Biological activity of the extracts of *Capsicum annuum* and *Strophantus hispidus* on the development of fruit flies (*Bactrocera dorsalis*) from egg to emergence

Abstract

Bactroceradorsalis and *Ceratitiscosyra* are the major constraintsof the mangosector in Burkina Faso. Variousmethods are used to control theseinsectpests, including the use of chemicalpesticides, which are expensive for farmers. The overuse of thesechemicals leads to environmental pollution, foodpoisoning and resistanceamonginsects. For thisreason, researchisincreasinglyfocusing on plant derivatives as an alternative. A study of the biologicalactivity of fractions of organicextracts of two plants, *Capsicum annum*and*Strophantus hispidus* on the development of *B. dorsalis*, wascarried out underlaboratory conditions in Burkina Faso. The plant fractions wereextractedusingsolvents of increasingpolarity: n-hexane, ethylacetate and methanol. The biologicalactivity test on the development of *B. dorsalis*wascarried out in a randomised Fisher block design with 25 treatments in 10 replicates. The active extractswerefractionatedusing the Nair method and the fractions obtainedweretested on the development of *B. dorsalis*. In the laboratory, hexane extracts of *C. annuum* and *S. hispidus*resulted in 100% inhibition of *B. dorsalis*development.

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Key words:*Capsicum annuum*, *Strophantus hispidus*, *Bactroceradorsalis*, plant extracts

Introduction

In recent decades, several species of the genus *Bactrocera*were accidentally introduced into different regions of the world where fruit industries are established, often with major economic consequences [1]. Fruit flies are known to spread through the movement of infested produce linked to weak phytosanitary control and surveillance systems for fruit and vegetable

exports between countries [2]. The impact caused by the accidental introduction and dispersal of *B. dorsalis* on fruit production in tropical Africa is huge[3]. *Bactrocera dorsalis* is a highly polyphagous species accidentally introduced into Burkina Faso. The sustainable management of these insect pests is very important because they are classified as quarantine pests in international trade. Fruit flies also constitute a limiting factor for exports of fruits and vegetables for certain countries and increase their costs for exportation because of the disinfection treatments applied [4]. Faced with the threat posed by this fly, several control methods were put in place, including: field hygiene, cultural control, mass trapping, the use of protein baits [5]which is very expensive and tedious for the farmer. It is in this contest that this study focused on the search for control alternatives through the use of pesticides based on less expensive plant fractions while preserving the health of the consumer and his environment.

The organic extracts of the two plant species, *Capsicum annum* and *Strophantus hispidus*, have an interesting biological activity on the two species of fruit flies [6]. Thus, it appears necessary to carry out more in-depth studies by evaluating the biological activity of various fractions of the extracts of these plants. The insecticidal, larvicidal, ovicidal, sterilizing, anti-palatable and repellent effects of *C. annuum*were extensively investigated. Indeed, *C. annuum* fruit powder alone or mixed with *Allium sativum* oil or powder is effective in protecting cowpea grains in storage against *Callosobruchus maculatus*[7].The insecticidal, larvicidal, ovicidal, sterilizing, anti-palatable and repellent properties of *S. hispidus*were the subject of numerous studies. Indeed, according to [8], the powders and extracts of *S. hispidus* tested against *Sitotrogacerealella* caused 100% mortality of adult butterflies after 24 hours of application. Extracts of *S. hispidus* with n-hexane and ethyl acetate showed 100% mortality on four larval stages of *Ochlerotatustriseriatus* at a concentration of 250 «microgram/ml». In addition, the methanol extract of *S. hispidus* showed anti-palatable activities of *Helicoverpazea* by reducing the larval mass by 74% [9]. Furthermore, the effects of *S. hispidus* extracts have potential to control lepidopteran pests and whiteflies [10].

The objective of this study is to determine the active ingredients of the main chemical groups contained in the different fractions of *C. annuum*, *S. hispidus* and to specify the insecticidal effects of these compounds on the different developmental stages of *B. dorsalis*.

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1. Material and methods

1.1. Material

The laboratory equipment used included:

- Hemolysis tubes for the preparation of extracts;
- A vortex to properly homogenize the solutions;
- Vials for biological tests;
- A muslin cloth to cover the bottles;
- Hydrophilic cotton for tests;
- Elastics to hold the bottles;
- Thin layer chromatography tanks;
- Silica gel sheets for thin layer chromatography;
- Binocular magnifiers for observing insects;
- A UV chamber for reading from chromatographic plates;
- A camera for taking different pictures.

