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MN Mounika
Department of Entomology, COH, Dr. YSRHU, Venkataramannagudem, Andhra Pradesh, India

Emmanuel Nathala
Associate Professor, Department of Entomology, COH, Dr. YSRHU, Venkataramannagudem, Andhra Pradesh, India

DRS Sunnetha
Professor, Department of Biochemistry, COH, Dr. YSRHU, Venkataramannagudem, Andhra Pradesh, India

Corresponding Author:
MN Mounika
Department of Entomology, COH, Dr. YSRHU, Venkataramannagudem, Andhra Pradesh, India

Antifeedant, toxic and biochemical effects of sweet flag, *Acorus calamus* against diamondback moth, *Plutella xylostella* (Linn) (Plutellidae: Lepidoptera)

MN Mounika, Emmanuel Nathala and DRS Sunnetha

Abstract
The present research work was carried at College of Horticulture, Dr. YSRHU, Venkataramannagudem. Hexane, methanol and aqueous extracts of *Acorus calamus* were tested against third instar larvae of diamondback moth, *Plutella xylostella* through leafdip method to assess the toxic and biochemical effects. AI_{50} values for hexane, methanol and aqueous extract were 0.022, 0.019 and 0.030%, respectively. Methanol extract was reported to show 100.00 per cent mortality with LC_{50} value of 0.019% at 5.0% concentration followed by hexane (LC_{50} =0.027%) and aqueous extracts (LC_{50} = 0.033%) at the same concentration. Total protein and carbohydrate content was highly reduced to 32.30 mg/g and 47.63% in the methanol extract of *A. calamus* at 5.0% concentration.

Keywords: *Acorus calamus*, leaf dip method, toxic, biochemical, *Plutella xylostella*

Introduction
India is next to China in area and production of vegetables. Cole crops are rich in vitamin C, A and contains indoles and dithiolthions which have been linked with the prevention of colon, rectum and breast cancers. The lepidopteran pest, diamondback moth, *Plutella xylostella* (Linn.) is one of the most destructive pest in cole crops throughout the world, as its management is very difficult [1]. The larvae of diamondback moth initially feeds on the leaves and later penetrate inside the head in case of cabbage and destroy it completely. *P. xylostella* management is currently based on heavy use of many neurotoxic insecticides. Consequently, it has developed resistance to almost every group of synthetic insecticide including *Bacillus thuringiensis* Berliner (*Bt*) formulations [2, 3]. The misuse and extensive use of synthetic insecticides cause undesirable effects not only to the agricultural ecosystem but also to human health due to the insecticide residues in food.
Pesticides derived from plants have the potential to play a major role in pest management for sustainable agriculture production [4]. Plants produce a wide range of chemicals to protect themselves from insect. Such chemicals are secondary metabolites like alkaloids, terpenoids, flavonoids and acetogenins, to protect themselves from insect pests [5]. It is difficult for the insects to develop resistance to these secondary chemicals.
In the pursuit of naturally occurring phytochemicals in botanicals, secondary metabolites of sweetflag, *Acorus calamus* is found to have insecticidal properties. Sweet flag is probably indigenous to India and it is a tall perennial wetland monocot plant from the Acoraceae family. The rhizomes of *A. calamus* contain β-asarone, cis-asarone, tran-asarone, acoramone and a bitter glycoside, acorine along with eugenol, pinene and camphene which possess insecticidal and antifeedant activity [6].
With this background, in the present study, it was proposed to extract the biological active compounds from the rhizomes of *A. calamus* and also evaluating its bioefficacy and biochemical effects against *P. xylostella*.

2. Materials and methods
The studies on toxicity and biochemical effects of *A. calamus* against *P. xylostella* was conducted at College of Horticulture, Venkataramannagudem, Andhra Pradesh.

2.1 Rearing of *P. xylostella*

A continuous culture of *P. xylostella* was developed in an insectary having controlled environment, i.e. 27 ± 1 ºC temperature and 60 ± 5 per cent relative humidity, a photo phase of 14 hours and scotophase of 10 hours. For this purpose, larvae and pupae of the *P. xylostella* were collected from the cauliflower and cabbage fields from the vegetable farm of the College. These were brought to insectary for mass rearing. *P. xylostella* and was maintained on mustard seedlings and cabbage leaves and reared in insectary at laboratory conditions from larval to adult stage [7]. The adults were held in oviposition cages (27× 21 × 21 cm) and provided with 10.00 per cent sugar solution as food in cotton swabs. Five day old mustard seedlings in cups were exposed overnight to adults for oviposition and allowed to hatch. Three day old larvae were transferred to cabbage leaves kept in plastic jars (25 cm × 20 cm). The larvae were regularly provided with fresh leaves without removing the infested one so as to enable them to shift to fresh leaves on their own, to improve their survival rate and reduce the handling damage considerably. The seedlings were exposed daily and larvae were maintained separately for each exposure date to get specific stage of larvae regularly throughout the study period.

2.2 Collection and processing *A. calamus* rhizomes

The rhizomes of *A. calamus* were collected from tribal areas viz., Rampachodavaram of East Godavari and Buttayyagudem, Jeelugumilli of West Godavari districts of Andhra Pradesh. The rhizomes were shade dried and later crushed in grinder to fine powder. The grounded powder was thus used for solvent extraction.

