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Efficacy of Botanicals on Parasitoids of Mango Fruit Flies in Burkina Faso

Rabieta Simde[1], Delphine Ouattara[1], Elias Mano[2], Georges Kambou[1], Souleymane Nacro[3*]

[1]Institute of Environment and Agricultural Research (INERA), Central Horticulture Laboratory of the Regional Center of Excellence in Fruits and Vegetables of Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso
[2]Institute of Research in Applied Sciences and Technologies (IRSAT), Laboratory of Natural Substances and Technologies of Natural Products and the Environment (LABTECH-PRONE), Ouagadougou, Burkina Faso
[3]Kamboinsé Environmental, Agricultural Research and Training Center, Ouagadougou, Burkina Faso
Email: *snacro2006@yahoo.fr

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Abstract

The effect of *Capsicum annuum* and *Strophantus hispidus* formulations was evaluated on *Diachasmimorpha longicaudata*. The formulations were obtained from the crude total extracts of *C. annuum*; *S. hispidus* and the adjuvant previously developed. The formulations were prepared according to three concentrations of extracts of each plant species (low, medium and high). These concentrations were calculated on the basis of 1.5 mg/mL of each formulation causing 18% and 15% mortality respectively in *Ceratitis cosyra* within 72 h. The effect of the formulations on *D. longicaudata* was tested using a Fisher block design with 8 treatments (*C. annuum* (3.75; 7.5; 12.5 g/L); *S. hispidus* (4.5; 9; 15 g/L); Success bait 2.4 g/L; control) and 5 replicates. For each formulation, 1.5 mL was poured into a vial containing 0.25 g of cotton. Then 20 adults of the parasitoid were added and the whole was covered with muslin and held in place with rubber bands. The parasitoids were examined after 24 and 72 hours, and those that did not react to the touch of a fine brush were considered dead. The results showed a difference between the treatments. After 24 hours of exposure, the parasitoid showed high mortality at the *S. hispidus* concentrations (15 g/L; 4.5 g/L) followed by the Success bait. These concentrations resulted in mortality rates of 22.50% for *S. hispidus* and 20.50% for Success bait. After 72 h, low parasitoid mortality (35.81%) was obtained with 3.75 g/L *C. annuum*, but high mortality was observed with *S. hispidus* (59.95%; 64.20%; 57.15%) and Success bait (54.80%). The use of *C. annuum* formulations at 3.75 g/L could be recommended for conserving *D. longicaudata* in the nature.

Keywords

Concentrations, Exposure, Mortality, Parasitoid, Formulations

1. Introduction

In Burkina Faso, mango is subject to fruit fly attacks, which in 2017 led to a 15% drop in production on a volume of around 200,000 tonnes [1]. *Ceratitis cosyra* (Hendel) (Diptera: Tephritidae) and *Bactrocera dorsalis* (Walker) (Diptera: Tephritidae) are the two main fruit fly species responsible for significant damage to the mango industry and food security in Africa, Asia, the Pacific and parts of South America [2]. They can cause losses ranging from 50 to 85% if no appropriate phytosanitary controls are put in place [3].

