



## Extracts of tropical African spices are active against *Plutella xylostella*

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### Abstract

Extracts from *Piper guineense*, *Aframomum melegueta*, *Aframomum citratum* and *Afrostryrax kamerunensis* were investigated for their antifeedant, lethal and developmental effects against *Plutella xylostella* larvae through laboratory dual-choice tests and topical application. Water and ethanol extracts of *P. guineense* were dose-dependent antifeedants at concentrations  $\geq 300$  and 500 ppm, respectively, whilst methanol extracts required  $\geq 1,000$  ppm. Methanol and hexane extracts of *A. melegueta* acted at  $\geq 100$  ppm and water extracts at  $\geq 300$  ppm, but ethanol extracts were deterring feeding only slightly at  $\geq 1,000$  ppm. Hexane and methanol extracts of *A. citratum* inhibited feeding at  $\geq 300$  ppm and water extracts did so at  $\geq 500$  ppm. None of the *Afrostryrax kamerunensis* extracts deterred feeding at any of the concentrations tested. No mortality was observed at any of the concentrations after topical application of the extracts on the larvae. However, the effects on larval development varied with extract concentration and larval age. Ingestion of the water and ethanol extracts of *P. guineense* caused 100% mortality of second instars at  $\geq 100$  ppm two to three days after infestation (DAI). Methanol and water extracts of *A. melegueta* and *A. citratum*, respectively, achieved  $\geq 80\%$  mortality of larvae at concentrations of  $\geq 500$  ppm and  $\geq 1,000$  ppm, respectively. With third instars, the mortalities were significantly lower; however, the *P. guineense* water or ethanol extracts caused 100% mortality two to four DAI. Larvae that survived till pupation had significantly longer larval periods compared with the control after application of *A. melegueta* extracts. We concluded that potent extracts from *Aframomum melegueta*, *Aframomum citratum* and especially *P. guineense* could be used as complementary measures in the management of *P. xylostella* by subsistence farmers.

**Key words:** *Piper guineense*, *Aframomum melegueta*, *Aframomum citratum*, *Afrostryrax kamerunensis*, crude extracts, biological activity, diamondback moth.

### Introduction

Plants are a rich source of natural products and many of these chemicals can protect plants against a variety of chewing and sucking insects. Given the array of selective pressures that pests exert on plants, it is not surprising that the plant kingdom offers a tremendous diversity of bioactive phytochemicals against diseases and pests<sup>1,2</sup>. Plant extracts or their active constituents such as derris, rotenone, pyrethrum, nicotine, sabadilla, physostigmine, quassin or azadirachtin, have been used as insecticides for decades<sup>3,4</sup>. The use of botanicals as sources of novel compounds or products in their own rights will likely increase given the need for more sustainable farming practices and a growing awareness of potentially negative impacts of synthetic pesticides on the environment<sup>3,5</sup>. Botanical products, as shown in the case of neem-based insecticides, are known to have negligible effects on beneficial insects and lower environmental impacts<sup>6,7</sup>. As botanicals are easily affordable and available, they may supplement imported synthetic pesticides and play a useful role in integrated pest management programmes in developing nations<sup>8</sup>.

The diamond back moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) is a major pest of vegetable brassica crops, and is one of the most important pests of cruciferous crops worldwide as its numerous larvae can cause complete defoliation and economic losses if not controlled<sup>9,10</sup>. *Plutella xylostella* has

developed resistance to most conventional pesticides in common use<sup>11</sup> and even to toxins of the bacterium *Bacillus thuringiensis*<sup>12</sup>. The rapid development of resistance by *P. xylostella* to most insecticides coupled with the environmental and consumer health hazards due to insecticide residues in plants underscore the importance of searching for alternative methods to control this pest. Potent plant extracts may be more effective in controlling *P. xylostella* and retard development of resistance than synthetic pesticides or purified derivatives due to synergistic or potentiating interactions among fairly complex bioactive phytochemicals<sup>13</sup>. The objectives of this study were therefore to determine the antifeedant and lethal effects of crude extracts of seeds of the widely used spices *Piper guineense* Schum et Thonn (Piperaceae), *Aframomum melegueta* (Rosk) K. Schum (Zingiberaceae), *Aframomum citratum* (Pereira) K. Schum (Zingiberaceae) and *Afrostryrax kamerunensis* Perkins and Gilg (Huaceae) on larvae of *P. xylostella*. This study extends our previous work where we tested the hypothesis that highly aromatic plants can serve as antifeedants and/or growth retardants of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)<sup>14</sup>.

