



Insecticidal and Anti-Feedant Activity of *Clerodendrum* Extracts on Growth and Development of Castor Spiny Caterpillar, *Ariadne merione*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The castor spiny caterpillar *Ariadne merione* (F.) is a major lepidopteran pest affecting castor (*Ricinus communis*) crops mainly by causing defoliation which in turn reduces the photosynthesis and yield. The conventional pest control methods have been based on chemical control which although has been effective, has its drawbacks of being hazardous to the environment and human health besides leading to resistance by the pests. The present work aims at investigating the

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possibility of using *Clerodendrum inerme*, a plant belonging to the Lamiaceae family, as an eco-friendly solution for pest management. Insecticidal and anti-feedant efficacy of *C. inerme* leaf extracts, which were prepared using solvents hexane, acetone, and petroleum ether, was tested against *A. merione* larvae using leaf dip bioassay under no-choice situation. The findings showed that the hexane extract had the highest insecticidal (73%) and anti-feedant (97%) action. The petroleum ether extract also showed moderate insecticidal (50%) and anti-feedant activity (48%) but not as potent as the acetone extract having insecticidal and anti-feedant action of 60 and 74 per cent respectively. The results imply that *C. inerme* extracts, especially the hexane extract, could be used as botanical insecticides in IPM programs as a natural replacement for chemical insecticides.

Keywords: *Clerodendrum inerme*; *Ariadne merione*; anti-feedant; mortality; integrated pest management.

1. INTRODUCTION

Ariadne merione (F.) (Nymphalidae: Lepidoptera) is responsible for causing severe harm to the yield and quality of castor (*Ricinus communis*). Its larvae, being an economically significant pest, feed extensively on castor leaves, resulting in defoliation and drop in photosynthetic ability, which ultimately causes a major yield loss. When tilling crops in tropical and subtropical areas, the pest is challenging to manage because of its wide host range and ability to thrive in a variety of environmental circumstances.

Synthetic pesticides are the mainstay of traditional pest management strategies. Although the effectiveness of these compounds in controlling *A. merione* has been demonstrated, their usage is linked to a number of drawbacks, including environmental contamination, toxicity to organisms that are not the intended targets, and the possibility of encouraging the emergence of resistance in pest species (Navatha et al., 2011). These issues have forced the farming community to search for safer alternatives for pest management [1,2].

Another set of research idea can be associated with the administration of plant origin substances known as botanicals, as opposed to synthetic compounds. It is important to note that the insecticidal qualities of the plant species of the current generation that belong to the genus *Clerodendrum inerme* (Lamiaceae: Lamiales) have attracted a lot of attention. *C. inerme* is found throughout the world's tropical and subtropical regions are distinguished by an abundance of bioactive substances. These substances have been used in numerous medicinal procedures in the past, and recent studies have shown that they may also be effective natural pesticides.

Various alkaloids, flavonoids, terpenoids and phenolic acids isolated from the extracts of *C. inerme* genus demonstrated the general biological activities such as insecticidal, anti-feedant and repellent activities [3] These properties make *C. inerme* extracts a viable additive to Integrated Pest Management (IPM) regimes designed to mitigate dependence on synthetic insecticides.

In the current study, we aim to evaluate the insecticidal and anti-feedant properties of *C. inerme* extracts against the castor spiny caterpillar (*Ariadne merione*). Our goal is to determine the effectiveness of these extracts as a sustainable pest control method along with their influence on its feeding behaviour. This research seeks to identify potent botanicals that can be integrated into Integrated Pest Management (IPM) strategies, offering farmers an eco-friendly alternative to chemical insecticides. The findings from this study could pave the way for developing new bio-insecticides derived from *C. inerme* species, potentially enhancing agricultural practices while minimizing the environmental impact of pest control methods.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extracts

The fresh leaves of *C. inerme* were harvested from the Botanical garden of PGP College of Agricultural Sciences, Namakkal, Tamil Nadu, India situated in the geographical co-ordinates of 11.21° N Latitude and 78.16° E longitude. The leaves were washed with distilled water several times and then dried under the shade and powdered mechanically. The powdered plant material was sealed in an air-tight container

before extraction and kept at ambient temperature.

2.2 Preparation of Crude Plant Extracts

The cold extraction method was carried out using solvents of increasing polarity such as acetone, petroleum ether and hexane. For extraction, 100g of the powdered leaf material was mixed with 500ml of the respective solvent and shaken in a shaker at room temperature for 48 hours. These were followed by filtration through Whatman no 1 filter paper and removal of solvents by evaporation under reduced pressure in a rotary evaporator at 40°C. The dried extracts were then weighed and kept in a closed glass containers in refrigerator at 4°C until analysis.

