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Evaluation in the Laboratory of the Effects of Natural Substance Fractions, *Cleome viscosa*, *Capsicum annuum* and *Strophantus hispidus* on *Diachasmimorpha longicaudata* Parasitoid of the Fruit Flies, *Bactrocera dorsalis* and *Ceratitis* spp.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diachasmimorpha longicaudata is successfully used to control *Bactrocera dorsalis* and *Ceratitis* species. Despite its widespread use as a biological agent, a limited number of studies reflect its pesticide-induced mortality. This scarcity highlights the need for studies on pesticide toxicity for the successful implementation of biological programs. Hence, the study of mortality tests on *D. longicaudata* through different fractions of *C. viscosa*, *C. annuum* and *S. hispidus* was carried out. The aim of the study was to determine the mortality of *Diachasmimorpha longicaudata* in relation to each fraction. 1.5ml of each fraction was poured into a vial containing 0.25g of cotton. Then, 20 parasitoids were sucked in and placed in the flasks, which were then covered with canvas and held in place with rubber bands. This operation was repeated 5 times for each fraction. The insects were observed after 24h and 72h, considering that insects which did not respond to the touch of a fine brush were dead. *Cleome viscosa* fractions were not toxic to *D. longicaudata* after 24h. The insects died because of the chloroform and methanol fraction of *Cleome viscosa* (2.15%) after 72h. The fraction of *C. annuum* in acetone (13.8%) and *S. hispidus* in methanol (9.2%) caused parasitoid mortality. After 72 h, high mortality of *D. longicaudata* was observed with *S. hispidus* in ethyl acetate with a sensitivity rate of 33.35%, *C. annuum* in chloroform (24.5%) and *S. hispidus* in methanol (20.45%). These tests revealed that the ethyl acetate fraction of *S. hispidus* was highly toxic to *D. longicaudata*.

Keywords: Fractions; Capsicum annuum; Cleome viscosa; Strophantus hispidus; Diachasmimorpha longicaudata.

1. INTRODUCTION

Fruit flies are invasive pests that damage the quality of fruits in horticultural crops and cause considerable loss of value. Managing fruit flies is difficult because of their biology, their adaptation to different regions and the diversity of their hosts. The oriental fruit fly *Bactrocera dorsalis* (Hendel), is a notorious global pest infesting fruits and vegetables. It has developed a high level of resistance to many commonly used insecticides [1]. The later author showed that tyrosine hydroxylase (TH) is required for cuticle tanning (sclerotization and pigmentation) in many insects and could be a potential target in the control of *B. dorsalis*. In addition to that, the use of deep-acting insecticides, mainly organophosphates, is not permitted in many crops, because of the risk of toxic residues in fruits [2]. Thus, the integration of management practices, such as the use of toxic baits and the action of parasitoids, has become an interesting alternative [3]. Spinosyne (spinosad), neurotoxic acetylcholine agonists, have attracted attention because they are more selective to beneficial insects than organophosphates [4,5,6,7], and have therefore become alternatives for the management of the Mediterranean fruit fly.

Diachasmimorpha longicaudata (Ashmed) (Hymenoptera: Braconidae) is an important parasitoid of fruit flies worldwide, mainly because of its ease of rearing and intensive feeding of host-seeking females [8,9]. This parasitoid species has been successfully used in many countries to control *Anastrepha*, *Bactrocera* and *Ceratitis* species [10,11,12,13].

This parasitoid is native to the Indo-Australian region and is a koinobiont endoparasitoid of various Tephritidae species. It is considered extremely effective in biological control programs against *Anastrepha* spp. and *C. capitata* [8,9].

Indeed, it has been introduced in Latin American countries [14], in various tropical and subtropical regions to control *Anastrepha* (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) species [15]. The success of augmentative biological control depends on the ability of released parasitoids to disperse and locate adult food, shelter and hosts [16].

Because of the potential use of *D. longicaudata* in biological control of *C. capitata* and spinosynes as lethal agents in toxic bait formulations, the toxicity of spinosad, associated with food lures, was evaluated in the laboratory, as well as the effects of spinosynes on the parasitoid [17]. Authors [17] worked on the concentration and lethal duration of spinosyn-based toxic baits on *Ceratitis capitata* and *Diachasmimorpha longicaudata.* The aim of this study was to assess the lethal concentration (LC) and lethal time (LT) of spinosad and spinetoram,

combined with the feed lures 7% sugarcane molasses, 3% Biofruit, 1.5% Ceratrap®, 1.25% Flyral®, 3% Isca Samaritá® and Samaritá Tradicional® on *C. capitata*, in the laboratory, as well as their effects, at a concentration of 96 mg L-1, on *D. longicaudata* [18,19].

