Phytochemical Composition and Insecticidal Effects of Aqueous Spice Extracts on Insect Pests Found on Green Beans (*Phaseolus vulgaris*) in Burkina Faso

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Keywords: Spices- Insects- Green bean-Yield- Burkina Faso

Summary

*This study focused on the insecticidal activity of aqueous spice extracts on pest insects (*Bemisia tabaci*,* Caliothrips impurus*,* Caliothrips occipitalis *and* Nisotra *spp.) found on green beans on farms in the Kou Valley, Burkina Faso. The pest insects were counted using transparent cylindrical cages, which were placed over 20 green bean plants per useful plot. The phytochemical analysis of aqueous extracts was based on the chemical method described by Ciulei I (1982). The spices contain chemical*s *(piperidine alkaloids, saponosides, anthraquinones and triterpenes), which have insecticidal properties. The differents extracts applied at the dose of 400 l/ha (100 g powder/1 l water) reduced total infestation populations of whitefly by 61-76%, thrips by 67-75% and coleoptera by 72-78%, compared to the untreated control. Aqueous extracts from black pepper (*Sinapis nigra*,* Xylopia aetiopica*), industrial tobacco (*Nicotiana tabacum*) make it possible to produce yields equivalent to those after the application of Deltamethrin and therefore represent an alternative to chemical insecticides.*

Résumé

Composition phytochimique et effets insecticide d'extraits aqueux de substances épicées sur les insectes ravageurs du haricot vert (*Phaseolus vulgaris*) au Burkina Faso

*Une étude de l'activité insecticide d'extraits aqueux de substances épicées sur les ravageurs (*Bemisia tabaci*,* Caliothrips impurus*,* Caliothrips occipitalis *et* Nisotra *spp.) du haricot vert a été réalisée en milieu paysan (Vallée du Kou) au Burkina Faso. Le dénombrement des ravageurs a été effectué à l'aide de cages cylindriques transparentes placées sur 20 plants du haricot vert par parcelle utile et l'analyse phytochimique des extraits a été réalisée par la méthode chimique de Ciulei I (1982). Les substances épicées renferment des constituants chimiques (Alcaloïdes pipéridiniques, saponosides, anthraquinones et triterpènes) à propriétés insecticides. Les différents extraits appliqués à la dose de 400 l/ha (100 g de poudre/1 litre d'eau) ont entraîné une réduction du cumul des populations infestantes de mouches blanches de 61-76%, des thrips de 67-75% et des coléoptères de 72 à 78% par rapport au témoin non traité. Les extraits aqueux du poivre noir (*Sinapis nigra*), du piment noir (*Xylopia aetiopica*), du tabac industriel (*Nicotiana tabacum*) assurent des rendements équivalents à la Deltaméthrine, constituants des alternatives aux insecticides chimiques.*

Introduction

Bemisia tabaci (Homoptera, Aleyrodidae) or whitefly cause considerable damage to vegetable crops (particularly green beans and tomatoes), which leads to significant or even total losses, due to the viruses (9, 16) that they transmit.

Losses are also caused by attacks of thrips and small coleoptera on green beans, which are an export crop for Burkina Faso.

In order to rectfiy this problem, agronomic methods used to combat pests include intercropping, biological control (8, 11, 18) by means of parasitoids, physical control (22) using cages to catch thrips and whitefly (24), while neem almond extracts have been considered (4, 13).

However, neem almonds are not easily available in all parts of Burkina Faso and a large number of farmers continue to use chemical insecticides, without always adhering to the relevant instructions due their being illiterate.

Based on the phytosanitary sampling that we conduct in vegetable crop fields every year, we have observed that onion leaves (purple Galmi variety) are not attacked by whitefly.

On the other hand, the growers themselves were producing spices, such as yellow and red chili peppers, ginger, garlic, etc for sale at market, for their families' consumption or for medicinal use.

In addition, it was considered necessary to develop a

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control method that would be easily available to the farmers, by studying the insecticidal activity of spices on insect found on green beans, such as *Bemisia tabaci, Caliothrips impurus, Caliothrips occipitalis and Nisotra* spp*.* The objectives were to confirm that whitefly are repelled by the aromatic nature of the onion, to encourage growers to produce natural insecticides in response to the rising cost of synthetic pesticides and to minimise contamination risks caused by chemical insecticide residues in green bean pods, in order to meet the export quality standards required by the European Union (zero pesticide residues).

