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PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL EFFICIENCY OF *OCIMUM BASILICUM* L. LEAVES, *PARKIA BIGLOBOSA* (JACQ.) R. BR EX G. DON PODS, POWDERS ORGANIC EXTRACTS AGAINST *BEMISIA TABACI* BIOTYPE B (GENNADIUS) (HEMIPTERA: ALEYRODIDAE) OF TOMATO (*LYCOPERSICUM ESCULENTUM* MILLER) (SOLANALES: SOLANACEAE), IN BURKINA FASO

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ABSTRACT

A study of six natural substances organic extracts (*Cleome viscosa L.*, *Mytragina inermis* (Wild) kuntze, *Ocimum basilicum* L., *Cassia nigricans* Vahl., *Cassia occidentalis* L. and *Parkia biglobosa* Jacq.) biological efficiency on white flies which cause big damages to tomato has been done in laboratory conditions. The extraction of the substances has been done with three solvents of increasing polarity (n-Hexane, ethyl- acetate, methanol) by Nair method. The experimental design was a randomized Fisher Block of 19 treatments in 5 replications. Phytochemical analysis of the active fractions of *O. basilicum* and *P. biglobosa* have been done by the Ciulei method. The best yield of apolar extracts was obtained with *C. occidentalis* (2.60%). The yield of medium polar extracts was obtained with *M. inermis* (2.10%). The best yield of polar extracts was obtained with *P. biglobosa* (44.09%). The highest mortalities after 24 hours'observations have been obtained with methanol extract of *O. basilicum* (38.30%), ethyl – acetate extract of *C. nigricans* (37%) and with hexane extract of *P. biglobosa*

(36.58%). After 72 hours'observations, the highest mortalities (87.42%) were observed at ethyl – acetate extracts of *O. basilicum*, *C. nigricans*, hexane extracts of *C. nigricans*, *C. occidentalis.* It was 86.32% at methanol extract of *O. basilicum*. Phytochemical analysis revealed more chemical groups in the methanol extract of *O. basilicum* than the one of *P. biglobosa* hexane extract. This explains the best insecticidal activity of the methanol extract of *O. basilicum* in comparison to the hexane extract of *P. biglobosa* against *B. tabaci* of tomato.

KEYWORDS: *Bemisia tabaci*, *Ocimum basilicum*, *Parkia biglobosa* tomato.

INTRODUCTION

In Burkina Faso, the fruit and vegetable sector is a source of agricultural growth and poverty reduction, which generates nearly 400000 jobs, of which 100000 are held by women.[1] In this sector, tomatoes production is growing. For example, from 1997-1998 crop year to 2004-2005 crop year, the areas of tomato production increased by 258%.[2] Tomato production has increased from 50 158 tonnes in 2004 to 157 086 tonnes in 2008[3] and tomatoes are producing in all provinces.[4]

According to a study conducted by Burkina Faso' Ministry of Agriculture[3], tomato was the most economically profitable vegetable market in Burkina Faso and this crop brought to farmers a net income of 1 504000 FCFA / ha in undeveloped area, against 2,377,400 FCFA / ha in developed area. However the tomato industry is constantly facing several constraints. These constraints include the presence of weeds, especially Cyperaceae, diseases as bacterial wilt caused by *Ralstonia solonacearum*[5], insect pests as the tomato moth *Helicoverpa armigera*, destructive *Tuta absoluta* and especially the whitefly (*Bemisia tabaci*) which causes a lot of direct and indirect damages by transmission of pathogenic viruses.[6] White fly (*B. tabaci*) is vector of about sixty viruses including Tomato Yellow Leaf Curl Virus (TYLCV) which causes the most damage. The damage caused by viruses on tomato is enormous and can sometimes annihilate all tomato's production.[7]

Many control methods have been developed to reduce the harmful effects of *B. tabaci*, between them, for a long time the research of resistant or tolerant varieties.[8] The study of its epidemiology[9], inoculation of bacteria like *Rhizobacteria* on tomato, to increase its resistance[10], the introduction of cultural technics as the association of aromatic plants with tomato[11], biological control by the use of auxiliary insects (*Encarsia formosa*, *Eretmocerus* *eremicus, Eretmocerus mundus, Macrolophus caliginosus*) against the insect pest has been used.[12] Entomopathogenic fungi as *Lecanicillium longisporum* and *L. muscarium* have been disseminated to reduce the population density of *B. tabaci*.[13] The compatibility of entomopathogens and plant extracts are used to control whitefly.[14] Similarly, the way of virus transmission has been studied and has shown that it is done either through eggs, nymphs or often not directly by adults.[15] However, short-term chemical control seems to be the method that has been and remains the most used.

