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Larvicidal, pupicidal, and ovicidal activities of hexane and methanol extracts of *Melochia Corchorifolia* against the castor semilooper, *Achaea Janata* (Lepidoptera: Noctuidae)

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Abstract
This study evaluated the larvicidal, pupicidal, and ovicidal effects of hexane and methanol extracts of *Melochia corchorifolia* (Malvaceae), commonly known as "chocolate weed," against third-instar larvae, pupae, and freshly laid eggs of the castor semilooper, *Achaea janata*, under laboratory conditions. Leaves were extracted successively with hexane and methanol, and the extracts were tested at concentrations of 50, 100, 200, 400, and 800 mg/L. Both extracts produced mortality that increased with concentration, and the methanol extract was significantly more toxic than the hexane extract. At 800 mg/L, the methanol extract caused 98.33 ± 1.45% larvicidal, 96.67 ± 1.67% pupicidal, and 95.00 ± 1.83% ovicidal activity within 72 hours, whereas the hexane extract produced lower rates. The LC_{50} and LC_{90} values were consistently lower for the methanol extract, indicating stronger bio-insecticidal activity. These findings suggest that *M. corchorifolia* extracts, especially the methanol extract, are promising eco-friendly options for controlling *A. janata* and support further isolation of active compounds and field testing.

Keywords: *Achaea janata*, *Melochia corchorifolia*, botanical insecticide, insecticidal activity, plant extracts

Introduction

Achaea janata (Lepidoptera: Noctuidae), commonly known as the castor semilooper or jute looper, is a highly polyphagous defoliator that attacks crops such as jute, cotton, castor, tobacco, and several vegetables. Its high fecundity, rapid larval development, and defoliating feeding habit cause severe yield loss, necessitating frequent insecticide applications. Conventional synthetic insecticides, however, have led to resistance, the resurgence of secondary pests, and adverse effects on beneficial organisms and human health. This has intensified the search for safer, plant-based alternatives in integrated pest management (IPM) strategies. Plant secondary metabolites, including alkaloids, terpenoids, flavonoids, and phenolic compounds, have demonstrated insecticidal, antifeedant, and growth-regulatory effects against several lepidopteran species. The choice of extraction solvent is critical because it influences the recovery of bioactive constituents. Hexane tends to dissolve nonpolar compounds such as sesquiterpenes, essential oil components, and long-chain hydrocarbons, which often act as contact toxins and antifeedants. Methanol, on the other hand, effectively extracts polar constituents such as phenolic acids, flavonoids, and glycosides, which can exert systemic toxicity and ovicidal activity.

Melochia corchorifolia L. (Malvaceae; commonly known as chocolate weed, bilpat, or thulak) is an annual herb or small shrub widely distributed in tropical and subtropical regions, including India. Traditionally, it is used in folk medicine for its anti-inflammatory and insecticidal properties. Aqueous and ethanolic extracts of *M. corchorifolia* have been reported to exert insecticidal and antifeedant activities against storage pests such as *Callosobruchus maculatus* and *Sitophilus oryzae*, with mortality increasing in a concentration-dependent manner. Furthermore, ethyl acetate and methanol extracts of *M. corchorifolia* leaves have shown strong antifeedant effects against *Helicoverpa armigera* and other noctuid larvae, supporting the presence of bioactive insecticidal principles. Despite these findings, there is limited information on the larvicidal, pupicidal, and ovicidal activities of its hexane and methanol extracts against *Achaea janata*. The present study aims to analyze the larvicidal, pupicidal, and ovicidal activities of hexane and methanol extracts of *M. corchorifolia* leaves against *A. janata* under laboratory conditions, and to compare the bio efficacy of the two solvent systems.

Materials and Methods

Fresh leaves of *Melochia corchorifolia* L. were collected from Guindy, *Chennai, Tamil Nadu, India,* authenticated using standard taxonomic keys, and air-dried in shade. The dried leaves were ground to a fine powder and subjected to successive extraction using hexane and methanol in a Soxhlet apparatus. The solvents were removed under reduced pressure, and the hexane and methanol extracts were stored at 4 °C until use.

Achaea janata colonies were maintained in the laboratory at 27 ± 2 °C, 60–70% relative humidity, and a 12:12 h (L:D) photoperiod. Larvae were reared on tender leaves of *Corchorus capsularis* in ventilated glass cages. Newly emerged pupae were transferred to separate cages, and adults were provided with 10% sucrose solution and moist cotton-wool wicks. Eggs were collected on wax-paper strips within 2 h of oviposition and used for ovicidal assays.