Materials and methods

Test environment

The tests were conducted on farming land with a ferruginous soil in the Kou Valley, which lies in the Sudanian zone. The green bean variety (Bravo), which is sensitive to pests, was sown in non-partitioned mounds, with 40 cm gaps between the pockets and 80 cm between lines. Two weedings were conducted on the 10^{th} and 30^{th} day after sowing (DAS). The recommended dose of 400 kg NPK mineral fertiliser (12.24.12) mineral fertiliser on the 14^{th} DAS, followed by 200 kg urea/ha (100 kg/ha on the 30^{th} day and 100 kg/ha after the 3^{rd} harvest). The test method was a block of FISHER consisting of 10 treatments (repeated 4 times) on plots measuring 6.80 m x 4 m = 27.20 m^2. The spice extracts were prepared at a concentration of 100 g powder or homogenate/1 litre of water. The solutions were kept for 24 hours, filtered using muslin sheets and Adhesol was added at a concentration of 30 cc/hl. Four hundert l/ha of the extracts were applied using an OSATU regulated pressure knapsack sprayer. The various insect numbers (*B. tabaci, C. impurus, C. occipitalis* and *Nisotra* spp.) were obtained before and on the 3^{rd}, 7^{th}, 14^{th}, 21^{st}, 28^{th} and 35^{th} day after the extracts were applied using transparent cylindrical cages placed on 20 green bean plants and each useful plot. After each count was completed, the aqueous spice extracts were reapplied. The applications were halted 10 days after harvesting. The biological effectiveness coefficients were calculated using the following formula devised by Afanasseva *et al.* (1).

$$C = 100 \text{ X } \left(\frac{A - B}{A} - \frac{a - b}{a}\right)$$

C= Biological effectiveness coefficient (%)
A – Number of insects before application of extracts on the treated plot .
B – Number of insects after application of extracts on the treated plot.
a – Number of insects before application of extracts on the untreated control plot.
b – Number of insects on the untreated control plot after application of extracts on the treated plots.

The yield was assessed according to weight and per hectare. The data obtained was subjected to a variance analysis, followed by the Newman-Keuls test using STAT ITCF software and correlations using Origin 3.0 software.

Phytochemimcal analysis of spice extracts

Collection and treatment of samples
For this study, spice samples were collected from the market in Bobo-Dioulasso, a town in western Burkina Faso. These samples were dried at room temperature (approx. 30° C) on ventilated racks, which were sheltered from sunlight. After drying, the samples were pulverised using a mixer. The resulting powders were stored in bags suitable for phytochemical analyses.

Phytochemical analysis of samples
This involved chemical screening in order to determine the major active phytochemical groups present in extracts taken from the various samples.

The chemical screening was conducted using the qualitative method described by Ciulei *et al*. (3). The principle of this method is based on the affinity of the active substances according to their polarity and the capacity of the active substances to react with certain chemical reagents.

The presence of a substance or group of active substances is indicated by the development of a colour reaction or characteristic precipitation.

Preparation of extracts
Thirty grammes of each sample were placed in a Soxhlet cartridge. Each test sample was extracted semi-continuously from the Soxhlet with methylene chloride (DCM) and methanol diluted with distilled water according to a ratio of 80:20 v/v. The residual marc from each sample was infused with distilled water for 5 hours and filtered using absorbent cotton. The filtrates obtained were centrifuged at 2000 rpm for 5 minutes. The organic extracts were concentrated under reduced pressure in the Rotavapor and the aqueous extracts were dried in a ventilated oven at a temperature of 45-50 °C. The various extracts were stored in coloured flasks for phytochemical screening.

Phytochemical screening of extracts
The active phytochemical substances, such as flavonoids, base and salt alkaloids, sterols and triterpenes, coumarins and derivatives, saponosides and tannins were determined in the various extracts tested. For this purpose, the following characterisation tests were used. Shibata or cyanidin test, Dragendorff and Mayer, Liebermann - Burchard, Feigl's test and fluorescence at 254 - 366 nm, foam index and haemolytic test described by Paris *et al.* (17), 2% ferric chloride solution (m/v).

The qualitative assessment of the test results was conducted by assigning the following ratings, according to the intensity of the reaction obtained: (++) = abundant, (+) = low quantity, (+/-) = trace, (-) = not detected.

Results

Phytochemical composition of spices

Phytochemical analysis of the substances studied reveals that saponosides, volatile oils, anthraquinones, triterpenes and alkaloids are present as chemical constituents (Table 1). Apart from garlic (*Allium sativum*), which does not contain saponosides, saponosides, volatile oils, sterols and triterpenes are common to all spices in abundant or significant quantities. Alkaloids are present in garlic, ginger and both types of tobacco. Tannins are present in black pepper (*Sinapis nigra*), as well as in traditional and industrial tobacco (cigarettes). Anthraquinones are present to a significant extent in the Jaune du Burkina (large yellow pepper) and virtually absent from extracts taken from other substances. The identification of active substances in the phytochemical groups, using organic solvents with different polarities, followed by fractionation and purification did not take place at this stage.