### Material and Methods

**Plant materials and plant extracts:** Seeds of *Piper guineense*, *Aframomum melegueta*, *Aframomum citratum* and *Afrostryrax*

*kamerunensis* were purchased in Bamenda market, North West region of Cameroon. These spice seed samples were authenticated by comparing with herbarium specimens at the Limbe Botanic Garden, Limbe, Cameroon. The samples were air-dried and finely ground in a Janke and Kunkel A10 grinder (IKA Labortechnik, Staufen, Germany).

Water extracts of each spice were prepared as previously described<sup>14</sup>. Briefly, ground samples (25 mg) were extracted with water (150 ml) for 1 min, left standing at room temperature for 24 h, shaken again and then filtered. The water was removed using a rotary evaporator at 40°C. The residue (15 mg) was dissolved in water (15 ml) to obtain a 1,000 ppm stock solution; this solution was diluted further to give 100, 300 and 500 ppm solutions.

The seed powders were also extracted sequentially with organic solvents in a Soxhlo extractor as described previously<sup>14, 15</sup>. Powdered samples (5.0 g) were eluted successively with 70 ml each of hexane, ethyl acetate, acetone, ethanol and methanol. The solvents were evaporated *in vacuo* at ca. 35°C. The resultant residues were dissolved in acetone/water (2/3 v/v) as described previously<sup>14</sup> and diluted as the above water extracts to obtain 1,000, 500, 300 and 100 ppm solutions.

**Bioassays:** The bioassays were conducted with third instar larvae of *P. xylostella* obtained from a culture maintained on Chinese cabbage leaves at the Department of Agriculture, University of Reading. All larvae were starved for 24 hours before being subjected to each test. Test plant parts were excised fully expanded leaves of Chinese cabbage, *Brassica pekinensis* Rupr (Cruciferae) that had been harvested from five to six week-old glasshouse-grown plants.

Preliminary no-choice test experiments were conducted using the stock solution (1,000 ppm) from each plant extract. Stock solution (2 ml) was placed into a Petri dish and a Chinese cabbage leaf was dipped into it, and the set-up then placed in a fume cupboard for 20 minutes to dry. Controls were treated with the corresponding solvents only. There were 8 replicates for each treatment. One *P. xylostella* third instar larva was then added to each Petri dish to feed for 24 h before observations were made for mortality and/or proportion of leaf remaining. The treatments and controls were visually compared by placing each leaf on a graph paper and counting the number of 1 mm squares consumed. Bioactive extracts were then used in dose response dual-choice tests, topical applications and developmental studies as described below.

**Dual-choice test:** Dual-choice tests used concentrations of 100, 300, 500 and 1,000 ppm of each extract plus the corresponding solvent controls. These were prepared by transferring 1, 3 and 5 ml from the 1,000 ppm stock solutions into separate vials containing 9, 7 and 5 ml of acetone-water (2:3 v/v) to avoid damaging the leaves with organic solvents; all water extracts were diluted with water. Each treatment was applied with the corresponding control by dipping one half of the fully opened leaf blade up to the mid-rib in 2 ml of the treatment solution and the other half in the control solution. Thereafter, the leaf was placed in a tray padded with filter paper and kept in a fume cupboard for ca 20 min to dry. Subsequently, the leaf was transferred into a Petri dish lined with a moist filter paper and marked on one side to indicate which half of the leaf had been treated. One third instar *P. xylostella* larva

previously starved for 24 h was then introduced and the dishes kept in the insectary at a constant temperature of 25°C and 70% relative humidity for 36-48 h when approximately 50% of one of the leaf portions had been consumed. The treatments and controls were replicated 8 times and the experiment was repeated once giving a total of 16 insects for each treatment. The percentage of the leaf area eaten was recorded separately for the treated leaf portion (T) and the control (C) using graph paper with 1 mm squares. The percentage antifeedant effect was calculated using the formula  $[(C-T)/(100-C) \times 100]$ <sup>14</sup>. All negative values were considered as zero and all antifeedant indices were expressed as percentages. A high antifeedant index indicates strong antifeedant activity.

