Study of Various Extracts of *Ayapana triplinervis* for their Potential in Controlling Three Insect Pests of Horticultural Crops

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Summary

Chemical groups of Ayapana triplinervis*, extracted successively with hexane, petroleum ether, methanol, chloroform: methanol (1:1), and chloroform: methanol (4:1) were studied for their effects on* Plutella xylostella*,* Crocidolomia binotalis *and* Myzus persicae*, three serious pests of horticultural crops in Mauritius. The most bioactive extracts were further fractionated into groups using Thin Layer Chromatography, and seven of those exhibiting strongest activity were tested on each of the three test insects. Results showed that the alkaloids and tannins exhibited greatest feeding deterrence in* P. xylostella *and* C. binotalis*, followed by phenols and flavonoids. In the case of* M. persicae*,* A. triplinervis *extracts disrupted growth and development of the nymphs, had significant pest control properties, and were good candidates for further study on their potential as botanical pesticides, in the context of an organic farming/ sustainable agriculture system, as an environmentally-friendly alternative to synthetic insecticides.*

Résumé

Étude de divers extraits de Ayapana triplinervis pour leur pouvoir de contrôle de trois ravageurs de cultures horticoles

Les groupes chimiques de Ayapana triplinervis*, extraits successivement au moyen de l'hexane, de l'éther de pétrole, du méthanol, de la chloroforme: méthanol (1:1) et de la chloroforme: méthanol (4:1) ont été étudiés pour leurs effets sur le* Plutella xylostella*, les* Crocidolomia binotalis *et les* Myzus persicae*, trois insectes nuisibles aux cultures horticoles à l'Ile Maurice. Les extraits bioactifs ont été fractionnés davantage en divers groupes en utilisant la chromatographie et sept d'entre eux montrant l'activité la plus forte ont été examinés sur chacun des trois insectes. Les résultats ont montré que les alcaloïdes et les tannins sont les groupes chimiques ayant la plus grande dissuasion d'alimentation dans les larves de* P. xylostella *et de* C. binotalis*, suivis des phénols et des flavonoïdes. Dans le cas des* M. persicae*, les extraits de* A. triplinervis *ont perturbé la croissance et le développement des nymphes, et sont des bons candidats pour les études plus poussées dans le contexte d'agriculture biologique et durable.*

Introduction

The knowledge that plants exhibit pesticidal properties is not new; it has been known and used since immemorial time for protecting grains and other foodstuffs (8, 19). Plants are a very rich source of bioactive organic chemicals and more than 400,000 secondary metabolites may be present in the plant kingdom (34). Extracts from tobacco, rotenone, and pyrethroids have been studied exhaustively for their pesticidal activities. Alkaloids, sesquiterpenes, flavonoids, limonoids, phenols, coumarins, and stilbenes of plant origin are known to possess toxic, antifeedant, and growth regulating effects against a wide range of insect pests (14, 20, 24, 26, 33). Use of allelochemicals in the form of pesticidal treatments (1, 7, 16, 23) within an integrated pest, disease and weed management programme (19), or as part of cropping systems for sustainable agriculture (2, 6, 27, 30), has several advantages – some of them are cheap, effective, environmentally friendly, less hazardous to human and animal health, non-toxic to non-target species, and less likely to result in resistance in the target organism (13). Synthetic modification of phytochemicals has resulted in more effective and improved bioactive compounds (32). Synthetic pyrethroids such as cypermethrin, cyahalothrin and deltamethrin based on the natural pyrethrum structural models, have become quite popular and occupy a large share of the pesticide market, mainly because of their broad-spectrum activity and low mammalian toxicity.

Some of the local plants in Mauritius have been shown to have potential in agricultural pest control (10, 12). Other allelopathic effects of plant extracts include the role of neem (*Azadirachta indica*) cake as a soil bactericide and for improving efficiency of fertiliser use (22).

