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Rabièta Simde
Environmental and Agricultural Research Institute. Research Laboratory, Bobo-Dioulasso, Burkina Faso

Georges Kambou
Environmental and Agricultural Research Institute. Research Laboratory, Bobo-Dioulasso, Burkina Faso

Elias Mano
Apply Science and Technology Institute. Bobo-Dioulasso, Burkina Faso

Antoine Sanon
Pr. Joseph Ki Zerbo University, Burkina Faso

Corresponding Author:
Georges Kambou
Environmental and Agricultural Research Institute. Research Laboratory, Bobo-Dioulasso, Burkina Faso

Kamboubactroceraticide 1 and 2: New hydroalcoholic formulations based on natural substances (*Capsicum annuum* L., *Strophantus hispidus* dc) against mango fruit flies (*Bactrocera dorsalis* (Hendel) in Burkina Faso

Rabièta Simde, Georges Kambou, Elias Mano and Antoine Sanon

Abstract
A study of hydro- alcoholic formulations efficiencies based on *Capsicum annuum* L. and *Strophantus hispidus* vegetables products, named Kamboubactroceraticide 1 and 2 respectively, against mango fruit flies, *Bactrocera dorsalis* (Hendel) which causes big damages to farmers, have been done in laboratory conditions, in Burkina Faso. The experimentation was a randomized Fisher block design of 8 treatments (Untreated control, control product GF 120, three concentrations rates of *C. annuum* and three concentrations of *S. hispidus* hydroalcoholic formulations) in five replications. The *S. hispidus* hydroalcoholic formulations at 9.00 g/L and 12.50 g/L concentrations gave highest rates mortality of 90% against female's adults of *B. dorsalis*. The *C. annuum*at the concentration of 12.50 g/L gave 82.64% of mortality. After 72 hours, all the insecticides formulations gave 90% mortality. Against *B. dorsalis* adult's males, *S. hispidus* and *C. annuum* hydroalcoholic formulations got 90% mortality after 24hours. After 72 hours, all the insecticides formulations gave 90% mortality against the males' adults. According to *B. dorsalis* females and males together, the higher mortality has been obtained with *S. hispidus*hy droalcoholic formulations at 9.00 g/L and 12.50 g/L concentrations (100% mortality) after 24 hours. The one of *C. annuum* gave 84.84% mortalityat the concentration of 12.50 g/L. After 72 hours, all the insecticides gave 100% mortality against females and males adults of *B. dorsalis.* These results augur the possibility to use Kamboubactroceraticide 1 and 2 as perspectives bio-pesticides against mango fruit flies.

Keywords: *Capsicum annuum*, *Strophantus hispidus*, *Bactrocera dorsalis*, mango

Introduction
In Burkina Faso, marketing of fresh mango and processing generated in 2018, nearly 15 billion CFA francs, with an estimated annual production of 197,000 tons for an area of 33,701 ha[1]. Mango plays an important role in the national economy. It is a source of income diversification and improved nutrition for the sector's stakeholders [25].
Unfortunately, mangoes are subject to fruit fly attack, which led to 15% production lost in 2017 with arounda volume of 200,000 tones [1]. *Ceratitis cosyra* and *Bactrocera dorsalis* are responsible for damages to mango sector and food security in Africa, in Asia, in the Pacific and parts of South America [20]. They can lead to losses which varied from 50 to 85% if appropriate phyto-sanitary control is not put in place [4].
In response to the threat caused by these flies, several control methods have been implemented, including: prophylactic control, chemical control, biological control as *Diachasmimorpha longicaudata* against *B. dorsalis* with success in many countries, by combination with sterile insect technic (TIS) [3, 4, 19, 24], mass trapping, use of protein baits [15, 2]. Unfortunately, some methods (those using synthetic insecticides or smoke) have disadvantages which their use. This chemical controls led sometime to resistance from fruit flies (in pulp and on mango). For example *B. dorsalis* resisted to Malathion, β-cyperméthrine and abamectine in few years [26, 8]. Some pesticide residues causing food poisoning [11] and environmental pollution [23]. These disadvantages are due to the relatively high prices of good quality control products on local markets. To avoid this, it became necessary to find alternatives which would be more environmentally friendly and meets the requirements of markets (where traceability and quality control standards are increasingly stringent) is essential [9].