2.3 Preparation of *A. calamus* rhizome extracts

The preparation of indigenous plant extracts with the solvents viz., hexane, methanol and aqueous extracts were made [8].

2.3.1 Hexane extract

Finely grounded rhizomes (500.00 g) were extracted with 2l hexane, using mechanical stirrer for half an hour and the blend was kept overnight for 24 hours. The supernatant was filtered and the rhizomes were extracted two more times with hexane. The pooled extract was subjected to vacuum distillation at 40 ºC temperature to obtain hexane concentrate.

2.3.2 Methanol extract

The rhizomes were further extracted with 2l methanol using the mechanical stirrer. The blend was thoroughly stirred for half an hour and left overnight for 24 hours. The supernatant was then filtered and subjected to vacuum distillation at 40 ºC temperature to obtain methanol concentrate.

2.3.3 Aqueous extract (fresh plant material)

In a separate experiment, freshly grinded rhizomes were suspended in 2l distilled water and the rhizomes were kept for 24 hours as such. After it was filtered and concentrated under vaccum at 50 ºC temperature to obtain viscous material.

In leaf dip method, cabbage leaf discs of approximately 5 cm diameter were cut and different solvent concentrations *viz*., 5.0, 3.0, 1.0, 0.5, 0.1, 0.05 and 0.01% were prepared. The leaf discs of appropriate size were first treated with the different concentrations of the hexane, methanol and aqueous solvents of *A. calamus* and then transferred individually to clean petriplate (8 cm x 1.5 cm) and ten, third instar larvae (5 day old) of *P. xylostella* were released on leaf disc which were present in the petriplate. Each treatment and control was replicated three times. Further, observations like leaf area fed in one day after treatment, antifeedancy and larval mortality count were recorded. The data was analyzed statistically using completely randomized block design.

2.4 Per cent antifeedancy

The leaf area fed by larva on treated and control disc were recorded and the per cent antifeedance in treated disc was calculated [9].

$$\text{Per cent leaf protection} = \frac{\text{Leaf area given} - \text{Leaf area consumed}}{\text{Leaf area given}} \times 100$$

$$\% \text{ Antifeedance} = \frac{\% \text{ leaf protection in treatment} - \% \text{ leaf protection in control}}{100 - \% \text{ leaf protection in control}} \times 100$$

2.5 Biochemical analysis

Treated and untreated *P. xylostella* larvae were used to estimate the total protein and carbohydrate content. Total protein content (mg/g) in treated and untreated *P. xylostella* larvae was estimated [10]. Carbohydrate content (%) of P. *xylostella* was estimated by using Anthrone reagent [11].

2.6 Statistical analysis

The data was analyzed statistically using completely randomized block design. The data recorded for various parameters viz., antifeedancy, larval mortality and normal adult emergence inhibition, were subjected to probit analysis for the calculation of AI_{50}, LC_{50} and I50 by using SAS 9.3 software program (AI_{50}- Antifeedancy index (%), LC_{50}- Lethal concentration (%).

3. Results and Discussion

Antifeedent effect, biological activity and biochemical effects of *A. calamus* extracts viz., hexane, methanol and aqueous, were tested against the test insect *P. xylostella*. The observations made on leaf area consumed, larval mortality were presented as under.

3.1 Antifeedant and toxic effects

3.1.1 Hexane extract

The antifeedance calculated at 5.0, 3.0, 1.0, 0.5, 0.1, 0.05, and 0.01% concentration was 87.06, 80.39, 76.08, 67.45, 55.69, 45.10 and 39.61 per cent, respectively. The concentration required to give 50.00 per cent antifeedance (AI_{50}) being 0.022%.The maximum cumulative larval mortality was observed at 5.0% (90.00 per cent). The median lethal

concentration (LC_{50}) for causing 50.00 per cent larval mortality being 0.027% (Table 1).

3.1.2 Methanol extract

The antifeedancy effect was concentration dependent and maximum of 87.50, 79.17, 67.50, 58.75 and 54.17 per cent was observed at 5.0, 3.0, 1.0, 0.5 and 0.1% concentrations, respectively (Figure 1). AI_{50} value *i.e*., the concentration calculated to give 50.00 per cent antifeedance was found to be 0.019%. Hundred per cent larval mortality was recorded at 5.0% concentration followed by 93.33, 83.33, 73.33, 63.33, 53.33 per cent mortality at 3.0, 1.0, 0.5, 0.1, 0.05% concentrations, respectively. The LC_{50} values for causing 50.00 per cent larval mortality was being 0.019% (Figure 2).

