

Biological Activity of *Capsicum annum* Extracts on the Developmental Stages of Mango Fruit Fly *Bactrocera dorsalis* (Egg and Pupa).

Abstract

Bactrocera dorsalis and *Ceratitiscosyra* are the major constraints of the mango sector in Burkina Faso. Various methods are used to control these insect pests, including the use of chemical pesticides, which are expensive for farmers. The overuse of these chemicals leads to environmental pollution, food poisoning and resistance among insects. For this reason, research is increasingly focusing on plant derivatives as an alternative. A study of the biological activity of fractions of organic extracts of one plant, *Capsicum annum* on the development of *B. dorsalis*, was carried out under laboratory conditions in Burkina Faso. The plant fractions were extracted using solvents of increasing polarity: n-hexane, ethyl acetate and methanol. The biological activity test on the development of *B. dorsalis* was carried out in a randomised Fisher block design with 25 treatments in 10 replicates. The active extracts were fractionated using the Nair method and the fractions obtained were tested on the development of *B. dorsalis*. The highest yield (77.42 %) was observed with toluene and the lowest was observed with methanol (11.70 %). The highest yield (59.44%) was observed with *C. annum* in chloroform and the lowest was observed with *C. annum* in ethyl acetate (0.13%). The fractionation of the most active extracts (hexanic and ethyl acetate) of *C. annum* made it possible to obtain fractions which were the subject of insecticidal tests. The methanol and chloroform fractions of *C. annum* resulted in the highest hatching rates with respectively 27.1% and 23.02%, i.e. inhibition rates of 67.5% and 72.27% respectively for effects of natural substance fractions on *Bactrocera dorsalis* eggs after 48 hours of exposure. The methanol fraction of *C. annum* resulted in a higher hatching rate of 63.5%, i.e. an inhibition of 36.5%, followed by the toluene fraction of *C. annum* (41%), i.e. a reduction 59% compared to the untreated control for effects of natural substance fractions on *Bactrocera dorsalis* eggs after 72 hours of exposure. The methanol fraction of *C. annum* 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. annum* (80.5%), i.e. reductions of 19.50% compared to the untreated control for effects of natural substance fractions on *Bactrocera dorsalis* eggs after

96 hours of exposure. 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 for effects of natural substance fractions on *Bactrocera dorsalis* eggs after 120 hours of exposure. 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. The toluene, chloroform and methanolic fractions of *C. annuum* are total inhibitors of *B. dorsalis* pupation. The fractions tested could be used to combat fruit flies, which are responsible for major economic losses in the mango sector in Burkina Faso.

Key words: *Capsicum annuum*, fraction, *Bactrocera dorsalis*, 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 (Potential quarantine pests are particularly dangerous harmful organisms for which it has yet to be determined whether they meet the criteria of a quarantine pest. Until such clarification is available, they are treated 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 context 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 one plant species chilli, *Capsicum annum*, have an interesting biological activity on the one 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. annum* were extensively investigated [6]. Indeed, *C. annum* 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 [6]. 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» [9]. 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. annum* and to specify the insecticidal effects of these compounds on the different developmental stages of *B. dorsalis*.

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 ± 1 Celsius; a photoperiod of 12 hours of light and 12 hours of darkness and a relative humidity of $70\% \pm 10$ [6].

1.3. Vegetable Powders Extraction

Different extracts were obtained from the plant powders. The extractions were carried out according to Nair's method [9]. The principle of this method is based on the ability of bioactive substances of plant origin to be carried out by solvents of different polarities.

For the present study, a sample of each vegetable powder (400 gr) was macerated with 1200 ml for 24 hours, three times, at room temperature of the laboratory, and then extracted by successive percolation with solvents of increasing polarity, until completely exhaustion. The successive extracting solvents used were n-hexane, ethyl acetate and methanol (VWR Prolabo Chemicals, France). The obtained extracts were then concentrated under reduced pressure using a rotavapor Büchi R 110 with a marry-regulated bath at an appropriate temperature of each extracting solvent. The concentrated extracts were then dried in a Cole Parmer Instrument brand ventilated oven set at 45° C. The mass of dry extracts obtained was determined by weighing using a Radwag precision analytical balance Model AS 110.R1.

