



Original Research Article

Phytochemical composition and biological efficiency of *Cleome viscosa* L. leaves, *Parkia biglobosa* (Jacq.) Br. Ex G. Don pods powders extracts against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) of tomato

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A study of biological efficiency of six natural substances (*Cassia nigricans*, *Cassia occidentalis*, *Cleome viscosa*, *Myragina inermis*, *Ocimum basilicum*, *Parkia biglobosa*) organic extracts against *Helicoverpa armigera* (Hubner) which causes big damages to tomato has been done on laboratory conditions. The extraction of plant materials has been done using solvents with increasing polarity according to Nair's method. The biological tests on *H. armigera* larvae at a dose of 100 µg / ml of nutritive diet, has been done according to a Fisher block design of 19 treatments in 4 replications. The number of dead and dwarf larvae has been counted by direct observation of the larvae using a binocular magnifying glass. Phytochemical analysis of the more active extracts of *C. viscosa* and *P. biglobosa* was done according to the described method of Ciulei. The pods of *P. biglobosa* contained more polar constituents and fewer apolar constituents than *C. viscosa* leaves. Efficacy tests showed that the methanolic extracts of *C. viscosa* and *P. biglobosa* led to the highest mortality rates of 42.65% and 21.65% respectively. The main nanism rate was at *M. inermis* with 68.20%. Extracts from other vegetable powders gave a lower rate of nanism which varied from 0% to 27.5%. Phytochemical analysis of active extracts revealed that *C. viscosa* and *P. biglobosa* contain some steroids, triterpenes, tannins, flavonoids, saponosides, anthocyanins and anthraquinons which could have insecticidal properties. The development of formulations based on methanolic extracts of *C. viscosa* and *P. biglobosa* would make it possible to develop bio-insecticides for an integrated pest management against *H. armigera*.

Key words: *Cleome viscosa*, *Parkia biglobosa*, *Helicoverpa armigera*, tomato, Burkina Faso.

INTRODUCTION

In Burkina Faso, tomato (*Lycopersicon esculentum*) production occupy second place after bulbous onions in terms of area planted and quantity of production. It contributes to food security by providing high-quality food products, rich in vitamins and trace- elements,

cardiovascular diseases prevention, vitamin deficiency and certain cancers (FAO / WHO 2004).

According to WHO (2002), a low fruit and vegetable consumption led for about 31% of ischemic heart disease and 11% of cerebrovascular accidents, in the world. In

Burkina Faso, the consumption of fruits and vegetables is low and estimated at about only 55-63 g / d / capita (CPF., 2011). In these recent years, tomato production has decreased considerably from 289,572 tones on 11,766.39 ha in 2013-2014 (MARHASA., 2014) to 200,518.93 tons on 23,054.45 ha in 2016-2017 (MAAH., 2017). This is a decrease production in about 89,000 tons. This decline is correlated with serious constraints including parasite pressure due to pests as *Helicoverpa armigera* (Hübner), *Bemisia tabaci* (Gennadius), which cause 50% to 100% loss of crop and pathogens as *Ralstonia solanacearum* Smith, *Fusarium oxysporum* Schltld (IFDC, 2007; Blancard et al., 2009; Ouattara et al., 2017). This leads to a decline of the production and of income from market gardeners. *Helicoverpa armigera* (Lepidoptera: Noctuidae) is one of the most important insect pests of tomato. The caterpillars of *H. armigera* cause big damages to tomato: the leaves are gnawed, flowers are cut and the fruits are full of dug galleries rot or fall if young, they were attacked.

Chemical control based on the use of synthetic insecticides has long been used. Thus, the efficacy of certain insecticides and bioinsecticides as chlorpyrifos, spinosad, abamectin (Carneiro et al., 2014, Vojoudi et al., 2011), Emamectin-benzoate, Spinetoran, Indoxacarb (Hamdi et al., 2013) and other insecticides as Flubendiamide, acephate, methomyl, chlorantraniliprole, etc. (Wakil et al., 2012; Perry et al., 2011; Hamed et al., 2003; Zahid et al., 2003) against *H. armigera* has been studied. However, resistance phenomena to certain insecticides as indoxacarb (Bird et al., 2017), Fenvelerate (Jou et al., 2012), Endosulfan (Torres-Vila et al., 2002), Emamectin-benzoate as well as BT compounds (*Bacillus thuringiensis*) have been underlined. Only recent insecticides of the diamide family are still effective against *H. armigera*. The mechanisms and causes of some of these resistances have been studied by Ahmad (2007). The side effects of certain molecules on some *H. armigera* parasitoids as *Habrobracon hebetor* have been evaluated by Dastjerdi et al. (2008).

