and placed in plastic containers with vermiculite, remaining until the emergence of adults, which were transported to the cage, suitable for breeding, mating and oviposition, and fed with diet based on sugar and yeast extract (Biones YE MF), (Silva-Neto *et al*., 2012), offered on filter paper. Cages were kept in air-conditioned room kept at average temperature of 25 ± 2ºC and relative humidity of 70%.

**Toxicity of Insecticides and Botanical Extracts on *Diachasmimorpha longicaudata* Adults**

A completely randomized design with six treatments and five replications was used, totaling 30 plots, each consisting of 10 parasitoids (females). The six treatments studied were: 1) Ethanolic extract of *Conchocarpus mastigophorus* Kallunki (EECM) leaves; 2) Ethanolic extract of *Metrodorea maracasana* Kaastra bark (EEMM); 3) Azamax® neem-based commercial botanical insecticide (Azadiractin A/B 12 g L-1) from DVA Brasil; 4) Neem-based commercial botanical insecticide, Natuneem® (emulsified neem seed oil 1.5 g L-1), from Natural Rural; 5) Control treatment with the solvente, and 6) Control treatment without application.

The plant material of *C. mastigophorus* was collected in a specimen (3 m in height) located on Alto da Caixa D’Água Farm (13°71’80.4”S and 39°62’81.5”W), in a small fragment of Ombrophilous Forest, located in the municipality of Itamari, BA. Leaves of approximately 20-25 cm (adult) were harvested. The species *M. maracasana* was collected on Brejo Novo Farm (13º56’41”S and 40º06’33.9”W), between 617 m and 755 m altitude, 9 km from Jequié, BA. The bark was collected from a trunk with the help of a machete. The extracts were prepared from the leaves and bark of the plants. The material was oven-dried at 40ºC for 48 hours and subjected to cold extraction with ethanol by maceration, following the methodology described by Gomes (2014).

The extracts were evaluated for toxicity by topical application on parasitoids, adapting to the methodology used by Siskos *et al*. (2009). The extracts were diluted with ethanol at a concentration of 70 mg mL-1. Neem-based commercial botanical insecticides were diluted according to the manufacturer’s recommendation for pest control (Azamax® at5 mL L-1 and Natuneem® at10 mL L-1).

Ten *D. longicaudata* females were used in each plot, in which treatments were applied with the aid of a 10 µL graduated micro syringe. The parasitoids were immobilized in the freezer for about one minute before application and 1 µL of the extract solution was then applied to the dorsal region of the insect. After application, the parasitoids were transferred to containers containing the diet and a cotton pad moistened with water. Mortality occurrence assessments were made 24, 48, 72 and 96 hours after application. Data were analyzed considering corrected mortality rates in relation to ethanol control using Abbott’s formula (1925).

**Sublethal Effects of Botanical Insecticides on *Diachasmimorpha longicaudata***

A completely randomized design was used, with ten treatments and five replications, totaling 50 plots. The treatments consisted of the use of two botanical insecticides (Azamax® and Natuneem®), e and ethanolic extract of *M. maracasana* (EEMM); varying the application, namely: T1- Natuneem® application on parasite unit and parasitoid – double application; T2- Natuneem® application on parasitoid; T3- Natuneem® application on parasite unit; T4- Azamax® application on parasite unit and parasitoid – double application; T5- Azamax® application on parasite unit; T6- Azamax® application on parasitoid; T7-EEMM application on parasite unit and parasitoid – double application; T8- EEMM application on parasitoid; T9- EEMM application on parasite unit and; T10-water application - control. Only the EEMM extract was used in the sublethal effects test, it's has toxic action on two species of fruit flies of quarantine importance, showing promise for studies of isolation of substances and formulation. (Gomes, 2014; Costa, 2016).

The application of insecticides on the parasitoid was performed following the methodology of the previous experiment and, after four days, the surviving parasitoids were used in this experiment. Thirty third-instar *C. capitata* larvae from laboratory breeding were placed in organza tissue, forming the parasitism units, which were immersed for 30 seconds in the products. The units were then dried at room temperature and placed in cages containing five females and five males parasitoids aged ten days for a period of one hour, adapting to the methodology of França *et al*. (2010). After exposure, the parasite units were transferred to plastic pots containing vermiculite, closed with paper towels and fastened with elastic, with daily observation of parasitoid and/or fly emergence.

