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Bioativity of *Agave sisalana* Perrine Ex Engelm (Asparagaceae) Aqueous Residual Extract on *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) †

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Abstract: The parasitoid *Diachasmimorpha longicaudata* is an important fruit fly control agent, being responsable for the pest population reduction in the field and, therefore, insecticide mitigation. The control based on natural products, as the aqueous extract produced from *Agave sisalana* dried residue, are candidates to be integrated to fruit flies management. However, little is known about the influence of such extracts on natural enemies. Thus, this investigation evaluated the influence of the extract from *A. sisalana* dried residue on *D. longicaudata.* The experiement was carried out following a randomized block design. For each repetition, it was used 24 guava fruits (*Psidium guajava* L.), all previously infested by *Ceratitis capitata.* The fruits were treated with the 5% extract (12 fruits). Additionally, 12 fruits treated only with water were considered the control set. All the fruits were offered in a cage for three hours, period when it was observed and quantified the number of female parasitoid visits on them. This experiment was repeated for 5 days. The quantification and sex separation of emerged parasitoid adults were made 14 days after offering the fruits. To the data analysis, as the total number of parasitoids and number of its females, it was applied the General Linear Model with Poisson distribution. There was no difference between the treatments about the number of emerged parasitoids. However, the mean of visits on the fruits differed between the tretaments, showing 3.8 to control and 2.6 to the extract treatment, suggesting a toxic effect in the last one.

Keywords: Botanical insecticides, vegetable extracts, parasitoids

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1. Introduction

The occurrence of fruit fly species (Diptera: Tephritidae) as *Ceratitis capitata* (Wiedemann, 1824), *Anastrepha fraterculus* (Wiedemann, 1830), *Anastrepha obliqua* (Macquart, 1835) and others, has resulted in severe damages to Brazilian fruits production. [1,2]. Generally, the control of such insects is based on toxic baits associated to chemical insecticides and coverage spraying, being eventually effective [3,4,5].

Although, for reasons as exportation obstacles, changes in the producers and market mentality about the acquisition of contaminant-free products, and environment preservation, there is a higher demand for pest control methods which are healthier for the consumer and safer for the environment [6].

In integrated pest management programs (IPM), biological control and application of biopesticides have showed as promising and even more advantageous than the chemical control [7]. For fruit flies biological control, the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) is the most applied, especially for its effectiveness, specificity and feasible to be reared in laboratory [8,9]. It is a solitary and koinobiont endoparasitoid of fruit flies larvae from the last stages [10,11,12]. The preservation of these natural enemies is an economical tactic and highly important for the environment, since it reduces the pest populations and minimize the pesticides use. However, little is known about the action of biopesticides on natural enemies of fruit flies.

Agave sisalana (Perrine ex Engelm) is a quite important culture in Brazil [13], considered the only species cultivated in the country that is destinated to the production of hard fibers [14]. Such fibers are especially valorized by their easy cultivation [15]. In the Northwest of Brazil, Bahia state is responsible for 95% of the national production, generating jobs for more than 800 thousand people [16]. By-products from *A. sisalana* after shredding can be utilized. They are called residues, but have been used as biopesticides [17], antiparasitic [13] and antimicrobial [18], especially for presenting tannins, alkaloids, saponin and coumarin [19].

In this context, considering that the association between natural enemies and the use of plant extracts can be constructed as an alternative for the integrated management of fruit flies, this study was carried out with the goal of verifying the selectivity of *D. longicaudata* for the extract of *A. sisalana* residue.

2. Materials and Methods

2.1. Location.

The experiment was carried out in the Entomology Laboratory from Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil (12º40'12''S, 39º06'07''W, 220m), under controlled conditions (25 ± 1ºC, 60-80% RU and 12 hours of photophase).

2.2. A. sisalana aqueous Extract.

The dried residue from *A. sisalana* was obtained from the city Valente, in Bahia state. The aqueous extract was acquired by infusion. The proportion used to obtain the aqueous extract from dried *A. sisalana* was 200 g of it for 1000 ml of distilled water. The extract was prepared, covered up until cool down and filtered in voil filter. After this, it was diluted for 5% concentration to be used.

2.3. Fruits.

Arthropod-free guava fruits (*Psidium guajava* L.) were submitted to selectivity tests with *Diachasmimorpha longicaudata* parasitoids under laboratory conditions.

2.4. Biological Material.

C. capitata and *D. longicaudata* adults were obtained in the insect creation from the Entomology Laboratory of Embrapa Mandioca e Fruticultura, Cruz das Almas, BA.