Also more and more, in order to avoid these secondary effects on beneficial entomological fauna, consumer's health as well as on the environment, the use of natural substances against *H. armigera* appeared, mostly with neem products as Neemazal (1% EC azadirachtin) Neemix (0.25% EC azadirachtin), Neemarin (0.15% EC azadirachtin) (Ahmad et al., 2015). Biological efficacy studies of *C. viscosa* and *Sinapis alba* seeds organic extracts were conducted by Sivaraman et al. (2014). Therefore in this way, for the first time, a study of the biological efficiency of organic extracts of *P. biglobosa* pod powders and leaves of *C. viscosa*, *C. occidentalis*, *C. nigricans* and *M. inermis* against *H. armigera* of tomato has been done.

MATERIALS AND METHODS

Plant material

The plant material was consisted of *P. biglobosa* pods

powder and leaves powder of *O. basilicum*, *C. occidentalis*, *C. nigricans*, *M. inermis* and *C. viscosa*. Plant material was collected in 2016 in western Burkina Faso, for their biocidal properties. These plant materials were dried at room temperature (about 30 °C) on ventilated racks, protected from light and then reduced to powder using a blender (BLG-450).

Animal material

Collection of caterpillars: *H. armigera* (Hübner) caterpillars stage L2, L3, L4 or L5 were collected using soft forceps. They were then placed in stalls previously equipped with nutritious diets, prepared with agar, corn flour, brewer's yeast and wheat germ the day before each collection. Two collections were made: the first at the end of the dry season (May 2017) on tomato fields in Di region, and the second during the late raining season (October 2017) on cotton field at Datome / Safane, in the western region of Burkina Faso.

Larva breeding: these collected caterpillars were breed in the laboratory (at the temperature of 25° C, at a relative humidity of 70% and with 12 hours of periodicity period) on entomological containers with a nutritive diet, which is stocked every two, three days. The diet is stocked till the caterpillars appear. At each time, when the caterpillars appeared, they were identified and sexed with the help of a binocular microscope. They were kept in different laboratory' conditions (males, at 25 ° C, females in a phytotron at 15 ° C for 72 hours). After this conditioning, they are transferred into transparent jars covered with a lid of toilet paper until the adults emerge (72 hours later). At each emergence, 50% of females and 50% of males are recovered and gathered in jars covered with canvases, in which mating and laying have been done. Infertile eggs (1st to 3rd day) are eliminated. The fertile eggs of the 4th, 5th and 6th days are transferred to a hatching pot. The L1 larvae at the end of the hatching (after 72 hours) constituted the animal material for organics extracts tests.

Preparation of plants extracts

The extracts studied were obtained according to the current method of solid-liquid extraction by successive exhaustion with solvents of increasing polarity (Kambou et al., 2008). A mass of 150 g of each vegetable powder was macerated in a 1 liter Erlenmeyer flask with analytical n-hexane as the initial extracting solvent in 1: 10 ratios; m / v at room temperature of the laboratory (30 ± 1 ° C) and with mechanical stirring for 24 hours. The plant material and the extracting solvent after maceration were transferred to a percolator and then leached with small portions of extracting solvent. The successive leaching of each vegetable powder was carried out with analytical n-hexane, ethyl acetate and methanol. The various organic extracts obtained were concentrated under reduced pressure in a rotary evaporator and then dried in a ventilated oven at 45 ± 1 ° C. The masses of the dry extracts obtained as well as

the extraction yields were determined.

Biological tests on *H. armigera* larva

1.5 mg of each dry extract obtained was crushed and dissolved in 1.5 ml of PBS buffer. A volume of 0.1 ml of this stock solution was pipetted into each of the 15 wells of a plastic box previously containing 1 ml of diet. Excess moisture from the contaminated diet (0.1 mg / ml) was removed by evaporation.

