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Biocidal and growth inhibitory effects of hexane leaf extract of *Anisomeles malabarica* on *Spodoptera litura*

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Abstract
The present study was carried out to identify the biocidal effects of hexane leaf extract of *Anisomeles malabarica* on *Spodoptera litura* under laboratory condition. Bioassays on third instar larvae were done to assess mortality, developmental abnormalities, and growth parameters. The result of the study exhibited that no larval mortality was observed; however, significant developmental disruptions occurred at later stages. Pupal malformation ranged from 11.11 to 55.56 per cent, with the highest in elution 5, while adult malformation reached 55.56 per cent in elution 2. Significant reductions in pupal weight (up to 64.15%) and length (~28%) indicated strong growth inhibitory effects. Phytochemical screening revealed the presence of flavonoids, phenols, saponins, proteins, and carbohydrates, while tannins, steroids, and terpenoids were absent. The findings suggested that hexane-soluble compounds of *A. malabarica* acted primarily as insect growth regulators rather than acute toxins, highlighting their potential as eco-friendly botanical agents for sustainable pest management.

Keywords: *A. malabarica*, *Spodoptera litura*, hexane extract, insect growth regulator, pupal malformation, botanical insecticide, phytochemicals, sustainable pest management

Introduction
The increasing reliance on synthetic insecticides for pest management has led to several ecological and environmental concerns, including resistance development, non-target toxicity, and environmental contamination. These challenges have prompted the search for alternative, eco-friendly pest control strategies, particularly those derived from plant-based secondary metabolites. Botanical insecticides have gained considerable attention due to their biodegradability, target specificity, and reduced risk of resistance development (Isman, 2020; Pavela and Benelli, 2016) [5, 10]. *Spodoptera litura* (Fabricius), a polyphagous lepidopteran pest, is a major agricultural threat affecting a wide range of economically important crops. Its high reproductive potential and adaptability to diverse environmental conditions make it difficult to control using conventional methods. Moreover, the species has developed resistance to several classes of synthetic insecticides, further complicating its management (Ahmad *et al*., 2019; Shad *et al*., 2012) [1, 14]. Therefore, exploring plant-derived compounds with growth regulatory and developmental disruptive properties offers a promising avenue for sustainable pest management. Plants synthesize a diverse array of secondary metabolites such as flavonoids, phenolics, saponins, and terpenoids, which play a crucial role in plant defence against herbivores. These compounds are known to interfere with insect physiology by affecting feeding behaviour, digestion, hormonal balance, and metamorphosis. The efficacy of such compounds often depends on the extraction solvent used, as different solvents isolate specific classes of phytochemicals based on their polarity (Miresmailli and Isman, 2014; War *et al*., 2012) [6, 8, 17]. *A. malabarica*, a medicinal plant, has been reported to possess various bioactive compounds with potential insecticidal properties. However, limited information is available regarding its effect on insect growth and development, particularly in non-polar solvent extracts. Therefore, the present study was undertaken to evaluate the bioefficacy of hexane leaf extract of *A. malabarica* against *S. litura*, focusing on developmental abnormalities, growth inhibition, and phytochemical composition. The study also aims to elucidate the potential of hexane-soluble compounds as insect growth regulators for integrated pest management programs.

Materials and Methods
Collection of plant materials
The leaves of *A. malabarica* were collected from Azhagar hills, Madurai, Tamilnadu, India. 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] (Fig 1).

Rearing of test insects
Mass rearing of *S. litura* was carried out under controlled laboratory conditions to ensure a uniform and homogeneous larval population for bioassay studies. Larvae were reared on fresh castor leaves (*Ricinus communis*) until pupation. During the final instar, larvae were transferred to plastic rearing trays, wherein approximately half of the tray was filled with fine, dried sand, and fresh castor leaves were provided as food. Upon completion of feeding, larvae entered the soil medium for pupation. Pupae were collected four days after pupation and subjected to surface sterilization using 10 % formaldehyde solution, followed by thorough rinsing with tap water to remove residual formaldehyde. The treated pupae were then placed on a thin