Indeed, fruit fly control technologies based on the use of herbal substances extract have attracted attention because of their availability and biodegradability, thus preserving the environment and human health as *Ocimum basilicum* against *B. dorsalis* [7], and *citrus aurantium* (L.) against *Bactrocera olea* [22]. The collected results in orchards from experiments based on vegetable substance extracts show a decrease in the loss caused by fruit flies, evaluated in terms of the number of bites [4], hence the interest of evaluating the biological efficiencies of hydroalcoholic extracts of vegetable substances against mango fruit flies, *B. dorsalis* (Hendel). The main objective of this study is to increase the production of healthy mangoes by reducing the population of the main fruit flies (*B. dorsalis*) to an economic acceptable level pest through the use of herbal substances formulations.

Materials and Methods

Study site

The study has been done at the biological control laboratory of the Fruits and Vegetables National Specialization Center (F.V.N.S.C)

Materials

The animal material concerned *B. dorsalis* adults. These flies were bred in the laboratory. The plant material used concerned ripe fruits of *Capsicum annuum* obtained from producers and leaves of *Strophantus hispidus* harvested in Gaoua area, located at the South – West of Burkina Faso. This plant material was dried in the shade under a greenhouse.

Methods

***B. dorsalis* rearing in the laboratory.**

Adults of *B. dorsalis* were obtained from rearing in the biological control laboratory the Fruits and Vegetables National Specialization Center (F.V.N.S.C). It was carried out in an air-conditioned room with a 12-hour photoperiod. The relative humidity was maintained at 70 ± 10% and the temperature at 26 °C ± 1 °C.

Preparation of formulations

Preparation of *C. annuum* and *S. hispidus* extracts.

The aqueous extracts of *C. annuum*, were obtained from 1.1 kg of ripe fruit powder of *C. annuum*. These ripe fruits were dried in the shade under a greenhouse and then finely ground using a micro mill. The resulting shred (1.1 kg) was macerated in 2 liters' of ethyl acetate and left for 24 hours at room temperature. After 24 hours, the mixture was then filtered through muselin placed on a bucket and now with an elastic band. The ethyl acetate suspension was vacuum evaporated at 76.9 to 77.4 °C using a Rotavapor R-110 to separate the crude extract from the solvent. This procedure resulted in 200 mL of crude extract of *C. annuum* from the starting volume.

Total extracts of *S. hispidus* were obtained from 3.1 kg of *S. hispidus* leaf powder. The leaves were also dried in the shade under a greenhouse well finely ground using a micro mill. The extraction procedures for *S. hispidus* were identical to those for *C. annuum*, the only difference being that the solvent for *S. hispidus* was ethanol with a volume of seven (07) liters. The ethanol suspension was vacuum evaporated between 64 and 65 °C using a Rotavapor R-110 to separate the crude extract from the solvent. After extraction, the total volume of the crude extract of *S. hispidus* with ethanol was 720 mL.

Preparation of formulations

The different formulations were obtained from the raw extracts of *C. annuum* and *S. hispidus* and an attractant. The formulations were prepared in three concentrations: the recommended concentration, the half concentration of this, the concentration + 2/3 of the concentration, packaged in water containers and kept cool. The different concentrations had a volume of 100 mL.

C1 = The recommended concentration of *C. annuum* was formulated with 1.50 mL of crude extract + 98.50 mL of an attractant.

C2 = The half recommended concentration of *C. annuum* with 0.8 mL of crude extract + 99.20 mL of an attractant.

C3 = The recommended concentration + 2/3 of the recommended concentration of *C. annuum* with 2.65 mL of crude extract + 97.35mL of an attractant.

C4 = The recommended concentration of *S. hispidus* was formulated with 2.8L crude extract + 97.20 mL of an attractant

C5 = The half recommended concentration of *S. hispidus* with 1.40 mL of crude extract + 98.60 mL of attractant

C6 = The recommended concentration+ 2/3 the recommended concentration of *S. hispidus* with 4.70 mL of crude extract + 95.30 mL of attractant

The following formula has been used to obtain the stock solution

- CiVi = CfVf with
- Vf= Vi + Vs [17].
- Ci = concentration of the formulation to be used for the preparation of the stock solution;
- Vi = volume of the formulation to be pipetted;
- Cf = concentration of the stock solution;
- -Vf = volume of the stock solution;
- Vs=volume of the solvent.