Author [20] has shown that organic extracts of three plant species, *Cleome viscosa*, *Capsicum annum* and *Strophantus hispidus* have interesting biological activity on both species of fruit fly. Studies are needed to evaluate the biological activity of various fractions of these plant extracts on natural enemies. Studies are therefore needed to determine their possible sublethal effects on *D. longicaudata.* In Burkina Faso, little information is available on the toxicity of plant extract-based products on *D. longicaudata* parasitoids of fruit flies. Hence, the need for studies on the toxicity of plant extract-based products on *D. longicaudata* parasitoids of fruit flies for the successful implementation of biological programs. The aim of this study was to determine the mortality of *Diachasmimorpha longicaudata* associated with each fraction (*Capsicum annuum*, *Cleome viscosa*, *Strophantus hispidus*).

2. MATERIALS AND METHODS

2.1 Location of the Tests

Our work was carried out at the insectarium of the Institut de l'Environnement et de Recherches Agricoles (INERA) in Farako-Bâ (Burkina Faso). Rearing of *B. dorsalis*, *D. longicaudata* and laboratory tests were carried out at the biological control laboratory of the Centre National de Spécialisation en Fruits et Légumes (CNS-FL). Aqueous extracts were extracted in the ecotoxicology laboratory of INERA in Bobo-Dioulasso.

2.2 Equipment

The laboratory equipment included:

- ✓ Hemolysis tubes for extract preparation ;
- ✓ A vortex to homogenize solutions ;
- ✓ Vials for biological tests ;
- ✓ Muslin cloth to cover vials ;
- ✓ Elastics to hold flasks ;
- ✓ Thin-layer chromatography bowls ;
- ✓ Silica gel sheets for thin-layer chromatography ;
- ✓ Binoculars for insect observation ;
- ✓ UV chamber for reading chromatographic plates ;
- ✓ A camera for pictures.

2.3 Methods

2.3.1 Rearing *B. dorsalis* in the laboratory

Rearing room conditions were 12 h/12 h photoperiod (dark/light), temperature 25-28°C, relative humidity 60-70%.

Rearing consisted in preparing egg-laying trays (height 11cm; 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 then introduced into 25x25x25 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 holes in the nesting boxes.

After 24 hours' exposure to the females, the egg-laying devices were removed from the various rearing 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 the nutrient medium for *B. dorsalis* larvae. The whole was placed in a large transparent basin (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 a fine-mesh muslin cloth 5mm in diameter 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).

2.3.2 Method of rearing *D. longicaudata* in the laboratory

Diachasmimorpha longicaudata was reared under the same conditions as *B. dorsalis.* The rearing was carried out using *B. dorsalis* L3 stage larvae, 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 oviposition units were then exposed in a cage to sexually mature females of the parasitoid. The egg-laying units were felt by *D. longicaudata* females, who positioned their ovipositors in the *B. dorsalis* larvae to deposit their eggs.

Diachasmimorpha longicaudata eggs hatch inside *B. dorsalis* larvae and feed on *B. dorsalis* larvae, which then become parasitized larvae. After 24 h exposure, the egg-laying units were removed from the *D. longicaudata* rearing cages. *Bactrocera dorsalis* larvae parasitized by the presence of *D. longicauadata* eggs were removed from the egg-laying units and transferred to tanks containing sterilized, slightly moistened 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 second and third larval development stages of *B. dorsalis*, then transform into parasitoid pupae and subsequently emerge as parasitoid adults).