**Topical application:** Extracts that showed antifeedant activity were prepared as described above and the controls were the corresponding solvents. Using a micro-applicator, extracts, 3 µl of each extract were applied separately on the dorsum of a third instar larva placed in a Petri dish. A Chinese cabbage leaf was then added and the dishes kept in the insectary for 24 h after which records of mortality were taken. Each treatment and control was replicated 30 times.

**Larval development:** Extracts that were antifeedant and/or toxic to the larvae were also tested in larval development studies. Adults of *P. xylostella* were put in rearing cages that contained potted glasshouse-grown Chinese cabbage plants and observed daily for oviposition. One day after oviposition, the plants were removed from the cages and kept in the insectary for egg incubation and development of first instars in mines created in the leaves. Second instars that emerged from the mines were transferred to fresh leaves to feed and to develop within two days into third instars. Thereafter, five of the third instar larvae were transferred into a Petri dish, each lined with moist filter paper and supplied with a Chinese cabbage leaf treated with either a 500 or 1,000 ppm solution of an active extract of either *P. guineense*, *A. melegueta* or *A. citratum*. Records of mortality were taken on alternate days and the leaves were also changed. This continued until pupation when the total larval period was recorded and each pupa weighed. The pupae were then kept until adult emergence to record the pupal duration.

**Data analysis:** Data were analyzed by ANOVA after an arcsine transformation of the antifeedant indices. A separate analysis was done for the extract of each plant species. Differences between means were assessed for significance using the LSD test at  $P = 0.05$ .

## Results

**Dual choice tests:** Preliminary bioassays revealed that the ethyl acetate and acetone extracts of all plants had no antifeedant effects at 1,000 ppm and were therefore not tested in subsequent experiments. In the dual-choice tests (Tables 1a and 1b), the water and ethanol extracts of *P. guineense* showed antifeedant activity at  $\geq 300$  ppm, but the hexane and methanol extracts were only slightly antifeedant at  $\geq 1,000$  ppm. *A. melegueta* significantly reduced feeding on the treated leaf portions at  $\geq 100$  ppm for hexane and methanol extracts but the water extract at  $\geq 300$  ppm. Table 1 shows that the antifeedant activity was dose-dependent. The

**Table 1a.** Effect of *Piper guineense* and *Aframomum melegueta* extracts on the antifeedant index [(C-T)/(100-C)] x100 of the third instar larvae of *Plutella xylostella* by dual-choice tests.

Plant species	Solvent	Concentration (ppm)				LSD (P = 0.005)
		100	300	500	1,000	
<i>P. guineense</i>	Water	45.7Ba (40.5)	55.1Ba (48.9)	76.3Bc (61.4)	69.5Bc (57.4)	(7.3)
	Hexane	13.9Aa (5.8)	16.5Aa (10.9)	15.3Aa (10.8)	21.6Ab (17.9)	(6.9)
	Ethanol	47.3Ba (41.6)	35.5Ba (34.5)	68.1Bb (56.1)	77.3Bb (63.7)	(12.3)
	Methanol	10.9Aa (13.9)	15.5Aa (16.9)	18.0Aa (20.1)	27.1Ab (25.3)	(8.6)
	LSD (P=0.05)	(13.6)	(10.4)	(12.8)	(13.2)	
<i>A. melegueta</i>	Water	38.1Ba (35.7)	51.4Bb (43.9)	53.6Bb (47.2)	56.6Bb (48.9)	(7.7)
	Hexane	52.0BCa (46.2)	73.5Cb (59.5)	83.4Cc (68.3)	81.0Cc (66.3)	(6.3)
	Ethanol	7.1Aa (9.2)	9.9Aab (15.5)	8.6Aab (14.7)	12.4Ab (18.8)	(5.1)
	Methanol	60.8Ca (53.6)	68.3Ccb (56.6)	74.9Cc (62.3)	66.9BCab (55.4)	(7.9)
	LSD (P=0.05)	(11.5)	(10.8)	(11.8)	(10.4)	