Crop protection is critical for 2-6 weeks after transplanting tomato. Also, many molecules have been used from pyrethrenoid family to neo-nicotinoids. However, the resistance phenomena of deltamethrin or Thiamethoxam[16,17,18], the inhibitors growth as pymetrozine, and juvenile hormones as Mimic pyriproxifa have been notified.[19] The resistance phenomena of some insecticides to the insect were also mentioned.[20,21] The management of this resistance[22,23] by the development of new molecules from different families[24] and by different ways of action as paralysis, knock-down and systemic action effects were applied to see the effects on the density of *B. tabaci* eggs and nymphs. If chemical control can have immediate results, the risk of harmful side effects on users, consumers and the environment remains, following the bad applications of synthetic pesticides by the illiterate farmers.[25] cited by.[26] Also, in order to minimize this risk, to find some alternatives with the use of natural products became necessary. The repellent and toxic effects of *Couripita guianensis* to reduce *B.tabaci* population density is studied.[27] Studies showed that aqueous extracts of certain spicy substances controlled 61 to 76% of white flies' infestations on green beans.[28] Organic extracts of *Swartzia madagascariensis* are 80% effective in the control of *B.tabaci*.[29] According to a study, a formulation based on aqueous extracts of *Cassia nigricans* applied resulted in an efficiency of 35.23 to 79.38% against *B. tabaci* on tomato crops.[30] It is in this same line of research that the present study deals with the biological efficacy of new organic extracts of *O. basilicum* and pods of *P. biglobosa* against *B. tabaci* on tomato.

MATERIAL AND METHODS

Material

The plant material was constituted of *P. biglobosa* pods, dried leaves of *O. basilicum*, *C. viscosa*, *C. occidentalis*, *C. nigricans* and *M. inermis* fine powders which were obtained by electric grinding in the laboratory, after drying in the shade. The animal material was white flies caught on tomato leaves. Three extracting solvents of different polarities were used to extract the substances which have to be tested on white flies: n-hexane, ethyl- acetate and methanol. The other part of the extraction material was hydrophilic cotton, rotary evaporator and laboratory glasswares.

Methods

Extraction and biological efficacy of plant products

The extracts were obtained from natural substances using standard methods, used in phyto-chemistry to extract bioactive substances according to their affinity with solvents of different polarities.[1] 150 mg each plant samplewas macerated for 24 hours successively in 1500 ml of each of these solvents: n-hexane, ethyl acetate and methanol-water (80:20, v / v). Each macerate was placed in a percolator and extracted by successive exhaustion with extraction solvents. The various extracts obtained were concentrated under reduced pressure in a rotary evaporator. The concentrated extracts obtained were dried in an oven ventilated at 45 ± 5 ° C. The yields of the various extracts were calculated from the following formula:

Yield = (ES / MS) * 100 where ES = mass of dry extract and MS = mass of dry matter.

For the study of natural substances extracts biological efficiency against *B. tabaci*, eighteen (18) plant extracts and a control in five replications were used for this test. 50 mg of each solid extract were dissolved in 1 ml of acetone in a microtube using a Vortex. Then the whole was poured into an Erlenmeyer flask containing 99 ml of distilled water. The white flies were captured by suction in micro-tubes and transferred to the tubes which are placed one to two minutes in a refrigerator, to immobilize the white flies (by the low temperature) to prevent them from flying, when they were introduced into Petri dish boxes containing the leaves, already imbibed with extracts. The tomato leaves were soaked with solutions of these extracts, ventilated and placed in Petri dishes. In each box, twenty white flies were introduced. The mortality was evaluated after 24^{th} hour and 72^{th} hour.