layer of absorbent cotton in Petri plates and transferred to rearing cages for adult emergence. Within the cages, adult moths were provided with a 10% honey solution supplemented with a few drops of Health OK multivitamin, supplied in a penicillin vial as a nutritional source. Following mating, a tender shoot of nerium was placed in a 100 ml conical flask containing water and introduced into the cage to serve as an oviposition substrate. The tender nerium leaves acted as a preferred site for egg laying by *S. litura*. Egg masses deposited on the leaves were carefully collected and stored in plastic containers under refrigerated conditions until further use. For subsequent rearing, eggs were incubated under high relative humidity (approximately 80%) in plastic trays, with tender castor leaves provided as a food source for the emerging neonate larvae (Jeyasankar *et al*., 2016) [7].

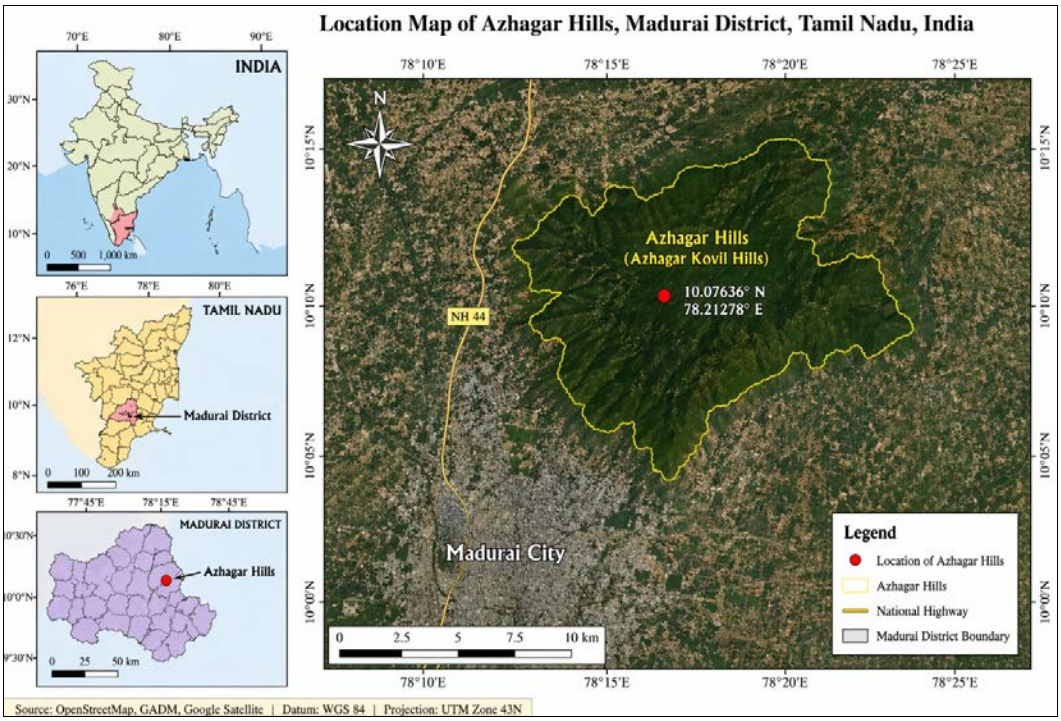

Fig 1: Location map of Azhagar hills

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 hexane 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 hexane (68-70 °C) was fixed in a heating mantle. (Patil and Chavan, 2010) [9]. 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) [13].

Purification by column chromatography

Glass columns were taken and rinsed with 1 ml of hexane 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 1/3 rd 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) [13].

Bioefficacy of leaf extract of *A. malabarica* by hexane solvent

The five elutions of hexane 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) [10] (Plate 1).

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) [10].

Effect of leaf extract of *A. malabarica* by hexane 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.

Phytochemical analysis of alkaloids

Phytochemical screening of the hexane leaf extract of *A. malabarica* was carried out following the procedures described by Thavapudalvi *et al*. (2022) [16]. Both qualitative and quantitative analyses were performed to determine the presence and concentration of major secondary metabolites, including flavonoids, phenols, saponins, tannins, and terpenoids.