3.1.3 Aqueous extract

The antifeedancy effect at 5.0, 3.0, 1.0 and 0.5% concentrations was more than 50.00 per cent. The AI_{50} value calculated was 0.030%. The highest larval mortality of 92.89 per cent was recorded when treated

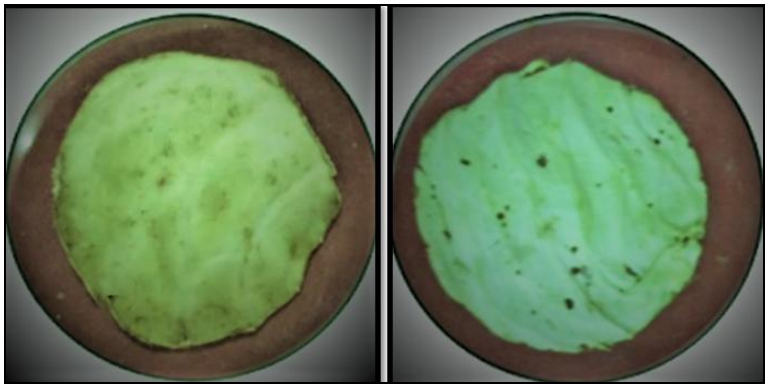

Fig 1: Antifeedance at 5.0% and 1.0% concentrations of methanol extract

Fig 2: Larval mortality of third instar *P. xylostella*

With 5.0% concentration. LC_{50} value required to calculate 50.00 per cent mortality of *P. xylostella* larvae was 0.033%.

All the extracts of *A. calamus viz.,* hexane, methanol and aqueous extracts possess antifeedant and toxic activity and the results are in accordance with 100.00 and 67.00 per cent larval mortality of *P. xylostella* due to cis-asarone and trans-asarones of *A. calamus* [12]. Petroleum ether extract of *A. calamus* at 10,000 ppm concentration yielded 88.00 and 29.00 per cent mortality of *Helicoverpa armigera* and *P. xylostella* [13]. Hexane extract of *A. calamus* rhizome caused 44.70 and 100.00 per cent mortality of *Trogoderma granarium* and *Spodoptera litura* at 4.0% concentration [14, 15]. *A. calamus* bait at 6.0% concentration gave highest mortality of housefly adults [16]. Larval mortality of 92.50 per cent was observed when second instar larvae of *S. litura* was treated with various concentrations of *A. calamus* [17].

3.2 Biochemical effects

Heamolymph protein and carbohydrate third instar larvae of *P. xylostella* were evaluated at 5.0% and 1.0% concentrations of *A. calamus* hexane, methanol and aqueous extracts. Total protein and carbohydrate was significantly decreased in all the three solvents tested whereas methanol extract of showed 32.30mg/g and 47.63% reduction at 5.0% concentration (Table 2).

Table 1: Antifeedant and Toxic effects of *A. calamus* solvents against *P. xylostella*

Conc. (%)	Hexane		Methanol		Aqueous	
	Antifeedant activity (%)	Larval mortality (%)	Antifeedant activity (%)	Larval mortality (%)	Antifeedant activity (%)	Larval mortality (%)
5.0	87.06	90.00	87.50	100.00	92.89	100.00
3.0	80.39	83.33	79.17	93.33	80.89	90.00
1.0	76.08	73.33	67.5	83.33	62.67	80.00

0.5	67.45	70.00	58.75	73.33	53.78	63.33
0.1	55.69	63.33	54.17	63.33	41.78	56.67
0.05	45.09	53.33	40.83	53.33	36.00	53.33
0.01	39.61	43.33	35.00	34.22	32.00	46.67
Control		3.33		3.33		3.33
	AI_{50}= 0.022	LC_{50}= 0.027	AI_{50}= 0.019	LC_{50}=0.019	AI_{50}= 0.030	LC_{50}= 0.033

AI_{50}-Concentration calculated to give 50.00 percent antifeedance.
LC_{50}-Median lethal concentration to give 50.00 percent mortality.

Table 2: Biochemical effects of *A. calamus* solvents against *P. xylostella*

Conc. (%)	Hexane		Methanol		Aqueous	
	Protien mg/g	Carbohydrate (%)	Protein mg/g	Carbohydrate (%)	Protein mg/g	Carbohydrate (%)
5	32.97	48.25	32.35	47.63	33.57	48.82
1	33	48.53	32.41	47.94	33.64	49.1
control	38.42	58.68	38.54	58.34	37.98	57.89

The results of the biochemical analysis are in accordance with the decreased total protein content in third instar larvae of *Musca domestica*, when treated with neem azal [18]. Essential oils of *Thymus vulgaris* and *Origanum vulgare* reduced the total protein and carbohydrate content in lesser mulberry pyralid, *Glyphodes pyloalis* [19]. Total protein was reduced in the mango leaf webber, *Orthaga exvinacea* with methanol extract of *V. negundo* leaves at 5.0% concentration [20].

5. Conclusion

All the three extracts of A. calamus viz., hexane, methanol and aqueous extracts showed more than 50.00 per cent antifeedant effect. AI_{50} values for hexane, methanol and aqueous extract was 0.022, 0.019 and 0.030%, respectively. The LC_{50} values were 0.027, 0.019 and 0.033% for hexane, methanol and aqueous extracts, respectively. All the three solvents reduced protein and carbohydrate content of the treated *P. xylostella* larvae whereas lowest (32.30mg/g and 47.63%) was recorded at 5.0% concentration of methanol extract.

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7. Reference

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