1.4. Fractioning of total extracts of *Capsicum annuum*

1.4.1. *Capsicum annuum*

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

Fractioning of the total extracts obtained with hexane

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.

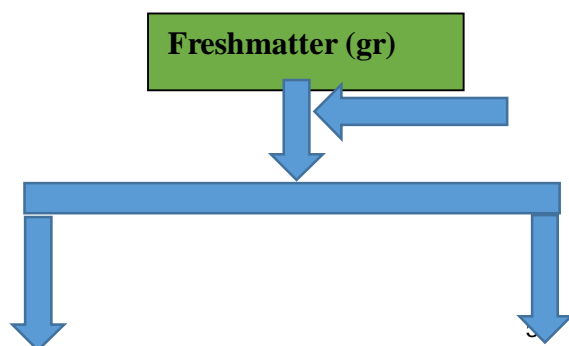
The collected fractions of each extract sample were concentrated under reduced pressure on a rotary evaporator then dried and weighed. The yield of each extract fraction was determined as a percentage relative to the test portion of the initial extract according to the following formula:

$$\text{Yield (percent)} = \frac{\text{Mass of dry extract}}{\text{Mass of dry matter}} \times 100 \quad [6]. \quad (1)$$

Fractioning of the total extracts obtained with ethyl acetate, chloroform, acetone, methanol

A test portion of 14.72 gr 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 °C to 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.

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



- Add solvent 1:n-hexan;
- Macerate for 24h;
- Place in the percolator and percolate.