QualitativeAnalysis

Flavonoids were detected by treating 1 mL of extract with sodium hydroxide, producing a yellow colour that disappeared upon acidification. Phenols were confirmed by the formation of a violet colour after addition of ferric chloride and ethanol. Saponins were identified by the formation of a stable froth/emulsion upon addition of water and coconut oil. Tannins were indicated by a greenish-black precipitate with 5% ferric chloride. Terpenoids were detected by grey colour formation after chloroform treatment, evaporation, and addition of concentrated sulfuric acid.

QuantitativeAnalysis

Flavonoid content was determined by repeated extraction of 1 g sample with various solvents, followed by filtration, evaporation, and gravimetric measurement. Phenolic content was estimated after ether extraction, with absorbance measured at 550 nm. Saponins were quantified by ethanol extraction, purification using diethyl ether and n-butanol, and gravimetric determination. Tannins were estimated spectrophotometrically after reaction with ferric chloride and potassium ferrocyanide. Terpenoid content was determined by solvent partitioning with petroleum ether and expressed as percentage using:

$$\text{Terpenoid content (\%)} = \frac{W_i - W_f}{W_i} \times 100$$

Where:

W_i=Initial weight of sample

W_f= Final weight after extraction

Statistical analysis

The experimental data obtained from laboratory studies were analyzed using a Completely Randomized Design (CRD). Analysis of variance (ANOVA) was performed using the "F" test to determine the significance of treatment effects. The standard error of difference [S.E. (d)] and critical difference (CD) values were calculated at a 5% probability level. Treatment means were compared using Duncan's Multiple Range Test (DMRT). One-way ANOVA was employed to assess statistical differences among treatment groups, following the methodology described by Steel and Torrie (1960) [15].

Results and discussion

Bioefficacy of hexane leaf extract of *A. malabarica*

Hexane leaf extracts of *A. malabarica* exhibited pronounced stage-specific effects on *Spodoptera litura*, with no larval mortality or deformities, indicating absence of acute toxicity. However, significant developmental disruption was observed during pupal and adult stages. Pupal malformation ranged from 11.11 to 55.56%, with the highest incidence in hexane elution 5, followed by elution 2. Adult malformation reached 55.56% in elution 2, while elutions 1 and 3 showed moderate effects (22.22%) compared to controls (Table 1) (Plate 1 & 2).

The absence of pupal mortality despite high malformation suggests sublethal activity characteristic of insect growth regulators (IGRs), which interfere with molting and metamorphosis rather than causing immediate lethality (Dhadialla *et al.*, 1998) [3]. The observed deformities may result from disruption of endocrine regulation, particularly the balance of ecdysteroids and juvenile hormones, or from interference with chitin synthesis and cuticular formation (Retnakaran *et al.*, 1985) [12]. Such effects are consistent with the activity of lipophilic phytochemicals, including terpenoids, commonly extracted in hexane fractions (Isman, 2006) [4]. Variation in bioactivity among elutions suggests differential distribution of active constituents, a pattern widely reported for plant-derived insecticidal compounds (Pavela R. and Benelli G., 2016) [10].

The absence of larval mortality highlights the suitability of these extracts for integrated pest management, as they suppress pest populations through developmental disruption while minimizing non-target toxicity and resistance risk (Isman and Grieneisen, 2014) [6]. Confirmation assays corroborated the preliminary results, with pupal malformation ranging from 11.11 to 55.56% and adult malformation from 0.00 to 55.56%.

Overall, hexane leaf extracts of *A. malabarica* act primarily as growth-disrupting agents and represent promising candidates for eco-friendly pest management. Further studies on compound isolation and mode of action are warranted.

Effect of hexane leaf extract of *A. malabarica* on growth parameters of *Spodoptera litura*

Hexane elution 2 of *A. malabarica* leaf extract significantly reduced pupal biomass and size of *Spodoptera litura*. The lowest pupal weights (0.21 and 0.19 g) corresponded to reductions of 58.17 per cent and 64.15 per cent in preliminary and confirmation screenings, respectively. Pupal length was also reduced to 1.13 and 1.11 cm (≈28% reduction), indicating consistent growth inhibition across trials (Table 2) (Plate 3).