The biological efficacy test has been done in four replications of Fisher block design, which consisting of 19 treatments including 18 organic extracts and an untreated control (PBS buffer). L1 larvae between 1 mm and 1.20 mm high in the entomology laboratory were individually placed, using soft forceps, in 15 stalls per repetition containing the contaminated nutrient diet. The cubicles were then closed hermetically with parafilm designed for this purpose and then placed under breeding conditions in the entomology laboratory (25 ° C, 70% RH, PP: 12h).

The treated larvae were observed every 24 hours in the laboratory, for seven days. A larva is said to be dead if it does not turn around after it is placed on its back; a larva is called dwarf if its size, after seven days remains less than or equal to 2 mm, 10% of the normal size (L: 15-20 mm). Cumulative numbers of dead or dwarf larvae collected during the seven days of observation were used to calculate larval mortality and nanism.

Phytochemical analysis of the active extracts of *C. viscosa* and *P. biglobosa*

The chemical screening of the most active extracts was carried out by the qualitative method described by (Ciulei, 1982). The aim is to highlight the bioactive phytochemical groups involved in the observed insecticidal activity.

Statistical analysis and data processing

A variance analysis of data (Dospiehov., 1979), at the level of 5% followed by Newman-Keuls test has been done using Genstat Discovery Ed. 4 and XLStat version 2015 softwares.

RESULTS

Yields of organic extraction

The extraction yield with n-hexane varied from 0.45% - 2.60%. The highest rate of hexanic extracts was observed with *C. occidentalis* and the lowest with *P. biglobosa*. That with ethyl acetate fluctuated from 0.54% - 2.10%. *M. inermis* gave the highest rate in ethyl acetate extracts while the lowest rate was obtained with *P. biglobosa*.

The extraction yield with methanol was 5.65% - 44.09%. *P. biglobosa* recorded the highest rate in methanolic extracts and *O. basilicum* the lowest.

The results obtained generally showed that the vegetable

powder of *C. occidentalis* contains more apolar substances unlike that of *P. biglobosa* which has more polar substances. Also, *O. basilicum* leaves contain more apolar substances compared to *P. biglobosa* pods in which the polar substances were more dominant (Table I).

Larvicidal activity of organic extracts on *H. armigera*

The variance analysis showed that, the studied organic extracts significantly affected the mortality of *H. armigera* larvae (Figure 1).

According to the organic extracts of *O. basilicum*, the average effect was an increase of 9.84% mortality compared to the untreated control. The mortality rate caused by *O. basilicum* extracts varied from 8.80% - 17.90%. The lowest mortality rate was obtained with ethyl acetate (8.80%) while the high rate was recorded with n-hexane (17.90%) in comparison with the untreated control.

In *C. Nigricans* extracts, the average recorded effect was 103.48% more than the untreated control. The mortality rate varied from 20.20% - 33.98%. The lowest mortality rate 20.20% was obtained with the n-hexane extract while the high rate was recorded with the ethyl acetate extract is 33.98% compared to the untreated control.

With *C. viscosa*, the average effect of organic extracts was 133.20% higher than the untreated control. The mortality rate caused by *C. viscosa* extracts varied from 19.50% to 42.65%. The lowest mortality rate was obtained with hexane extract (19.50%) while the high rate was recorded with methanol (42.65%).

The activity of *C. occidentalis* extracts showed an average effect of 18.64% more than the untreated control. The mortality rate from extracts of *C. occidentalis* ranged from 3.75% to 20.82%. The lowest mortality rate was obtained with the methanol extract (3.75%) while the high rate was recorded with that with n-hexane (20.82%).