2.5. Bioessays.

To the statistical analysis of the data, it was applied a randomized block design. The score scale data were submitted to GLM test (General Linear Model) Gamma distribution. To the total number of parasitoids and number of female parasitoids, it was used GLM with Poisson distribution.

The treatments were divided in aqueous extract from *A. sisalana* dried residue at 5%, and control (distilled water), with four repetitions (number of cages). For 5 days, groups of 32 guava fruits were exposed to a cluster of *C. capitata* (10 ± 2 days) for infestation. Each group remained in the cage for 24 h. During such period, the fruits were turned in order to permit a uniform oviposition. The insects were fed with artificial diet made of brown sugar, white sugar, brewer's yeast, Sustagen, protein hydrolysate and honey [20].

After the exposition to *C. capitata*, the fruits were placed in plastic trays and covered by paper. A 10-day interval were applied before the exposition of infested fruits to the parasitoid *D. longicaudata.* The fruits infested by *C. capitata* were submerged in the aqueous extract from *A. sisalana* dried residue at 5%, left to dry and, then, kept in four cages (50 cm x 50 cm x 50 cm) with 140 females and 115 males of 7-days *D. longicaudata* each. The adults of *D. longicaudata* were fed with artificial diet composed by ascorbic acid, nipagin, honey, agar and water [20]. Three treated fruits and three control fruits were placed in each cage, summing 24 fruits per day. It was created a score scale, based on the number of parasitoids in oviposition position (Table 1).

Table 1. Score scale to evaluate the number of parasitoids in oviposition position on the guava fruits.

Score	Parasitoid number
1	0 - 4
2	5- 9
3	10- 14
4	15 - 19
5	≥ 20

The fruits which were treated and exposed *D. longicaudata* adults were removed after three hours. The change for new fruits was made in the next day, summing five days of evaluation. Once they were removed from the cage, the fruits were placed in plastic pots with vermiculite and covered with paper. After 14 days, the number of females and males adult parasitoids that emerged from the fruits were acquired.

3. Results

For the general analysis of the score scale, it was verified difference between the treatments. The fruits treated with water presented the highest means. In other words, they showed a higher number of parasitoids on the infested guavas (3.8). The aqueous extract from *A. sisalana* dried residue at 5% negatively affected the parasitoid while locating and laying the eggs in the treated fruits with fruit flies larva (2.6).

About the number of *D. longicaudata* females, it was verified interaction between the treatment and the cage. In all cages, the treatments did not differ between them, except for the fruits treated with water and placed in the cage B. In this case, it is possible to observe that the quantity of female parasitoids found on these fruits was much higher than the others (6.8) (Fig. 1).

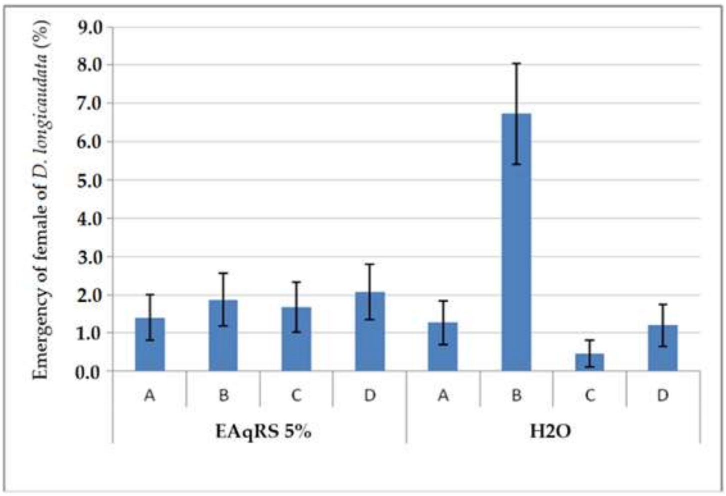

Figure 1. Percentage of *D. longicaudata* females emergence in guava fruits treated with aqueous extract from *A. sisalana* dried residue at 5% and distilled water.

On the other hand, the total number of *D. longicaudata*, to the fruits treated with the aqueous extract, in the cages A, B, C and D, it was not found differences (Fig. 2). Similar to the number of female parasitoids, the total number of parasitoids was equally higher in the fruits treated only with water and placed in the cage B (11.7).