Fig 2: Overall Graphical representation of methodology

Plate 1: Effect of hexane extraction of *A. malabarica* leaf on *S. litura*

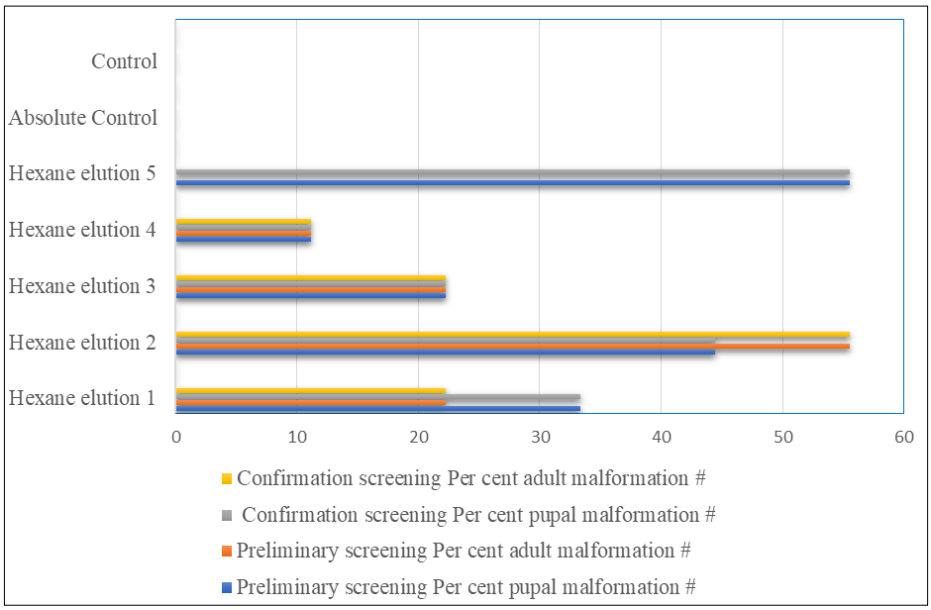

Plate 2: Bioefficacy of leaf extract of *A. malabarica* by hexane solvent

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

SI.No.	Treatments (%)	Preliminary screening		Confirmation screening	
		Per cent pupal malformation #	Per cent adult malformation #	Per cent pupal malformation #	Per cent adult malformation #
1	Hexane elution 1	33.33 (35.26)^{c}	22.22 (28.12)^{b}	33.33 (35.26)^{c}	22.22 (28.12)^{b}
2	Hexane elution 2	44.44 (41.81)^{b}	55.56 (48.20)^{a}	44.44 (41.81)^{b}	55.56 (48.20)^{a}
3	Hexane elution 3	22.22 (28.12)^{d}	22.22 (28.12)^{b}	22.22 (28.12)^{d}	22.22 (28.12)^{b}
4	Hexane elution 4	11.11 (19.47)^{e}	11.11 (19.47)^{c}	11.11 (19.47)^{e}	11.11 (19.47)^{c}
5	Hexane elution 5	55.56 (48.19)^{a}	0.00 (2.87)^{d}	55.56 (48.19)^{a}	0.00 (2.87)^{d}
6	Absolute Control	0.00 (2.87)^{f}	0.00 (2.87)^{d}	0.00 (2.87)^{f}	0.00 (2.87)^{d}
7	Control	0.00 (2.87)^{f}	0.00 (2.87)^{d}	0.00 (2.87)^{f}	0.00 (2.87)^{d}
	S.E (d)	0.63	0.64	0.63	0.64
	F – test	**	**	**	**
	C.D. (P=0.005)	1.35	1.38	1.35	1.38
	C.V.	3.02	3.11	3.02	3.11

Values in the parentheses Arcsine transformed values
- Mean of 3 replications
**Significant at P=0.01

In a column mean followed by the common letter are significantly different by DMRT (P=0.005) by one way ANOVA,