1.2. Study conditions

The study was carried out at the eco-toxicology laboratory of INERA in Bobo-Dioulasso. The fruit fly rearing conditions were:a temperature of 26□± 1Celsius; a photoperiod of 12hours of light and 12 hours of darkness and a relative humidity of 70% ± 10.

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1.3. Fractioning of total extracts of *Capsicum annuum* and *Strophantus hispidus*

1.3.1. *Capsicum annuum*

Two types of total extracts were fractionated for *C. annuum.*

Fractioning of the total extracts obtained with hexane

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A test portion of 15.81 gr of the hexanic extract fraction of *C. annuum* was dissolved in a minimal volume of extractant solvent. The extract solutions obtained were each mixed with silica gel for column in the ratios 1/5; m/m; (100gr for *C. annuum*). The silica and extract mixture of each plant drug was homogenized using a spatula, then dried at room temperature in the laboratory (30□ C). After evaporation of the extracting solvent, a series of solvents of increasing polarity were successively added to the silica and dried extract mixture and were transferred to a one-liter Erlenmeyer flask and 750 ml of the first solvent (toluene) of the series were added. Thus, the hexanic extract of *C. annuum* was successively fractionated by percolation with toluene, chloroform, n-hexane and methanol.

The collected fractions of each extract sample were concentrated under reduced pressure on a rotary evaporator then dried and weighed. The yield of each extract fraction was determined as a percentage relative to the test portion of the initial extract according to the following formula:

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$$\text{Yield (percent)} = \frac{\text{Mass of dry extract}}{\text{Mass of dry matter}} \times 100 \qquad (1)$$

Fractioningof the total extracts obtained with ethyl acetate

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A test portion of 14.72 g of the most active ethyl acetate extract of *C. annuum* was dissolved in 150 ml of extractant solvent (analytical ethyl acetate). The extract solution was mixed with a mass of 140 g of silica gel for column chromatography (Silica Gel 60; 0.063-0.20 ml; Merck) in ratios 1/10; m/m. The silica gel and extract mixture were homogenized using a spatula, then placed in a ventilated oven at a temperature of 45□ Cto eliminate the extractant solvent. The silica and dried extract mixture were transferred into an Erlenmeyer flask and a volume of 250 ml of analytical acetone was added to it. The extract and solvent mixture were macerated for 1 hour then transferred to a percolator in the form of a glass column. After percolation by successive leaching until exhaustion with a total volume of 750 ml of acetone, the mixture of silica gel and residual extract was macerated and percolated successively until exhaustion with 750 ml of chloroform, acetone, methanol and ethyl acetate. The collected fractions of each extract sample were concentrated under reduced pressure on a rotary evaporator then dried and weighed. The yield of each extract fraction was determined as a percentage relative to the test portion of the initial extract.

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1.3.2.*Strophantus hispidus*

Comment [AK11]: Provide references for all these process

The total extracts obtained from methanol were fractionated as follows: a mass of 8.59 g of dried methanol extract of *S. hispidus* was dissolved in 100 ml of analytical methanol. The extract solution was mixed with a mass of 90 g of column silica gel. The mixture was homogenized and placed in an oven at 45□C to remove the solvent. After evaporation of the solvent, the silica and extract mixture were placed in a cylindrical glass percolator and then successively leached to exhaustion with chloroform, ethyl acetate, acetone and methanol. The collected fractions of each extract sample were concentrated under reduced pressure on a rotary evaporator then dried and weighed. The yield of each extract fraction was determined as a percentage relative to the test portion of the initial extract.

The method for extracting the active fractions was carried out according to the following diagram (Fig. 1):

