Effect of Acetone leaf extract of the Malabar Catmint *Anisomeles malabarica* Linnaeus against the tobacco leaf caterpillar *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera)

Abstract

Spodoptera litura is one of the serious and ubiquitous pests and causes severe damage that leads to yield loss. Synthethic chemicals causes severe effects to the natural enemy and serious hazardous to human health. Botanicals are gaining popularity in organic farming due to their safety profile on crop consumption, and consumers are willing to pay a premium price for organic produce. *A. malabarica* is an aromatic, densely pubescent, perennial herb which contains numerous secondary metabolites. In this study, we used acetone leaf extract of *A. malabarica* Acetone elution 3 showed highest amount of pupal malformation of 55.56 per cent and pupal mortality of 44.44 per cent. We also pupal weight and length, in which acetone elution 3 recorded the lowest pupal weight of 0.19 g with a per cent reduction of 63.40 and pupal length of 1.20 cm with a per cent reduction of 21.57. Similar trend of results was also observed in confirmatory screening. The lowest pupal weight of 0.21 g with a per cent reduction of 61.11 and pupal length of 1.22 cm with a per cent reduction of 21.79. we also noted the presence of secondary metabolites, tannin (1.32±0.12) and saponins (1.11±0.09) were observed in acetone leaf extract. Flavonoids, phenols and terpenoids were absent in acetone leaf extract.

Keywords

Spodoptera litura, Anisomeles malabarica, Acetone elution 3, pupal mortality, malformation

1. Introduction

The tobacco leaf caterpillar, *S. litura* is a ubiquitous, polyphagous, multivoltine and dreaded lepidopteran pest that feeds on nearly 112 species of cultivated crops all over the world and on about 60 species from India (Garad *et al*., 1984). It is one of the significant pests found in Southeast Asia, Africa, America, India, China, Japan and Australia. *S. litura* attacks several crops namely cotton, tomato, potato, soybeans, barley, sweet potato, groundnut and several horticultural crops (Ramzan *et al*., 2020). Botanical pesticides were widely utilized for millennia in both subsistence and commercial farming before the development of synthetic pesticides. Botanical pesticide ingredients are naturally occurring chemical derivatives of plants that act as repellents, attractants, antifeedants, and growth inhibitors (Mahmood *et al*., 2016). In this study, we use acetone leaf extract to treat *S. litura* (Poison food bioassay) in which acetone elution 3 registered 55.56 per cent pupal malformation and 44.44 per cent pupal mortality. Here in this paper, we have described briefly about the procedures and protocols that we have carried out.

2. Materials and Methods

2.1. Collection of plant materials

The leaves of *A. malabarica* were collected from Azhagar hills, Madurai, Tamilnadu, India (latitude is 10.094288 N and the longitude is 78.223316 E). The collected leaves were washed with distilled water and chopped into small pieces with a sharp knife. The chopped leaves were ground with the help of electric blender into paste like and shade dried in enamel trays. Dried samples were powdered and stored in an airtight container by covering it with aluminium foil. The container was placed in a refrigerator at 20°C until the powdered plant materials were used for further studies (Bakavathiappan *et al.,* 2012).

2.2. Rearing of test insects

Mass production of *S. litura* was done in laboratory conditions to maintain the homogenous population of larvae throughout the bioassay experiments. The larvae were fed with castor leaves (*Ricinus* communis) till pupation. In the last instar, half of the plastic rearing tray was filled with fine dried sand in which fresh castor leaves were placed and then larvae were released. After the completion of feeding the larvae took pupation in the soil. Four days after pupation, pupae were collected and washed in 10 per cent formaldehyde solution and then washed in tap water to remove the traces of formaldehyde. The pupae were placed on a thin layer of absorbent cotton kept in the petriplate which was placed in the cage for the emergence of moths. In the cage, a pencilin vial containing 10 per cent honey solution with a few drops of Health OK multivitamin drops was placed as food for adult moths. After the completion of mating by moths, a tender shoot of nerium was kept in a 100 ml conical flask containing water which was placed in the cage. The tender leaves of nerium acted as a preferred substratum for *S. litura* moths to lay eggs. The nerium leaf with egg mass was removed and kept in a plastic container which was placed in the refrigerator, until the egg mass was used for mass production. The eggs were incubated at a high relative humidity of about 80 per cent maintained in plastic trays with tender castor leaves as food for neonates (Jeyasankar *et al*., 2016).