These allowed getting the different treatments of hydroalcoholic formulations based on *C. annuum* and *S. hispidus*. (Untreated control, *C. annuum* 3.75 g/L, *C. annuum* 7.50 g/L, *C. annuum* 12.50 g/L, *S. hispidus* 4.50 g/L, *S. hispidus* 9.00 g/L, *S. hispidus* 15.00 g/L).

Biological efficiency of hydroalcoholic formulations against *B. dorsalis* (males + females) adults

For the test, 1.50 mL of each formulation was collected using a syringe and poured into vials containing 0.25g of cotton. A pair of 10 *B. dorsalis* females' adults and 10 *B. dorsalis* males' adults were aspirated using a mouth vacuum and placed into the vials. The whole thing was covered with a muslin canvas and held by rubber bands. This operation was repeated in five (5) replications for each dose of the different formulations based on extracts of *S. hispidus* and *C. annuum*, and for success bait and the untreated control. A total of 8 treatments were performed with five (5) replications. All tests were lunched simultaneously. Each of the vials containing *B. dorsalis* was placed on shelves and kept in the fly room. Observations on *B. dorsalis* mortality have been done after 24h and 72h, considering that insects, which did not respond to the touch of a fine brush, died.

Statistical analyses

The Microsoft Excel 2016 spreadsheet was used to enter the collected data. Variance analysis (ANOVA) was done with

the GenStat software (11th edition). The data relating to the proportions (or percentage) have been transformed by angle arcsin√P. When the analysis of variance reveals a significant difference, the Student-Newman-Keuls multiple comparison test was used to compare the means of the different variables used at 5% level.

Results

Biological efficiency of *C. annuum* and *S. hispidus* hydro-alcoholic formulations against *B. dorsalis* female's adult after 24 hours.

After 24 hours, the average effect of the different concentrations of the *C. annuum* and *S. hispidus* formulations (80.86%) is an increase of *B. dorsalis* adult female mortality of 898.44% compared to the untreated control (fig. 1).

Between the different concentrations of *C. annuum* and *S. hispidus*, the *S. Hispidus* concentration of 9.00 g/L and the one of 15g/L led to a mortality rates of 1000% compared to the untreated control and had no significant difference. The *C. Annuum* hydro-alcoholic concentration of 7.50g/L, 12.50g/L and the one *S. hispidus* 4.5g/L got 900%; 918.22% and 918.22% mortality respectively compared to the untreated control. The statistical analysis did not show any significant difference between them at the 5% level. The bait success and half-dose of *C. annuum* (3.75g/L) showed mortality rates of 693.33% and 859.11% respectively compared to the untreated control.

The best mortalities has been obtained with the average concentration and the high concentrations of *S. hispidus* hydro-alcoholic formulations. The hydro-alcoholic formulations of *C. annum* were more efficient than the control formulation GF 120.

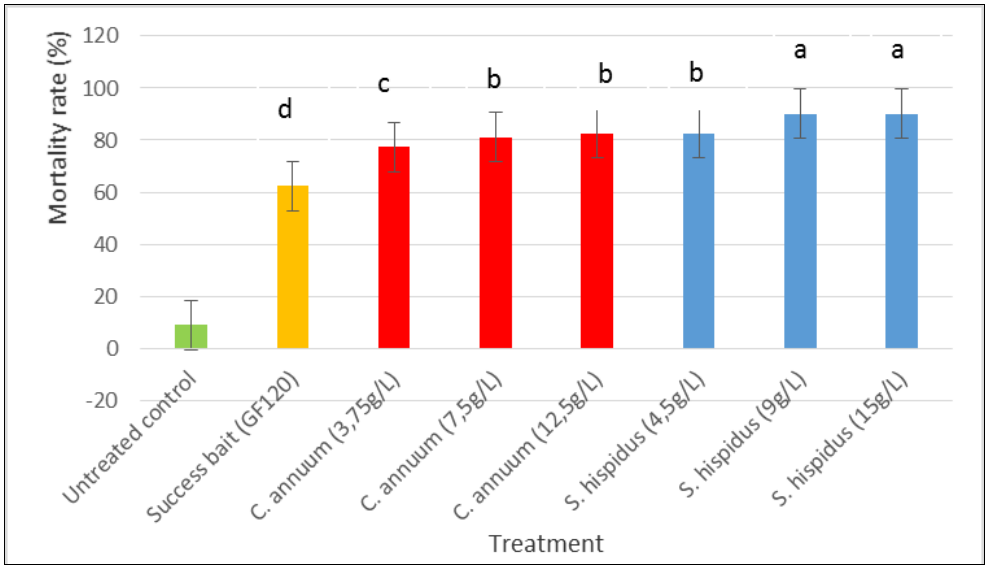

Fig 1: Efficacy of *C. annuum* and *S. hispidus* hydroalcoholic formulations against *B. dorsalis* females' adults after 24 hours of observations