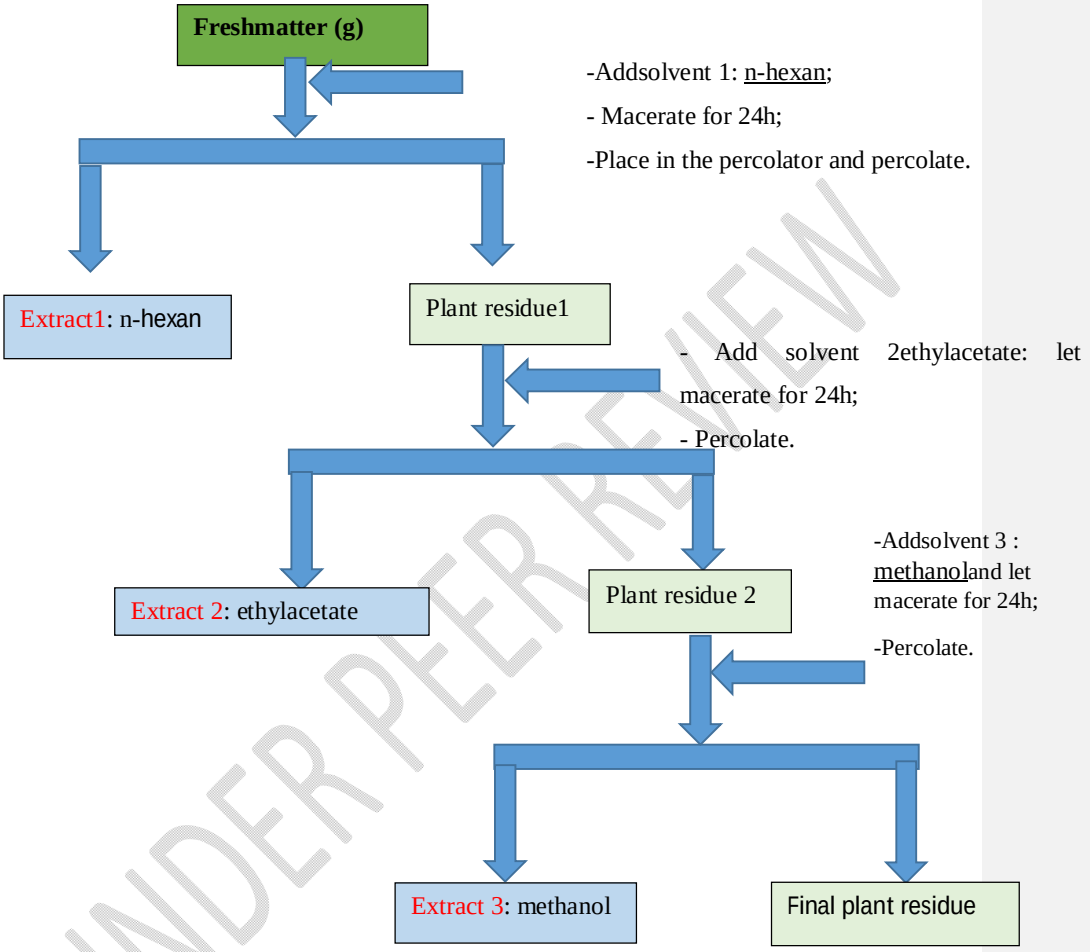

Figure 1: Extract fractionspreparation flowchart

1.3.3.Tests on the biological activity of fractions of total extracts of *C. annuum* obtained on eggs and the development of *B. dorsalis*

1.3.3.1.Collection of *B. dorsalis* eggs

1.3.3.1.1.Biological activity of total extracts on eggs, pupation and emergence of *Bactrocera dorsalis*

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Collection of *Bactrocera dorsalis* eggs

The eggs of *Bactrocera dorsalis* were obtained from the introduction of six nest boxes for 2 hours into breeding cages containing adults of *B. dorsalis* (200 males and 200 females) aged at least 15 days. Using a graduated test tube, 5,000 eggs were collected for carrying out various tests in the laboratory.

Preparation of modern brewery yeast waste

A nutrient medium for *B. dorsalis* larvae is necessary to carry out the biological activity test of total extracts on eggs and the development of *B. dorsalis.*

The preparation of the nutrient medium for the larvae of *B. dorsalis* is as follows: the brewer's yeast waste was heated until boiling with a total heating time of 1 h30 mn, stirred frequently to prevent the yeast from sticking to the bottomfrom the pot. After boiling, the yeast thus obtained was cooled completely for 24 h. After this cooling, a quantity of 0.92 g of papain was added to 11.5l of modern brewery yeast waste (at the proportion of 8g of papain per 100 l of yeast waste). Everything was mixed well and transferred to a container then placed in an incubator set at 65□ C for 24 h. A quantity of 69g of potassium sorbate was added to the mixture at the rate of 6g/l, for the preservation of the product which was then packaged in cans. The yeast waste preparation technique is summarized in Fig. 2:

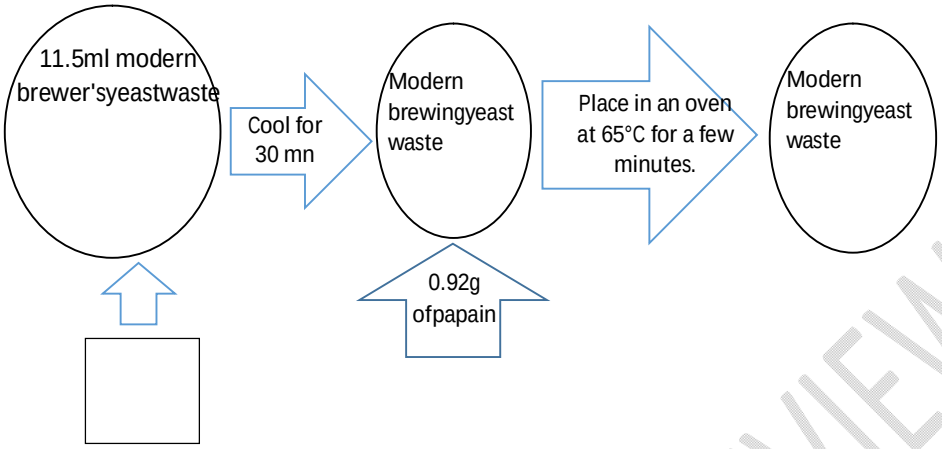

Figure 2 : Preparation of modern brewer'syeastwaste

Preparation of the larval nutrient medium

A protein-rich nutrient medium was then prepared and used for the development of *B. dorsalis* larvae using the following technique. The preparation of 1kg of nutrient medium requires:

– Corn flour: 59g;

– Soy flour: 112.10g;

– Sugar: 108.17g;

– Rice bran: 68.83g;

– Rice litter: 29.5g;

– Nipagin: 0.98g;

– Brewer's yeast waste: 295 ml;

– HCL: 2.95 ml;

– Distilled water: 295 ml.

Preparation of the medium (Fig. 3):add 295 ml of distilled water to the brewer's yeast waste, heat to boiling for 30 mn and cool completely. Then measure 295 ml of waste brewer's yeast, add beforehand to 295 ml of distilled water then add the different quantities of the components of the nutrient medium mentioned above while stirring and in the following order: sugar, bran rice, rice litter, corn flour, soy flour, nipagin. Measure 2.95 ml of HCL and

add to the middle, then stir. Finally, condition this medium in a plastic pot and keep it in the refrigerator for the various tests on eggs and the development of *B. dorsalis.*

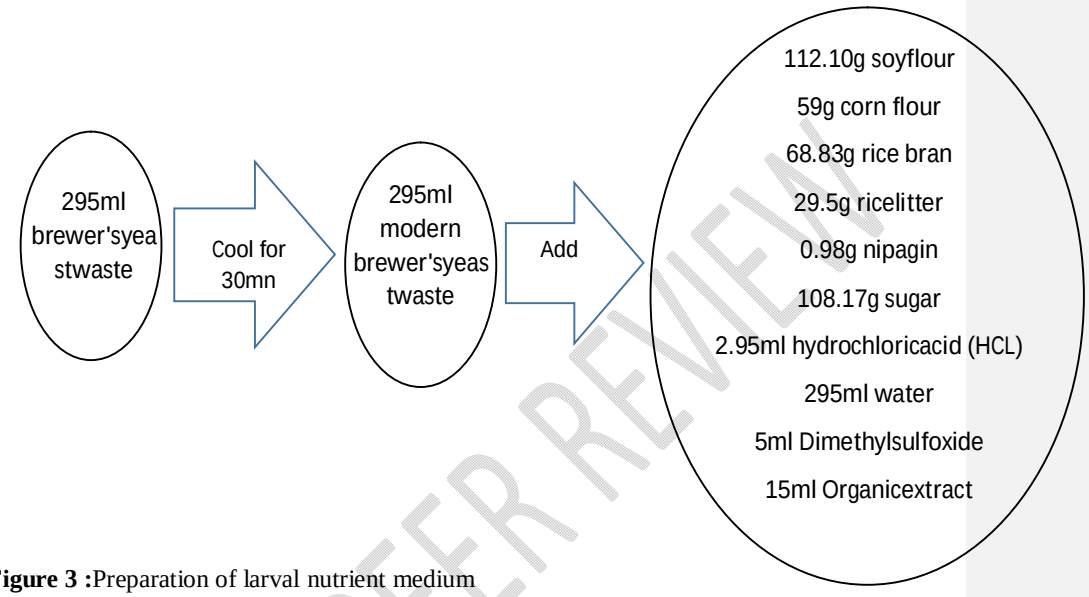

Figure 3 : Preparation of larval nutrient medium

Incorporation of the nutrient medium rich in proteins with eight organic extracts of natural substances