2.3. Extraction of leaf by Soxhlet method

About 10 g of a powdered leaf sample was weighed and packed in kada cloth as thimbles using a stapler. 250 ml of conical flasks were taken and five thimbles of leaf powder were kept inside and 200 ml of acetone is added. Then, the mouths of conical flasks were closed with non-absorbent cotton and again the top of conical flasks was wrapped with aluminium foil and tightly secured with a rubber band. These conical flasks were kept overnight for soaking 12 hrs – 14 hrs. Next day, the thimbles were taken from the conical flasks and placed in the Soxhlet extractor unit and the soaked solvent was transferred from conical flask to bottom flask. Further, the required quantity of solvent was added. The boiling point of acetone (56.2 °C) was fixed in a heating mantle. (Patil and Chavan, 2010). The solvents in the bottom flasks were allowed to get boiled, evaporated, condensed and the

thimble in the extractor was soaked and waited for 6-7 hours. The extract was collected in bottom flasks and transferred to glass bowls which were kept under open sun-light in order to evaporate the solvent. When the solvent reduced to 20 ml in the glass bowl, silica gel was added to make it into paste form (miscella) (Senhaji *et al*., 2005).

2.4. Purification by column chromatography

Glass columns were taken and rinsed with 1 ml of acetone thoroughly and packed with a loose mat of non-absorbent cotton near the spout using a glass rod gently. The glass columns were made with three layers. The first layer was filled with silica gel of 60-120 mesh up to 20 cm height and gently packed it by tapping with a glass rod. Similarly, a layer of 5 cm activated charcoal, miscella with silica gel and silica gel of 3 cm were packed one over the other and 1/3rd of glass column was left free. The solvent was poured on the top of the column and allowed to run. Purified extract was collected in small vials to a quantity of 5 ml as elutions. There were five elutions of solvent done. For each elution nearly 3-4 hours intervals were taken (Senhaji *et al*., 2005).

2.5. Bioefficacy of leaf extract of *A. malabarica* by acetone solvent

The five elutions of acetone solvent was taken. Bioassay was carried out with seven treatments *viz*. five elutions, an absolute control (Solvent) and a control (Distilled water), replicated thrice. In each replication, three third instar larvae were released. The bioassay experiments were carried out only during evening hours only, since larvae are nocturnal in habit (Ray *et al*., 2009).

The 3 cm² diameter of castor leaf disc was taken and 10 µl of the diluted elution was pipetted out with the micropipette and smeared on both adaxial and abaxial surface and dried. The larvae were prestarved for 3 hours in petridish plates with filter paper before the experiment. The treated leaf discs were placed over the filter paper in the petriplates and then the prestarved larvae were released and allowed to feed for 24 hours (1 day). From the next day of treatment, daily fresh leaves were supplied for the treatments and control until the larvae reached pupation. Growth regulatory activities *viz.,* larval malformation, larval mortality, pupal malformation, pupal mortality and adult malformation were also recorded (Ray *et al*., 2009).

2.6. Effect of leaf extract of *A. malabarica* by acetone solvents against the growth parameters of *S. litura*

Growth parameters *viz.,* pupal weight and pupal length were recorded in preliminary and confirmatory screenings. Pupal weight was recorded by using digital balance and expressed grams (g) per two pupae, because two larvae were used per replication. Pupal length was also measured with a measurement scale and expressed in centimetre (cm) per two pupae.

2.7. Phytochemical analysis of alkaloids

By following the procedures of Thavapudalvi *et al*. (2022) both qualitative and quantitative analysis (Flavonoids, proteins, saponins, tannins and terpenoids) were carried out for acetone leaf extract of *A. malabarica.*

2.7.1. Qualitative test for alkaloids

Flavonoids

A few drops of sodium hydroxide solution were added to 1 ml of the leaf extract. Flavonoids were detected by the formation of a bright yellow colour that turned colourless when diluted acid was added.

Phenols

About 1 ml of leaf extract was taken in a test tube in which few drops of $FeCl_3$ and ethanol were added. Formation of violet colour indicated the formation of phenols

Saponins

When two drops of coconut oil and a few drops of water were applied to 1 ml of leaf extract taken in a test tube, appearance of a layer or foam showed the presence of saponins.

Tannins

About 5 ml of the leaf extract was mixed with 2 mL of 5 per cent of $FeCl_3$ in a test tube. The formation of greenish-black precipitation showed presence of tannins.

Terpenoids

About 5ml of leaf extract was taken in a test tube in which two millilitres of chloroform was added and evaporated over a water bath, and then heated with two millilitres of concentrated H_2SO_4. Terpenoids generated a grey colour, which served as a sign of their presence.