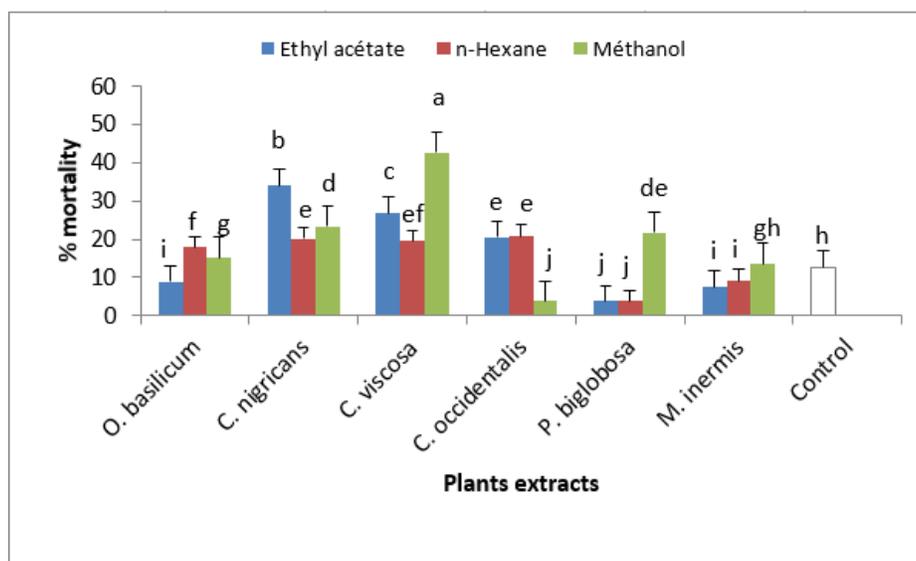
The mortality rate caused by *P. biglobosa* extracts varied from 3.75% to 21.65%. The lowest mortality rate was obtained with the n-hexane extract (3.75%) while the high rate was recorded with the methanol extract (21.65%). The average effect recorded was 23.49% less than the untreated control.

With *M. inermis*, the average recorded effect of the extracts was 20.87% less than the untreated control. The mortality rate caused by extracts of *M. inermis* varied from 7.50% - 13.55%. The lowest mortality rate was observed with the ethyl acetate extract (7.50%) while the high rate was with the methanol extract (13.55%).

Overall, at the rate of 0.1 mg dry extract per ml of nutritious diet, the studied extracts induced variable larval mortality rates between 3.75% and 42.65% with an average increase of 36.80% compared to the untreated control (12.7%). The most lethal extracts were the methanolic extracts and ethyl acetate of *C. viscosa* with 42.65% respectively of 335.83% and 26.7% or 210.24% in comparison with the untreated control. The lethality of the methanolic extract of *P. biglobosa* pods was 21.65%, ie 170.47% in comparison with the untreated control. The

Table 1. Yields of vegetable powders extracts

Plant species	N-Hexane		Yields		methanol	
	Mass (g)	Yield (%)	Mass (g)	Yield (%)	Mass (g)	Yield (%)
<i>O. basilicum</i>	6.71	2.24	5.77	1.92	16.95	5.65
<i>C. viscosa</i>	3.67	1.47	4.16	1.66	24.29	9.72
<i>C. occidentalis</i>	7.79	2.60	2.81	0.94	53.00	17.66
<i>C. nigricans</i>	5.43	2.17	3.80	1.52	55.83	22.33
<i>M. inermis</i>	3.27	2.31	2.96	2.10	31.56	22.43
<i>P. biglobosa</i>	0.62	0.45	0.75	0.54	60.72	44.09

**Figure 1.** Effect of organic extracts on larval mortality of *H. Armigera*

mortality rate of the other extracts remained low between 3.75% and 20.83% (Figure 1).

Growth inhibitory activity of organic extracts on *H. armigera* larvae

The average growth inhibitory effect caused by *O. basilicum* was 21.94% higher than the untreated control. The rate of nanism varied from 3.75% to 12.85%. The lowest rate of nanism was obtained with ethyl acetate extracts (3.75%) while the high rate was recorded with hexane extracts (12.85%).

In *C. nigricans* extracts, the average effect recorded was 190.83% more than the untreated control and the dwarf count ranged from 12.20% to 27.50%. The lowest rate of dwarfism was obtained with ethyl acetate extracts (12.20%) while the high rate was recorded with methanol extracts (27.50%).

At the level of *C. viscosa*, the average effect was an increase of 83.61% in comparison with untreated control. The rate of nanism caused by *C. viscosa* extracts varied by 0.00% to 18.90%. The lowest rate of nanism was obtained with hexane extract (0.00%) while the high rate

was recorded with ethyl acetate extract (18.90%).