Once the protein-rich nutrient medium has been prepared, the procedure for its incorporation into each of the 8 organic extracts is as follows:

- 15ml of each of eight organic extracts of natural substances (*Capsicum annum* L, *Cleome viscosa* L, *Mytragina inermis* (Wild) kuntze, *Strophantus hispidus* A. DC, *Ocimumbasilicum*L, *Cassia nigricans*Vahl., *Cassia occidentalis* L., *Pseudocedrela*kotchyi) was first taken and then dissolved in 1ml of Dimethyl Sulfoxide (DMSO) in a microtube. The mixture was stirred using a vortex for good dissolution;
- Then, 29g of the local nutrient medium was taken and then added to 1ml of organic extract of each natural substance. The untreated control received 29g of nutrient medium to which 1ml of DMSO was added (i.e. 0.517ml/g concentration). The mixture of each natural substance thus obtained was placed in Petri dishes (in total 25 dishes) previously containing a piece of lotus paper.

Carrying out biological activity tests of total extracts of eight plants on the eggs and development of *B. dorsalis*

In each of the boxes, 20 eggs were placed. This was repeated 10 times for each of the eight extracts, for a total of 250 Petri dishes used for this experiment. Daily observations were performed and the number of eggs hatched was counted every 48 hours, 72 hours and 96 hours respectively. The experiment was monitored until the 25th day of the *Bactrocera dorsalis* cycle. It was then possible to determine the rates of egg hatching, pupation and emergence of flies. In this test, we were not separately interested in larval mortality because 1rst instar larvae does not burrow into the nutrient medium to feed on it and therefore are not visible under a magnifying glass once dead.

These rates were calculated based on the following formulas:

$$\text{Hatching rate} = \frac{\text{number of eggs hatched}}{\text{total number of eggs placed}} \times 100 \quad (2)$$

$$\text{Pupation rate} = \frac{\text{number of pupae collected}}{\text{number of eggs incubated}} \times 100 \quad (3)$$

$$\text{Emergence rate} = \frac{\text{number of pupae emerged}}{\text{number of emerging pupae}} \times 100 \quad (4)$$

1.3.3.2. Carrying out tests on the biological activity of active extract fractions on eggs and the development of *B. dorsalis*

Comment [AK13]: Reference for this process??

In the laboratory, the active fractions (*Capsicum annuum* and *Strophantus hispidus*) were previously incorporated into the nutrient medium rich in proteins prepared as described earlier. The eggs were exposed in these environments and the hatching rates were determined after 24 h, 48 h, 72 h, 96 h and 120 h. The development of *B. dorsalis* was monitored for 25 days at the end of which the pupation and emergence rates of *B. dorsalis* were determined.

1.3.4.Data processing and analysis

The Microsoft Office 2019 Excel spreadsheet was used to enter and process the collected data and create the various graphs. R software version 3.6.2 was used for statistical analyses.

Comment [AK14]: provide reference for R software and package if used.

When the data distribution did not follow the normal distribution, a nonparametric Kruskal-Wallis analysis was performed to detect differences between treatments. When there was a significant difference between the treatments, the pairwise comparison of the means was made with the pairwise t-test at the 5% threshold.

2. Results

2.1.Yields and chemical composition of active extract fractions

Fractions obtained from hexanic extracts of *Capsicum annuum*

The yield of the fractions obtained varied from: 77.42 % to 11.70 %. The highest yield (77.42 %) was observed with toluene and the lowest was observed with methanol (11.70 %). The n-hexane fraction of *C. annuum* gave insignificant masses of extracts not quantifiable by weighing. The extraction yield of the different sub-fractions is given in Table 1.

Table 1: Yield of fractions of *C. annuum*

Fractions	Extract mass (g)	Yield (%)
	Fractions n-Hexane *C. annuum*	
Toluene	124	77.2
Chloroform	23	17.90
n-Hexan	Traces	Traces
Methanol	1.75	11.0

Fractions obtained from ethyl acetate extracts of *Capsicum annuum*

The fractionation yield varied from: 0.13% to 59.44%. The highest yield (59.44%) was observed with *C. annuum* in chloroform and the lowest was observed with *C. annuum* in ethyl acetate (0.13%). The extraction yield of the different sub-fractions is given in Table 2.