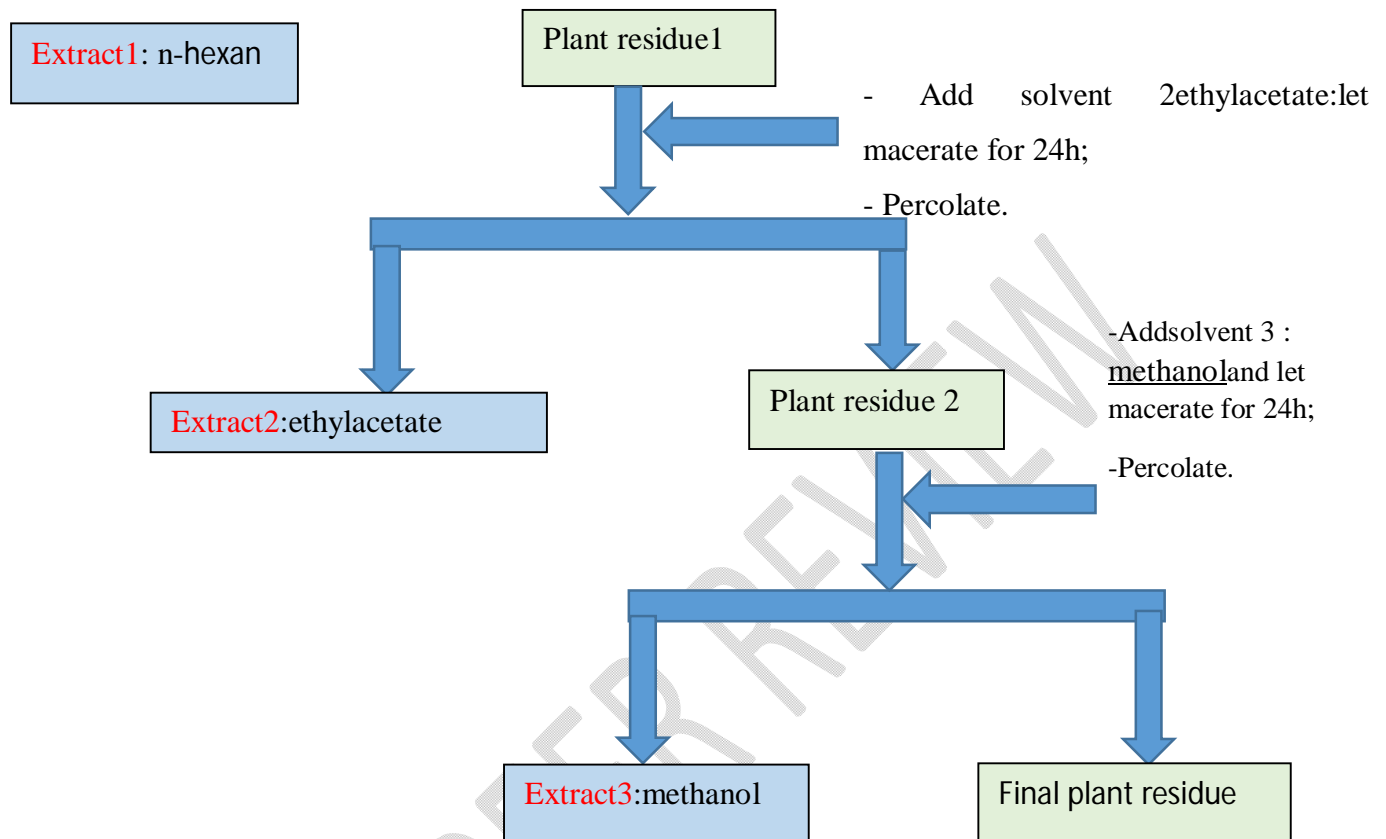


Figure 1: Extract fractions preparation flowchart

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

1.4.3.1. Collection of *B. dorsalis* eggs

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

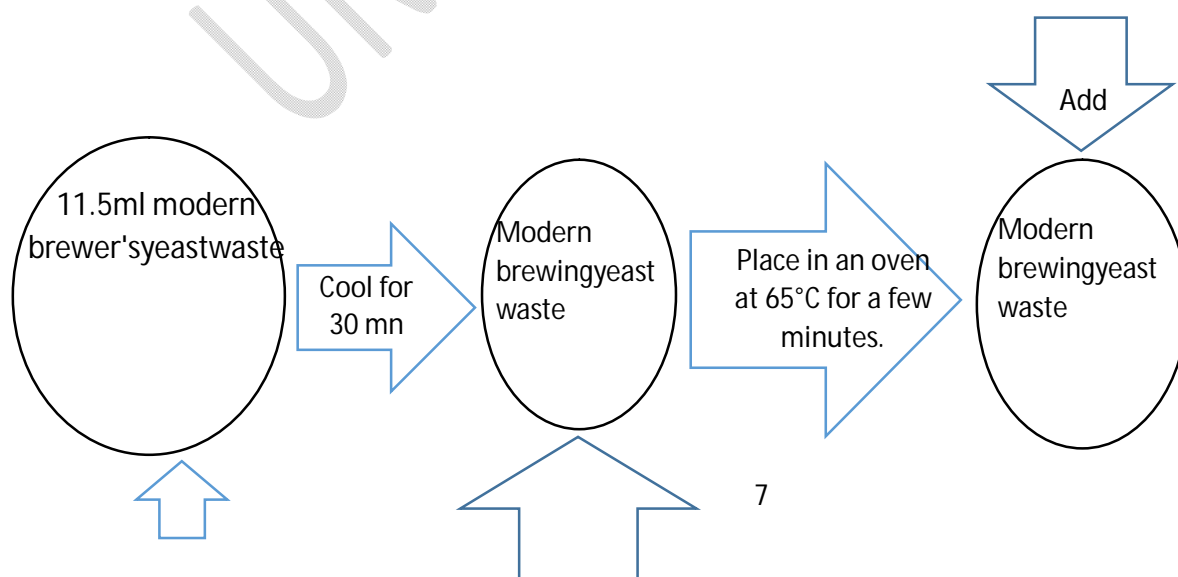
Collection of *Bactrocera dorsalis* eggs

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 bottom from the pot. After boiling, the yeast thus obtained was cooled completely for 24 h. After this cooling, a quantity of 0.92 gr 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 69gr 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:



0.92grofp
apain



Figure 2 :Preparation of modern brewer's yeastwaste

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: 59gr;
- Soy flour: 112.10gr;
- Sugar: 108.17gr;
- Rice bran: 68.83gr;
- Rice litter: 29.5gr;
- Nipagin: 0.98gr;
- 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*.