Fractionation of *O. basilicum* and *P. biglobosa* active extracts

For the determination of the profile, on the thin layer chromatography, test samples of 6.72 gextracts of the active extracts of *O.basilicum*methanolic and of *P. biglobosa* hexanic were dissolved in 50 ml of extracting solvent. Each extracted solutions was mixed with 50 g of silica gel for column, in the ratios 1/10 (m/m). The silica gel and extract mixture was homogenized with a spatula and then dried in a ventilated oven at 40-50 ° C. To the dried mixture, it was added 700 ml of the less polar extracting solvent of the series. The methanolic extract of *O. basilicum* was successively fractionated with chloroform (1250 ml); ethyl acetate (1250 ml), acetone (1750 ml) and methanol (1250 ml). The n-hexane extract of *P. biblobosa* was successively fractionated with toluene (700 ml) and ethyl acetate (500 ml).

The fractions of eachobtained extract were concentrated under reduced pressure in a rotavapor and dried under ventilation in an oven at a temperature of 35-40°C. The phytochemical profile of each extract fraction has been determined by thin-layer chromatography to target groups of substances that may be isolated. A volume of 10 µl of each extract fraction was deposited on a stationary phase of silica gel (Silicagel, G-60, F 254). The chromatoplates were then migrated into two solvent systems of different polarity (apolar and polar).

Medium polar to polar constituentsof the extracted fractions migrated into a mobile phase composed of: ethyl acetate; methanol and distilled water in the proportions: 77: 15: 8; v / v / v, while apolar ones of the extract fractions have been in a solvent systemconsisting of: toluene and ethyl acetate in the proportions: 93: 7; v / v. After migration, the chromatoplates were dried (using a hair dryer), then observed in daylight and under ultraviolet radiation at 254 nm and 366 nm before and after revelations ofbioactive chemicals compounds spots withspecific chemical reagents.

Phytochemical analysis of active extracts

For the extracts preparation which will be have to be screened, the method of CIULEI[31] has been used. A test of 15 g of each plant material was successively exhausted by percolation with n-hexane, chloroform, ethyl acetate, acetone and methanol. The most active identified extract fractions, namely: *P. biglobosa* n-hexane and *O. basilicum* methanol were subjected to phytochemical screening.

A portion of the extracts were used to identify some chemical groups as polyphenolic compounds (tanins), saponosides, reducing compounds, alkaloid salts. Another portion of the methanol extracts was hydrolyzed in an acidic medium to extract total genomes for steroid and triterpenic glycosides, flavone glycosides, anthracenosides (anthraquinones), coumarins and anthocyanosides.

Data processing and analysis

The collected data during these different studies were processed with the Microsoft Office 2013, Excel software. The statistical analyses were carried out with the GenStat Edition 11 software. Analysis of variance and comparison of averages were made according to the Newman-keuls test at the 5% probability level.

RESULTS

Yield of extraction of plant products

The yield of apolar lipophilic extracts (with n-hexane) varied from 0.45% (*Parkia biglobosa* extract) to 2.60% (*Cassia occidentalis* extract). The yield of medium polar extracts (with ethyl acetate) varied from 0.54% (*Parkia biglobosa* extract) to 2.10% (*Mytragina inermis* extract). The yield of polar extracts (with methanol) varied from 5.65% (*Ocimum basilicum* extract) to 44.09% (*Parkia biglobosa* extract) (Table I).

Table I: Yield of plant extracts.

Samples	Taking (g)	n-Hexane		Ethyl acetate		Methanol	
		Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	yield (%)
Ocimum basilicum	300	6.71	**2.24**	5.77	**1.92**	16.95	**5.65**
Cleome viscosa	250	3.67	**1.47**	4.16	**1.66**	24.29	**9.72**
Cassia occidentalis	300	7.79	**2.60**	2.81	**0.94**	53.00	**17.66**
Cassia nigricans	250	5.43	**2.17**	3.80	**1.52**	55.83	**22.33**
Mytragina inermis	150	3.25	**2.31**	2.96	**2.10**	31.56	**22.43**
Parkia biglobosa	150	0.62	**0.45**	0.75	**0.54**	60.72	**44.09**

Biological efficiency of natural substances organic extracts against *B. tabaci*.