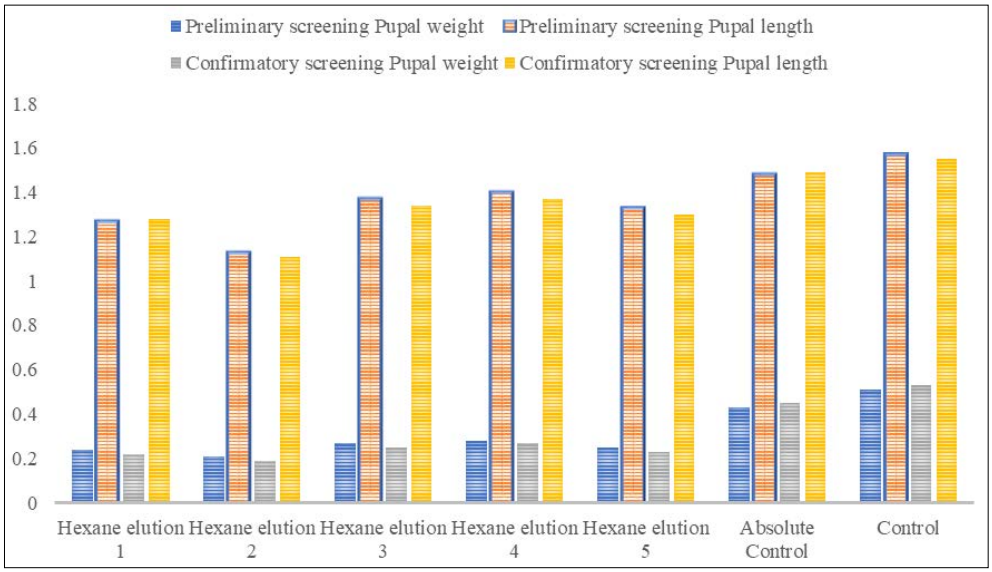

Plate 3: Effect of leaf extract of *A. malabarica* by hexane solvents against the growth parameters of *S. litura*

Table 2: Effect of hexane 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 confirmation screenings)

SI.No.	Treatments (%)	Preliminary screening				Confirmation 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	Hexane elution 1	0.24 [b] ±0.01	52.29	1.27 [b] ±0.03	19.32	0.22 [b] ±0.02	58.49	1.28 [b] ±0.03	17.42
2	Hexane elution 2	0.21 [a] ±0.01	58.17	1.13 [a] ±0.06	28.24	0.19 [a] ±0.03	64.15	1.11 [a] ±0.01	28.39
3	Hexane elution 3	0.27 [dc] ±0.01	47.71	1.37 [c] ±0.09	12.95	0.25 [cd] ±0.01	52.83	1.34 [c] ±0.07	13.55
4	Hexane elution 4	0.28 [d] ±0.01	45.75	1.40 [cd] ±0.06	10.83	0.27 [d] ±0.02	49.06	1.37 [c] ±0.04	11.61
5	Hexane elution 5	0.25 [bc] ±0.01	50.33	1.33 [bc] ±0.03	15.07	0.23 [bc] ±0.03	56.60	1.30 [bc] ±0.02	16.13
6	Absolute Control	0.43 [e] ±0.02	-	1.48 [cd] ±0.10	-	0.45 [e] ±0.02	-	1.49 [dc] ±0.6	-
7	Control	0.51 [f] ±0.03	-	1.57 [d] ±0.02	-	0.53 [f] ±0.02	-	1.55 [d] ±0.01	-
	S.E (d)	0.01	-	0.01	-	0.01	-	0.01	-
	F – test	**	-	**	-	**	-	**	-
	C.D. (P=0.05)	0.02	-	0.03	-	0.02	-	0.03	-
	C.V.	4.05	-	2.16	-	2.14	-	2.20	-