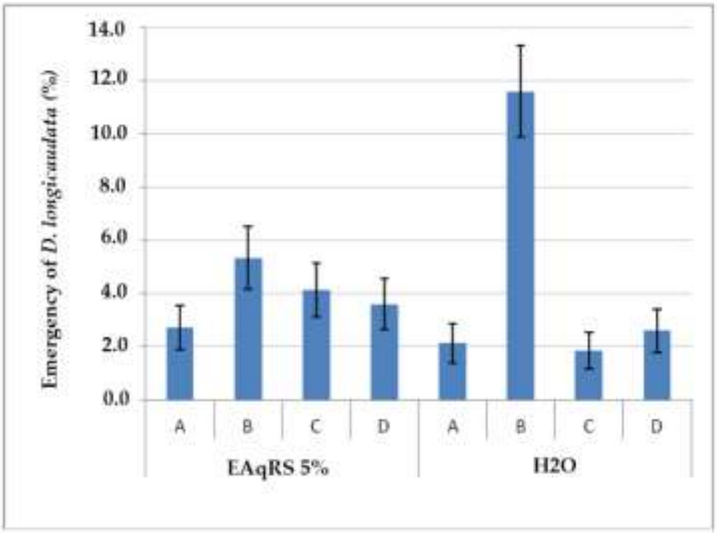

Figure 2. Percentage of *D. longicaudata* males and females emerged from the guava fruits treated with the aqueous extract from *A. sisalana* dried residue at 5% and distilled water.

4. Discussion

The reduction of parasitism in treated fruits possibly happened because of the strong odors released by the extract, since the female parasitoids guide themselves by olfactory stimuli while seeking the host [21]. So, such smell could act as a repellent, considering that some compounds found in *A. sisalana* can work as so. *A. sisalana* presents tannin, alkaloids, saponin and coumarin, and some of them act as repellent for some insects [22,23,24,19]. The volatiles released by the *A. sisalana* extract, in this case, can interfere in the chemical compounds naturally released by the fruits.

The chemical stimuli which provide information about an organism to another are called semiochemicals [25]. The ones which transmit information between individuals from different species are called allelochemicals, and, more precisely, when these chemicals positively favor the receptor and negatively the releaser, they are called kairomones [26,27,28]. *D. longicaudata* females use the infested fruits volatiles as kairomones, in order to locate their hosts [27]. The same happen for parasitoids from other groups, as Oppinae [29].

On the other hand, there is another possible explanation for the lower preference of the parasitoids for fruits treated with *A. sisalana* extract. It could happen due to the lower number of *C. capitata* larva found inside these fruits. Or even the extract could be affected the larva in the fruits, altering their development and, consequently, reflecting in the parasitoid seek by the host chemical cues. Bioinsectides can affect the development of the fruit flies larva, as shown for *Anastrepha* spp. when in contact with Natuneem® (azadirachtin) sprayed in guava fruits [30].

The higher number of parasitoids found in the cage B with fruits treated only with water could be a consequence of guava fruits more attractive to the parasitoids or even with a higher number of larva inside of them, since it is impossible to control the quantity of flies. Fruit flies parasitoids rely on some environmental cues to find the host, as visual, color, shape and size of the fruit, as well as vibrations and chemical compounds coming from the infested fruits [31]. The parasitoids sense such stimuli by receptors found especially in their eyes and antenna, utterly reflecting in modifications in behaviors as flight and oviposition [32]. However, in this case, all laboratory conditions were controlled, excluding possible bias caused by environmental cues.

To achieve a successful IPM program, it is necessary to deeply know about the pest, its natural enemies and the plant, for then deciding which management tactic to apply [33]. Thus, traps using aqueous extract from A. sisalana dried residue at 5% must be installed far from the productive place, along the borders of orchids, or any place that only catch the flies, not negatively interfering in the parasitoid role. New investigations must be done in order to verify if this extract can affect other beneficial insects, as pollinators and other natural enemies. Ideal insecticides must be the ones that are toxic only for the pest and not for the natural enemies [34].

Investigations which tested the oil extracted from the tree *Azadirachta indica*, showed that when this oil is sprayed on *C. capitata* larva, it works as repellent for the parasitoid *D. longicaudata* in different concentrations. However, it neither damaged the *C. capitata* larva, nor their adults emerged from this situation [35]. In a study which investigated the interaction of *D. longicaudata* and the toxicity of *A. indica* extract, it was showed that such bioinsecticide had a negative effect over the emergency of *C. capitata* adults, but also the same results for the natural enemies [36]. Overall, the results presented here can be explained by the fact that the parasitoids are eventually affected due to being exposed to the chemical characteristics of the plant extract, especially because they forage by seeking the host [37,38,31].

5. Conclusion

The aqueous extract from *A. sisalana* dried residue at 5% interfered in the location and oviposition rate of the natural enemy *Diachasmimorpha longicaudata* and possibly in the reduction of parasitism rate, since the treatment had a toxic effect on them.

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