Effects of spices on the density of insect populations on green beans

As confirmed by the variance analysis, spices have influenced the density of *B. tabaci, C. impurus, C. occipitalis* and *Nisotra* spp. populations (Table 2).

With reference to whitefly, the mean effect of all products (19.46 flies/plant) is a reduction of 71.82% compared to the untreated control. Garlic, ginger, yellow pepper, red chilli, black pepper and traditional tobacco do not differ significantly from Deltamethrine. Nor is there any significant difference between spice extracts, which cause, however, a 61.72 - 76.15% reduction in whitefly infestation populations.

In terms of thrips, the mean effect of the products tested (8.18/plant) was a reduction of 73.76% compared to the untreated control (Table 2). No significant difference could be identified between extracts taken from different spices. These substances reduce *C. impurus* and *C. occipitalis* by 66.63 - 75.01%. Compared to Deltamethrin, a double – threefold increase could be observed.

The same trend could be seen in small coleoptera (*Nisotra* spp.). The mean effect of the products tested (10.87 coleoptera/plant) was a reduction of 77.15%. Deltamethrin caused a fall of 90.8% compared to the untreated control. The various spices, which are indistinguishable, caused reductions ranging from 71.8% to 77.91%. Compared to Deltamethrin, a double – threefold increase could be observed.

Biological effectiveness coefficients for spice extracts

After being evaluated using the formula described by Afanasseva *et al*. (1), compared to infestations on the untreated control, various treatments before the application of products and total infestations of treated plots after the application of products, the effectiveness coefficients for spices in terms of whitefly ranged from 46% - 69.15%, while the coefficient for Deltamethrine rose to 78.33% (Table 2).

In terms of small coleoptera, of the spices, only red chilli, black pepper, garlic and yellow peppers produced positive and low coefficients. Only Deltamethrine

Table 1
Phytochemical composition of spices

Spices	Chemical constituents					
	Alkaloids	Tannins	Anthraquinones	Saponosides	Volatile oils	Sterols and triterpenes
Garlic (*Allium sativum*)	+++	-	-	-	+++	+++
Black pepper (*Sinapis nigra)*	-	++	-	++	+++	+++
Ginger (*Zinziber officinale)*	++	-	-	++	+++	+++
Red chilli (*Capsicum fructescens*)	-	-	-	+++	++	+++
Large yellow pepper (*Capsicum* spp.)	-	-	+++	++	+++	+++
Senegal pepper (*Xilopia aetiopica*)	-	-	+/-	+++	+++	+++
Traditional tobacco (*Nicotiana tabacum*)	+++	++	-	+++	+++	+++
Industrial tobacco (*Nicotiana tabacum)*	+++	+/-	-	+++	++	+++

Key: - absent +/- doubtful presence ++ significant presence +++abundance.

Table 2
Effects of aqueous spice extracts on green bean infestations and their biological effectiveness coefficients (%)

Treatment	Total infestations (average/plant)			Biological effectiveness coefficients for extracts		
	Whitefly	Thrips	Coleoptera	Whitefly	Thrips	Coleoptera
Untreated control	69.08 a	31.17 a	47.56 a	-	-	-
Decis (Deltamethrine)	11.90 c	3.05 c	4.38 c	78.33	88.54	69.89
Garlic (*A. sativum*)	16.63 bc	8.01 b	12.33 b	69.15	82.49	15.84
Ginger (*Z. officinale*)	16.48 bc	9.86 b	12.89 b	62.60	80.80	-45.05
Yellow pepper (*C.* spp.)	19.56 bc	9.17 b	11.05 b	47.09	83.41	2.34
Red chilli (*C. fructescens*)	21.54 bc	7.79 b	10.51 b	67.95	83.50	34.54
Senegal pepper (*X. aetiopica*)	17.33 bc	8.48 b	11.14 b	64.30	97.10	-3.55
Industrial tobacco (*N. tabacum*)	26.45 bc	10.40 b	13.41 b	46.63	96.45	-28.02
Traditional tobacco (*N. tabacum*)	22.05 bc	8.59 b	10.96 b	50.31	97.81	-7.71
Black pepper (*S. nigra*)	23.22 b	8.26 b	11.23 b	61.39	94.41	26.57
Mean	24.42	10.51	14.52	N.B: The – symbol indicates an increase in pests.		
CV (%)	19.00	18.30	18.00			
RSD (ddl= 27)	4.64	10.51	14.52			
ETM (Sx)	2.32	5.26	7.26			

produced a coefficient as high as 79%. However, in terms of thrips, the spices have high biological effectiveness coefficients.
Senegal pepper (*X. aetiopica*), traditional tobaccos, industrial tobacco (*T. nicotiana*) and black pepper (*S. nigra*) have higher coefficients than Deltamethrine (94% - 97% compared to 87.5% respectively).