2.3 Leaf Dip Bioassay

To evaluate the toxicity and anti-feedant activity of *C. inermis* extracts to *A. merione* larvae, leaf dip method under no choice condition was employed. The prepared crude acetone, petroleum ether and hexane extracts were taken and six different concentrations viz., 0.7, 1.0, 3.0, 5.0, 7.0 and 10.0 per cent were prepared using the respective solvents. Each concentration was prepared and added with 0.1 per cent Triton X-100 as a sticking agent so that the extracts stick to the leaves and did not drain out from the leaves. Triton X-100 with respective solvents were kept as control. The castor leaf discs with known size (4 cm diameter) were dipped in different concentrations of acetone, petroleum ether and hexane crude extracts for about 30 seconds. The leaf discs were taken out using forceps and placed in filter paper and shade dried. After drying, the leaves were kept in the Petri dishes and 4 h pre-starved larvae were released into each Petri dish and allowed to feed under no choice conditions. For each concentration, three replicates with ten larvae per replication were used. The experiment was done under Completely Randomized Design. The mortality due to direct toxicity and anti-feedant activity to the larvae were calculated at different time intervals starting with 24 h after treatment up to the treated larva stops feeding [4]. The mortality percentage and the anti feedant index [5] was calculated by using the formula.

$$\text{Mortality (\%)} = \frac{\text{Number of insects dead}}{\text{Total number of insects released}} \times 100$$

$$\text{Antifeedant index (\%)} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treated}}{\text{Leaf area consumed in control} + \text{Leaf area consumed in treated}} \times 100$$

All collected data were analysed using analysis of variance (ANOVA) on a Statistical Package for Social Science (SPSS 2020) software. Differences of greater magnitude between the treatments were established using Duncan's Multiple Range Test at 5 % level of significance.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield of *C. inermis* Extracts

The highest yield was obtained with hexane (9.6 ± 0.22%) while petroleum ether yielded 6.2 ± 0.14% and acetone yielded 2.9 ± 0.03% (Table 1). The variation in extraction yield of different solvents can be attributed to the polarity of the solvents because the solubility of bioactive compounds in the plant leaves varies with polarity of the solvent used. The yield of hexane is higher than that of the other solvents and this indicates that non polar compounds form a large proportion of the bio active compounds in *C. inermis* leaves.

Table 1. Extraction yield of *C. inermis* extracts

Solvent	Extraction yield (%)
Acetone	2.9 ± 0.03 ^c
Petroleum ether	6.2 ± 0.14 ^b
Hexane	9.6 ± 0.22 ^a

All values are mean ± SD for triplicate experiments (n=3). Different letters within the same column indicate significant difference between the values (p<0.05).

Extraction yield (%) calculated as g of dried extract/500 g DW

3.2 Anti-Feedant Activity of *C. inermis* Extracts

The antifeedant activity of *C. inermis* extracts was tested with petroleum ether, hexane and acetone as solvents. Out of all the extracts, hexane gave the highest results at 10% concentration with the highest AFI of 87% followed by Acetone (74%) and Petroleum ether (48%). The similar trend was observed at the lowest concentration (1%) with Hexane (61%), Acetone (64%) and Petroleum ether (48%). The anti-feedant action increases with increasing concentration (Fig. 1). On the other hand, the control group exhibited low AFI suggesting that *C. inermis* extracts are efficient in reducing feeding by *A. merione* larvae with hexane as the best solvent.

Table 2. Lethal Concentration of Petroleum ether, Hexane and Acetone extracts of *C. inermis* on *A. merione*

Extracts	Lethal Concentration	Estimated value	Confidence interval	Slope ± SE	Chi square value
Petroleum ether	LC10	0.80	0.140 - 1.548	1.016 ± 0.267	1.993
	LC20	2.18	0.932 - 3.486		
	LC50	14.70	8.032 - 72.258		
Hexane	LC10	0.525	0.156 - 0.952	1.314 ± 0.252	5.012
	LC20	1.136	0.512 - 1.744		
	LC50	4.963	3.523 - 7.866		
Acetone	LC10	0.382	0.029 - 0.905	0.911 ± 0.246	2.848
	LC20	1.161	0.283 - 2.044		
	LC50	9.747	5.597 - 37.925		