Third-instar larvae (100 per bioassay) were exposed to hexane and methanol extracts at 50, 100, 200, 400, and 800 mg/L, prepared in acetone (0.1% final concentration), with distilled water + 0.1% acetone as the control. The solutions were sprayed onto fresh leaves, and 10 larvae were placed in each Petri dish (10 replicates per concentration). Mortality was recorded after 24, 48, and 72 h. Data were corrected using Abbott's formula and analyzed by probit analysis to determine LC_{50} and LC_{90} values.

Freshly formed pupae (within 6 h) were selected (10 per vial, 10 replicates per concentration) and treated with 1 mL

of extract solution (50–800 mg/L) in distilled water containing 0.1% acetone. Controls received only distilled water + 0.1% acetone. Pupal mortality was recorded after 24, 48, and 72 h, and LC values were calculated using probit analysis.

Freshly laid eggs (within 2 h, 100 per replicate, 10 replicates per concentration) were treated with 0.5 mL of extract solution (50–800 mg/L) in distilled water containing 0.1% acetone. Hatching was recorded at 24, 48, and 72 h. The percentage of unhatched eggs was calculated and LC values were derived using probit analysis.

Statistical analysis

Corrected mortality (%) was computed using Abbott's formula:

$$\text{Corrected mortality (\%)} = \frac{(\text{Treatment mortality} - \text{Control mortality})}{(100 - \text{Control mortality})} \times 100$$

LC_{50} and LC_{90} with 95% confidence intervals were calculated using probit analysis (e.g., SPSS). Differences among treatments were analysed by one-way ANOVA followed by Duncan's multiple-range test at $p < 0.05$.

Result and Discussion

Table 1: Larvicidal effects of hexane extract of *M. corchorifolia* against third instar larvae of *A, janata*

Exposure duration	Concentrations mg/L	Mortality%	LC_{50}	LC_{90}	R^2 Linear
24h	50	16.43±4.87[a]	410.946 (168.49-1025.25)	1068.702 (693.62-4269.81)	0.852
	100	21.74±3.04[b]			
	200	46.22±2,52[c]			
	400	55.91±2.11[d]			
	800	72.70±1.84[e]			
48h	50	17.48±5.86[a]	343.59 (1.957-1005.41)	942.73 (596.39-5639.22)	0.834
	100	23.70±4.41[b]			
	200	51.92±3.00[c]			
	400	63.01±2.78[d]			
	800	78.04±1.94[d]			
72h	50	18.01±3.59[a]	309.44 (0.391-709.279)	874.59 (568.03-3484.57)	0.855
	100	27.14±2.61[b]			
	200	53.12±2.25c			
	400	66.10±1.99[d]			
	800	81.54±1.79[e]			

Values are shown as mean ± Standard Deviation. Different letters within a column denote significant differences according to Tukey's test.

Table 1 shows that the hexane extract of *M. corchorifolia* causes increased mortality in *A,janata* larvae as the concentration rises from 50 to 800 mg/L. At 24 h, mortality increases from 16.43% at 50mg/L to 72.70% at 800 mg/L, while at 48 h it ranges from 17.48% to 78.04%. After 72 h, mortality further increases from 18.01% at 50 mg/L to 81.54% at 800 mg/L, indicating a clear time-dependent effect. The LC_{50} values decrease from 410.946 mg/L (24 h) to 343.59 mg/L (48 h) and 309.44 mg/L (72 h), indicating improved toxicity over time. Similarly, LC_{90} values decrease from 1068.702 mg/L to 874.59 mg/L, confirming the extract's strong larvicidal potential. This observed dose- and time-dependent increase in larval mortality aligns with previous findings on the efficacy of plant extracts as larvicides against various mosquito species (Arivoli and Tennyson, 2011; Raj *et al*., 2017) [4, 40]. For instance, studies evaluating the larvicidal activity of plant extracts have reported similar trends, with increased concentrations and prolonged exposure times resulting in higher mortality rates among mosquito larvae (Ashwini *et al*., 2017) [5].