On *C. occidentalis*, the average effect recorded was an increase of 118.33% compared to the untreated control. The rate of nanism due to *C. occidentalis* extracts varied from 10.70% to 14.45%. The lowest rate of nanism was obtained with ethyl acetate extract (10.70%) while the high rate was recorded with methanol extract (14.45%).

The average effect of recorded *P. biglobosa* extracts was 328.78% higher than the untreated control. The rate of nanism caused by extracts of *P. biglobosa* varied from 12.85% - 35.15%. The lowest level of nanism is in ethyl acetate extract (12.85%) while the high level is in methanol extract (35.15%).

The average effect of *M. inermis* extracts was 416.94% more than the untreated control. The rate of nanism caused by *M. inermis* extracts varied from 12.00% - 68.20%. The lowest rate of dwarfism was obtained with hexane extract (12.00%) while the high rate was recorded with methanol extract (68.20%).

Overall, the results on the inhibitory activity of natural extracts on larval growth of *H. armigera* showed a very highly significant difference ($P < .001$; Lsd: 1,886; Cv: 7.8%) compared to untreated control. The rate of larval nanism

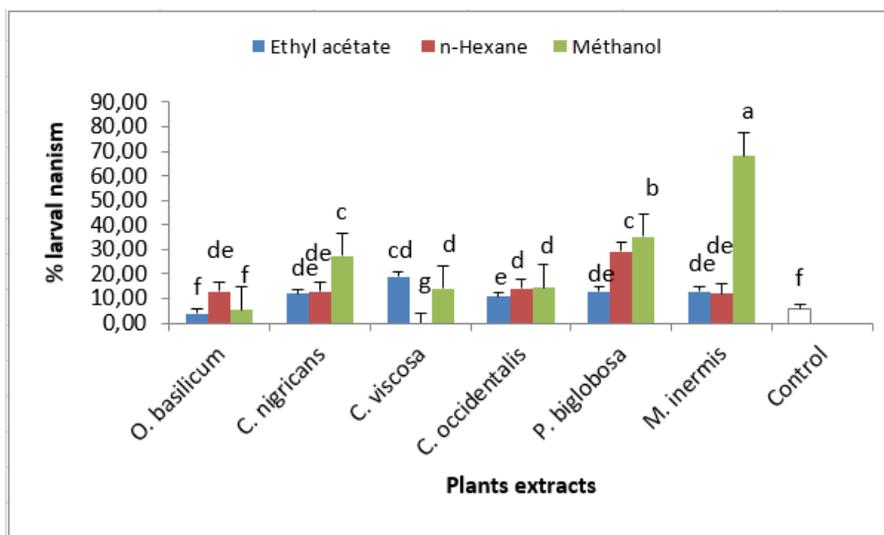


Figure 2. Effects of organic extracts on larval growth of *H. armigera*

Table 2. Chemical profile of the methanolic extracts of *P. biglobosa* and *C. viscosa*

Chemical groups	Methanolic extracts	
	<i>C. viscosa</i>	<i>P. biglobosa</i>
Sterols and triterpens	+	+
anthraquinons	+	(-)
Coumarins and derivatives	(-)	(-)
carotenoids	(-)	(-)
alkaloids	(-)	(-)
tanins	+	+
flavonoids	+	+
saponosides	+	+
anthocyanosids	+	+
Reducing compounds	±	+

Legend : + = present ; (-) = absent ; ± = traces.

varied from 0% - 68.20% (Figure 2) with a mean nanism up to 193.41% compared to the untreated control (6.00%).

The most inhibitory extracts of larval growth were obtained with the methanolic extracts of *M. inermis* (68.20% or 1136.66% in comparison with the untreated control) and *P. biglobosa* (35.15% or 585.83% in comparison with the untreated control) and *P. biglobosa* hexane extract (29.18% or 486.33% in comparison with the untreated control). The rate of other extracts nanism was low and varied between 0% and 27.5% (Figure 2).