In response to the threat posed by fruit flies, several control methods have been developed, including prophylactic control, chemical control, biological control, the sterile insect technique (SIT), mass trapping and the use of protein baits [4] [5]. Unfortunately, some methods (those using synthetic insecticides or fumigants) have drawbacks that limit their use. In the case of chemical control, the overuse of pesticides leads to resistance in fruit flies (in pulp and on mango), the presence of pesticide residues causing food poisoning and environmental pollution. This situation is exacerbated by the relatively high prices of good quality pest control products on local markets. To remedy this, the search is on for an effective control alternative that is more respectful of the environment and human and animal health, and that meets market requirements (where traceability and quality control standards are increasingly stringent) [6]. Combinations of integrated fruit fly management have been used to protect mango orchards in Côte d'Ivoire [7] and Burkina Faso [8]. In Burkina Faso, IPM has been implemented in 12 mango orchards in three provinces. The combination was sanitation + M3 food bait (SM), sanitation + GF-120 protein bait (SG), sanitation + Timaye + M3 food bait (STM) and sanitation + Timaye + GF-120 bait (STG). From this integrated pest management approach, it appears that the GF-120 STG bait was the most effective in reducing both the *B. dorsalis* and *C. cosyra* populations in these orchards. This technique has the advantage of prolonging the mango season, by protecting mangoes left on the trees after the export season. The sterile insect technique (TIS) [9] has proved effective against fruit flies in Montreal. Indeed, fruit fly control technologies based on the use of plant substance extracts have particularly attracted our attention due to their availability, biodegradability thus preserving human and animal health and the environment. This work was carried out with a view to finding alternatives to synthetic pesticides that would solve these thorny problems and contribute to sustainable environmental management. We evaluated the effect of hydroalcoholic formulations of *Capsicum annuum* and *Strophantus hispidus* on the mango fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). These two substances are generally selective insecticides, non-hazardous to humans and the environment, biodegradable and less costly than synthetic insecticides [5] [7] [8] [9].

The general aim of this study is to evaluate the sensitivity of the parasitoid *D. longicaudata* to hydroalcoholic formulations of *C. annuum* and *S. hispidus* un-

der laboratory conditions. Before using hydroalcoholic formulations of *C. annuum* and *S. hispidus* in an agricultural or ecologically sensitive ecosystem, it is necessary to measure their effects on beneficial organisms (parasitoids and predators). Our study measured the effects of hydroalcoholic formulations of *C. annuum* and *S. hispidus* on the parasitoid *D. longicaudata* used worldwide in biological control programs against fruit Tephritidae [10]. The following hypothesis of research is stated: there is an optimum effective concentration of each hydroa coholic formulation (*Capsicum annum* and *Strophantus hispidus*) that is less harmful or tolerated by *D. longicaudata*.

2. Methodology

2.1. Location of Study Area

The study was conducted at the eco-toxicology laboratory and the biological control laboratory of the Centre National de Spécialisation en Fruits et Légumes (CNS-FL) of the Institut de l'Environnement et de Recherches Agricoles (INERA) in Farako-Bâ, near Bobo-Dioulasso in western Burkina Faso. The rearing room conditions were 12 h/12h photoperiod (dark/light) with a temperature of 25˚C - 28˚C and a relative humidiy of 60% - 70%.

2.2. Composition of the Different Formulations Used in the Laboratory

The material used to apply the different formulations in the laboratory consisted of formulations based on *Strophantus hispidus*, *Capsicum annuum* and Success bait (GF120). The yellow pepper Capsicum annuum is available from market gardeners and Strophantus hispidus is a plant available in Burkina Faso in the south-west region and in Africa.

Strophantus hispidus belongs to the Apocynaceae family. This plant has been used to preserve stored foodstuffs [11]. *Capsicum annuum* belongs to the Solanaceae family and comprises up to 30 species, of which the five main cultivated species are *Capsicum annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens* Ruiz et Pav. *Capsicum annuum* was used by who tested the insecticidal activity of its extracts on the fall armyworm, *Spodoptera frugiperda* J.E Smith (Lepidoptera: Noctuidae) in maize cultivation and their effects on the microorganisms of a ferruginous soil, in Burkina Faso [12]. *C. annuum* extracts are rich in sterols, triterpenes and alkaloids, giving them insecticidal properties.

Strophantus hispidus DC belongs to the Apocynaceae family. Both root and leaf extracts have been shown to inhibit *Escherichia coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes in vitro*. Strains of these pathogens to which resistance against certain antibiotics has been induced in the laboratory were also sensitive to aqueous and ethanol extracts of *Strophanthus hispidus* [13]. *S. hispidus* extracts are not rich in sterols and triterpenes, but contain many anthraquinones, coumarins and cardenolide derivatives, alkaloids, tannins, fla-

vonoids and anthocyanocides. Certain compounds such as anthraquinones, saponins and flavonoids confer insecticidal properties.

Success bait (GF-120) is a concentrated, water-based suspension of 0.24 g/L spinosad and food attractants (proteins, sugar, fruit flavoring).