C and T are respectively the proportion of control and treated leaf portions remaining. A higher antifeedant index implies higher antifeedant activity. Means with the same upper case and lower case letters in a column and row, respectively, are not significantly different (P = 0.05). Values in brackets are the arcsine transformed data.

**Table 1b.** Effect of *Aframomum citratum* and *Afrostryax kamerunensis* extracts on the antifeedant index [(C-T)/(100-C)] x100 of the third instar larvae of *Plutella xylostella* by dual-choice tests.

Plant species	Solvent	Concentration (ppm)				LSD (P = 0.005)
		100	300	500	1,000	
<i>A. citratum</i>	Water	36.1Ba (32.8)	22.6Ba (29.4)	61.4Bb (50.2)	51.9Bb (46.4)	(9.6)
	Hexane	21.1Ba (28.2)	68.0Cb (50.6)	70.0BCb (57.3)	67.8Cb (58.3)	(10.1)
	Ethanol	4.2Aa (8.4)	6.3Aa (11.7)	6.7Aa (8.2)	19.3Ab (23.8)	(5.9)
	Methanol	10.0Aa (11.8)	56.6Cb (50.5)	76.1Cc (64.8)	60.5BCbc (56.8)	(16.3)
	LSD (P=0.05)	(10.7)	(12.1)	(14.5)	(10.9)	
<i>A. kamerunensis</i>	Water	21.2Aa (19.2)	23.9Aa (22.0)	25.1Aa (21.8)	27.3Aa (23.4)	(11.9)
	Hexane	12.1Aa (11.7)	16.5Aa (11.9)	19.4Aa (11.2)	15.3Aa (9.1)	(7.8)
	Ethanol	18.3Aa (10.3)	14.7Aa (13.9)	17.8Aa (11.6)	16.1Aa (11.5)	(7.1)
	Methanol	20.2Aa (19.8)	15.7Aa (14.5)	14.4Aa (15.6)	21.2Aa (17.8)	(8.1)
	LSD (P=0.05)	(8.6)	(9.4)	(8.9)	(8.8)	

C and T are respectively the proportion of control and treated leaf portions remaining. A higher antifeedant index implies higher antifeedant activity. Means with the same upper case and lower case letters in a column and row, respectively, are not significantly different (P = 0.05). Values in brackets are the arcsine transformed data.

ethanol extract was only slightly antifeedant at 1,000 ppm. The hexane and methanol extracts of *A. citratum* inhibited feeding at  $\geq 300$  ppm and the water extract acted at  $\geq 500$  ppm; the ethanol extract was antifeedant only at  $\geq 1,000$  ppm. In general, antifeedant activity increased with concentration. However, none of the *Afrostryax kamerunensis* extracts was antifeedant.

**Topical application tests and larval development studies:** No significant mortalities were recorded when extracts that had given

potent anti-insect activity was applied topically on the larvae (Table 2). However, larval development was affected by extract concentration and the effect also depended on larval age. When second instar larvae were transferred onto leaves treated with active extracts (500 ppm or 1,000 ppm) from each plant, at least 80% of them died two to three days after infestation (DAI) (Table 2). However, the *P. guineense* 500 ppm water or ethanol extracts caused 100% mortality within the same period.

When third instar larvae were used, all extracts resulted in

**Table 2.** Percent mortalities (mean  $\pm$  s.e.) of second and third instar *Plutella xylostella* larvae respectively after 2-3 days feeding on treated leaves and 24 hours post topical application of *Piper guineense*, *Aframomum melegueta* and *Aframomum citratum* extracts.