**Statistical Analyses**

For the toxicity study, data from all evaluations were used, looking for a statistical model that described the factors that influenced the survival of *D. longicaudata*. A likelihood ratio test was performed for a GLM (Generalized Linear Model), with 5% significance, using the R software, version 3.2.2 (R Core Team, 2015). Based on parasitoid mortality, the products tested were classified as: 1- harmless (<30% mortality); 2- slightly harmful (30-79%); 3- moderately harmful (80-99%) and 4- harmful (>99% mortality) (Sterk *et al*., 1999); (Harbi *et al*., 2017).

The data from the study of sublethal effects were analyzed taking into account the methodology proposed by Stupp *et al*., 2020 and Bernardi *et al*., 2019, which estimates the reduction in parasitism capacity for each treatment by comparing with the control, using the formula: RP = [1 - (P/p) \*100].

Where, RP is the percentage of parasitism reduction, P is the average parasitism value for each product and p is the average parasitism observed for the control treatment. As a function of the results obtained, each product was classified into classes: 1, harmless (PR < 30%); class 2, slightly harmful (30% ≤ PR ≤ 79%); class 3, moderately harmful (80% ≤ PR ≤ 99%); and class 4, harmful (PR > 99%) (Bernardi *et al*., 2019).

The data were submitted to Bartlett and Shapiro-Wilk tests to evaluate the homocedastity assumptions of treatment variances and normality of residues, respectively and subsequently submitted to analysis of variance (ANOVA) for comparison of means by the Tukey test (P <0.05) using the R software, version 3.2.2 (R Core Team, 2015).

**Results**

**Toxicity of Insecticides and Botanical Extracts on *D. longicaudata* Adults**

The statistical model found in the likelihood test with the best data fit was Survival ~ Time + Azamax® + Natuneem® + ethanolic extract of *M. maracasana*, where survival occurs as a function of time interval and among extracts that showed significant results (Table 1).

The selection of this model indicates that Azamax®, Natuneem® and ethanolic extract of *M. maracasana* provided survival of parasitoid *D. longicaudata* of 58%, 76% and 80%, respectively, significantly lower compared to the control (94%), while the ethanolic extract of *C. mastigophorus* provided higher percentage of survival (92%), similar to the control treatment.Based on parasitoid mortality percentages, Azamax® was classified as slightly harmful (class 2); Natuneem®, ethanolic extract of *M. maracasana* and ethanolic extract of *C. mastigophorus* were classified as harmless (class 1) (Table 2).

The highest mortality rates were registered within 24 hours for all extracts and insecticides botanical, mainly for Azamax® reaching 28% mortality (Table 2).

**Sublethal Effects of Botanical Insecticides on *D. longicaudata***

The number of emerged parasitoids was influenced by the control versus factorial v4 E Finteraction (F = 18.4479; P <0.0001), with averages of 10.20 and 6.35, respectively (Table 3). Unfolding the interaction, when the products were applied only in the parasitoid, the ethanolic extract of *M. maracasana* differed from the other treatments (F = 28.638; P < 0.0001), presenting a greater emergence of parasitoids (average of 23.0 ± 1.41) followed by Azamax® (6.2 ± 3.4) which did not differ from Natuneem® (5.8 ± 3.0) parasitoids. When the products were applied in the parasitism units, the ethanolic extract of *M. maracasana* provided greater emergence of parasitoids 7.4 ± 1.01 not differing from Azamax® 4.4 ± 6.3 and Natuneem® which presented lowest number of parasitoids 0.6 ± 1.3 (F = 3.3124; P = 0.0467). Considering only the double application, the neem-based treatments and the ethanolic extract of *M. maracasana* showed a similar effect in relation to the number of parasitoids (F = 1.6885; P = 0.1977) (Table 3).

Analyzing the type of application for each product, only the application of the ethanolic extract of *M. maracasana* was significant (F = 29.0818; P < 0.0001), [in other words](https://www.linguee.com.br/ingles-portugues/traducao/in%2Bother%2Bwords.html), and provided greater emergence of parasitoids when the application was only in the parasitoid (average of 23.0 ± 1.41), differing from the application in the parasitism unit (7.4 ± 1.01) and, also, from the double application (1.8 ± 3.49).