Effects of spice extracts on green bean yields

By modifying the infestation population of green bean pests, the application of spice extracts influenced pod quantities and green bean yields (Table 3). The mean effect of the products applied (812,340 pods/ha) represents an increase of 248.13% compared to the untreated control.
The spices caused pod increases of 208% - 296.57% compared to the untreated sample, but caused significant reductions compared to Deltamethrine.
In terms of yield factor, the mean effect of the products is an increased yield (215.51 kg/ha) of 222.78% compared to the control. Deltamethrine tripled the yield compared to the control. On average, the spices doubled the yield compared to the control. However, there is no significant difference between black pepper, Senegal pepper, industrial tobacco and Deltamethrine.

Table 3
Effects of aqueous spice extracts on green bean yields

Treatments	Quantity of pods (1000/ha)	% of control	Yield (kg/ha)	% of control
Untreated control	327.39 d	-	965.77 c	-
Decis (Deltamethrine)	1262.45 a	385.61	2995.06 a	310.12
Garlic (*A. sativum*)	682.86 c	208.83	1812.94 b	187.72
Ginger (*Z. officinale*)	726.07 c	221.78	1930.03 b	199.84
Yellow pepper (*C.* spp.)	716.07 c	218.72	2036.84 b	210.90
Red chilli (*C. fructescens*)	696.29 c	212.88	1748.49 b	181.05
Senegal pepper (*X. aetiopica*)	970.95 b	296.57	2350.70 ab	243.40
Industrial tobacco (*N. tabacum*)	833.50 bc	254.59	2328.69 ab	241.12
Traditional tobacco (*N. tabacum*)	660.40 c	201.72	1746.24 b	180.81
Black pepper (*S. nigra*)	762.45 c	232.84	2414.60 ab	250.02
Mean	763.84		2032.94	
CV (%)	14.10		18.30	
RSD (ddl= 27)	107.63		372.36	
ETM (Sx)	53.82		186.18	

Discussion

The antimicrobial activity of certain spices has been proven by authors such as Benkeblia (2), Erdogan *et al*. (6), Mahadi *et al.* (14) and Nikolic *et al.* (15), while their anti-carcinogenic activity has been demonstrated by Ejaz *et al.* (5) and Thomson and Ali (23).
Chemical analyses conducted using gas chromatography or mass spectrometry by Erdogan *et al.* (6) and Qin *et al.* (19) reveal that the active compounds contained in spices are found in the volatile oils. Alkaloids, aldehydes, ketones, esters and hydrocarbons are the main examples. In 1995, Robert *et al.* (20) showed that extracts of *Nicotiana gossei* controlled whitefly. Stephen *et al.* (21) confirmed that sugar esters from *Nicotiana glutinosa* 24, *N. langsdorffii* and *N. trigonaphylla* are biologically effective when applied to *B. tabaci*. Esters from *N. gossei* at a concentration of 10 at 0.05 mg/ml lead to high nymph mortality on *B. tabaci* (Gennadius) (Homoptera: Aleyrodidae).
In our study, the variations in yield were due to those seen to affect the population densities of green bean pests following the application of extracts, as witnessed by the different correlations determined between them and the yield (Figures 1, 2, 3).

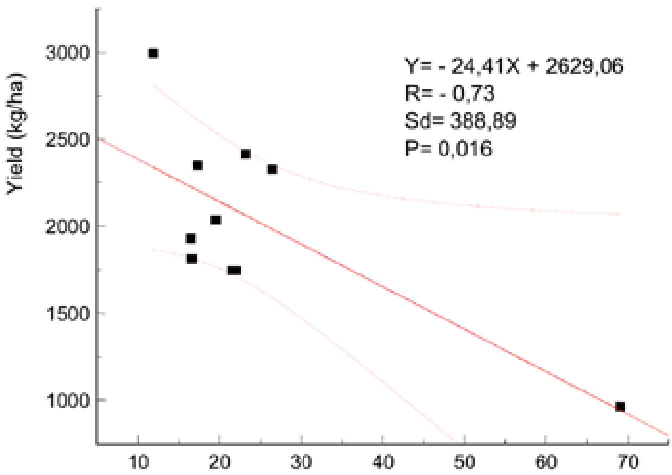

Figure 1: Correlation between thrips infestation and bean yields.