2.3.3 Effects of *C. viscosa*, *C. annuum* and *S. hispidus* fractions on *Diachasmimorpha longicaudata* mortality

2.3.3.1 Extraction method for active fractions

Preparation of *Cleome viscosa* and *Capsicum annuum* n-Hexane extract fractions : A test portion of 6.28 g of the hexane extract fraction of *C. viscosa* and 15.81 g of *C. annuum* were each dissolved in a minimal volume of extraction solvent. The resulting extract solutions were each mixed with silica gel for columns in ratios of 1 : 5 w/w (75 g for *C. viscosa* and 100 g for *C. annuum*). The mixture of silica and extract of each plant drug was homogenized with a spatula, then dried at laboratory room temperature (30°C). After evaporation of the extracting solvent, a series of solvents of increasing polarity was successively added to the silica and dry extract mixture and transferred to a one-liter Erlenmeyer flask, where 750 mL of the first solvent (toluene) in the series was added. Thus, the hexane extract fraction of *C. viscosa* and C. *annuum* was successively sub-fractionated by percolation with toluene; chloroform; n-hexane and methanol.

The fractions collected from each extract sample were concentrated under reduced pressure in the rotary evaporator, then dried and weighed. The yield of each extract fraction was determined as a percentage of the initial extract fraction test sample.

2.3.4 Preparation of *Capsicum annuum* ethyl acetate extract and *Strophantus hispidus* methanolic extract fractions

- ***Capsicum annuum* ethyl acetate extract :**

A 14.72 g test portion of the most active *C. annuum* ethyl acetate extract was dissolved in 150 mL of extractor solvent (analytical ethyl acetate). The extract solution was mixed with 140 g of silica gel for column chromatography (Silica gel 60 ; 0.063-0.20 mm ; Merck) in 1 :10 m/m ratios. The silica gel/extract mixture was homogenized using a spatula, then placed in a ventilated oven at a temperature of 45°C to remove the extracting solvent. The dried silica-extract mixture was transferred to an Erlenmeyer flask and 250 mL of analytical acetone was added.

The solvent-extract mixture was macerated for 1 h, then transferred to a glass column percolator. After percolation by successive leaching to exhaustion with a total volume of 750 mL of acetone, the silica gel and residual extract mixture was macerated and percolated successively to exhaustion with 750 mL of chloroform, acetone, methanol and ethyl acetate. Fractionation of the ethyl acetate extract of *C. annuum* yielded four extract fractions (chloroform: 8.75g; ethyl acetate: 0.02g; acetone: 2.88g; methanol: 0.23g).

- **Methanol extract of *Strophantus hispidus:***

A mass of 8.59 g of dry methanol extract of *S. hispidus* was dissolved in 100 mL of analytical methanol. The extract solution was mixed with 90 g of silica gel column material.

The mixture was homogenized and placed in an oven at 45°C to remove the solvent. After evaporation of the solvent, the silica-extract mixture was placed in a cylindrical glass percolator and successively leached to exhaustion with chloroform, ethyl acetate, acetone and methanol. Fractionation of the *S. hispidus* methanol extract yielded four extract fractions (chloroform : 6g ; ethyl acetate : 1.78g; acetone: 1.71g; methanol : 7.13g).

The extraction method for the active fractions was performed according to the following diagram:

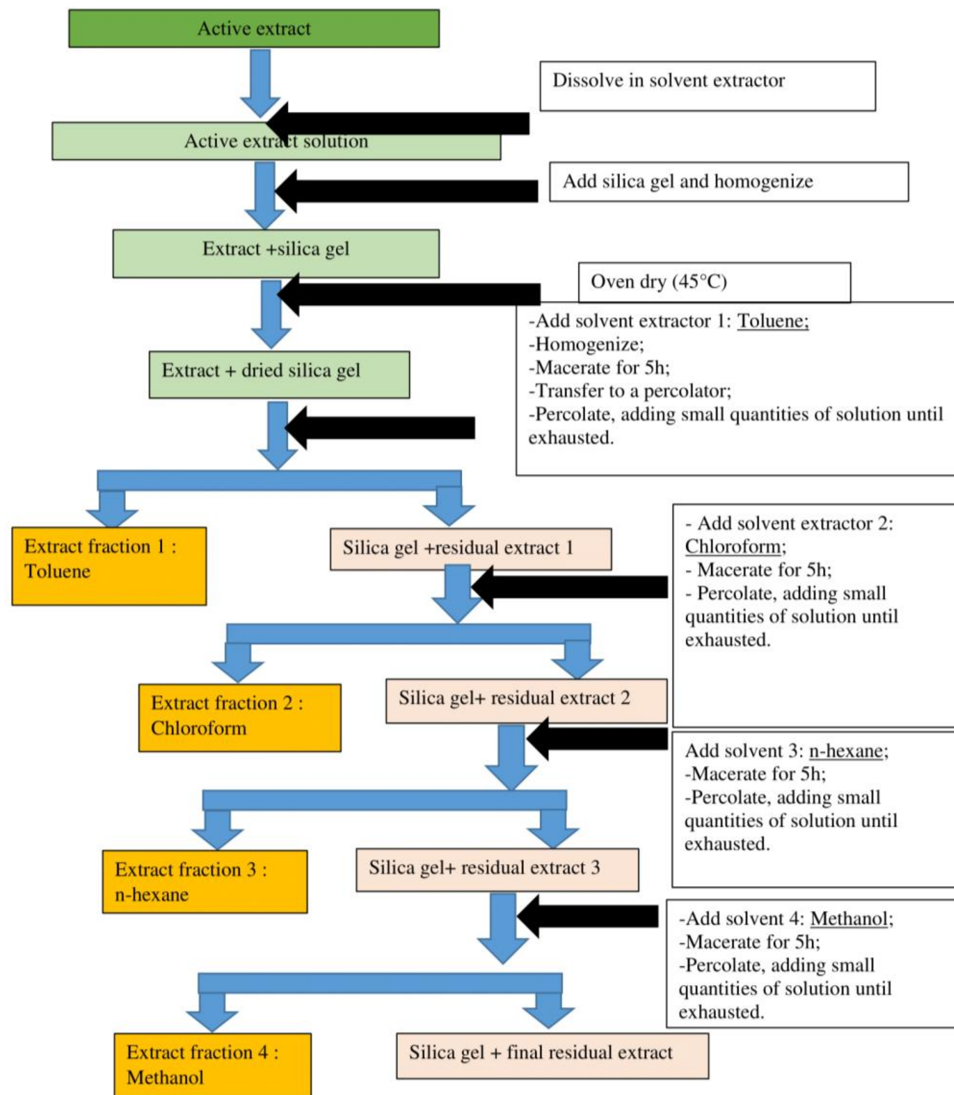

Fig. 1. Diagram of the preparation of the study extract fractions

2.3.4.1 Testing active fractions on Diachasmimorpha longicaudata adult mortality

The *C. annuum* fractions were obtained by isolation and purification with chloroform of the ethyl acetate extract of *C. annuum* by preparative chromatography, sufficiently isolated and purified.

The same applies to the *C. viscosa* fraction and the chloroform fraction of the *S. hispidus* methanol extract. The various fractions we used were previously obtained and stored in the refrigerator.

The evaluation of the active fractions of organic plant extracts (*C. annum* L, *C. viscosa* L, *S. hispidus* A. DC) on *D. longicaudata* adults was done as follow : 100 mg of extracts from each sample were weighed and placed in a microtube to which 1mL of acetone was added. The mixture was dispersed using a vortex. The mixture of organic extract and acetone from each microtube was then poured into 99 mL of water (1mg/mL concentration). 20 5-day-old *D. longicaudata* adults were sucked into cages deprived of food for 12 h. These 20 adults were placed in vials which received a drop of 1.5 mL of each fraction on 0.25 g of cotton containing 1 g of sugar.

Mortality of *D. longicaudata* adults caused by the extract fractions was assessed by the mortality rate of parasitoids at 24h and 72h after exposure, considering that insects which did not respond to the touch of a fine brush were dead. The

experiment was repeated 5 times for each organic extract fraction. In this case, we carried out a technical repetition, which consisted in running the different tests at the same time.

2.3.5 Data processing and analysis

The Microsoft Office 2019 Excel spreadsheet 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. Analyses were performed for the following parameters :

Mortality rate :

- The percentage of observed mortality in control and treated adults is estimated by applying the following formula :

Mortalities in treated boxes (Mo) were expressed as corrected mortalities (Mc) according to Abbott's formula [21], taking into account natural mortalities observed in control boxes (Mt)

MC%= (Mo-Mt*100)/ (100-Mt)

Observed mortality= [Number of dead individuals/Total number of individuals] × 100.