Among the insecticides that affected the parasitism of *D. longicaudata* about larvae of *C. capitata*, highlight the treatments with Natuneem® T1- double application and T3- application in the parasitism unit, which reduced parasitism by 84.3% and 94.1%, respectively (Table 3). The ethanolic extract of *M. maracasana* reduced parasitism only in the double application (T7) by approximately 82.3%. Based on the reduction of parasitism capacity, the treatments with ethanolic extract of *M. maracasana* with application in the parasitoid (T8) and in the parasitism unit (T9) were considered harmless (class 1), while all treatments with Azamax® (T4, T5 and T6) and Natuneem® with application in the parasitoid (T2) were classified as slightly harmful (class 2), Natuneem® with double application and in the parasitism unit (T1 and T3) and ethanolic extract of *M. maracasana* also with double application were moderately harmful (Class 3).

**Discussion**

The toxicity obtained in adults of the parasitoid *D. longicaudata* allowed it possible to classify the ethanolic extract of *M. maracasana* and ethanolic extract of *C. mastigophorus* and the Natuneem® product as harmless and Azamax® as slightly harmful. These results are of great importance for the biorational management of pest insects, because in studies with fruit flies, such extracts presented toxic effects on pests of economic importance and quarantine worldwide, *C. capitata* (EEMM) (Gomes, 2014) and *A. obliqua* (EEMM and EECM) (Costa, 2016), highlighting the ethanolic extract of *M. maracasana*, responsible for corrected mortality rates the flies of 77.7% and 73.0%, respectively. The selectivity obtained by the ethanolic extract of *M. maracasana* and ethanolic extract of *C. mastigophorus* was similar (innocuous) to that of seed extracts of *Annona mucosa* Jacq and *D. longicaudata* (Stupp *et al*., 2020).

The effects of extracts or botanical insecticides on parasitoids vary function of the type of extract, concentration of the active substance, the targets studied and their stages of development, exposure methodology, among others. Thus, in the case of neem-based products, selectivity in favor of parasitoids can be observed (Viñuela *et al*., 2001, Silva and Bueno, 2015, Silva *et al*., 2016; Asma *et al*., 2018) or not (Gonçalves-Gervásio and Vendramim, 2004; Nogueira *et al*., 2011; Harbi *et al*., 2017).

Regarding the evaluation time, the results evidenced that mortality was significantly higher in the first evaluation (24 hours after topical application) in relation to others, enabling the discrimination between treatments with only one evaluation. This result was similar to that obtained by other researchers for *D. longicaudata* (Bernardi *et al*., 2019), and can assist in the screening phase of bioactive compounds with optimization of time and labor effort.

An interesting aspect verified right after the topical application of the treatments on the parasitoids tergo was the exhibition of the cleaning behavior, in the sense of removing liquids on the body. This behavior was observed by Ruiz *et al*. (2008), in studies with topical application of the orginosynthetic insecticide spinosad in *D. longicaudata*, fact that led authors to lifted the hypothesis that such behavior may take to a reduction in the deposit of the product in the insect's body, reducing the lethal effect of the product. Self-cleaning is a common behavior in social insects, especially ants, removing organisms or potentially pathogenic substances, present on their body surface. (Camargo *et al*., 2017). Some parasitoids of the Braconidae family may also exhibit this behavior (Bilodeau *et al*., 2013).

In the selection of an extract, compound or substance with bioactivity against pest insects, the ideal would be to associate high toxicity and high selectivity to natural enemies and indicate as potentials for formulation those that combine these two characteristics. Considering global trends in the management of agricultural and forestry pests, biorational products must be aligned with the trend of compatibility with natural biological control and applied to the detriment of organosynthetic products with high toxicity, broad spectrum of action and persistence in the environment. However, mostly, studies are restricted to toxic action of the pest and the natural enemy, taking the situation of secondary importance to the generation of knowledge about sublethal effects to natural enemies.

The sublethal effects caused by insecticides and botanical extracts are very variable among non-target species, with emphasis to reduction of longevity, fecundity, pupal viability and parasitism capacity, in addition to malformations (Alvarenga *et al*., 2012; Fontes *et al*., 2018; Parreira *et al*., 2018).