Table 2: Yield of fractionations of *C. annuum* fraction

Fractions	Extract mass (g)	Yield (%)
Fractions of *C. annuum* in ethylacetate		
Chloroform	8.75	59.44
Ethylacetate	0.02	0.13
Acetone	2.88	19.57
Methanol	0.23	1.56

Fractions obtained from methanol extracts of *Strophantus hispidus*

The fractionation yield varied from19.90% to 69.85%. The highest yield was observed with *C.annuum* in methanol (83%) and the lowest was observed with *C. annuum* in acetone (19.90%). The extraction yield of the different sub-fractions is given in table 3.

Table 3: Yield of fractions of *S.hispidus*

Fractions	Extract mass (g)	Yield (%)
Fractions of *S. hispidus* in methanol		
Chloroform	6.00	69.85
Ethylacetate	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 *C. annum* and *S. hispidus* methanol made it possible to obtain fractions which were the subject of insecticidal tests.

2.2.Biological activity of the fractions obtained

2.2.1.Effects of natural substance fractions on *Bactrocera dorsalis* eggs

2.2.1.1.Effects of natural substance fractions on *Bactrocera dorsalis* eggs after 48 hours of exposure

Figure 4 shows the effect of fractions of natural substances on the hatching of *B. dorsalis* eggs after 48 hours of exposure. The analysis of variance revealed a very high significant difference between treatments. The toluene fraction of *C. annuum* showed a low egg hatching

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rate (9.5%), i.e. a reduction in hatching of 88.55% compared to the untreated control. On the other hand, the methanol and chloroform fractions of *C. annuum*resulted in the highest hatching rates with respectively 27.1% and 23.02%, i.e. inhibition rates of 67.5% and 72.27% respectively.

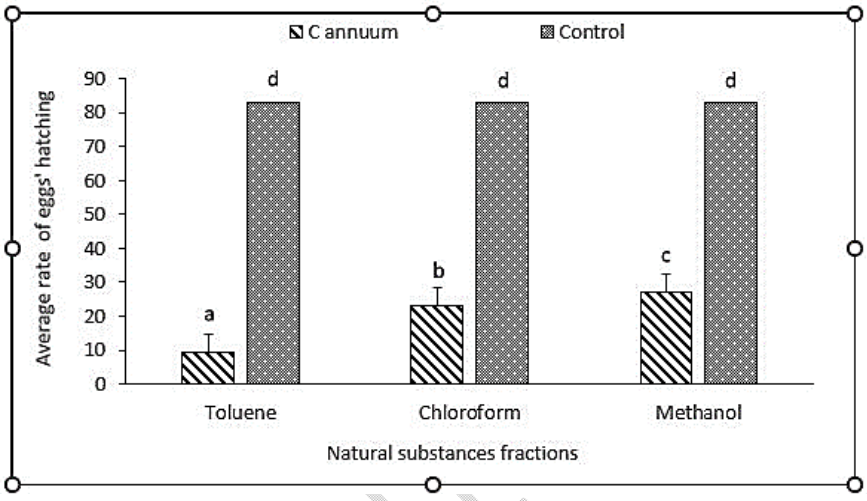

Figure 4: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 48 hours of exposure

2.2.1.2.Effects of natural substance fractions on *Bactrocera dorsalis* eggs after 72 hours of exposure

Figure 5 shows the effect of fractions of natural substances on the hatching of *B. dorsalis* eggs after 72 h of exposure. The analysis of variance showed a very high significant difference between treatments. The lowest hatching rate was recorded with the chloroform fraction of *C. annuum* (35.3%), i.e. an inhibition of the hatching rate of 64.70% compared to the untreated control. On the other hand, the methanol fraction of *C. annuum* resulted in a higher hatching rate of 63.5%, i.e. an inhibition of 36.5%, followed by the toluene fraction of *C. annuum* (41%), i.e. a reduction 59% compared to the untreated control.