Specifically, a positive correlation between larval mortality and increased extract concentrations and prolonged exposure times has been consistently observed (Ghebriel and Adugna, 2017; Yagoo *et al*., 2023) [20, 47]. This aligns with research demonstrating that the efficacy of botanical insecticides is often contingent on both the dosage and the duration of exposure, with higher concentrations and longer contact periods enhancing their biological activity (Ersino *et al*., 2020) [15]. Regression analysis has further substantiated these observations, revealing a positive correlation between mortality rates and exposure duration, often with a regression coefficient approaching unity, while LC_{50} values concurrently decrease with extended exposure periods (Chowdhury *et al*., 2008) [10]. This time-dependent toxicity profile suggests a gradual accumulation or sustained action of the active compounds within the larval system, leading to enhanced efficacy over time (Zulhussnain *et al*., 2020) [53]. Such findings are consistent with prior research indicating that the lethal concentration values (LC_{50} and LC_{90}) for various plant extracts decrease progressively with increasing exposure duration, demonstrating heightened larvicidal potential over time (Aziz, 2013; Mondal *et al*., 2023; Rawani *et al*., 2017) [6, 35, 41].

Table 2: Larvicidal effects of methanol extract of *M. corchorifolia* against third instar larvae of *A, janata*

Exposure duration	Concentrations mg/L	Mortality%	LC_{50}	LC_{90}	R^2 Linear
24h	50	17.23±4.73[a]	379.72 (101.22-1012.82)	1033.12 (661.88-4870.37)	0.842
	100	24.08±3.99[b]			
	200	49.25±2.50[c]			
	400	58.14±1.11[d]			
	800	74.61±0.80[d]			
48h	50	19.41±3.00[a]	271.323 (295.64-804.56)	805.320 (501.32-6539.26)	0.826
	100	27.58±2.62[b]			

	200	57.83±2.98[b]			
	400	71.72±2.19[c]			
	800	84.00±1.26[d]			
72h	50	21.07±3.94[a]	192.410 (96.89-287.26)	493.540 (370.447-842.00)	0.968
	100	33.45±3.62[b]			
	200	60.61±2.89[c]			
	400	83.11±2.19[c]			
	800	98.33±1.45[d]			

Values are shown as mean ± Standard Deviation. Different letters within a column denote significant differences according to Tukey's test.

Table 2 shows that the methanol extract of *M. corchorifolia* causes maximum mortality in *A. janata* larvae as the concentration rises from 50 to 800 mg/L. At 24 h, mortality increases from 17.23% at 50 mg/L to 74.61% at 800 mg/L, while at 48 h, it ranges from 19.41% to 84.00%. After 72 h, mortality further increases from 21.07% at 50 mg/L to 98.33% at 800 mg/L, indicating a clear time-dependent effect. The LC_{50} values decrease from 379.72 mg/L (24 h) to 271.323 mg/L (48 h) and 192.410 mg/L (72 h), indicating improved toxicity over time. Similarly, LC_{90} values decrease from 1033.12 mg/L to 493.540 mg/L, confirming the extract's highest larvicidal potential. This enhanced efficacy over time and concentration is consistent with observations in other studies where plant-derived compounds, such as those from *T. diversifolia* and *R. communis*, demonstrated increased mortality against *An. gambiae* s.s. mosquitoes with prolonged exposure (Wachira *et al*., 2014) [48]. This trend, where increased extract concentration leads to higher mortality rates, is a common finding in larvicidal studies (Marc *et al*., 2021; Seiyaboh *et al*., 2020) [34, 42]. For example, research has shown that mortality among Culex and Anopheles mosquitoes increased with extended exposure periods and elevated extract concentrations (Sharawi, 2024) [43]. This dose- and time-dependent larvicidal activity underscores the potential of *M. corchorifolia* methanol extract as a promising agent for mosquito control, echoing similar efficacy profiles reported for other botanical extracts against various mosquito species also (Agwu *et al*., 2018; Mang'era *et al*., 2018) [1, 33]. For instance, *Rosmarinus officinalis* extracts have demonstrated comparable dose- and time-dependent larvicidal effects, achieving over 90% mortality at higher concentrations and prolonged exposure, along with decreasing LC_{50} values over time (Alhaithloul *et al*., 2023) [2]. Furthermore, the observed reduction in LC_{50} and LC_{90} values with increased exposure duration indicates greater potency of the extract over time, aligning with similar findings in which extended exposure to botanical compounds enhanced larvicidal activity (Ali *et al*., 2018) [3]. The dose-dependent mortality observed here is further supported by studies showing a direct correlation between higher concentrations of plant extracts and increased larval mortality across various mosquito species (Podder & Ghosh, 2019) [39]. These findings are further corroborated by studies indicating that an extract is considered to have high larvicidal activity when its LC_{50} is less than 50 ppm, and moderate activity when between 50 and 100 ppm, thus contextualizing the potency of the *M. corchorifolia* methanol extract (Jaqueline *et al*., 2018) [24].