After 24 hours' observations, the average effect of all organic extracts (28.88% mortality) is an increase of 185.13% incomparison with the untreated control. The best mortality rate was obtained with the methanol extract of *O. basilicum* which is 2.5times higher thanthe one of the untreated control. It is followed by the ethyl acetate extract of *C. nigricans* which is not different with hexane extract of *P. biglobosa* (figure 1).

Hexane extracts of *C. occidentalis*, methanol extracts of *C. viscosa*, *M. inermis*, *C. nigricans* and the hexane extract of *M. inermis* are not statistically different each other. The lowest rates, which varied from 23.58% to 24.20%, are in the ethyl acetate extract of *C. viscosa*, the hexane extract of *O. basilicum* and the methanol extract of *C. occidentalis*.

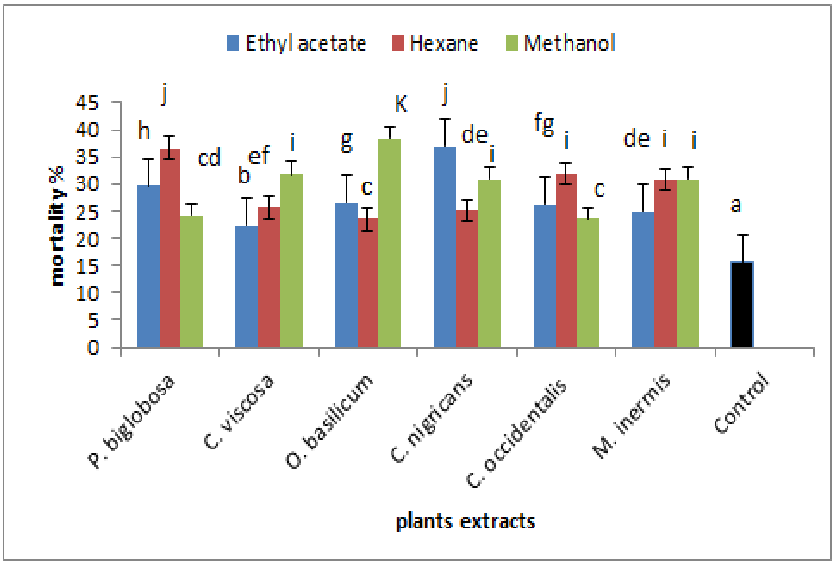

Figure 1: Effects of organic extracts on white flies' mortality after 24 hours.

The means presenting the identical letters in the same columns are not statistically different at the 5% threshold according to the Student-Newman-Keuls test.

After 72 hours' observations, themortality average effect of all organic extracts on *B. tabaci* (78, 92% mortality) is an increase of 118.36% compared to the untreated control.

The methanol extracts of *O. basilicum*, *P. biglobosa*, hexane extracts of *C.occidentalis*, *C.nigricans*, the ethyl acetate extracts of *C. nigricans* and *O. basilicum*, are not significantly different from each other. These extracts resulted in a 131.10% mortality rate compared to the untreated control. The lowest rates are situated on the*M. inermis* hexane extractand that of *C. viscosa*, which is not differentwith the untreated control (figure 2).

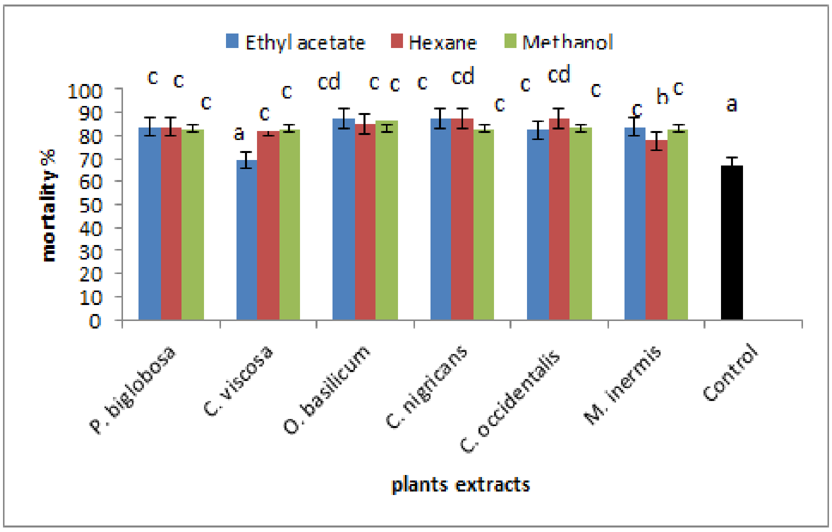

Figure 2: Effects of organic extracts on white flies' mortality after 72 hours.