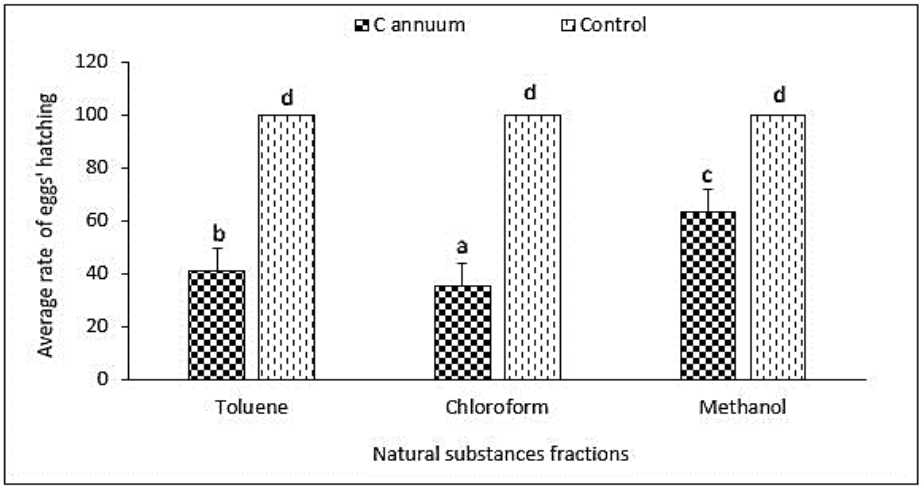

Figure 5: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 72 hours of exposure

2.2.1.3.Effects of natural substance fractions on *Bactrocera dorsalis* eggs after 96 hours of exposure

Figure 6 shows the effect of the effectiveness of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 96 hours of exposure. The analysis of variance revealed a very high significant difference between treatments. The lowest hatching rate was recorded with the Chloroform fraction of *C. annuum* (50.40%), i.e. a reduction in the hatching rate of 49.60% compared to the untreated control. On the other hand, the methanol fraction of *C. annuum* resulted in higher hatching rates of 94%, i.e. a reduction of 6.00%, followed by the methanol fraction of the toluene fraction of *C. annuum* (80.5%), i.e. reductions of 19.50% compared to the untreated control.

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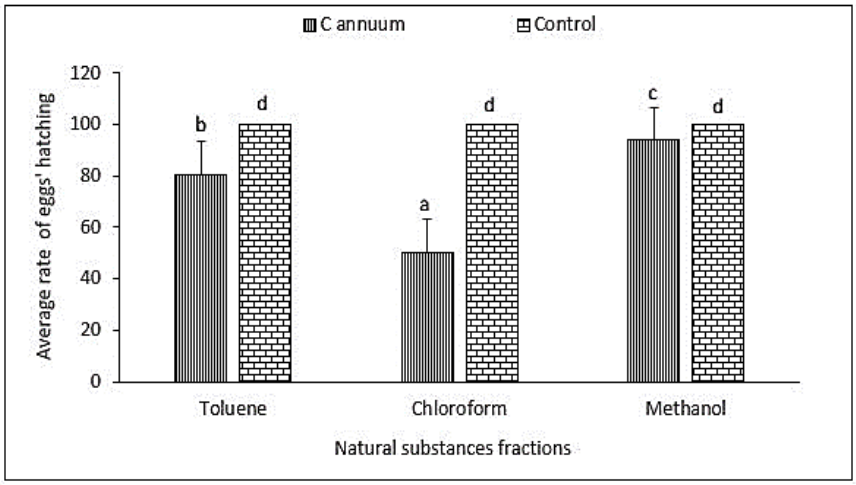

Figure 6: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 96 hours of exposure

2.2.1.4.Effects of natural substance fractions on *Bactrocera dorsalis* eggs after 120 hours of exposure

Figure 7 illustrates the effect of fractions of natural substances on the hatching of *B. dorsalis* eggs after 120 hours of exposure. The analysis of variance showed that there was a very high significant difference between treatments. The lowest hatching rate was observed with the Chloroform fraction of *C. annuum* (51.00%), a reduction of 49.00% compared to the untreated control. On the other hand, the methanol fraction of *C. annuum* resulted in a higher hatching rate with respectively 99.50%, i.e. a reduction of 0.50%, followed by the toluene fraction of *C. annuum* 96.00%, i.e. a reduction of 0.50% reduction of 4.00% compared to the untreated control.