The formulas below were used for the various calculations:

3. RESULTS AND DISCUSSION

3.1 Extraction of Plant Substances

The most active extracts identified, i.e. the n-Hexane fraction of *C. viscosa* and *C. annuum*, were fractionated to give sub-fractions ranging from:

- The toluene fraction, from 77.42% to 69.90%. The highest yield was observed with *C. annuum* and the lowest with *C. viscosa* ;
- The chloroform fraction, from 46.34% to 17.90%. *Capsicum annuum* gave the highest extraction yield ;
- The n-Hexane fraction of *Cleome viscosa* and *C. annuum* yielded insignificant extract masses that could not be quantified by weighing;
- The methanol fraction varied from 19.75% to 11.07%. *C. viscosa* gave the highest extraction yield.

Extraction yields for the various sub-fractions are given in Table 1.

3.2 Yields from fractionation of the Ethyl Acetate Extract of *C. annum*

Fractionation of the most active extract (ethyl acetate) of *C. annum* yielded extract fractions ranging from 0.57% to 66.43%.

The highest extract fraction yield was recorded with acetone, and the lowest with ethyl acetate and 1-butanol (Table 2).

Table 1. Extraction yields of the n-Hexane fractionations of *C. viscosa* and *C. annuum*

Fractions	Extract masses (g)	Yield (%)
	Fractions n-Hexane of *C. viscosa*	
SF1.1 (toluene)	4.39	69.90
SF2.1 (Chloroforme)	2.91	46.34
SF3.1 (n-Hexan)	Traces	ND
SF4.1 (methanol)	1.24	19.75
	Fractions n-Hexan of *C. annum*	
SF1.2 (toluene)	12.24	77.42
SF2.2 (Chloroform)	2.83	17.90
SF3.2 (n-Hexan)	Traces	Traces
SF4.2 (methanol)	1.75	11.07

***SF1**= hexanic fraction of C. visosa; **SF2** = hexanic fraction of C. annum*
1 = toluene ; 2= chloroform ; 3= hexan ; 4= methanol

Table 2. Yields of *C. annuum* ethyl acetate extract fractionation

Fractions	Extract masses (g)	Yield (%)
Acetone (F1)	3.74	66.43
Methanol (F2)	0.36	6.38
Ethyl acetate (F3)	0.03	0.58
1-butanol (F4)	0.03	0.57

3.3 Yields and Chemical Composition of Active Extract Fractions

- **Fractions recorded from hexanolic extracts of *Capsicum annuum:***

The yield of recorded fractions ranged from 77.42% to 11.07%. The highest yield was observed with toluene (77.42%) and the lowest with methanol (11.07%). The n-Hexane fraction of *C. annuum* yielded insignificant extract masses that could not be quantified by weighing. Extraction yields for the various sub-fractions are shown in Table 3.

Table 3. Fractionation yields for the *C. annuum* fraction

Fractions	Extract masses (g)	Yield (%)
n-Hexane fractions of *C. annuum*		
Toluene	12.24	77.42
Chloroform	2.83	17.90
n-Hexane	Traces	Traces
Methanol	1.75	11.07

- **Fractions recorded from ethyl acetate extracts of *Capsicum annuum***

Fractionation yields ranged from 0.13% to 59.44%. The highest yield was observed with *C. annuum* in chloroform (59.44%) and the lowest with *C. annuum* in ethyl acetate (0.13%). Extraction yields for the various sub-fractions are given in Table 4.

Table 4. Fractionation yields for the *C. annuum* fraction

Fractions	Extract masses (g)	Yield (%)
Fractions of *C. annuum* in ethyl acetate		
Chloroform	8.75	59.44
Ethyl acetate	0.02	0.13
Acetone	2.88	19.57
Methanol	0.23	1.56

- **Fractions recorded from methanol extracts of *Strophantus hispidus:***

Fractionation yields ranged from 19.90% to 69.85%. The highest yield (83%) was observed with *C. annuum* in methanol and the lowest with *C. annuum* in acetone (19.90%). Extraction yields for the various sub-fractions are given in Table 5.