The means presenting the identical letters in the same columns are not statistically different at the 5% threshold according to the Student-Newman-Keuls test.

Biological efficiency of methanol faction of *O. basilicum* and hexane faction of *P. biglobosa* against *B.tabaci*

The variance analyzes showed that the different active fractions influenced the mortality of*B. tabaci* adults after 24 hours and 72 hours observations (Table II).

The average effect of all the fractions after 24 hours (41.75%) is an increase of 67% mortality rate in comparison with the untreated control. The mortality rates of *O. basilicum*fractions, varied from 136% to 220% compared to the untreated control. The acetone fraction of *O. basilicum* got amortality of 220% in comparison with the untreated control.

After 72 hours, the average effect of the active fractions (66.75%) is an increase of 30.88% compared to the untreated control. Between the fractions, only the acetate-ethyl fraction of *P. biglobosa* is not differentwith the untreated control. In *O. basilicum*fractions, the mortality rates varied from 125.49% to 152.94%. The best rate is at the acetone fraction of *O. basilicum*with a rate of 152.94% compared to the untreated control. It is followed by the methanol extract of *O. basilicum* with a rate of 143.14%.

Table II: Biological efficiency of active fractions of *P. biglobosa* and *O. basilicum* on*B. tabaci.*

Treatements	Mortality after 24h	% to the untreated control	Mortality Afer 72h	% to the untreated control
Untreated control	25.00 a	-	51.00 a	-
P. biglobosa(ethyl acetate)	28.00 b	112.00	52.00 a	101.96
O. basilicum (ethylacetate)	34.00 c	136.00	64.00 b	125.49
O. basilicum (methanol)	50.00 d	200.00	73.00 c	143.14
O. basilicum (acetone)	55.00 e	220.00	78.00 d	152.94
Mean	41.75		66.75	
CV (%)	4.30		3.50	
S.e. (df = 16)	1.643		2.12	
e.s.e	0.74		0.95	

The means presenting the identical letters in the same columns are not statistically different at the 5% level according to the Student-Newman-Keuls test.

Phyto-chemical analysis of the active extracts of *O. basilicum* and *P. biglobosa.*

Tests of chemical characterizations in solution in test tubes made it possible to highlight groups of potentially bioactive phytochemicals in the fractions of n-hexane extracts of *P. biglobosa*, and the methanol fractions of *O. basilicum* (Table III).

Table III: **Phytochemical characterization of the n-hexane fraction of *P. biglobosa* and methanol fraction of *O. basilicum*.**

phytochimical groups	***P.biglobosa* hexane fraction**	***O.basilicum* methanol fraction**
Sterols/triterpens	+	+
Anthraquinons	**(-)**	+
Coumarins and derivatives	+	**(-)**
Carotenoids	**(-)**	**(-)**
Alkaloids	**(-)**	**(-)**
Tannins	**(-)**	+
Flavonoids	**(-)**	+
Saponosides	**(-)**	+
Anthocyanosides	**(-)**	+
Reducing compound	**(-)**	±

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

The hexane extract of *P. biglobosa* contains only sterols and triterpenes as well as coumarins and derivatives between the studied chemical groups. On the other hand, the methanol extract of *O. basilicum* is rich in triterpenes and sterols but also in anthraquinones, tannins, flavonoids, saponosides, anthocyanosides and traces of reducing compounds.

DISCUSSION

The yields of the different extractions varied from one natural substance to another. They also varied according to the types of solvent used. These results are similarto those obtained according to the extraction methods (maceration, with hot water and decoction with boiling water) and to the types of extracting solvents used.[32] Similar results were obtained toowith rosmarinic acid extracted from lemon balm with ethanol.[33] The highest yield in our testhas been obtained with methanol extract of *Parkia biglobosa* because of the presence of polarmore important constituents in this natural substance compared to the others.