This aligns with other studies reporting low LC_{50} values for botanical extracts, such as the methanolic extract of *Ricinus communis* seeds against *Aedes aegypti* larvae (Ekpoma *et al*., 2022) [14]. Similarly, the methanolic extract of *Moringa oleifera* has demonstrated comparable LC_{50} values against *Anopheles stephensi* larvae, further supporting the potent larvicidal efficacy observed in the current study (Kolandhasamy *et al*., 2011) [29]. Indeed, the efficacy of plant extracts as mosquito control agents is widely recognized, with several botanical families, including Asteraceae, Cladophoraceae, Lamiaceae, Meliaceae, Oocystaceae, and Rutaceae, identified as particularly promising (Kim and Ahn, 2017; Perumalsamy *et al*., 2015) [28, 37]. Given the growing threat of insecticide resistance and the environmental toxicity of synthetic alternatives, these plant-derived agents represent a sustainable approach for vector control programs (Mandal, 2012) [32].

Table 3: Pupicidal effects of hexane and methanol extracts of *M. corchorifolia* against freshly formed pupae of *A, Janata*

Exposure duration	Concentrations mg/L	Mortality (%)			
		Hexane		Methanol	
24h	50	14.01±2.93[a]	LC_{50} -472.302	20.33±3.44[a]	LC_{50} -275.33
	100	29.86±2.87[b]		36.09±2.91[b]	
	200	45.19±2.64[c]	LC_{90} – 1330.52	54.27±2.40[c]	LC_{90} -919.04
	400	51.65±2.20[d]		68.60±1.47[d]	
	800	63.84±1.45[e]		79.81±0.38[e]	
48h	50	19.46±2.74[a]	LC_{50} -239.922	27.31±1.95[a]	LC_{50} -161.79
	100	35.81±2.53[b]		44.00±1.83b	
	200	63.74±1.98[c]	LC_{90} -867.913	63.74±1.60[c]	LC_{90} -654.75
	400	72.01±1.40[d]		78.90±1.04[d]	
	800	80.93±0.59[e]		91.65±0.14[e]	
72h	50	24.80±2.83[a]	LC_{50} -151.015	33.89±2.92a	LC_{50} -80.288
	100	46.77±2.64[b]		59.26±2.07[b]	
	200	69.05±2.20[c]	LC_{90} -666.705	70.77±1.83[c]	LC_{90} -529.00
	400	77.36±1.28[d]		82.14±1.71[d]	
	800	89.94±021[e]		96.67±1.63[e]	

Values are shown as mean ± Standard Deviation. Different letters within a column denote significant differences according to Tukey's test.

Table 3 indicates that both hexane and methanol extracts of *M. corchorifolia* increase pupal mortality in *A. janata* at higher concentrations and longer exposure times. At 24 h, hexane causes 14.01% mortality at 50 mg/L and rises to 63.84% at 800 mg/L, while methanol shows higher toxicity, ranging from 20.33% to 79.81%. At 48 h, mortality further increased, with hexane reaching 80.93% and methanol 91.65% at 800 mg/L. By 72 h, the highest mortality is observed, with hexane at 89.94 and methanol at 96.67%. The LC_{50} values decrease over time for both extracts at (hexane: 472.302 to 151.015 mg/L; methanol: 275.33 to 80.288 mg/L), indicating improved effectiveness, with methanol extract showing comparatively stronger pupicidal activity.

(Kavallieratos *et al*., 2023) [26] This enhanced efficacy of methanol extracts as pupicides is consistent with previous research, which found methanol extracts to be more effective than hexane and chloroform extracts in controlling pupae (Yagoo *et al*., 2023a [47] & b). The solubility of active compounds responsible for larvicidal, pupicidal, and ovicidal activities tends to be higher in methanol compared to less polar solvents like chloroform and n-hexane (Vilvest *et al*., 2023) [47].

Table 4: Ovicidal effects of hexane and methanol extracts of *M. corchorifolia* against freshly laid eggs of *A, Janata*