In this study, *Ayapana triplinervis* (also known as *Eupatorium ayapana*), which plays a traditional role in Mauritian pharmacognosy, was subjected to a comprehensive extraction system with solvents of different polarity to obtain a series of fractions which were then individually tested against *Plutella xylostella* (Lepidoptera: Plutelldiae)*, Crocidolomia binotalis* (Lepidoptera: Pyralidae), the major lepidopteran pests of crucifers for their larvicidal, antifeedant, and growth regulating effects, and against *Myzus persicae* (Homoptera: Aphididae) for their insecticidal and growth regulating properties.

Materials and methods

Plant and insect materials

Ayapana triplinervis is a perennial, slightly erect shrub-like, medicinal plant with reddish twigs, sessile and ovoid leaves, having a length of 5-8 cm and a width of 0.8-1.7 cm. 500 g of fresh young leaves of *A. triplinervis* obtained from the University farm were gently washed with distilled water, dried at 50 °C and ground to pass a 1.5 mm mesh sieve. 150 g of the powder was then successively extracted with 500 ml each of hexane, petroleum ether (60-80 °C), methanol, choloroform: methanol (1:1), and chloroform: methanol (4:1) in a Soxhlet extractor.

The various extracts obtained were rotoevaporated under vacuum, and kept as a 10% w/v solution at 4 °C for further analyses.

Extracts were tested for alkaloids, terpenes, sterols, phenols, tannins, flavonoids and anthraquinone heterosides (Table 1).

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Table 1
Results of chemical tests on extracts of *Ayapana triplinervis*

Test for	Reagents	Extracts				
		Hexane	Petroleum ether	Methanol	Chloroform : methanol (1:1)	Chloroform : methanol (4:1)
Alkaloids	Draggendorf	+	+	-	++	+++
Sterols	Acetic anhydride	+	++	-	+	+
Terpenes	Acetic anhydride	+	++		+	+
Phenols	Iron III chloride	+	++	+	++	+
Tannins	Iron III chloride	+	+	+	+++	+++
Flavonoids	Shinoda reaction	+	-	-	+	++
Anthraquinone heterosides	Iron III chloride	+	+			+

+ indicates a weak reaction; ++ indicates a strong reaction; +++ indicates a very strong reaction.

Plutella xylostella, Crocidolomia binotalis and *Myzus persicae* were reared separately under laboratory conditions at 25 ± 2 °C, in wooden cages (75 x 50 x 50 cm) covered with fine nylon mesh. The lepidopteran larvae were fed with clean untreated leaves of cabbage (*Brassica oleraceae capitata*). A cotton swab soaked in 10% honey solution was provided as food for the adults, and untreated cabbage leaves were provided for oviposition. Third-instar larvae from these cultures were used for the bioassays. In the case of *M. persicae*, the nymphs and adults were fed and bred on potted cabbage seedlings. Third-instar nymphs were used for the bioassays.

Bioassays

The extracts were bioassayed by a no-choice disc assay method, using young cabbage (*Brassica oleraceae capitata*) leaves. 2.5 cm discs from untreated cabbage leaves were dipped for 30 seconds in one of the plant extracts (10% w/v), air-dried and weighed. Five such treated leaf discs were placed in a petridish lined with moistened filter. Five larvae of either *Crocidolomia binotalis* or *Plutella xylostella*, or seventy five nymphs of *Myzus persicae*, starved for 2 h in order to equilibrate their level of hunger, were introduced into each petridish. Antifeedant activity on the lepidopteran larvae was measured by estimating the amount of leaf area eaten in 48h, and the percentage mortality noted. In case of *M. persicae*, the number of deformed and/or dead nymphs, as well as the number of deformed and/or dead adults was noted.

Each treatment was replicated five times, with a replicated control (leaf discs dipped in solvent alone).