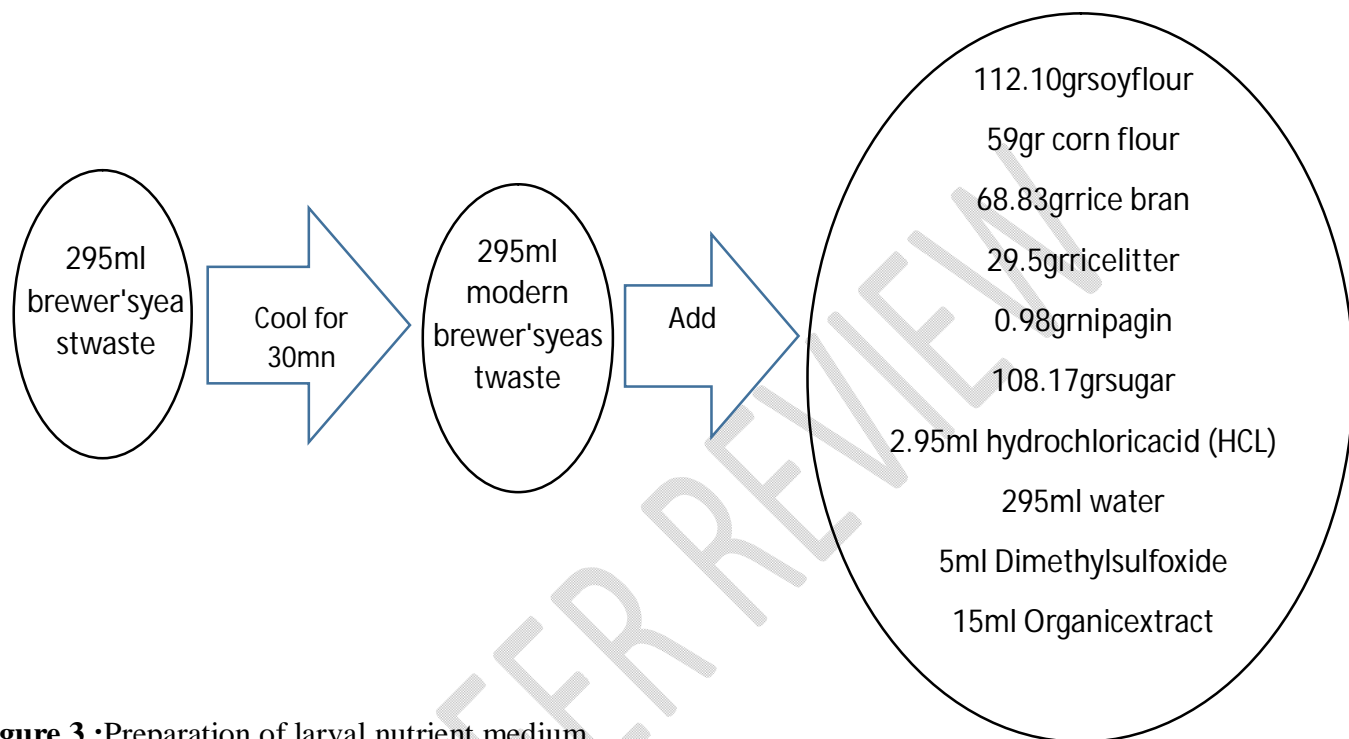


Figure 3 :Preparation of larval nutrient medium

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 1 organic extract is as follows:

- 15ml of each of one organic extract of natural substances (*Capsicum annum* L) 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, 29gr of the local nutrient medium was taken and then added to 1ml of organic extract of each natural substance. The untreated control received 29gr of nutrient medium to which 1ml of DMSO was added (i.e. 0.517ml/gr 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 1st 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:

$$\text{Hatching rate} = \frac{\text{number of eggs hatched}}{\text{total number of eggs placed}} \times 100 \quad [6] \quad (2)$$

$$\text{Pupation rate} = \frac{\text{number of pupae collected}}{\text{number of eggs incubated}} \times 100 \quad [6] \quad (3)$$

$$\text{Emergence rate} = \frac{\text{number of pupae emerged}}{\text{number of emerging pupae}} \times 100 \quad [6] \quad (4)$$

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

In the laboratory, the active fractions (*Capsicum annum*) were previously incorporated into the nutrient medium rich in proteins prepared as described earlier [6]. 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.