Success Appat (S.A.) is a mixture of food attractants and a spinosad-based insecticide (0.24 g/L) capable of attracting and killing fruit fly species present in the intervention zone. The natural insecticide spinosad is obtained by fermentation of a soil bacterium, *Saccharopolyspora spinosa*.

Animal material: The animal material consisted of adults of the fruit fly *B. dorsalis* and its parasitoid, *D. longicaudata*.

2.3. Preparation of Crude Total Extracts of *C. annuum* and *S. hispidus*

The various total extracts were obtained from plant powders [14]. Total extracts of *C. annuum*, were obtained from 3.1 kg of *C. annuum* ripe fruit powder and those of *S. hispidus* were obtained from 3.1 kg of *S. hispidus* leaf powder.

Ripe *C. annuum* fruits and *S. hispidus* leaves were shade-dried in a greenhouse, then finely ground using a microgrinder. Extractions were carried out using the Nair method [15], based on the ability of bioactive substances of plant origin to be carried away by solvents of different polarities. For this study, the crushed *C. annuum* fruit (3.1 kg) was macerated in 2 liters of ethyl acetate, stirred well with a spatula and left for 24 hours at room temperature. The crushed *S. hispidus* leaves (3.1 kg) were macerated in 7 liters of ethanol and left for 24 hours at room temperature, after thorough stirring. After 24 hours, the different macerates were then filtered through 5 mm fine mesh muslin placed on a bucket and held in place by an elastic band, then each substrate was rinsed several times with the solvents concerned in reserve to extract the maximum amount of active ingredient.

The filtrates obtained were then concentrated under reduced pressure using a rotavapor. Pressure reduction is facilitated by a device associated with the Büchi R 110 R steam rotavapor, regulated at an appropriate temperature for each extraction solvent, enabling a vacuum to be created at the surface of the liquids (solvent plus solute) to bring them to boiling point at a temperature where the molecules are not destroyed by heat. Thus, for our study, the boiling temperature thanks to the pressure reduction system was located around 50˚C to 60˚C. This enabled evaporation of solvents and concentration of solutes in the rotating flask in the Marie bath.

At the end of the processes, the total crude extract obtained was 200 mL for *C. annuum* from the starting volume. For *S. hispidus*, the volume of crude extract obtained was 720 mL. The extracts were then dried in a ventilated Cole Parmer oven, set at 45˚C. The masses of the dry extracts obtained were determined by weighing using a Radwag AS 110.R1 precision analytical balance.

Success bait preparation: Success bait (GF-120) is a concentrated, water-based suspension of 0.24 g/L spinosad and food attractants (proteins, sugar, fruit fla-

voring).

It is used to control fruit flies in organic production.

For the tests, it was recommended to dilute 1 L of Success bait in 5 L of water.

The preparation and production of hydroalcoholic formulations based on the extracts obtained enabled us to move on to the next stage of the study, which involved testing the effect of the formulations in the laboratory on the parasitoid *D. longicaudata*. The aim of this stage was to determine the effect of the hydroalcoholic formulations on the parasitoid in a controlled environment, thus ensuring their application in orchards.

A personal protective equipment (overalls + gloves + boots + masks + hats) was used. The maceration time was 24 h.

2.4. Efficacy Testing of Hydroalcoholic Extracts on the Parasitoid

B. dorsalis was reared to obtain male and female adults for the test. Rearing room conditions were 12 h/12h photoperiod (dark/light), temperature 25˚C - 28˚C, relative humidity 60% - 70%.

Rearing consisted in preparing egg-laying trays (height 11 cm; diameter 10 cm), which are yellow funnels perforated at regular intervals and lined with a black cloth soaked in mango juice. The prepared nests were introduced into the various 25 × 25 × 25 cm breeding cages containing sexually mature females and males (15 days old). The scent of mango juice diffused throughout the cage, attracting female *B. dorsalis* to deposit their eggs through the egg-laying holes. After 24 hours' exposure to the females, the nesting boxes were removed from the various cages and the eggs were collected by rinsing the funnels in water using a soft brush. The collected eggs were placed on toilet paper and deposited in small bowls containing *B. dorsalis* larval nutrient medium.