The most bioactive extract (chloroform: methanol 1: 1 in the case of *P. xylostella* and chloroform: methanol 4: 1 in the case of *C. binotalis*) was subjected to Thin Layer Chromatography (TLC) on a 250 µm thick silica gel plate using a hexane: acetone: chloroform (4: 3: 3) solvent system. The individual spots were sprayed with various chemical reagents (Table 1). Those giving a positive reaction were scraped off the plate, dissolved in 20 ml chloroform: methanol (1: 1), concentrated by rotoevaporation to 10 ml and bioassayed as above, using *Plutella xylostella, Crocidolomia binotalis* and *Myzus persicae* as test species. Controls consisted of chloroform: methanol (1: 1) with a drop of the same reagent that was sprayed on the TLC plate.

Results and discussion

Extraction and rotoevaporation gave yields of 3% with hexane, 0.8% with petroleum ether, 0.33% with methanol, 0.53% with chloroform: methanol (1: 1) and 0.2% with chloroform: methanol (4: 1).

Results of the chemical tests on the various extracts of *A. triplinervis* are shown in table 1, while tables 2 to 7 show the antifeedant, insecticidal and growth regulating effects of these extracts.

The petroleum ether fraction showed higher amounts of sterols, terpenes and free phenols, whereas the chloroform–methanol fractions had higher concentrations of alkaloids, tannins, phenols and flavonoids (Table 1). The hexane fraction exhibited weak reactions for all the tests, while only phenols and tannins in low amounts were present in the methanol fraction.

Plutella xylostella* and *Crocidolomia binotalis

All the fractions exhibited significant antifeedant properties (Tables 2 and 4). The percentage reduction in feeding by *P. xylostella* larvae was highest in the chloroform: methanol (1: 1) fraction (69.2%), being more than twice that of the methanol extract (30.9%) (Table 2), while percent reduction

Table 2
Antifeedant effect of *Ayapana triplinervis* extracts on *Plutella xylostella* larvae

Extracts	Treated leaves		Untreated leaves		Reduction in feeding (%)
	Amount eaten (mg/ larva)	No. of dead larvae (n= 25)	Amount eaten (mg/larva)	No. of dead larvae (n= 25)	
Hexane	8.7 ± 1.5* b#	7	20.3 ± 1.2 ab	1	57.1 **
Petroleum ether	13.0 ± 2.0 a	6	21.7 ± 0.8 a	2	40.0 **
Methanol	14.3 ± 1.7 a	6	20.7 ± 1.2 ab	3	30.9 **
Chl: Meth 1:1	6.0 ± 2.3 b	6	19.5 ± 1.8 b	4	69.2 **
Chl: Meth 4:1	6.9 ± 1.7 b	7	19.9 ± 0.9 ab	3	65.3 **

* Mean ± s.d.

Figures followed by the same letter down a column are not significantly different at p= 0.05 with DMRT

** Significantly different at p= 0.05 with t-test

Table 3
Antifeedant effect of chemical groups in C: M 1: 1 fraction of *Ayapana triplinervis* on *Plutella xylostella* larvae

Rf values	Group	Amount eaten (mg/larva)	No. of larvae dead (n= 25)	Reduction in feeding (%)
	Control	22.2 ± 1.3* a#	2	
0.11-0.14	Sterols	13.4 ± 1.6 b	2	39.6 ab
0.17-0.20	Phenols I	13.8 ± 0.7 b	5	37.8 a
0.90-0.92	Phenols II	9.8 ± 1.3 de	7	55.9 d
0.37-0.39	Tannins I	10.5 ± 1.4 cde	6	52.7 cd
0.77-0.87	Tannins II	12.3 ± 1.3 bc	5	44.6 b
0.54-0.65	Alkaloids I	8.9 ± 1.2 e	4	59.9 d
0.70-0.72	Alkaloids II	11.1 ± 0.9 cd	3	50.0 c

* Mean ± s.d.
# Figures followed by the same letter down a column are not significantly different at p= 0.05 with DMRT

in feeding by *C. binotalis* larvae was highest in the chloroform: methanol (4: 1) fraction (61.6%), being about 10 times more potent than the methanol extract (6.6%) (Table 4).

The high activity exhibited by the chloroform: methanol fraction suggests that the bioactive groups responsible for the antifeedant activity of *A. triplinervis* could be alkaloids and/or tannins, and to a lesser extent phenols and/or flavonoids in varying combinations. Also, the extracts were observed to have differential antifeedant effects on the different test species.