During this experiment, all the extracts had more or less insecticidal activity compared to the untreated control. The efficiency of pesticides, whether synthetic or plant-based, is related to their active ingredients, their way of action, the type of application, their persistence action, but also depending to the morphological structure of insects, their ability to resist to the products toxicity.

Between the organic extracts, the insecticidal properties of *Cassia nigricans* against *B. tabaci* had already been demonstrated because of the presence of three anthraquinones and a flavonoid. This is one of the reasons that during this experimentation, a deep study has been not done.[29,30]

The aqueous extracts of *C. occidentalis* dried leaves is very rich in flavonoids compound and a little quantity in anthracene derivatives of chrysophanol type (https: africaine2ie.monsite.com medicine). Thiscould also explain its efficiency against *B. tabaci*. The insecticidal properties of *C. viscosa* were further demonstrated in this experimentation, confirming works previously done against *Spodoptera littura*[34] and *S. frugiperda*.[35]

Between the organic extracts, besides *C. nigricans* and *C. occidentalis*, hexane fractions of *P. biglobosa* and methanol fraction from *O. basilicum* proved to be better both after 24hours and after 72hours of observations. Phytochemical analysis have shown that the hexane extract of *P.biglobosa* contains only sterols and triterpenes as well as coumarins and derivatives. Herbicidal properties of *P. biglobosa* pods powder against *Striga hermonthica* which cause big damage to corn are showed.[36] The phyto-chemical analysis showed that this powder is rich in triterpenes of stereodic type which is beta-sitosterol. It is not excluded that this active ingredient is responsible for this insecticidal activity against *B. tabaci*on tomato.

The methanol fraction of *O. basilicum* is rich in chemical constituents (Table III). Despite some diversity due to their origin, soil and climatic conditions and different crops, the dried leaves of *O. basilicum* contain 0.25% of essential oil that can reach to 2% of the dry product. The one of the Indian Ocean would contain mainly estragol with other terpenes in small quantities (cineole, fenchol, linalool and methyl eugenol). In Europe, it contains more linalool or methyl eugenol.[37] In Africa, it found that oxygenated monoterpenes including estragol (38.78%), linalool (19.45%) and methyl eugenol (9.98%) predominate in the essential oil of *O. basilicum.*[38] In Cameroon, *O. basilicum* had insecticidal properties against *Anopheles funestus* vector of malaria[39], also this study noted that it would contain hydrocarbon monoterpenes (56,20%). It showed that oxygenated monoterpenes and / or, the association to phenol compounds toterpinene could be responsible for this efficacy. In Senegal, it noted that *O. basilicum* contains estragol (27.85-38.78%), linalool (18.45 - 18.87%), methyl eugenol

(9.98 - 14.40%) sesquiterpene hydrocarbon and berganotene (8.48 - 10.46%).[40] In Togo, it has been mentioned that *O. basilicum* contains a high content of estragole or metyl-chavicol (66%), linalool (15%) and a significant content of cineole (1.8 -7.6%).[41] They finally characterized *O. basilicum* as a chemotype of methyl-chavicol and linalool.

In all cases, all of these analyzes confirm the phytochemical analysis of the main chemical constituents of the methanol fraction of *O. basilicum*, during this experimentation. On the other hand, the presence of anthraquinones and other constituents whose insecticidal properties have been highlighted against other insect pests have also undoubtedly contributed to this efficacy against *B. tabaci* of tomato. In Burkina Faso, against mango fruit flies, methyl-eugenol is used as an attractant for these insects, associated with Diclorophos fumigant in traps, placed under mango trees; in an integrated pest control program against these insect pests. So, methyl eugenol cannot be considered as an active ingredient with insecticidal property against tomato white flies.

CONCLUSION

The extracts of *Ocimum basilicum* and *Parkia biglobosa* contain insecticides properties against white flies. They caused respectively 38.3 and 36.58% mortality of these tomato pests after 24 hours of application. This mortality increased respectively to 86.32% and 83.74% after 72 hours of application. Purification and characterization of these active substances and a field test could lead to the development of biopesticides against tomato white flies preserving consumers and environment health.

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