The whole was placed in a large transparent dish (height 11 cm; diameter 15 cm) containing sterilized sand, from which the L3 stage larvae of *B. dorsalis* would jump and fall to become pupae. The contents were covered with 5 mm fine-mesh muslin to allow air to circulate and prevent the larvae from emerging. The nutrient medium for *B. dorsalis* larvae was watered every two days. After 12 to 15 days of incubation, the sand was sieved to recover the pupae, which were then placed in the cages for emergence. After emergence of *B. dorsalis*, they were kept in rearing cages and fed with a mixture of yeast hydrolysate enzymatic (3 measures of cane sugar and 1 measure of yeast hydrolysate enzymatic) and drinkers (water-filled bottles with a piece of water-soaked cotton in the lid).

Diachasmimorpha longicaudata was reared in the laboratory to test the effect of hydroalcoholic formulations on it. It was reared under the same conditions as *B. dorsalis*. The rearing was carried out using *B. dorsalis* L3 stage larvae (in their third developmental stage) previously obtained from the rearing of the aforementioned pest.

These larvae were placed in oviposition units containing a nutrient medium for future *D. longicaudata* larvae. The egg-laying units were then exposed in a cage to sexually mature females of the parasitoid. The egg-laying units were

probed by *D. longicaudata* females, who positioned their ovipositors in the *B. dorsalis* larvae to deposit their eggs. *D. longicaudata* eggs hatch inside *B. dorsalis* larvae and feed on the latter, which then become parasitoid larvae. After 24 h of exposure, the egg-laying units were removed from the *D. longicaudata* rearing cages. Parasitized *B. dorsalis* larvae were removed from the egg-laying units and transferred to tanks containing lightly-wetted sterilized sand, where pupation took place. After 7 days, the sand was sieved and the pupae collected were placed in cages in which water-soaked cotton was placed and honey droplets (on the upper wall of the cage) for the feeding of future parasitoids (they feed on the 2nd and 3rd larval development stages of *B. dorsalis*, then transform into parasitoid pupae and subsequently emerge as parasitoid adults).

Methods for obtaining crude extracts: Ripe *C. annuum* fruits and *S. hispidus* leaves were shade-dried in a greenhouse, and then finely ground using a micro-grinder. Extractions were carried out using the Nair method [15], based on the ability of bioactive substances of plant origin to be carried away by solvents of different polarities. The substances were extracted using solvents of increasing polarity (n-hexane, ethyl acetate, methanol). Phytochemical analysis of the active sub-fraction of *C. annuum* was carried out using the Romanian method of [14] [16]. These studies led to the development of the *C. annuum*-based hydroalcoholic formulation.

At the end of the processes, the total crude extract obtained was 200 mL for *C. annuum* from the starting volume. For *S. hispidus*, the volume of crude extract obtained was 720 mL. The extracts were then dried in a ventilated Cole Parmer oven, set at 45˚C. The masses of the dry extracts obtained were determined by weighing using a Radwag AS 110.R1 precision balance.

2.5. Method of Determining Test Concentrations

For success bait, the concentration considered in this study is that recommended by the manufacturer (0.24 g/L). For hydroalcoholic formulations, 3 different concentrations of total crude extracts were determined and considered for each plant substance. These were low, medium and high concentrations. The different concentrations of hydroalcoholic formulations were calculated based on the work of [14]. According to these authors, masses of dry extracts containing 1.5 mg of *C. annuum* and 1.5 mg of *S. hispidus*, *i.e.* concentrations of 1.5 mg/mL each, caused 18% and 15% mortality respectively in adult *C. cosyra* in the laboratory over 72 hrs. From these values it was possible to calculate the concentrations of these extracts that could cause 90% mortality in the pests (**Table 1**). We used the rule of 3 to calculate extracts capable of 90% pest mortality.