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

The reduction in pupal weight and size suggests impaired larval feeding efficiency, nutrient assimilation, and metabolic disruption. Despite the absence of larval mortality, such sublethal effects are characteristic of botanical insecticides acting as antifeedants or growth regulators (Isman, 2006) [4]. The pronounced decrease in pupal biomass further indicates possible interference with endocrine regulation, particularly the ecdysteroid–juvenile hormone balance governing larval–pupal transition (Dhadialla *et al.*, 1998) [3]. Reduced pupal length may reflect impaired morphogenesis due to disrupted chitin synthesis or protein metabolism (Retnakaran *et al.,* 1985) [12]. The reproducibility of these effects suggests the presence of stable, non-polar bioactive compounds, likely terpenoids, known for their insect growth regulatory activity (Pavela and Benelli, 2016) [10]. Such reductions in pupal size may adversely affect adult fitness and reproduction, contributing to long-term population suppression (Isman and Grieneisen, 2014) [6]. Overall, hexane elution 2 exhibits strong growth-disrupting activity and holds promise as a botanical insect growth regulator.

Qualitative analysis and Quantitative analysis of secondary metabolites of *A. malabarica* leaf in Hexane solvents

Phytochemical screening of the hexane leaf extract of *A. malabarica* revealed the presence of carbohydrates, proteins, flavonoids (1.31 ± 0.16), saponins (1.07 ± 0.12), and phenols (1.37 ± 0.17), while tannins, steroids, and terpenoids were absent. Although hexane is a non-polar solvent, the detection of some polar metabolites may be due to trace co-extraction or assay limitations (Table 3). Flavonoids and phenolic compounds are likely responsible for the observed bioactivity, as they are known to disrupt insect feeding, digestion, and endocrine processes, leading to reduced growth and developmental abnormalities (Isman, 2006) [4]. Saponins, owing to their amphiphilic nature, may contribute to membrane disruption and impaired nutrient absorption. These combined effects align with the previously observed reductions in pupal weight and increased malformations.The absence of terpenoids, commonly associated with non-polar extracts and insect growth regulation, suggests that the biological activity is primarily driven by non-terpenoid compounds such as flavonoids and phenolics. Similarly, the lack of tannins and steroids indicates a distinct mode of action compared to polar extracts. The phytochemical profile supports the role of the hexane extract as a growth-disrupting agent acting through metabolic and endocrine interference. These findings highlight the importance of solvent-specific extraction in identifying bioactive compounds and reinforce the potential of *A. malabarica* as a source of eco-friendly insect growth regulators (Isman and Grieneisen, 2014) [6].

Table 3: Quantitative Analysis of secondary metabolites of *A. malabarica* leaf in Hexane solvents

SI.No.	Secondary metabolites	Hexane #
1	Flavanoids	1.31±0.16
2	Phenols	1.37±0.17
3	Saponins	1.07±0.12
4	Tannins	-
5	Terpenoids	-

- Mean of 3 replications (mg/g)
± Standard error difference

Conclusion

The present investigation demonstrates that hexane leaf extract of *A. malabarica* exhibits significant growth regulatory activity against *Spodoptera litura*, primarily affecting pupal and adult developmental stages without causing larval mortality. The observed pupal and adult malformations, along with substantial reductions in pupal

weight and length, indicate strong interference with physiological and hormonal processes governing insect development. Among the tested fractions, hexane elution 2 and 5 showed pronounced bioactivity, suggesting the presence of potent growth-disrupting compounds. Phytochemical analysis revealed that the hexane extract predominantly contains flavonoids, phenolic compounds, and saponins, which are likely responsible for the observed biological effects. The absence of terpenoids and other commonly reported non-polar bioactive compounds suggests a distinct mode of action driven by non-terpenoid constituents. These compounds appear to exert sublethal effects by disrupting metabolic and endocrine pathways, ultimately impairing morphogenesis and adult emergence.

The findings underscore the potential of *A. malabarica* hexane extracts as eco-friendly insect growth regulators with applications in sustainable pest management. Their ability to induce developmental abnormalities without causing immediate mortality highlights their suitability for integration into integrated pest management (IPM) programs. Further studies focusing on isolation, characterization, and mode-of-action analysis of the active compounds are essential to advance their practical application and commercial development.

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