The methanol extract did not show significant feeding deterrence in the test insects. The fact that the fraction tested was very weak for the different bioactive groups indicates extraction of very few or little of the compounds responsible for the bioactivity against the two test species.
Further fractionation of the chloroform: methanol extract yielded 14 different compounds, of which 7 tested positive for one or more of the chemical groups listed in table 1. Bioassays of these 7 fractions confirmed their excellent antifeedant properties (Tables 3 and 5), with biological activity ranging from 37.8% to 59.9%, and 39.9% to 65.4% feeding reduction in *P. xylostella* and *C. binotalis* larvae, respectively. TLC showed that two different groups of alkaloids, with different Rf values, were present in the fraction. Furthermore, their antifeedant effect was also noted to be significantly different on *Plutella* larvae, with one group having a strong feeding deterrence of 59.9%, and the other 50%. In the case of *C. binotalis*, the 2 alkaloid groups, exhibited maximum feeding reduction (64.7 and 65.4%). Alkaloids, along with terpenoids and flavonoids, are well known phytochemicals having biocidal activity (3, 9, 25) and have potential in crop protection. Tomatine, an alkaloid found in tomato plants, considerably reduced the rate of increase in larval growth of the larvae (17).
The order of the antifeedant property of the different solvent extracts in the case of *P. xylostella* was chloroform: methanol (1: 1) > chloroform: methanol (4: 1) > hexane > petroleum ether > methanol.

Table 4
Antifeedant effect of *Ayapana triplinervis* extracts on *Crocidolomia binotalis* larvae

Extracts	Treated Leaves		Untreated Leaves		Reduction in feeding (%)
	Amount eaten (mg/larva)	No. of dead larvae (n= 25)	Amount eaten (mg/larva)	No. of dead larvae (n= 25)	
Hexane	22.1 ± 2.1* c#	2	31.5 ± 2.7 a	1	29.8 **
Petroleum ether	25.9 ± 1.3 b	4	32.6 ± 2.6 a	2	20.6 **
Methanol	29.8 ± 2.9 a	2	31.9 ± 2.4 a	2	6.6
Chl:Meth 1:1	20.4 ± 1.7 c	6	33.7 ± 2.6 a	3	39.5 **
Chl:Meth 4:1	13.2 ± 1.4 d	4	34.4 ± 2.4 a	1	61.6 **

* Mean ± s.d.
# Figures followed by the same letter down a column are not significantly different at p= 0.05 with DMRT
** Significantly different at p= 0.05 with t-test

Table 5
Antifeedant effect of chemical groups in C: M 4: 1 fraction of *Ayapana triplinervis* on *Crocidolomia binotalis* larvae

Rf values	Group	Amount eaten (mg/larva)	No. of larvae dead (n= 25)	Reduction in feeding (%)
	Control	42.8 ± 1.4* a#	1	
0.11-0.14	Sterols	19.9 ± 2.9 c	3	53.5 b
0.17-0.20	Phenols I	24.5 ± 2.4 b	6	42.8 a
0.90-0.92	Phenols II	26.0 ± 2.1 b	5	39.3 a
0.37-0.39	Tannins I	23.7 ± 1.7 b	7	44.6 a
0.77-0.87	Tannins II	18.0 ± 3.6 cd	6	57.9 b
0.54-0.65	Alkaloids I	14.8 ± 2.3 d	5	65.4 c
0.70-0.72	Alkaloids II	15.1 ± 1.9 d	5	64.7 c

* Mean ± s.d.
# Figures followed by the same letter down a column are not significantly different at p= 0.05 with DMRT

The order of the antifeedant property of the different solvent extracts in the case of *C. binotalis* was slightly different, being chloroform: methanol (4: 1) > chloroform: methanol (1: 1) > hexane > petroleum ether > methanol.
None of the fractions extracted in this study produced significant mortality nor growth disruption in the lepidopteran species, the effect was mainly one of reduction in feeding. However, other studies (28) have demonstrated insecticidal effect of *Ayapana triplinervis* extracts on *Sitophilus* spp. and *Tribolium* spp., which suggests that the active fractions from *A. triplinervis* show different activities on different insects