2.7.2. Quantitative test for alkaloids

Flavonoids

About 1 g of leaf extract was weighed and repeatedly extracted at room temperature with 100 ml each of 80 per cent acetone, water, ethanol, and methanol. A Whatman no.1 filter paper was used to filter the mixture into a pre-weighed 250 ml beaker. The filtrate was put into a water bath, allowed to dry completely, and then weighed.

Phenols

The phenolic component was extracted from the fat-free samples by boiling them in 50 cc of ether for 15 minutes. A 50 mL flask was filled with 5 mL of the appropriate extract, followed by 10 mL of distilled water. Additionally, 5 mL of concentrated amyl alcohol and 2 mL of NH_4OH solution were added. Acetone leaf extract was prepared according to specifications and allowed to respond for 30 minutes before being coloured. At absorbance of 550nm was measured by using Systronics spectrophotometer 166.

Saponins

The leaf sample *of A. malabarica* was ground separately. About 200 ml of 20 per cent ethanol was mixed with 20g of each leaf sample. In turn, the suspension was cooked at roughly 55°C over a hot water bath for 4 hours while being constantly stirred. The mixture was filtered, and the leftover material was extracted once more using 200 cc of 20 per cent ethanol. A water bath heated to roughly 90°C was used to decrease the combined extracts to 40 ml. Transferring the concentrated samples into a 250 ml separator funnel, 20 ml of diethyl ether was added, and the mixture was violently agitated. While the ether layer was discarded, the aqueous layer was recovered. The cleansing procedure was repeated. N-butanol 60 ml was added. Two separate washes with 10 ml of 5 per cent aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the residual solution was warmed. Following evaporation, the corresponding samples were baked to a consistent weight. The amount of saponins was calculated as a percentage.

Tannins

In a 50 ml plastic bottle, 500 mg of *A. malabarica* leaf extract was measured out, in which aqueous solvent and 50 ml of methanol were added, and the mixture was agitated for an hour in a mechanical shaker. This was built up to specification and filtered into a 50 ml volumetric flask. Then, 2 ml of 0.1M ferric chloride in 0.1 N hydrochloric acid and 0.008M $C_6N_6FeK_3$ were added to 5 ml of the filtrate in a test tube. Within 10 mm, the absorbance at 120 nm was measured by using Systronics spectrophotometer 166.

Terpenoids

About 100 mg of *A. malabarica* leaf extract was taken, and they were steeped for 24 hours in 9 ml of methanol and water. Following filtering, the extract was extracted using a separating funnel and 10 ml of petroleum ether. The plant ether extract was divided into pre-weighed glass vials, let to dry completely, and then the yield (percent) of the total terpenoids contents was calculated using the formula below.

(wi-wf/wi×100) Whereas wi – Initial weight; wf – Final weight

2.8. Statistical analysis

The data obtained from laboratory experiment were analysed in a Completely Randomized Block Design by "F" test for significance. Standard Error of difference (S.E (d)) and Critical difference values were calculated at 5 per cent probability level and the treatment means values of the experiments were compared using Duncan's Multiple Range Test (DMRT). The one-way anova is commonly used to test the statistical differences among the means of two or more interventions/ more groups (Steel and Torrie, 1960).

3. Results and discussion

In the preliminary and confirmatory screening, the effect of acetone extract of *A. malabarica* leaf exhibited that the acetone elutions recorded the pupal malformation ranged from 11.11 to 55.56 per cent, pupal mortality ranged from 0.00 to 44.44 per cent and adult malformation ranged from 0.00 to 33.33 per cent (Table 1). Acetone elution 3 recorded the high pupal malformation of 55.56 per cent, followed by acetone elution 1 (44.44%) and acetone elution 3 evinced the high pupal mortality of 44.44 per cent, followed by acetone elution 1 (33.33%) and high adult malformation of 33.33 per cent was recorded at acetone elution 4, followed by acetone elution 1 of (22.22%) compared to absolute control and control. Acetone elutions did not record any larval mortality and larval malformation. It is recorded with the dead pupa with constriction in abdomen and moth is unable to come from the pupa and moth with straight wing and coiled antennae (Fig. 1).

Sharma *et al.* (1981) reported that acetone extract of *A. malabarica* showed 100 per cent ovipositional deterrence activity against the potato tuber moth, *Phthorimoea operculella* (Zeller). Thakur *et al*. (2004) concluded that extract from sea cucumber body wall expressed larvicidal activity against the mosquito *Culex sp.* larvicidal activity due to presence of saponins. Leatemia and Isman (2006) reported that high concentrations of extracts of *Ocimum sp*. caused high mortality of larvae even though only very small portions of the leaf discs were consumed.