Exposure duration	Concentrations mg/L	Mortality (%)			
		Hexane		Methanol	
24h	50	10.73±3.77[a]	LC_{50} -647.764	13.44±5.75[a]	LC_{50} -424.634
	100	16.86±3.01[b]		20.13±4.09[b]	
	200	27.61±2.83[c]	LC_{90} -1432.21	32.09±4.93[c]	LC_{90} -966.144
	400	42.40±2.55[c]		58.83±3.11[d]	
	800	55.17±1.92[e]		76.27±2.87[e]	
48h	50	18.62±3.76[a]	LC_{50} -304.130	25.52±4.94[a]	LC_{50} -106.425
	100	39.58±2.45[b]		56.87±4.01[b]	
	200	50.00±1.82[c]	LC_{90} -988.274	69.43±3.56[c]	LC_{90} -656.431
	400	62.75±1.40[d]		81.70±3.00[d]	
	800	78.33±1.11[e]		90.36±2,30[e]	
72h	50	23.43±2.84[a]	LC_{50} -183.091	30.31±3.90[a]	LC_{50} -51.576
	100	45.61±1.63[b]		66.95±2.82[b]	
	200	62.96±1.01[c]	LC_{90} -740.462	73.27±2.05[c]	LC_{90} -526.692
	400	73.85±0.75[d]		86.39±1.58[d]	
	800	88.43±0.53[e]		95.00±1.83[d]	

Values are shown as mean ± Standard Deviation. Different letters within a column denote significant differences according to Tukey's test.

Table 4 represents the ovicidal activity of *M. corchorifolia against* the eggs of *A. janata.* It shows a steady increase in both concentration and exposure duration in *M. corchorifolia* extracts. In the hexane extract. mortality rises from 10.73% at 50 mg/L (24 h) to 55.17% at 800 mg/L, further reaches 88.43%after 72 h. The methanol extract shows a stronger effect, with mortality increasing from 13.44% (50 mg/L,24 h) to 76.27% and peaking at 95.00% at 800 mg/L after 72 h. A clear time-dependent improvement is observed, with LC_{50} values decreasing from 647.764 to 183.091 mg/L for hexane and from 424.634 to 51.576 mg/L for methanol. Overall, the methanol extract demonstrates higher ovicidal efficiency than hexane across all tested conditions. These findings are consistent with previous reports highlighting the superior efficacy of methanol extracts in disrupting insect development due to their ability to extract a broader spectrum of polar phytochemicals with insecticidal properties (Dutta and Chandra, 2023; Fernandes *et al*., 2021) [12, 17]. For instance, methanol extracts of *Urtica massaica* leaves have shown significantly higher ovicidal, larvicidal, and pupicidal activity compared to hexane extracts against *Anopheles gambiae* (Giles *and* Yugi, 2021) [21].

Similarly, the n-hexane extract of *M. grandiflora* leaves contains compounds such as palmitic acid, oleic acid, and linoleic acid, which demonstrate insecticidal effects, particularly against Spodoptera littoralis (Hussein *et al*., 2023) [22]. These findings collectively underscore the enhanced efficacy of methanol as an extraction solvent for isolating potent insecticidal compounds from plant matrices (Souza *et al*., 2023) [44]. This differential extraction efficiency aligns with studies showing that polar solvents, such as methanol, are often more effective at isolating bioactive compounds with insecticidal properties than non-polar solvents (Zaki *et al*., 2024) [52].

This enhanced solubility in polar solvents likely contributes to the higher efficacy observed in the methanol extracts, as these solvents can more effectively extract a wider range of bioactive phytochemicals, including steroids and alkaloids with larvicidal properties (Ejeta *et al*., 2021; Jayaraman *et al*., 2023; Mou *et al*., 2025) [13, 25, 36]. This solvent-dependent efficacy is further corroborated by prior research demonstrating that lipophilic compounds, which are often the active insecticidal agents, are more efficiently extracted into organic media, thereby enhancing the overall potency of the extract (Baz *et al*., 2023) [9].

For instance, comparisons have revealed that petroleum ether and ethyl acetate extracts often exhibit significantly higher mortalities in mosquito larvae than methanol or aqueous extracts, suggesting that apolar compounds may be more adept at penetrating larval cuticles (Falkowski *et al*., 2019) [16]. The varying polarities of solvent extracts have been shown to yield different suites of phytochemicals, directly influencing their bioactivity against mosquito vectors (Bansod *et al*., 2024) [7]. This divergence in efficacy based on solvent polarity highlights the importance of optimizing extraction protocols to maximize the yield of insecticidally active secondary metabolites (Baz *et al*., 2024) [8].

Conclusion

The extracts from *M. corchorifolia* leaves using hexane and methanol show notable larvicidal, pupicidal, and ovicidal effects against *A. janata*. The methanol extract is more potent than the hexane one. The results, including concentration-dependent mortality and low LC_{50} values, highlight the potential of *M. corchorifolia* as an eco-friendly bioinsecticide for integrated pest management (IPM). Future research should aim to isolate and identify active compounds, standardize formulations, and test their effectiveness in field conditions to establish sustainable control solutions for *A. janata* and similar noctuid pests.

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