Myzus persicae
The various extracts produced growth regulating and insecticidal effects on *M. persicae* nymphs, with a significant reduction in the proportion of nymphs developing normally to adulthood (Tables 6 and 7).
As in the case of *C. binotalis*, the chloroform: methanol (4: 1) fraction exhibited highest bioactivity (85.7% growth disruption) as compared to 44% by the methanol extract (Table 6). Further studies on the chloroform: methanol (4: 1) fraction (Table 7) showed that one from each of the phenol and alkaloid groups, and both tannin groups, reduced development by more than 80%. The growth disruption resulting from the extracts and fractions were manifested also in the form of deformities in the nymphal stages or in the adults. Severe deformities such as abnormal bodies, twisted legs and antennae, abnormal pigmentation caused death of the nymphs. While the relatively milder deformities (smaller bodies, thinner legs, disproportionate caudal filaments) allowed the nymphs to survive to adulthood. However, the nymphal abnormalities were observed to have been accentuated in the adults, and resulted in adult death in some cases.
Terpenoids from other plants, such as the azadirachtins from neem (*Azadirachta indica*) inhibited feeding in *Schistocerca gregaria* following either topical or systemic application to food substrates (4), as well as in the striped cucumber beetle (*Acalymma vittatum*) (29). The antifeedant, growth regulating as well as toxic effects of neem have been widely reported against a large variety of insects (11, 15, 31). Limonene and other monoterpenes have also been shown to exhibit potential for control of insect and other pests (18). Plant essential oils and their volatiles reduced feeding damage and decreased adult survival in *Thrips tabaci* in leaf disc bioassays (21).
The sterol group also exhibited significant antifeedant effect on *P. xylostella* and *C. binotalis* and growth disturbance in *M. persicae*. The feeding reducing effect of steroids in *Mamestra brassicae* and *Pieris brassicae* larvae has been reported by some workers (5).
The two different groups of phenols (Rf values 0.17-0.21 and 0.90-0.92) did not show any difference in their biological activity against *C. binotalis,* having more or less comparable influence on feeding, but was significantly different against *P. xylostella* and *M. persicae*. The two different tannin groups

Table 6
Growth disrupting effect of *Ayapana triplinervis* extracts on *Myzus persicae* nymphs (n= 75 per replicate)

Extracts	Treated			Untreated			Reduction in nymphs reaching adulthood (%)
	Nymphs reaching adulthood	Deformed nymphs	Deformed adults	Nymphs reaching adulthood	Deformed nymphs	Deformed adults	
Hexane	10.6 ± 2.7* b#	26.4	4.2	56.2 ± 7.0 ab	1.2	2.2	81.14 **
Petroleum ether	30.0 ± 5.2 a	15.6	7.0	64.0 ± 5.7 a	1.2	0.8	53.13 **
Methanol	30.8 ± 5.4 a	17.2	6.0	55.0 ± 5.3 b	2.2	0.6	44.0 **
Chl: Meth 1: 1	8.0 ± 2.1 b	28.0	2.4	52.4 ± 4.7 b	2.0	1.2	84.73 **
Chl: Meth 4: 1	7.2 ± 2.4 b	14.0	3.0	50.4 ± 5.7 b	0.6	1.0	85.71 **

* Mean ± s.d.
# Figures followed by the same letter down a column are not significantly different at p= 0.05 with DMRT;
** Significantly different at p= 0.05 with t-test

Table 7
Growth disrupting effect of chemical groups in C: M 4: 1 fraction of *Ayapana triplinervis* on *Myzus persicae* (n= 75 per replicate)