The acetone elution of *A. malabarica* leaf registered the pupal weight ranged from 0.19 to 0.54 and pupal length ranged from 1.20 to 1.56 (Table 2). In the preliminary screening, the acetone elution 3 recorded the lowest pupal weight of 0.19 g with a per cent reduction of 63.40 and pupal length of 1.20 cm with a per cent reduction of 21.57. Similar trend of results was also observed in confirmatory screening. The lowest pupal weight of 0.21 g with a per cent reduction of 61.11 and pupal length of 1.22 cm with a per cent reduction of 21.79

The presence of secondary metabolites *viz.,* tannin (1.32±0.12) and saponins (1.11±0.09) were observed in acetone leaf extract. Flavonoids, phenols and terpenoids were absent in acetone leaf extract (Table 3).

Thavapudalvi *et al*. (2022) studied the presence of secondary metabolites by the quantitative analysis of *A. malabarica* leaf extract in aqueous, benzene, chloroform, and methanol extracts and reported the presence of carbohydrate, flavonoids, proteins and saponins in chloroform and methanol leaf extract, The above studies are in agreement with the present findings.

Table 1. Effect of Acetone extraction of *A. malabarica* leaf on the biostage malformation and mortality of *S. litura* (Poison food bioassay – Preliminary and Confirmatory screenings)

SI.No.	Treatments (%)	Preliminary screening			Confirmatory screening		
		Per cent pupal malformation #	Per cent pupal mortality #	Per cent adult malformation #	Per cent pupal malformation #	Per cent pupal mortality #	Per cent adult malformation #
1	Acetone elution 1	44.44 (41.81)^{b}	33.33 (35.26)^{b}	22.22 (28.12)^{b}	44.44 (41.81)^{b}	33.33 (35.26)^{b}	22.22 (28.12)^{b}
2	Acetone elution 2	22.22 (28.12)^{d}	22.22 (28.12)^{c}	11.11 (19.47)^{c}	22.22 (28.12)^{d}	22.22 (28.12)^{c}	11.11 (19.47)^{c}
3	Acetone elution 3	55.56 (48.19)^{a}	44.44 (41.81)^{a}	0.00 (2.87)^{d}	55.56 (48.19)^{a}	44.44 (41.81)^{a}	0.00 (2.87)^{d}
4	Acetone elution 4	11.11 (19.47)^{e}	0.00 (2.87)^{d}	33.33 (35.26)^{a}	11.11 (19.47)^{e}	0.00 (2.87)^{d}	33.33 (35.26)^{a}
5	Acetone elution 5	33.33 (35.26)^{c}	0.00 (2.87)^{d}	11.11 (19.47)^{c}	33.33 (35.26)^{c}	0.00 (2.87)^{d}	11.11 (19.47)^{c}
6	Absolute Control	0.00 (2.87)^{f}	0.00 (2.87)^{d}	0.00 (2.87)^{d}	0.00 (2.87)^{f}	0.00 (2.87)^{d}	0.00 (2.87)^{d}
7	Control	0.00 (2.87)^{f}	0.00 (2.87)^{d}	0.00 (2.87)^{d}	0.00 (2.87)^{f}	0.00 (2.87)^{d}	0.00 (2.87)^{d}
	S.E (d)	0.66	0.50	0.47	0.66	0.50	0.47
	F – test	**	**	**	**	**	**
	C.D. (P=0.05)	1.42	1.08	1.01	1.42	1.08	1.01
	C.V.	3.17	3.70	3.64	3.17	3.70	3.64

In a column mean followed by the common letter are significantly different by DMRT (P=0.05) by one way ANOVA. Values in the parentheses Arcsine transformed values # - Mean of 3 replication **Significant at P=0.01

SI. No.	Treatments (%)	Preliminary screening				Confirmatory screening			
		Pupal weight #	Pupal weight per cent reduction over control	Pupal length #	Pupal length per cent reduction over control	Pupal weight #	Pupal weight per cent reduction over control	Pupal length #	Pupal length per cent reduction over control
1	Acetone	0.21 [ab]	58.82	1.24 [b]	18.74	0.23	57.41	1.26 [ab]	19.23