Method of preparing hydroalcoholic formulations: The various hydroalcoholic formulations were obtained from the crude total extracts of *C. annuum* and *S. hispidus* and the adjuvant (fruit fly attractant) (water, sugar, fresh bovine blood) previously developed. The adjuvant makes the hydroalcoholic extract suitable for practical and effective use [17]. According to these authors, it enhances the efficacy of chemical compounds. For example, the adjuvant ensures that the

product adheres to plant leaves, thus improving the effect of product duration and safety. The formulation thus improves the efficacy, shelf life and ease of use of the hydroalcoholic extract.

The formulations were prepared according to the 3 different concentrations of hydroalcoholic extracts of each plant species (*C. annuum* (3.75; 7.5; 12.5 g/L); *S. hispidus* (4.5; 9; 15 g/L)). The formulations were based on low, medium and high concentrations.

The different formulations were prepared in 1000 mL of solution according to the following formula:

$$CiVi = CfVf \text{ with } Vf = Vi + Vadj \quad [18]$$

where:

Vi = volume of formulation to be pipetted;

Cf = concentration of the stock solution;

Vf = volume of stock solution;

Ved = volume of adjuvant.

Ci = concentration of the formulation to be used to prepare the stock solution;

The formulations obtained are shown in Table 1 and Table 2 [19].

For packaging and storage of the formulations, empty cans of recovered water were used. As the maximum volume of these cans was 1.5 L, a 10-fold reduction factor was applied to the various hydroalcoholic formulations to obtain volumes of 100 mL. Experimental set-up and data analysis Bioassays were performed in a randomized Fisher block design with 8 treatments and 5 replicates. The treatments were:

Table 1. Concentrations of hydroalcoholic formulations and success bait tested.

Nom du produit	Concentrations		
	moderate	low	high
Success bait	0.24 g/L	-	-
C. annuum (Crude extract)	7.50 g/L	3.75 g/L	12.5 g/L
S. hispidus (Crude extract)	9.00 g/L	4.5 g/L	15 g/L

Table 2. Hydroalcoholic formulations based on crude total extracts and adjuvant.

Vegetale substance	concentration	Volume of extract to be taken (mL)	Volume of additive (mL)	Total volume (mL)	Content (g/L)
C. annum	low	7.97	992.03	1000	3.75
	moderate	15	985	1000	7.5
	high	26.5	973.5	1000	12.5
S. hispidus	low	14	986	1000	4.5
	moderate	28	972	1000	9
	high	47	953	1000	15

Capsicum annuum 3.75 (g/L) treatment.
Capsicum annuum treatment 7.5 (g/L).
Capsicum annuum treatment 12.5 (g/L).
Treatment *Strophantus hispidus* 4.5 (g/L).
Treatment *Strophantus hispidus* 9 (g/L).
Strophantus hispidus treatment 15 (g/L).
Success bait treatment 0.24 (g/L).
Untreated control treatment.

Testing the effects of different concentrations of hydroalcoholic formulations on the parasitoid *D. longicaudata* in the laboratory: The test consisted in syringing 1.5 mL of each concentration of hydroalcoholic formulation and pouring it into vials containing 0.25 g of cotton. Next, 20 parasitoid adults were sucked up with a mouth aspirator and placed in the vials, which were then covered with muslin and held in place with rubber bands. This operation was repeated 5 times for each concentration of the different hydroalcoholic formulations of *C. annuum* and *S. hispidus*, followed by Success bait and the control (water). All replicates were run simultaneously, and the vials containing the parasitoids were placed on shelves and kept in a room with the same facilities as the insect rearing room. Observations were made in 24 h and 72 h. considering that insects which did not respond to the touch of a fine brush were dead.