In the present study, the reduction in parasitism capacity was quantified in function of the parasitoid phase exposed to the treatment; adult and larva (parasitism unit). Considering the application only in adults, no treatment caused a reduction in the parasitism capacity and when the application was only in the parasitism unit (larvae), Natuneem® drastically reduced the parasitism capacity (94.1%), while the other non-treatments did not cause such an effect.

In the condition of double application, that is, exposure of the two phases (adult and larva) to the treatments, Natuneem® and the ethanolic extract of *M. maracasana* caused a reduction in parasitism capacity of 84.3% and 82.3%, respectively. This condition simulated, for example, an application in full coverage of the extract and the botanical inseticide in the field, reaching the adults of the parasitoids and the fruits, and indirectly, probable larvae of the parasitoid inside the larvae of the flies that feed of the fruit pulp. In this case, the treatments it should have a translaminar effect to reach the probable parasitoid larvae. However, the translaminar effect of that extract has not been proven ethanolic extract of *M. maracasana*.

On the other hand, the Azamax® product was harmless in terms of sublethal effects in all conditions of application. This difference in the reduction of parasitism capacity between botanical insecticides the base of neem may be related to the products composition, formulation and concentration of the active ingredient (Hohmann *et al*., 2010). The Natuneem® product may have caused repellency, as according to the product manufacturer (Natural Rural), Natuneem® has, in addition to azadiracthina, other bioactive substances that, when diluted, exhale accented odor, fact that can interfere parasitism, once the short distances the parasitoid uses visual stimuli and olfactory for host selection (Vinson, 1976). On the other hand, Smaniotto *et al*. (2013) observed that Natuneem® does not affect the percentage of parasitism and the emergence of adults *Telenomus podisi* Ashmead.

The form of product is applied can also interfere in the reduction of parasitism (Viñuela *et al*., 2001). The parasitism capacity of females of *Opius concolor* (Szèpligeti) was reduced by azadiractin per residual contact by 40.5% and ingestion by 32.5%, already by topical contact there was no interference in this parameter (Viñuela *et al*., 2001).

The effect of neem with application only on the host (eggs or larvae) are that most affect parasitism in *Trichogramma achaeae* Nagaraja and Nagarkatti (Fontes *et al*., 2018), *Trichogramma galloi* Zucchi (Parreira *et al*., 2018) and in *D. longicaudata* (França *et al*., 2010). Exposure to neem of fruit fly larvae already parasitized, also affects the emergence of adult parasitoids (Alvarenga *et al*. 2012).

Thus, among the treatments studied, the greatest advantages fall on the ethanolic extract of *M. maracasana*, for shown to be harmless to adults of *D. longicaudata* and did not determined sublethal effect when applied only to the adult or on unit of parasitism (larva), whereas neem-based products have been shown to be toxic to adults (Azamax®) or have caused a significant reduction in parasitism (Natuneem®). When practiced, the double application (adult and parasitism unit) of ethanolic extract of *M. maracasana* and Natuneem® extract resulted in reduction in the parasitism capacity of *D. longicaudata* considered moderately harmful, which is a condition of maximum exposure. In addition of the toxic or sublethal effects determined per base neem products the *D. longicaudata*, these insecticides presented low agronomic efficacy (12.5% Natuneem® and 25.5% Azamax®) in controlling *A. obliqua* (Costa, 2016), adding a further disadvantage to these products.

Chemical studies indicate that *M. maracasana* is a plant species rich in coumarins, mainly pyranocoumarins (Reis *et al*., 2010) the which are probably responsible for bioactivity. Isolated fractions of this substance, as isodentatin and citrusarina A and the mixture containing citrusarina A + dipetalolactone + isodentatin, provided mortality in *C. capitata* of 100, 96.7 and 96.7%, respectively (Gomes, 2014).

**Conclusion**

In summary, ethanolic extracts of *Metrodorea maracasana* is presented potential for bioprospecting, with the fractionation and detection of the bioactive substances to main species of fruit flies to later formulation, aiming the development of a botanical insecticide of high toxicity ace flies and selectivity to *D. longicaudata*. Considering the fundamental importance of study the effects of ethanolic extracts of *Metrodorea maracasana* on other species of fruit flies and parasitoids of this important group of pests, in addition to investigating its probable translaminar effect.