Phytochemical analysis of organic extracts of *C. viscosa* and *P. biglobosa*

The chemical characterization tests in solution of the active extracts made it possible to demonstrate, in the methanolic extracts of *C. viscosa* and *P. biglobosa*, groups of potentially bioactive chemical compounds, namely: steroids, triterpenes, anthraquinones, tanins, flavonoids,

saponosides and anthocyanosides. The presence of anthraquinones was particularly evident in the methanolic extract of *C. viscosa* but not in that of *P. biglobosa* (Table 2).

DISCUSSION

The biological efficiency of the various organic extracts of natural substances against *H. Armigera* depends on the chemical structure of their active ingredient, their mode of action, their application rate, their persistence, their exposure to the organism. It is also related to exogenous factors such as temperature but also the anatomy and morphology of the insect, the permeability of the cell envelope to the product. During this experiment, the natural substances efficiency was certainly determined by the factors mentioned above.

According to *C. nigricans*, all organic extracts were found to be effective against *H. Armigera*. However, the ethyl

acetate extract proved to be the most effective, confirming the work of KAMBOU et al. (2008 and 2015) concerning the presence of 3 anthraquinones (Emodin, citreorosein, Emodic acid) and a flavonoid (luteolin) with insecticidal properties, effective against the *Helicoverpa* genus and contained mainly in the ethyl acetate extract.

In *C. occidentalis* extracts, hexane and ethyl acetate extracts proved to be better, hence the presence of flavonoid glycosides (Yadava and Satmani, 2011). This experiment confirmed the recent work of Ankita and Sangeeta (2018) about the larval activity of *C. occidentalis* against *H. armigera* and *Spodoptera litura* of tomato.

The hexane and methanolic extracts of *O. Basilicum* resulted in significant mortalities due to the presence of secondary metabolites such as camphor, limonene and β -caryophyllene, geramacrene-D mentioned by Singh et al. (2014) that have insecticidal properties against *H. armigera* previously noted by Rozman et al. (2006) and Liska et al. (2010).

At *P. biglobosa*, only the methanolic extract was effective against *H. armigera*. It was known that aqueous extracts of *P. biglobosa* pods had herbicidal properties against *Striga hermonthica* (Kambou et al., 2000) and against *Striga gesteneroides* of cowpea (Lado et al., 2018) due to the presence of β -sistosterol as active ingredient. Indeed, during this experiment, the phytochemical analysis reveals the presence of sterols and triterpenes to which belongs β -sistosterol which is a steroidal glycoside, as well as tannins, flavonoids, saponosides, anthocyanosides but not anthraquinones.

It is not excluded that one of the compounds or the synergistic effect of these compounds is responsible for this biological activity. On the other hand, the phytochemical analysis reveals, in addition to these chemical compounds present, the absence of anthraquinones in *P. biglobosa*, known for their insecticidal properties (Table 2).

According to *C. viscosa*, only the methanolic extract of the leaf powder showed a significant efficacy on *H. armigera* because of the presence of chemical compounds large number likely to be responsible for this activity. This methanolic extract thus contains six compounds (Figure 2) against three identified by Sivaraman et al. (2014) in the seeds of *C. viscosa* and which are alkaloids, quinones and tannins.

According to the organic extracts of *M. inermis*, they have practically proved to be ineffective against *H. armigera*, probably due to the absence of toxic active ingredients against this pest. In fact, the study of *H. armigera* nanism reflects its high level in *M. inermis* (Figure 2) and less in the other organic extracts which previously displayed biological activities against this insect pest of tomato (Figure 1) This fact reflects the absence of an active ingredient in *M. inermis* able for irreversibly disrupting biochemical reactions or able to destroy enzymes in the insect cytoplasm which could lead to a high mortality rate. In the methanol extract of *M. inermis*, the rate of nanism was high. At the opposite, the rate of nanism remained low in the other plant materials organic extracts.

CONCLUSION

The powder of *C. viscosa* leaves contains more chemical compounds with insecticidal properties than those of the seeds tested by Sivaraman et al. (2014) against *H. armigera*. In addition to its herbicidal properties, the nere pods (*P. biglobosa*) powder also contains insecticidal properties against *H. armigera* of tomato. The fractionation, purification, identification and characterization of these active ingredients contained in the active fractions would make it possible to develop formulations that can be used in an integrated pest control program against *H. armigera*, preserving consumers' health and the environment.

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Competing interests

The authors declare that they have no competing interests.

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