Table 5. Fractionation yields for the *S. hispidus* fraction

Fractions	Extract masses (g)	Yield (%)
Fractions of *S. hispidus* in methanol		
Chloroform	6.00	69.85
Ethyl acetate	1.78	20.72
Acetone	1.71	19.90
Methanol	7.13	83.00

The fractionation of the most active extracts (hexanic and ethyl acetate) of Capsicum annum and S. hispidus methanol made it possible to obtain fractions which were the subject of insecticidal tests

3.4 Mortality of *Diachasmimorpha longicaudata* due to *C. viscosa* Fractions after 24 hours of Observation

The statistical analysis showed that there was a very high significant difference between the different fractions of *C. viscosa* ($p<0.001$). After 24 hours of exposure, the control showed no mortality of *D. longicaudata* adults (Table 6). The different fractions of *C. viscosa* showed each, mortality rates of 1.07% compared to the untreated control. The statistical analysis did not show any significant difference between them at the 5% threshold.

Table 6. Mortality of *D. longicaudata* due to *Cleome viscosa* fractions after 24 hours of exposure

Exposure periods	
24h	
Treatments	**Average mortality rate (%)**
Control	0.00 ±0.00a
C. viscosa chloroforme	1.07 ±0.40b
C. viscosa methanol	1.07 ±0.40b
C. viscosa toluene	1.07 ±0.40b
Probability	< 0.001

In each column, the values followed by the same letter are not statistically different at the 5% threshold according to the pairwise-t-test

3.5 Mortality of *Diachasmimorpha longicaudata* due to *C. viscosa* fractions after 72 Hours of exposure

The statistical analysis showed that there was a very high significant difference between the different fractions of *C. viscosa* ($p<0.001$) (Table 7). The different fractions of *C. viscosa*, showed mortality rates of 5% for *C. viscosa* chloroform and *C. viscosa* methanol compared to the untreated control. However, no mortality was observed with *C. viscosa* toluene as well as the control. Statistical analysis showed a significant difference between them at the 5% threshold.

Table 7. Mortality of *D. longicaudata* due to *Cleome viscosa* fractions after 72 hours of exposure

Exposure periods	
72h	
Treatments	**Average mortality rate (%)**
Control	0.00 ±0.00a
C. viscosa chloroforme	5.00±0.90b
C. viscosa methanol	5.00±0.90b
C. viscosa toluene	0.00±0.00a
Probability	< 0.001

In each column, the values followed by the same letter are not statistically different at the 5% threshold according to the pairwise-t-test

Table 8. Mortality of *D. longicaudata* due to fractions of *C. annuum* and *S. hispidus* after 24 hours of exposure

Exposure periods	
24h	
Treatments	**Average mortaity rate (%)**
Control	0.00±0.00 a
C. annuum chloroforme	4.60±0.14b
C. annuum acétone	13.80±2.01d
C. annuum méthanol	0.00±0.00a
C. annuum ethyl acetate	0.00±0.00a
S. hispidus chloroforme	4.60±0.14b
S. hispidus acétone	0.50±0.01a
S. hispidus méthanol	9.20±1.24c
S. hispidus ethyl acetate	4.60±0.14b
Probability	< 0.001

In each column, the values followed by the same letter are not statistically different at the 5% threshold according to the pairwise-t-test

3.5.1 Mortality of *Diachasmimorpha longicaudata* due to fractions of *C. annuum* and *S. hispidus* after 24 hours of exposure

The statistical analysis showed that there was a very significant difference between the different fractions of *C. annuum* and *S. hispidus* ($p<0.001$) (Table 8). The different fractions of *C. annuum* and *S. hispidus*, showed mortality rates of 9.83% compared to the untreated control. Statistical analysis revealed a significant difference between them at the 5% threshold. The *C. annuum* acetone fraction yielded the highest mortality rate of 13.8%, followed by the *S. hispidus* methanol fraction (9.2%) and no mortality (0%) was recorded with the *C. annuum* fractions with ethyl acetate and methanol as well as the untreated control.

Table 9. Mortality of *D. longicaudata* due to fractions of *C. annuum* and *S. hispidus* after 72 hours of exposure

Exposure periods	
72h	
Treatments	**Average mortality rate (%)**
Control	0.00±0.00 a
C. annuum chloroforme	24.50±3.04 e
C. annuum acetone	18.40±2.31 d
C. annuum methanol	9.20±1.25 b
C. annuum ethyl acetate	0.00±0.00 a
S. hispidus chloroforme	13.80±2.01 c
S. hispidus acetone	13.80±2.01 c
S. hispidus methanol	20.45±2.97 e
S. hispidus ethyl acetate	33.35±3.89 f
Probability	< 0.001