Extract	Concentration (ppm)	Feeding second instar	Topical application third instar
<i>P. guineense</i>			
Water	500	100 $\pm$ 0.0	10 $\pm$ 2.0
	1,000	100 $\pm$ 0.0	15 $\pm$ 4.1
Ethanol	500	100 $\pm$ 0.0	12 $\pm$ 3.2
	1,000	100 $\pm$ 0.0	16 $\pm$ 5.3
<i>A. melegueta</i>			
Water	500	82 $\pm$ 4.0	5.5 $\pm$ 5.2
	1,000	90 $\pm$ 3.2	7.8 $\pm$ 3.4
Hexane	500	85 $\pm$ 5.4	5.5 $\pm$ 4.2
	1,000	92 $\pm$ 3.5	7.2 $\pm$ 5.1
Methanol	500	87 $\pm$ 2.8	6.0 $\pm$ 4.8
	1,000	95 $\pm$ 3.6	8.4 $\pm$ 3.3
<i>A. citratum</i>			
Water	500	84 $\pm$ 4.8	5.1 $\pm$ 1.6
	1,000	88 $\pm$ 5.3	6.2 $\pm$ 2.1
Hexane	500	80 $\pm$ 4.1	4.4 $\pm$ 1.3
	1,000	94 $\pm$ 5.6	4.5 $\pm$ 2.8
Methanol	500	82 $\pm$ 6.2	6.2 $\pm$ 3.2
	1,000	94 $\pm$ 4.6	7.8 $\pm$ 4.1

significantly lower mortalities with the exception of *P. guineense* extracts. The water or ethanol extracts of *P. guineense* caused 100% mortality at 500 ppm two to four DAI. Generally, larvae that were exposed to *A. melegueta* water, hexane or methanol extracts and that survived till pupation took significantly longer to develop than control larvae (Table 3). Although pupal weights were slightly lower, this difference was not significant; similarly, there were no noteworthy differences in the total larval period or pupal weights of the other treatments albeit the slight variations (Table 3). *A. citratum* extracts produced no significant effect on larval or pupal periods, pupal weight or mortality (Table 3).

**Table 3.** Effect of antifeedant extracts from *Aframomum melegueta* or *A. citratum* on the development (mean  $\pm$  s.e.) of third instar larvae of *Plutella xylostella*.

Extract	Concentration (ppm)	Larval period (days)	Pupal weight (mg)	Pupal period (days)	Pupal mortality (%)
<i>P. guineense</i>					
Water	500	2-3*	-	-	-
	1,000	2-3*	-	-	-
Ethanol	500	2-3*	-	-	-
	1,000	2-3*	-	-	-
<i>A. melegueta</i>					
Water	500	11.7 $\pm$ 0.4b	5.1 $\pm$ 0.2a	4.6 $\pm$ 0.3a	30 b
	1,000	12.1 $\pm$ 0.6b	5.7 $\pm$ 0.5a	4.4 $\pm$ 0.5a	35 b
Hexane	500	11.8 $\pm$ 0.2b	5.5 $\pm$ 0.3a	4.5 $\pm$ 0.3a	30 b
	1,000	12.2 $\pm$ 0.4b	5.3 $\pm$ 0.4a	4.7 $\pm$ 0.5a	40 bc
Methanol	500	12.5 $\pm$ 0.3b	5.3 $\pm$ 0.4a	4.8 $\pm$ 0.3a	25 ba
	1,000	12.4 $\pm$ 0.2b	5.1 $\pm$ 0.3a	4.6 $\pm$ 0.2a	45 bc
<i>A. citratum</i>					
Water	500	10.2 $\pm$ 0.4a	5.9 $\pm$ 0.2a	4.5 $\pm$ 0.3a	10 a
	1,000	10.6 $\pm$ 0.2a	5.3 $\pm$ 0.4a	4.6 $\pm$ 0.3a	20 ab
Hexane	500	10.5 $\pm$ 0.1a	6.1 $\pm$ 0.2a	4.7 $\pm$ 0.3a	15 a
	1,000	10.2 $\pm$ 0.2a	6.9 $\pm$ 0.3a	4.9 $\pm$ 0.1a	18 a
Methanol	500	10.8 $\pm$ 0.2a	5.9 $\pm$ 0.2a	4.3 $\pm$ 0.2a	30 b
	1,000	10.2 $\pm$ 0.2a	5.5 $\pm$ 0.2a	4.2 $\pm$ 0.1a	40 bc
Control		9.8 $\pm$ 0.2a	6.8 $\pm$ 0.3a	4.4 $\pm$ 0.2a	20 ab
LSD, P=0.05		1.9	1.8	1.0	16

\*100% mortality was observed after 2-3 days. Means with the same lower case letter in a column are not significantly different (P = 0.05).