Table 2. Effect of Acetone extraction of *A. malabarica* leaf on the biostage against *S. litura* on the basis of pupal weight and pupal length (Poison food bioassay – Preliminary and confirmatory screenings)

	elution 1	±0.02		±0.04		[ab]±0.03		±0.02	
2	Acetone elution 2	0.27 [b] ±0.03	47.06	1.28 [bc] ±0.03	16.12	0.29 [bc]±0.04	46.30	1.29 [b] ±0.02	17.31
3	Acetone elution 3	0.19 [a] ±0.01	63.40	1.20 [a] ±0.06	21.57	0.21 [a] ±0.03	61.11	1.22 [a] ±0.04	21.79
4	Acetone elution 4	0.30 [c] ±0.03	41.83	1.33 [c] ±0.04	12.85	0.31 [c] ±0.02	42.59	1.34 [c] ±0.02	14.10
5	Acetone elution 5	0.31 [c] ±0.04	39.87	1.35 [c] ±0.04	11.76	0.33 [c] ±0.02	38.89	1.36 [c] ±0.03	12.82
6	Absolute Control	0.46 [d] ±0.02	-	1.43 [d] ±0.01	-	0.49 [d] ±0.01	-	1.45 [d] ±0.02	-
	Control	0.51 [d] ±0.01	-	1.53 [e] ±0.04	-	0.54 [d] ±0.02	-	1.56 [e] ±0.02	-
	S.E (d)	0.01	-	0.01	-	0.01	-	0.01	-
	F – test	**	-	**	-	**	-	**	-
	C.D. (P=0.05)	0.02	-	0.02	-	0.02	-	0.02	-
	C.V.	3.17	-	17.45	-	2.92	-	17.59	-

In a column mean followed by the common letter are significantly different by DMRT (P=0.05) by one way ANOVA

- Mean of 3 replications ± - Standard error of difference **Significant at P=0.01

Table 3. Qualitative and Quantitative Analysis of secondary metabolites of Acetone extract of *A. malabarica* leaf

SI.No.	Secondary metabolites	Qualitative test	Quantitative test #
1	Flavanoids	-	-
2	Phenols	-	-
3	Saponins	+	1.11±0.09
4	Tannins	+	1.32±0.12
5	Terpenoids	-	-

+ Presence - absence # - Mean of 3 replications (mg/g) ± Standard error difference

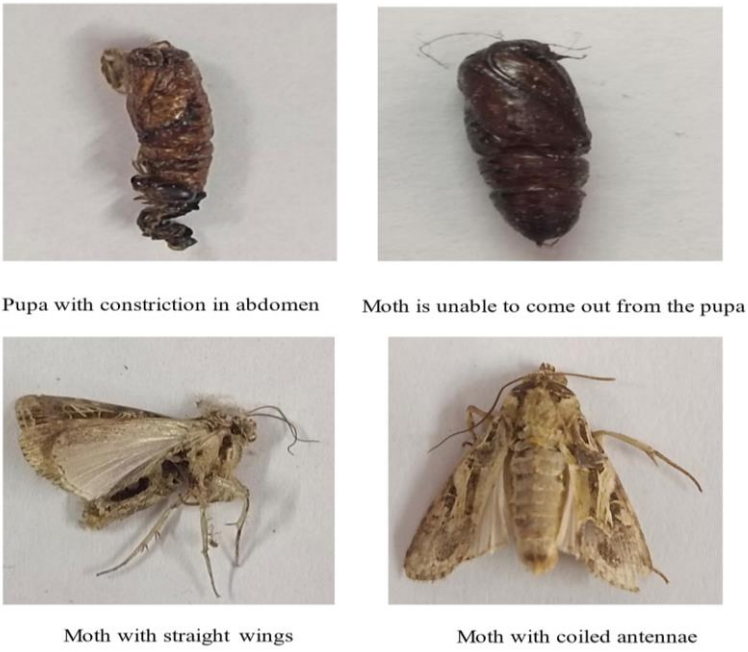

Fig 1. Effect of Acetone extraction of *A. malabarica* leaf on *S. litura*

(Poison food bioassay – Preliminary and Confirmatory screenings)

4. Conclusion

This study concludes that on five elution, Acetone elution 3 shows highest per cent of pupal malformation of 55.56 and pupal mortality of 44.44. It also registered that in the same elution (acetone elution 3) the pupal weight and length is very low. The lowest pupal weight of 0.21 g and pupal length of 1.22 cm. The presence of secondary metabolites, tannin (1.32±0.12) and saponins (1.11±0.09) were also observed in acetone leaf extract. Flavonoids, phenols and terpenoids were absent in acetone leaf extract.

Disclaimer (Artificial Intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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