The mathematical link between the total infestations of *B. tabaci* and the green bean yield is expressed by the following ratio: y= -24.41x + 2629.06 with r= - 0,73 (p= 0.016), that of thrip infestations and yield by y= -56.39x + 2623.74 with r= - 0.79 (p= 0.006) and that of coleoptera by the following regression equation: y= -35.44x + 2548.47 avec r= - 0.78 (p= 0.008).
This confirms the insecticidal effects produced by the chemical constituents contained in spice extracts. Piperidine alkaloids, tannins, anthraquinones, saponosides, sterols and triterpenes are chemical groups that are likely to have pesticidal properties (insecticides, vermicides, molluscicides, fungicides).
The high biological effectiveness of aqueous spice extracts on adult forms of whitefly (*B. tabaci*) and thrips (*C. impuris* and *C. occipitalis*) leads us to believe that some constituents have repellent properties. The low biological effectiveness on small coleoptera is probably linked to their protective shell, which hinders the penetration of extracts.
The yields produced using black pepper (*S. nigra),* Senegal pepper (*X. aetiopica)* and industrial tobacco are probably linked to the abundance of saponosides, volatile oils, sterols and triterpenes in these extracts. In addition, black pepper and tobacco appear to contain tannins, whose oxidant properties have been described by Field and Lettinga (7). Tobacco also contains alkaloids.
These factors have therefore helped make it possible to produce equivalent yields to those achieved when synthetic insecticides, such as Deltamethrine, are applied.

Conclusion

Spice extracts produce high biological activity on whitefly (*B. tabaci*), thrips (*C. impurus* and *C. occipitalis*) and low biological activiy on small coleoptera (*Nisotra* spp.). Applied at a concentration of 200-400 l/ha on a weekly basis, if attacks are observed, extracts of

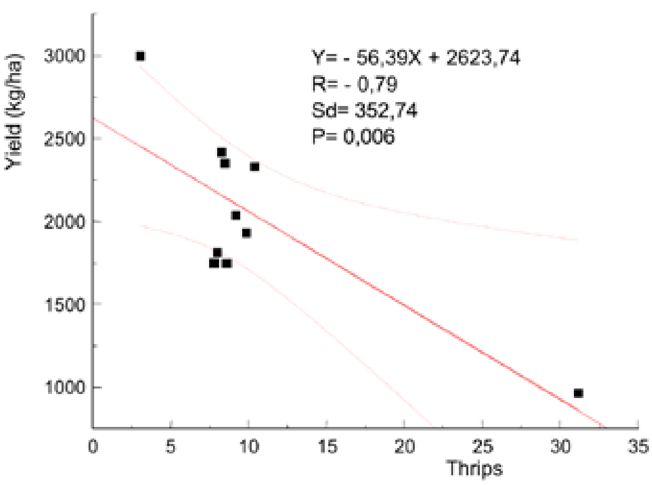

Figure 2: Correlation between thrips infestation and bean yields

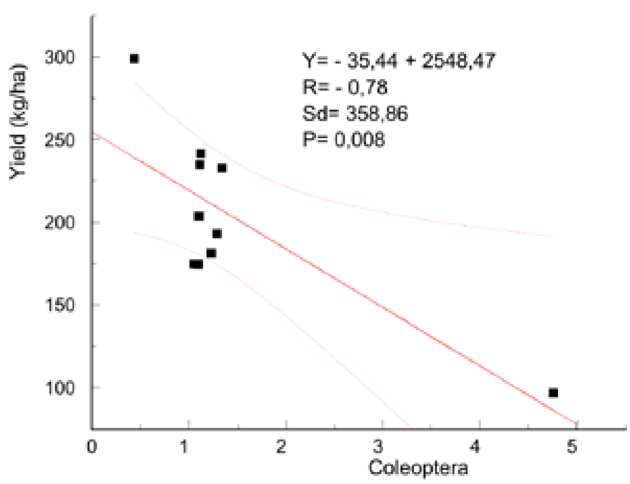

Figure 3: Correlation between coleoptera population density and bean yields

black pepper (*S. nigra*), Senegal pepper (*X. aetiopica*) and industrial tobacco make it possible to double green bean yields without harming the environment or consumer health.

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