## Discussion

The water and ethanol extracts of *P. guineense* had antifeedant activity against third instar larvae in a 24 hour bioassay (Table 1a) and led to 100% mortality within two to three days after infestation (Table 2). This could explain why farmers use *Piper guineense* seed powders to control insect pests during grain storage<sup>16-18</sup>. Indeed, a number of insecticidal unsaturated isobutylamides have already been identified in *P. guineense* and *P. nigrum*<sup>19,20</sup> and these might also be responsible for the observed mortality of *P. xylostella* larvae, but further tests would be needed to confirm this. Although a previous study<sup>21</sup> reported that seed extracts of the closely related *P. nigrum* acted as contact poison on *P. xylostella* larvae, none of the extracts of *P. guineense* caused mortality when applied topically in our study; a possible explanation could be a low penetration rate through the insect cuticle or a lower concentration of the bioactive ingredients in *P. guineense*.

In contrast, *A. melegueta* or *A. citratum* water, methanol and hexane extracts were potent antifeedants (Table 1) but did not kill all larvae after a four-day exposure during the larval developmental studies; these extracts only caused a delay in larval development (Table 3). This finding may be of practical interest as under field conditions, longer development times can provide more opportunities for larvae to experience unfavourable environmental conditions or increased parasitoid attack, which has been reported in *P. xylostella* larvae<sup>7,10</sup>.

*Aframomum melegueta* and *A. citratum* seeds produce pungent hot tasting extracts which may contribute to their antifeedant activities as observed against *Spodoptera littoralis*<sup>14</sup>. *Aframomum melegueta* also deters feeding in termites, where 6-gingerol and 6-shogaol were identified as strong antifeedant compounds<sup>22</sup>. Phytochemical analyses of other *Aframomum* species showed that they produce labdane diterpenoids which are *Spodoptera* antifeedants<sup>23,24</sup>.

Contrary to our hypothesis that volatile oils in aromatic plants act as potent antifeedants, the highly aromatic *A. kamerunensis*

did not deter feeding by *P. xylostella*. This finding agrees with our previous report that *A. kamerunensis* extracts did not have antifeedant activity against *Spodoptera littoralis*<sup>14</sup>. The present study also confirmed that water was a good solvent to extract compounds that deterred feeding by *Spodoptera littoralis* larvae<sup>14</sup>.

It has been suggested that mixtures of phytochemicals offer multifactorial selective pressures that retard the development of resistance in pests<sup>25</sup>. Therefore, plant based pesticides may have an advantage over synthetic pesticides against *P. xylostella*, which is known for its ability to develop resistance against most pesticides<sup>26,27</sup>. Whilst *Piper guineense* is cultivated in some parts of West Africa, *A. melegueta* and *A. citratum* are still harvested from the wild for their culinary and/or medicinal uses<sup>28</sup>. It is therefore important that these underutilised plants are studied further in order to stimulate public interest in their conservation, sustainable exploitation and possibly cultivation.

### Conclusions

Our study demonstrated the potent activities of *P. guineense*, *A. melegueta* and *A. citratum* seed extracts against *P. xylostella*. The fact that readily available solvents such as water, methanol and ethanol produced potent extracts may enable farmers or small enterprises to develop their own pesticide formulations. Technology based on local resources is important for subsistence farmers in developing countries and will conserve foreign cash reserves. Clearly, efficacy and safety tests will need to be done in field trials but given that these seeds have a long traditional use as culinary spices; it is unlikely that safety will be an issue.

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