1.4.4. 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. Shapiro's test was used to check the normality of the data. 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 (gr)	Yield (%)
Fractions n-Hexane <i>C. annuum</i>		
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 (gr)	Yield (%)
Fractions of <i>C. annuum</i> in ethylacetate		
Chloroform	8.75	59.44
Ethylacetate	0.02	0.13
Acetone	2.88	19.57
Methanol	0.23	1.56

The fractionation of the most active extracts (hexanic and ethyl acetate) of *C. annuum* 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 ($p=0.0001$). The toluene fraction of *C. annuum* showed a low egg hatching 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.

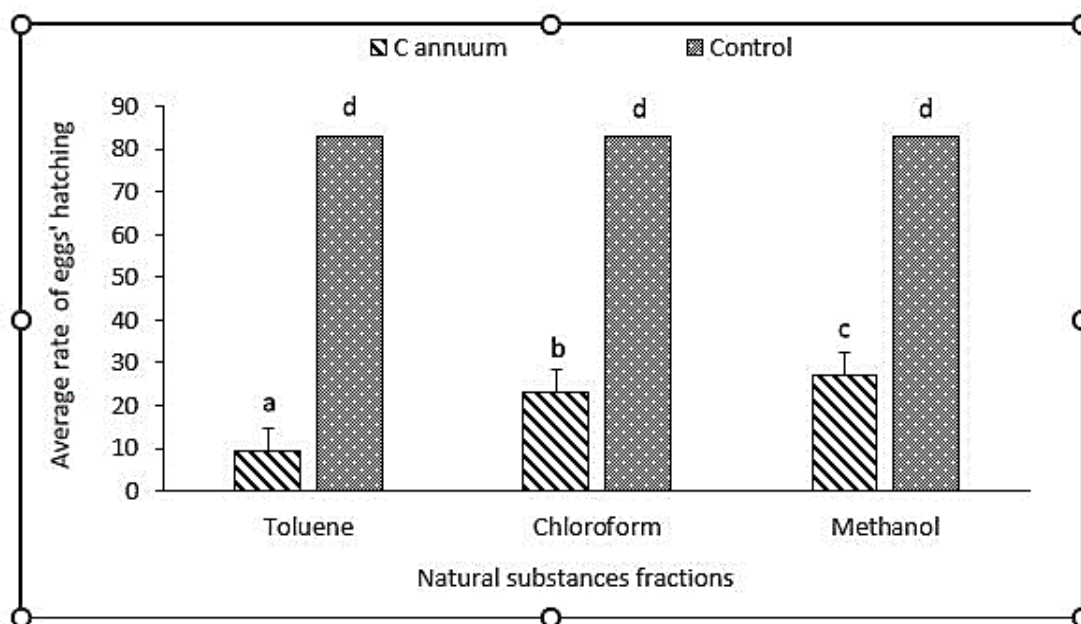


Figure 4: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 48 hours of exposure

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 ($p=0.0001$). 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.