Rf values	Group	Nymphs reaching adulthood	Deformed nymphs	Deformed adults	Reduction in nymphs reaching adulthood (%)
	Control	68.0 ± 2.9* a#	0.8	0.6	
0.11-0.14	Sterols	34.8 ± 6.0 a	2.6	5.4	48.8 a
0.17-0.20	Phenols I	24.0 ± 5.2 b	3.8	4.8	64.7 b
0.90-0.92	Phenols II	6.8 ± 2.4 d	2.8	1.0	90.0 c
0.37-0.39	Tannins I	10.6 ± 3.4 d	2.8	2.2	84.4 c
0.77-0.87	Tannins II	8.0 ± 2.7 d	5.0	3.8	88.2 c
0.54-0.65	Alkaloids I	11.6 ± 3.6 d	4.6	5.2	82.9 d
0.70-0.72	Alkaloids II	17.0 ± 5.0 c	2.6	3.0	75.0 c

* Mean ± s.d.
# Figures followed by the same letter down a column are not significantly different at p= 0.05 with DMRT

also showed a similar pattern.
The effects of *A. triplinervis* extracts appear to be different on the three test species studied, affecting mainly feeding in the holometabolous insects, and growth and development in the hemimetabolous insect. This difference may be partly due to inherent differences in the mode of action of the bioactive compounds in different insect groups, and partly due to the method of application (treated food in case of *P. xylostella* and *C. binotalis*, and contact and/or residual effect in case of *M. persicae*).
Another important finding of this study is the fact that when the extracts of *A. triplinervis* were further fractionated, there was a decrease in activity of the individual fractions in comparison to the complete extract in the case of *P. xylostella* and an increase in activity in the case of *C. binotalis*. For example, the complete chloroform: methanol (1:1) extract gave a maximum of 69.2% feeding reduction (Table 2), but the individual compounds (Table 3) produced feeding reduction ranging from 37.8 to 59.9 %. This indicates that some compounds within a fraction may have either a synergistic or simply an additive effect on each other, while others may act in an antagonistic manner.

Conclusion

Many phytochemicals are being studied with a view to developing alternatives to the highly toxic and persistent pesticides being used today. The present study reveals that there is a potential for obtaining antifeedant compounds from *Ayapana triplinervis*, either as botanical pesticides by themselves, or as models for the synthesis of pesticidal analogues. The biologically active compounds showing the highest activity need to be valuated under field conditions for persistence, and may be formulated with other natural compounds from other sources to increase the spectrum of activity. At a later stage conventional toxicology testing of these extracts can also be undertaken.
A. triplinervis is another of the vast array of tropical plants which can contribute significantly to the presently ongoing study of bioactive phytochemicals as alternatives to the highly toxic and persistent synthetic pesticides. The apparent difference in its mechanism of action (antifeedant, growth regulating) and/or differences resulting from application techniques can be used to make this potential pesticide specific to selected pest groups.

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AVIS

Nous rappelons à tous nos lecteurs, particulièrement à ceux résidant dans les pays en voie de développement, que TROPICULTURA est destiné à tous ceux qui oeuvrent dans le domaine rural pris au sens large.

Pour cette raison, il serait utile que vous nous fassiez connaître des Institutions, Ecoles, Facultés, Centres ou Stations de recherche en agriculture du pays ou de la région où vous vous trouvez. Nous pourrions les abonner si ce n'est déjà fait.

Nous pensons ainsi, grâce à votre aide, pouvoir rendre un grand service à la communauté pour laquelle vous travaillez.

Merci.

BERICHT

Wij herrineren al onze lezers eraan, vooral diegenen in de ontwikkelingslanden, dat TROPICULTURA bestemd is voor ieder die werk verricht op het gebied van het platteland en dit in de meest ruime zin van het woord.

Daarom zou het nuttig zijn dat u ons de adressen zou geven van de Instellingen, Scholen, Faculteiten, Centra of Stations voor landbouwonderzoekvan het land of de streek waar U zich bevindt. Wij zouden ze kunnen abonneren, zo dit niet reeds gebeurd is.

Met uw hulp denken we dus een grote dienst te kunnen bewijzen aan de gemeenschap waarvoor u werkt.

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