Biological efficiency of *C. annuum* and *S. hispidus* hydro-alcoholic formulations against *B. dorsalis* females' adults after 72 hours

Statistical analysis showed that there is a very significant difference between the different formulations of *C. annuum* and *S. hispidus* (p<0.001) (fig. 2).

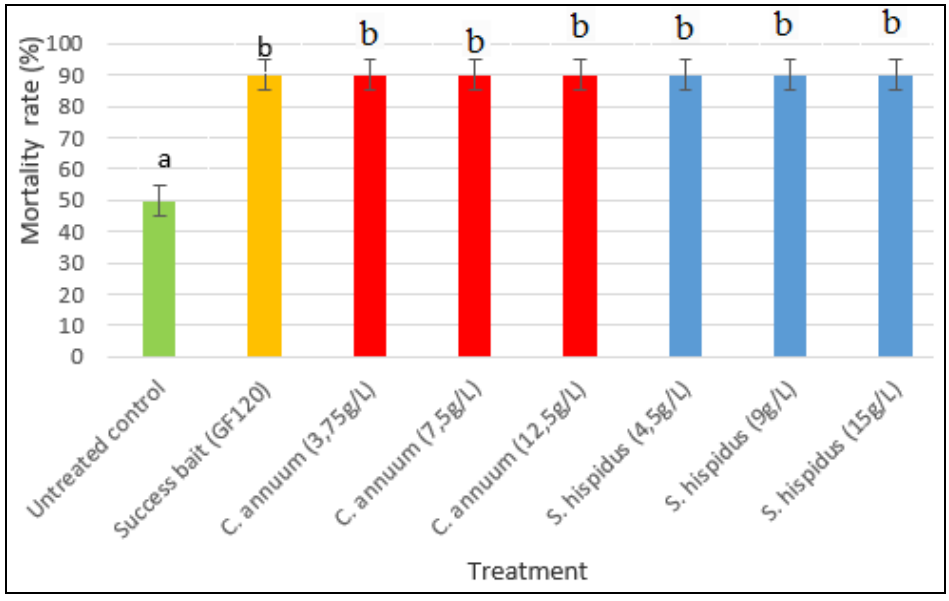

Fig 2: Efficacy of *C. annuum* and *S. hispidus* formulations on *B. dorsalis* females after 72 hours of observations.

After 72 hours, the average effect of the different insecticides (90%) is an increase of *B. dorsalis* females' adult mortality of 180.73% in comparison with the untreated control. The different concentrations of *C. annuum* and *S. hispidus*, led to a mortality of 180.73% compared to the untreated control and did not differ significantly between them at the 5% level by the Newman-keuls test.

Biological efficiency of *C. annuum* and *S. hispidus* hydro-alcoholic formulations against *B. dorsalis* adults' males after 24 hours and 72 hours.

Statistical analysis showed that there is a very significant difference between the different formulations of *C. annuum* and *S. hispidus* (Table 1).

Table 1: Efficacy of *C. annuum* and *S. hispidus* hydro-alcoholic formulations on adult males of *B. dorsalis*.

Treatment	Observations period					
	24h			72h		
	Without Transf. (%)	After anglarc sin√P	% to control	Without Transf. (%)	After anglarc sin√P	% to control
Untreated control	10.00	14.00 e	-	60.00	51.52 b	-
Successbait 0.24 g/L	76.00	60.76 d	434.00	100.00	90.00 a	174.68
C. annuum 3.75 g/L	94.00	81.00 c	578.00	100.00	90.00 a	174.68
C. annuum 7.50 g/L	98.00	86.32 b	616.57	100.00	90.00 a	174.68
C. annuum 12.50 g/L	100.00	90.00 a	642.86	100.00	90.00 a	174.68
S. hispidus 4.50 g/L	96.00	82.64 c	590.29	100.00	90.00 a	174.68
S. hispidus 9.00 g/L	100.00	90.00 a	642.86	100.00	90.00 a	174.68
S. hispidus 15.00 g/L	100.00	90.00 a	642.86	100.00	90.00 a	174.68
Mean		74.30			85.19	
CV(%)		4.00			5.40	
S.e.d (df=28)		3.00			4.56	
e.s.e (Sx)		1.34			2.04	
Probability		< 0.001			< 0.001	

N.B: The means which are assigned with the same alphabetic letters in the same column are not significantly different according to the Student Newman-Keuls test at the 5% level.