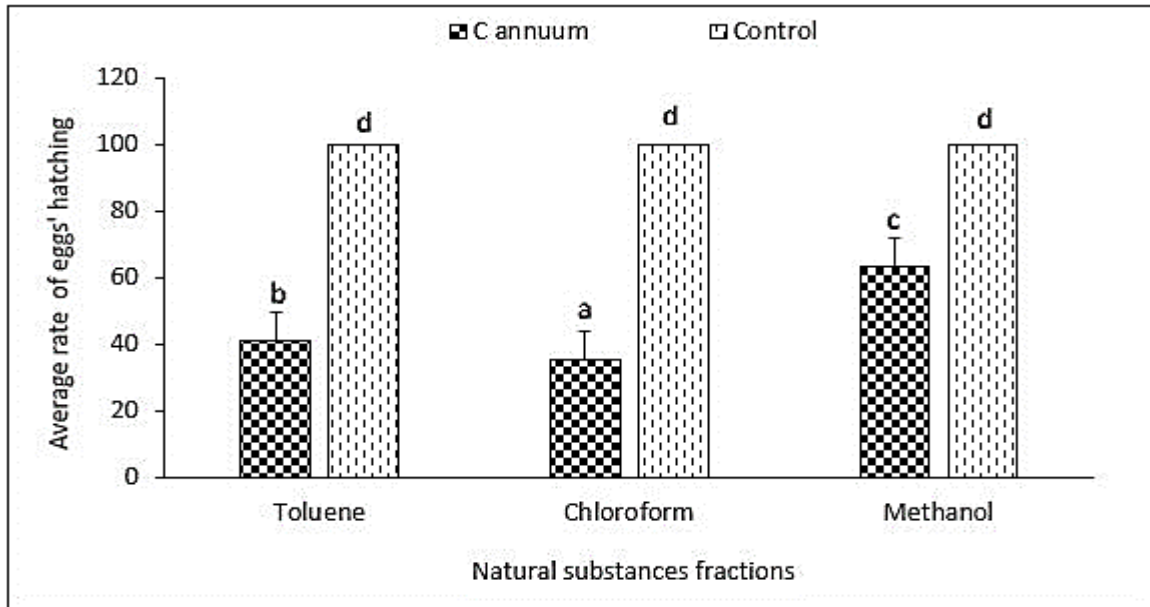


Figure 5: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 72 hours of exposure

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 ($p=0.0001$). 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.

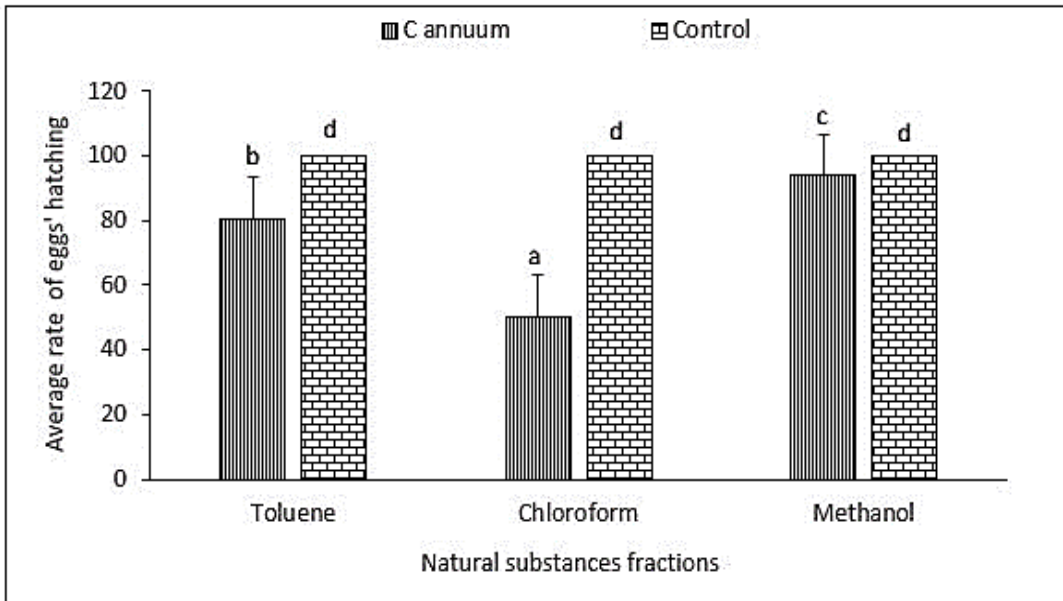


Figure 6: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 96 hours of exposure

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 ($p=0.0001$). 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.