In each column, the values followed by the same letter are not statistically different at the 5% threshold according to the pairwise-t-test

3.5.2 Mortality of *Diachasmimorpha longicaudata* due to fractions of *C. annuum* and *S. hispidus* after 72 hours of exposure

Statistical analysis showed that there was a very high significant difference between the different fractions of *C. annuum* and *S. hispidus* ($p<0.001$) (Table 9). The different fractions of *C. annuum* and *S. hispidus*, showed different mortality rates of which the highest one (33.35%) was observed with the fraction of *S. hispidus* ethyl acetate followed by *C. annuum* with chloroform (24.5%) and *S. hispidus* with methanol (20.45%). The lowest rate (0%) was observed with *C. annuum*

ethyl acetate as well as the untreated control. Statistical analysis showed a significant difference between the different fractions at the 5% threshold.

3.6 Discussion

The *C. viscosa* fractions caused less mortality in adults of *D. longicaudata* after 24 hours of exposure. This result could be explained by the fact that *C. viscosa* fractions are less toxic to *D. longicaudata* adults. Our findings are similar to that of [22] who showed that 3% of Isca Samaritá Tradicional and 7% of sugar cane molasses in formulations with the insecticides spinosad and spinetoram (0.096 g a.i. L- 1 or kg) were moderately harmful (class 3) to *D. longicaudata*. Our results are also similar to the findings reported by [23] who showed that after 72 h, relatively low mortality (35.81%) of parasitoids was recorded with 3.75 g/L of *C. annuum*. But after 72 hours of exposure, the chloroform and methanol fraction of *C. viscosa* caused mortality (2.15%) in adults of *D. longicaudata*. The chloroform and methanol fraction of *C. viscosa* had a small toxicity effect on *D. longicaudata* adults. But no mortality was observed with the toluene fraction of *C. viscosa*. *Cleome viscosa* fractions are less toxic towards *D. longicaudata* adults. The form and dose of application could be the cause of the difference in the recorded results.

The mortality of the adults of *D. longicaudata* due to the *C. annuum* acetone fraction was 13.8%, followed by the *S. hispidus* methanol fraction (9.2%). This result could be associated with the presence of triterpenes in these fractions. The fractions of *C. annuum* with ethyl acetate and methanol did not induce any mortality among adult parasitoids. These fractions were less toxic towards the parasitoids after 24 hours of exposure of the insects. These results are similar to those of [22] who showed that the food attractants Anamed, 3% Biofruit, 1.5% CeraTrap, 1.25% Flyral and 3% Isca Samaritá Tradicional in combination with spinosad and spinetoram and the Success 0.02CB formulation (0.096 g a.i. L-1 spinosad) were classified as harmless (<10% mortality up to 96%) to *D. longicaudata*. But after 72 hours, the fraction of *S. hispidus* with ethyl acetate caused high mortality (33.35%) to adults of *D. longicaudata* followed by *C. annuum* with chloroform (24.5%) and *S. hispidus* with methanol (20.45%). In fact, the ethyl acetate fraction of *S. hispidus* had a toxic effect on *D. longicaudata* adults. These results are close to those reported by [23] who showed that after 72 h, high mortality (59.95%; 64.20%; 57.15%) was observed with *S. hispidus* (4.5; 9; 15 g/L) and with the Success bait (54.80%). *Capsicum annuum* at 3.75 g/L could be recommended for the conservation of *D. longicaudata* in nature.

The ethyl acetate fraction of *C. annuum* did not cause any mortality in adults of the parasitoid because it is less toxic to *D. longicaudata* adults. These findings are similar to those of [24] who showed that azadirachtin, a limonoid tetranor-triterpenoid chemical used on *D. longicaudata* was less harmful to it. These results are also in agreement with those reported by [25] and [26] 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). Authors [22] reported that 4.0 g a.i. L-1 alpha-cypermethrin) showed high toxicity to adults of *D. longicaudata* (>90% mortality) after 96 h and were therefore classified as harmful (class 4).

4. CONCLUSION

These tests revealed that the fraction of *S. hispidus* ethyl acetate was very toxic to adults of *D. longicaudata*. The ethyl acetate fraction of *C. annuum* did not cause any mortality in adults of the parasitoid. This fraction could be used for further work.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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