After 24h, the average effect (82.96%) of the different concentrations of C. *annuum* and *S. hispidus* hydro-alcoholic formulations is *B. dorsalis* males' adults' mortality increase of 492.57% compared to the untreated control (table 1). Between the different hydro-alcoholicconcentrations, *S. hispidus* (15.00 g/L), *S. hispidus* (9g/L) and *C. annuum* (12.5g/L) showed each a mortality of 6.43 times the one of untreated control and did not differ significantly between them at the 5% level of Newman-Keuls test. The half concentration of *C. annum* (3.75g/L) and *S. hispidus* (4.50 g/L) got some mortalities of 578.00% and of 590.29% respectively compared to the untreated control. There is no significant difference between them. According to the control product, bait success and *C. annuum* (7.50 g/L) they showed a mortality rate of 434.00% and of 616.57% respectively compared to the untreated control. The hydro-alcoholic formulations of *S. hispidus* at 9.00 g/ L; 15.00 g/L and the formulation of *C. annum* 12.50 g/L were the most efficient. They led to an increase mortality of 48.12% in comparison with the control product Bait Success (GF120).

After 72 hours, the average effect of the different concentrations of the *C. annuum* and *S. hispidus* formulations (90%) is an increase in mortality of adult males of *B. dorsalis* of 174.68% compared to the untreated control (table 1)..The different hydro-alcoholic concentrations of *C. annuum* and *S. hispidus*, led to a mortality rate of 174.68% compared to the untreated control and did not differ significantly between them at the 5% level.

Biological efficiency of *C. annuum* and *S. hispidus* hydro-alcoholic formulations against *B. dorsalis* adults (males + females) after 24hours and 72 hours

The statistical analysis showed that there is a very significant difference between the different formulations of *C. annuum* and *S. hispidus* ($p<0.001$) (Table 2).

The average effect of the different concentrations of the *C. annuum* and *S. hispidus* formulations (80.50%) is an increase in adult mortality of *B. dorsalis* of 468.50% compared to the untreated control. *S. hispidus* 15.00 g/L, *S. hispidus* 9.00 g/L which are the most efficient led to an increase mortality of 535.59% in comparison with the untreated control, and an increase of 52.34% in comparison with the GF 120.

The half concentration of *C. hispidus* 4.50g/L, *C. annuum* 7.50 g/L and *C. annuum* 12.50 g/L which are not different mathematically between them, led to an increase mortality which varied from 473.16% to 499.16% in comparison with the untreated control. In comparison with the GF 120, this increase mortality varied from 37.37% to 43.60%.

The low concentration of *C. annuum* 3.75 g/L led to increase mortality of 439.41% in comparison with the untreated control and of 29.28% in comparison with the control product.

Success bait 0.24 g/L showed a mortality of 417.23 in comparison with the untreated control.

After 72 hours,the average effect (90%) of*C. annuum* and *S. hispidus* hydro – alcoholic formulations, is an increase of 77.87% *B. dorsalis* adult's mortality compared to the untreated control. Between The different concentrations of *C. annum* and *S. hispidus*, there is not a significant difference and with the control product Bait Success.

Table 2: Efficacy of *C. annuum* and *S. hispidus* hydro-alcoholic formulations on adults (males + females) of *B. dorsalis*.

Treatment	Observations period					
	24h			72h		
	Without Transf. (%)	After anglarc sin√P	% to control	Without Transf. (%)	After anglarc sin√P	% to control
Untreated control	8.00	14.16 e	-	59.00	50.60 b	-
Successbait 0.24 g/L	75.00	59.08 d	417.23	100.00	90.00 a	177.87
C. annuum 3.75 g/L	93.00	76.38 c	539.41	100.00	90.00 a	177.87
C. annuum 7.50 g/L	96.00	82.06 b	585.03	100.00	90.00 a	177.87
C. annuum 12.50 g/L	98.00	84.84 b	599.16	100.00	90.00 a	177.87
S. hispidus 4.50 g/L	96.00	81.16 b	573.16	100.00	90.00 a	177.87
S. hispidus 9.00 g/L	100.00	90.00 a	635.60	100.00	90.00 a	177.87
S. hispidus 15.00 g/L	100.00	90.00 a	635.60	100.00	90.00 a	177.87
Mean		72.21			85.08	
CV(%)		6.30			4.40	
S.e.d (df=28)		4.56			3.76	
e.s.e (Sx)		2.03			1.68	
Probability		< 0.001			< 0.001	