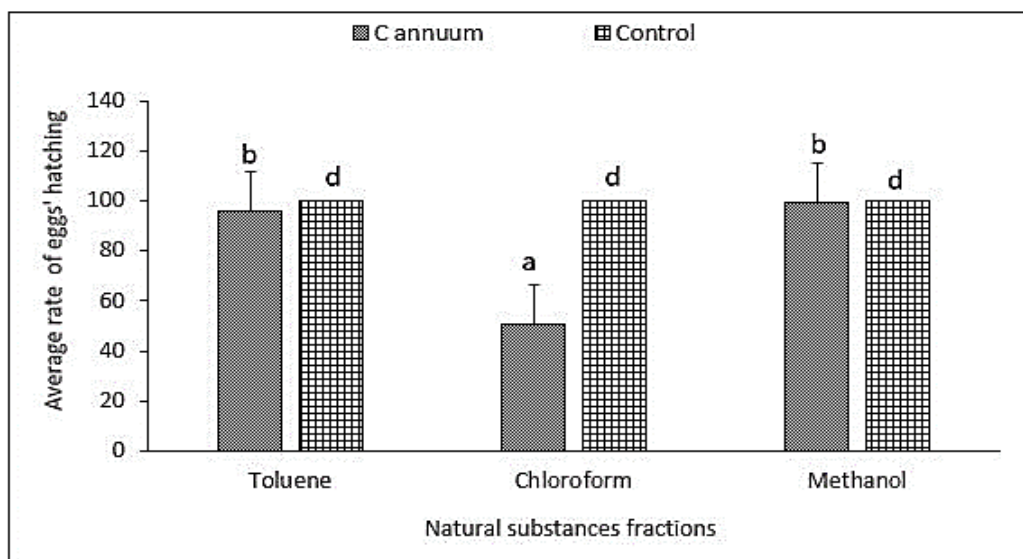


Figure 7: Effects of fractions of natural substances on the hatching of *Bactrocera dorsalis* eggs after 120 hours of exposure

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. Discussion and conclusion

The use of *C. annuum* fractions in fruit fly control could present a promising source for mango growers in Burkina Faso. Tests of *C. annuum* fractions on the eggs and developmental stages of *B. dorsalis* showed that the chloroform fraction of *C. annuum* had an inhibitory effect on the hatching of *B. dorsalis* eggs. This result could be explained by the presence of one or more active ingredients in the chloroform fraction disrupting biochemical reactions inside the eggs, thus blocking their development. Indeed, a phytochemical analysis of *C. annuum* was carried out by [11] and noted the presence of triterpenes, anthraquinones, flavonoids, alkaloids, saponosides, tannins and cardenolides. The efficacy of these fractions could be due to ovocidal, larvicidal and anti-appetent activity. [12] reported on phytochemical analysis of the methanolic extract of *Cleome viscosa* L. (Capparidaceae) leaves, which revealed the presence of steroids, triterpenes, anthraquinones, tannins, flavonoids, saponosides and anthocyanosides that are known to have insecticidal and larval growth inhibitory activities for *H. armigera*. In addition, [13] and [14] have shown anti-

appetent activity against *Helicoverpa armigera* larvae due to the presence of eight flavonoids with cytotoxic properties that have also been isolated from *C. viscosa* leaves. Authors such as [15] have reported ovicidal and anti-appetent activities of *C. viscosa* extracts against *S. oryzae*. [16] and [17] have also reported that the alkaloids of several plants have effective anti-appetent activities against *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and *Helicoverpa armigera* (Hübner). This inhibition of egg hatching could be explained by the fact that the fractions contain constituents with insecticidal and inhibitory properties responsible for inhibiting the hatching of *B. dorsalis* eggs. The ovicidal effects of *C. annuum* were evidenced by [7], [18] who showed the same effects on *Callosobruchus maculatus* eggs. This explains the total inhibition of their development. In addition, the *C. annuum* fractions may have blocked the moulting of *B. dorsalis* larvae. [19] also highlighted the terpenes in *C. viscosa* essential oils and their inhibitory properties on *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) hatchings.

The aim of the present study was 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 insecticidal effects of these compounds on the development of *B. dorsalis*. Tests on the active extract fractions revealed that the *C. annuum* fractions were effective in reducing egg hatching and inhibiting *B. dorsalis* development. The toluene, chloroform and methanolic fractions of *C. annuum* are total inhibitors of *B. dorsalis* pupation. The fractions tested could be used to combat fruit flies, which are responsible for major economic losses in the mango sector in Burkina Faso.

Disclaimer (Artificial intelligence)

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

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