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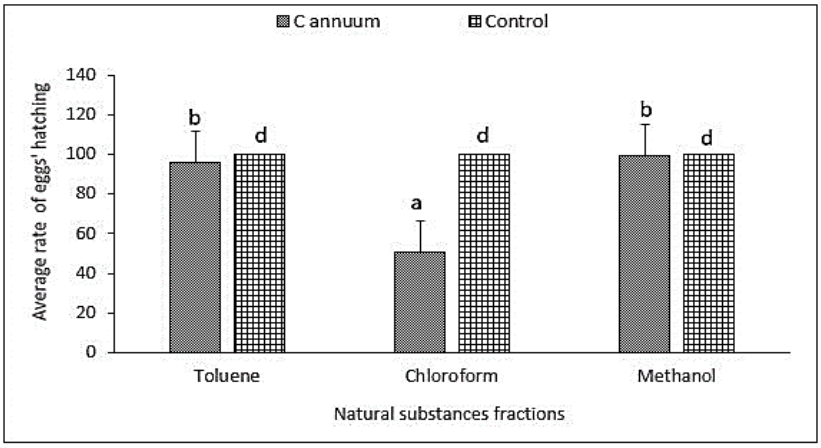

Figure 7: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 120 hours of exposure

2.2.1.5.Effects on pupation of *Bactrocera dorsalis*

The toluene, chloroform and methanolic fractions of *C. annuum* are 100% inhibitors of *Bactrocera dorsalis* pupation. No pupation took place. So, no emergence of adults was recorded.

3.Discussionandconclusion

The use of *C. annuum* fractions in fruit fly control couldpresent a promising source for mangogrowers in Burkina Faso. Tests of *C. annuum* fractions on the eggs and developmental stages of *B. dorsalis*showedthat the chloroform fraction of *C. annuum*had an inhibitoryeffect on the hatching of *B. dorsalis*eggs. This resultcouldbeexplained by the presence of one or more active ingredients in the chloroform fraction disruptingbiochemicalreactionsinside the eggs, thus blocking theirdevelopment. Indeed, a phytochemicalanalysis of *C. annuum*wascarried out by [11]and noted the presence of triterpenes, anthraquinones, flavonoids, alkaloids, saponosides, tannins and cardenolides. The efficacy of these fractions couldbe due to ovocidal, larvicidal and anti-appetentactivity. [12]reported onphytochemicalanalysis of the methanolicextract of *Cleomeviscosa*L. (Capparidaceae) leaves, whichrevealed the presence of steroids, triterpenes, anthraquinones, tannins, flavonoids, saponosides and anthocyanosidesthat are known to have insecticidal and larvalgrowthinhibitoryactivities for *H. armigera*.In addition, [13]and [14]have shown anti-

appetentactivityagainst*Helicoverpaarmigera*larvae due to the presence of eightflavonoidswithcytotoxicpropertiesthat have also been isolatedfrom*C. viscosa*leaves.Authorssuch as [15]have reportedovicidal and anti-appetentactivities of *C. viscosa*extractsagainst*S. oryzae*. [16]and[17]have alsoreportedthat the alkaloids of several plants have effective antiappetentactivitiesagainst*Spodopteralittoralis*Boisduval (Lepidoptera:Noctuidae), *Leptinotarsadecemlineata*(Say) (Coleoptera: Chrysomelidae) and *Helicoverpaarmigera*(Hübner). This inhibition of egghatchingcouldbeexplained by the factthat the fractions containconstituentswithinsecticidal and inhibitorypropertiesresponsible for inhibiting the hatching of *B. dorsalis*eggs. The ovicidaleffects of *C. annuum*wereevidencedby [7],[18]whoshowed the sameeffects on *Callosobruchus maculatus*eggs. This explains the total inhibition of theirdevelopment. In addition, the *C. annuum* fractions may have blocked the moulting of *B. dorsalis*larvae. [19]alsohighlighted the terpenes in *C. viscosa*essential oils and theirinhibitoryproperties on *Acanthoscelidesobtectus*(Say) (Coleoptera:Chrysomelidae) hatchings.

The aim of the presentstudywas to determine the active principles of the main chemical groups contained in the different fractions of *C.annuum and S.hispidus*, and to clarify the insecticidaleffects of these compounds on the development of *B. dorsalis*. Tests on the active extract fractions revealedthat the *C. annuum* fractions were effective in reducingegghatching and inhibiting*B. dorsalis*development. The toluene, chloroform and methanolic fractions of *C. annuum* are total inhibitors of *B.dorsalis*pupation. The fractions testedcouldbeused to combat fruit flies, which are responsible for major economiclosses in the mangosector in Burkina Faso.

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