2.6. Data Processing and Statistical Analysis

Microsoft Office 2019 Excel spreadsheet software was used to enter and process the collected data and produce the various graphs. R software version 3.6.2 was used for statistical analysis. When the distribution of data did not follow the normal distribution, a non-parametric Kruskal-Wallis analysis was performed to detect differences between treatments. When there was a significant difference between treatments, pairwise comparison of means was performed using the pairwise t-test at the 5% threshold. The analyses concerned the following parameters:

Mortality rate was calculated using the following formula:

Mortality rate = (Number of dead individuals)/(total number of individuals) × 100

3. Results and Discussion

3.1. Results

Effects of different hydroalcoholic formulations on mortality of *Diachasmimorpha longicaudata*

After 24 h exposure of *D. longicaudata* adults to the different formulations, a significant difference was found between the 3 *C. annuum* treatments (Table 3). Parasitoid mortality rates obtained with the high (12.5 g/L) and low (3.75 g/L) doses of *C. annuum* were 13.80% and 12.88% respectively. With the medium dose (7.5 g/L) of hydroalcoholic concentration of *C. annuum*, the corresponding

mortality was 4.60%. We also detected a significant difference between doses of *S. hispidus* in terms of the efficacy of this hydroalcoholic formulation against the parasitoid. An average mortality rate of 22.05% of *D. longicaudata* was obtained with the 15 g/L and 4.5 g/L doses of *S. hispidus*. The average parasitoid mortality rate recorded with the medium dose of *S. hispidus* (9 g/L) was 13.8%. Analysis of variance revealed a highly significant difference between the 8 treatments. The highest average parasitoid mortality rate was obtained with the high (15 g/L) and low (4.5 g/L) concentrations of *S. hispidus*, while the lowest average mortality rate (4.60%) of *D. longicaudata* was observed with the medium concentration (7.5 g/L) of *C. annuum*

Mortality effects of different formulations on *Diachasmimorpha longicaudata* after 72 h

A significant difference was detected between doses of *C. annuum* after 72 h of exposure of *D. longicaudata* adults (Table 4). Parasitoid mortality of 50.20% was observed with the 12.5 g/L dose of *C. annuum* (Table 4). Average mortality rates of 36.70% and 35.81% were recorded with doses of 7.5 g/L and 3.75 g/L of this hydroalcoholic concentration respectively. It was also possible to demonstrate a significant difference between the 3 *S. hispidus* treatments. Thus, a high average mortality rate for *D. longicaudata* (64.40%) was obtained with the 9 g/L dose, whereas the 4.5 g/L and 15 g/L doses of this hydroalcoholic concentration produced average mortality rates of 59.95% and 57.15% respectively. Analysis of variance showed a highly significant difference between the 8 hydroalcoholic concentration treatments. The highest mean mortality rate (64.40%) was obtained with the 9 g/L dose of *S. hispidus*, while the lowest mean mortality rate (35.81%) of *D. longicaudata* was observed with the 3.75 g/L concentration of *C. annuum*.

Table 3. Average mortality rate of *D. longicaudata* to formulations of *C. annuum* and *S. hispidus* 24 hours after exposure, Farakô-Ba, Burkina Faso.

Periods of exposure	
	24 hours
Treated	Average mortality rate
Untreated	0.00 ± 0.00[a]
Success bait (GF120)	20.45 ± 0.90[e]
C. annuum (3.75 g/L)	12.88 ± 0.13[c]
C. annuum (7.5 g/L)	4.60 ± 0.09[b]
C. annuum (12.5 g/L)	13.80 ± 0.14[c]
S. hispidus (4.5 g/L)	22.50 ± 1.02[f]
S. hispidus (9 g/L)	13.80 ± 0.14[d]
S. hispidus (15 g/L)	22.50 ± 1.02[f]
Probability	<0.001

Numbers followed by the same letters (a, b, c etc.) are not significantly different at 5% treshhold.

Table 4. Average mortality rate of *D. longicaudata* to formulations of *C. annuum* and *S. hispidus* 72 hours after exposure, Farakô-Ba, Burkina Faso.

Periods of exposure	
	72 hours
Treated	Average mortality rate
Untreated	0.00 ± 0.00[a]
Success bait (GF120)	54.80 ± 1.96[e]
C. annuum (3.75 g/L)	35.81 ± 1.65[b]
C. annuum (7.5 g/L)	36.70 ± 1.83[c]
C. annuum (12.5 g/L)	50.20 ± 1.95[d]
S. hispidus (4.5 g/L)	59.95 ± 2.01[g]
S. hispidus (9 g/L)	64.20 ± 2.45[h]
S. hispidus (15 g/L)	57.15 ± 1.98[f]
Probability	<0.001

Numbers followed by the same letters (a, b, c etc.) are not significantly different at 5% treshhold.