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**Author contributions**

DRC and MAC planned the experiments, JC and VFP Contributed with botanical extracts, DRC and RJSN statistically analyzed the data and made illustrations, DRC, MAC and SAL made the write up with contributions from AELR, AAM, RJSN, and VFP.

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**Table 1.** Likelihood ratio test (LRT) for the logistic model, of *D. longicaudata* survival as a function of insecticides and botanical extracts (\*) significant results

|  |  |
| --- | --- |
| **Causes of Variation** |  **G.L LRT P** |
| Azamax® | 1 |  | 22.768 | < 0.0001 \*\*\* |
| Natuneem® | 1 |  | 9.2009 | 0.002419 \*\* |
| EEMM | 1 |  | 6.6179 | 0.0101 \* |
| EECM | 1 |  | 0.23474  | 0.628 |
| Control (ethanol)  | 1 |  | 1.4325 | 0.2314 |
| Time | 4 |  | 80.189 | < 0,0001 \*\*\* |

Significant at 0.1%, (\*\*\*), at 1% (\*\*), at 5% (\*)

 **Table 2.** Mortality observed (%) as a function of evauation time (hours), total mortality (%), corrected mortality (%) of *D. longicaudata* and classification of insecticides and botanical extracts

**Control**

**Azamax®**

**Natuneem®**

**EMMB**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **¹Treatments** | **Observed Mortality (%)** | **Total Mortality (%)** | **²Corrected Mortality (%)** | **³Classification** |
| **24h** | **48h** | **72h** | **96h** |
| Azamax® | 28.0 | 34.0 | 34.0 | 42.0 | 42.0  | 40.8 | 2 |
| Natuneem® | 22.0 | 22.0 | 24.0 | 24.0 | 24.0  | 22.4 | 1 |
| EEMM | 18.0 | 18.0 | 20.0 | 20.0 | 20.0  | 18.4 | 1 |
| EECM | 2.0 | 6.0 | 8.0 | 8.0 | 8.0  | 6.1 | 1 |
| Control (ethanol) | 0.0 | 2.0 | 4.0 | 6.0 | 6.0  | - | - |
| Control | 2.0 | 2.0 | 4.0 | 4.0 | 4.0  | - | - |

1Treatment - EMMB- Ethanolic extract of *M. maracasana* bark, ECML- Ethanolic extract of *C. mastigophorus* leaves.

2Corrected mortality, obtained by Abbott’s formula (%).

3Classification: 1- harmless (<30% mortality); 2- slightly harmful (30-79%); 3- moderately harmful (80-99%) and 4- harmful (>99% mortality)

**Table 3.** Mean number of emerged parasitoids, reduction in parasitism capacity (RP) and class

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Parasitoid****(N°)** | **PR****(%)** | **Class\*** |
| T1-Natuneem® double application | 1.6± 2.3 | 84.3 | 3 |
| T2-Natuneem® application on parasitoid | 5.8±3.0  | 43.1 | 2 |
| T3- Natuneem® application parasitism unit | 0.6± 1.3 | 94.1 | 3 |
| T4-Azamax® double application | 6.4± 3.9 | 37.2 | 2 |
| T5-Azamax® application on parasitoid | 6.2± 3.4 | 39.2 | 2 |
| T6-Azamax® application parasitism unit | 4.4± 6.3 | 56.9 | 2 |
| T7- EEMM double application | 1.8±3.49  | 82.3 | 3 |
| T8- EEMM application on parasitoid | 23.0±1.41 | 1.25 | 1 |
| T9- EEMM application parasitism unit | 7.4±1.01 | 27.4 | 1 |
| T10-Control | 10.2± 3.5 | - | - |

\*Class 1- harmless (PR < 30%); 2- slightly harmful (30% < PR < 79%); 3- moderately harmful (80% < PR < 99%); and 4- harmful (PR < 99%).



 **Fig. 1.** Estimated survival of *D. longicaudata* as a function of insecticides and botanical extracts

**Control**

**Azamax®**

**Natuneem®**

**EMMB**