Discussion

The biological efficacy of the different formulations of *C. annuum* and *S. hispidus* depends on the nature of the active ingredients, their modes of action, theirapplication rates, and thepersistence of action and duration of exposure to the organism. It also depends on some exogenous factors such as temperature, insect anatomy and morphology, the permeability to the cytoplasmic cell for the organic fractions and the different formulations [13]. Added that the increase of the chemical compounds toxicity is in relationship with the presence or not of some atoms or toxic groups in the molecular structure of the active ingredient as Cl, Br, I, F, Hg, Cu, etc…

During these tests, the biological efficacy of the *C. annum* formulations (kamboubactroceraticide1) was weak in comparison with the one of *S. hispidus* (kamboubactroceraticide 2) after 24hours against male's adults, female's adults and against males + females adults (fig.1, fig 2, Table 1 and table 2).It could be related to their concentrations. However, the obtainedresults could depend on the nature of the active ingredients contained in these organic fractions used for the formulations on the one hand and on the other hand depend on *B. dorsalis* sensitivity to the products. The mortalities of *B. dorsalis* were total after 24 hours with the average and the high concentrations of hydro-alcoholic concentrations of *S. hispidus*. This level of toxicity could be getting with hydro-alcoholic concentrations of *C. annuum* only after 72 hours.

According to the formulations of *S. hispidus* the same efficacy has been obtained by Kambou and al [16] against white flies with methanol extracts of *S. hispidus* [6] also showed that *S. hispidus* is a toxic plant well known in Africa.

The various phytochemical compounds of *C. annuum* and *S. hispidus* could have toxic effects by contact and/or ingestion in adults of *B. dorsalis*. It is also not impossible that insects died by inanition due to repellent or anti-fouling effects [10]. This could justify this high adult mortality rate of *B. dorsalis* after 72 hours.

Indeed, the presence of an active substance is not the only determining factor; its concentration in the organ is a crucial point in achieving the blockage of food intake. This could explain the high mortality rate at the high concentrations of the different formulations based on extracts of *C. annuum* (12.5g/L) and *S. hispidus* (15g/L) after 24 hours. Anti-appealing effects are thought to cause paralysis of peristaltic movements of the insect's intestine [12]. This could explain the high adult mortality of *B. dorsalis* after the application of formulations based on extracts of *C. annuum* and *S. hispidus* in 24h and 72h.

In an experimentation of the natural substances organic fractions efficiency against *C. cosyra* [21] showed that the ethyl acetate fraction of *C. annuum* was very rich in sterols, triterpenes, Alkaloïds compounds. But the methanol extract of *S. hispidus* was very rich not in sterols and triterpens but contained a lot of anthraquinons, coumarins and derivated, cardenolids, alkaloids, tanins, flavonoïds, anthocyanocids. Some of these compounds as anthraquinons, saponins and flavonoïds are recognized to have insecticidal properties. For example according to Nozzolillon and Bouchelta [17, 5] saponins induce pesticidal and growth, ovogenesis inhibitory effects [1] against insects. This could justify the mortality of adults of *B. dorsalis* exposed to the different formulations based on extracts of *C. annuum* and *S. hispidus*.

Conclusion

The hydro-alcoholic formulations of *S. hispidus* (kamboubactroceraticide 2) at the concentration of 9g/L and 15 g/L were the most efficient against *B. dorsalis* females, males and females +males adults. They led to 100% mortality after 24 hours in comparison with the untreated control. The hydro-alcoholic formulations of *C. annuum* (kamboubactroceraticide 1) only at the concentrations of12.5 g/L got a mortality of 100% against the male adults of *B. dorsalis* in comparison with the untreated control. All these formulations were more efficient than the control product Bait success (GF 120). The experimentation of these formulations (efficacy, selectivity on mango' trees) in field conditions will help to choose the concentration and the right rate for an integrated pest program against mangoes fruit flies in the world.

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