3.2. Discussion

The various mortality tests of *D. longicaudata* to different formulations of *C. annuum* and *S. hispidus* enabled us to identify which formulations were toxic to the parasitoids.

The *S. hispidus* formulations (15 g/L and 4.5 g/L) and the success bait were toxic to *D. longicaudata* adults after 24 h of exposure. This result could be explained by the fact that the *S. hispidus* formulations (15 g/L and 4.5 g/L) act through active ingredients such as flavonoids, cardenolides, sterols/triterpenes, alkaloids and saponosides, which are responsible for the observed toxicity towards parasitoids. These results are in agreement with [20] who showed that flavonoids were found in the leaves and nowhere else in the other plant organs. [14] showed the presence of sterols/triterpenes, flavonoids, anthraquinones, cardenolides, alkaloids and saponosides in *S. hispidus* extracts. This presence also depends on exogenous factors such as temperature, insect anatomy and morphology. The success bait was toxic to *D. longicaudata* adults. This result could be associated with the insecticidal effect of spinosad on parasitoid adults. These results are in agreement with those reported by [21] [22] who showed that spinosad, a bacterial insecticide derived from the actinomycete *Saccharopolyspora spinosa* contained in the bait was slightly harmful to *D. longicaudata* (IOBC class 2). However, the half-dose formulation of *C. annuum* (3.75 g/L) was less toxic to *D. longicaudata* adults. This concentration of *C. annuum* can be recommended for fruit fly control, while preserving the *D. longicaudata* parasitoid in the wild after its release in orchards.

After 72 h, the *S. hispidus* formulations (9 g/L; 15 g/L) and the success bait were highly toxic to *D. longicaudata* adults. These results could be explained by

the presence of phytochemical compounds in *S. hispidus* that could have toxic effects on parasitoid adults through contact and/or ingestion. In contrast, *C. annuum* formulations proved less toxic to *D. longicaudata*. The phytochemicals in *C. annuum* may have had less toxic effects on adults of the parasitoid by contact and/or ingestion. This formulation of *C. annuum* can be recommended for fruit fly control, while preserving the *D. longicaudata* parasitoid in the wild after its release in orchards.

Successive baits were also found to be toxic to parasitoid adults. These results are in line with those of [23], who carried out a spinosad contact test with *Fopius arisanus* (Sonan) and *Pysttalia* (Silvestri). In this test, spinosad was toxic to parasitoids at concentrations above 500 mg/liter after application of aerosol baits. In addition, spinosad (not in bait-sprays) has been shown to be toxic to some hymenopterans in previous studies [24] [25] [26]. [27] found that malathion in GF-120 aerosol bait formulations, which contain spinosad, was toxic to the aphid parasitoids Aphytis melinus DeBach and Lysiphlebus testaceipes Cresson. [28] showed the toxicity of three treatments of spheres with imidacloprid (2 and 4% active ingredient [MA] Provado 1.6 F) to *D. longicaudata* and an untreated (non-toxic) control sphere over a 24 h - 72 h exposure period. There was no significant difference in mortality of *D. longicaudata* exposed to spheres treated with imidacloprid at 2% and 4% (AI) for 2 or 4 weeks. Although higher mortality of *D. longicaudata* was recorded with 4% compared with 2% (AI) after 24 h, it is possible that the seasonal population dynamics of fruit fly parasitoids inhabiting the different niches of Anastrepha suspensa (for feeding) is so limited that populations would not be significantly reduced [29]. [30] [31] also reported that sprays of spinosad or phloxine B baits had little or no effect on the *A. suspensa* parasitoid, *Fopius arisanus* (Sonan), the main parasitoid of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Hawaii. Nevertheless, further research in real-life conditions would be needed to determine the toxicity of hydroalcoholic formulations of *C. annuum* and *S. hispidus* to *D. longicaudata* under natural conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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