SE – Standard Error

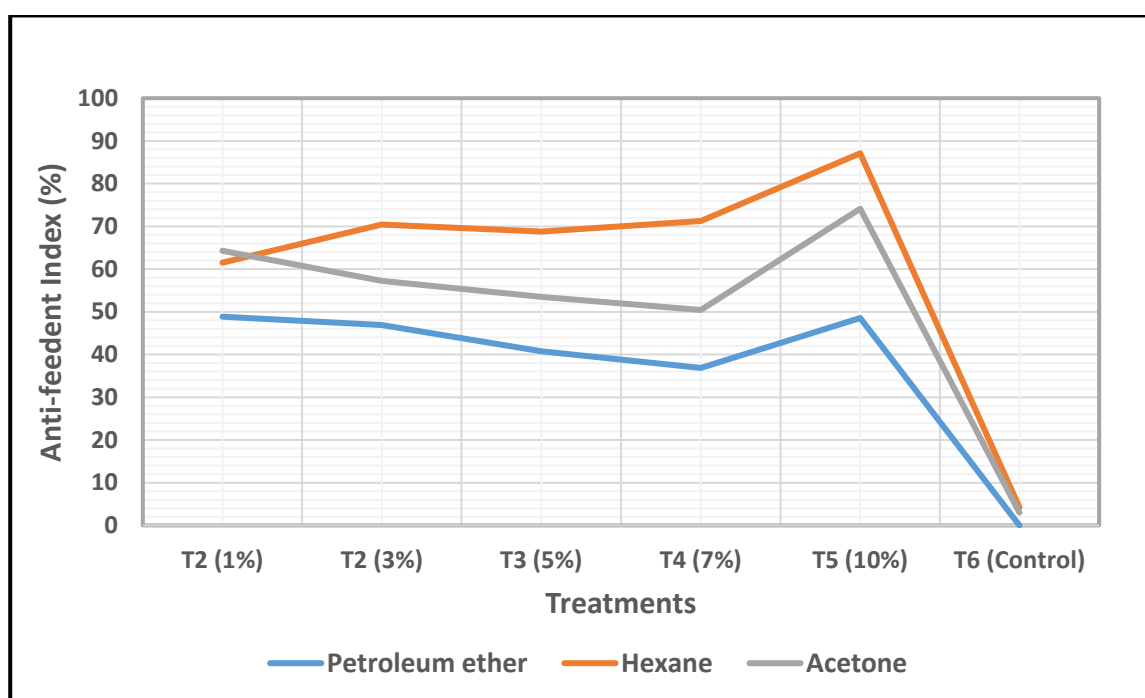


Fig. 1. Effect of different extracts of *C. inermis* on feeding action of *A. merione* larvae

3.3 Toxicity of *C. inermis* Extracts against *A. merione*

The results obtained from the experiment on the larval mortality of *A. merione* when treated with *C. inermis* extracts of various concentrations presented in Fig. 2 affirm the insecticidal property of the extracts. Out of the three extracts used, hexane extract was the most toxic followed by acetone extract and petroleum ether. The mortality gradually increased from day 2 and extended to day 5. At 10% concentration of hexane extract showed 73.33% mortality by day 5 as compared to Acetone (56%) and Petroleum ether (50%). This can be attributed to the fact

that hexane extract contains higher concentration of non-polar bioactive compounds that are soluble in non-polar solvents and possess good insecticidal properties.

The LC₁₀, LC₂₀ and LC₅₀ values also support the effectiveness of the hexane extract of the plant. It had the least LC₅₀ value (4.963%), this implying that it was the most toxic to *A. merione* larvae at lower concentration than acetone (LC₅₀ = 9.747%) and petroleum ether (LC₅₀ = 14.70%). The confidence intervals for these LC₅₀ values especially the narrow one for the hexane extract supports the claim that it could be a potent insecticide. The mortality rates obtained

for *A. merione* larvae decreased with time and with higher concentrations of *C. inerme* extracts, which indicates that *C. inerme* extracts have both acute and chronic toxic effects on *A. merione* larvae as was found in other plant-based pest control systems.

The findings of this study are evident to show that the extracts of *C. inerme*, especially the hexane and acetone extracts, have potent insecticidal and anti-feedant effects against the *A. merione*. The high mortality rates and strong feeding deterrence indicate that these extracts could be useful components of IPM. The reason for the better performance of hexane extract is due to the fact that it is capable of solubilizing non-polar compounds which possess high biological activity. These findings are in agreement with earlier studies that the presence of several bioactive compounds including terpenoids and flavonoids in *C. inerme* species exhibiting insecticidal and anti-feedant effects.

This is in concordance with our study, especially the work done by Talukdar et al. [6] who postulated that root and stem extracts of *C. viscosum* were toxic to *T. castaneum* and resulted upto 88% mortality. Azmi [7] also determined LC₅₀ value of 1460 µg/cm² for *C. inerme* against *Rhizopertha dominica*. In

addition, Adesina et al. [8] showed that *C. capitatum* extracts were efficacious against stored grain insects including *Sitophilus oryzae*, *R. dominica*, and *Tribolium castaneum*. They discovered that the ethyl extract had good repellent properties while the hexane extract was toxic through fumigation and killed 80%–90% of pests within 24 hours. Lu et al. [9] and colleagues also revealed that the essential oil of *C. bungei* was very effective as a repellent against stored product insects like *Tribolium castaneum* and the major active compounds were myristicin and linalool.

Nambiar and Ranjini, [10] on *C. infortunatum* and *Chromolaena odorata* revealed that these botanicals significantly reduced carbohydrate levels in *Orthaga exvinacea* larvae and the latter was more effective. Jadhav et al. [11] reported that *C. inerme*, *C. viscosum* and *C. philippinum* were found to be effective against *Spodoptera litura* and *Helicoverpa armigera* and were found to possess potent anti-feedant and toxic activity. Our findings on the efficacy of *C. inerme* against *A. merione* are in conformity with the result of Jadhav et al. [11] on *C. inerme*, *C. viscosum*, and *C. philippinum*. Talukdar et al. [12] also found that *C. viscosum* root extract had 44-84% mortality and strong repellent activity against rice weevils.

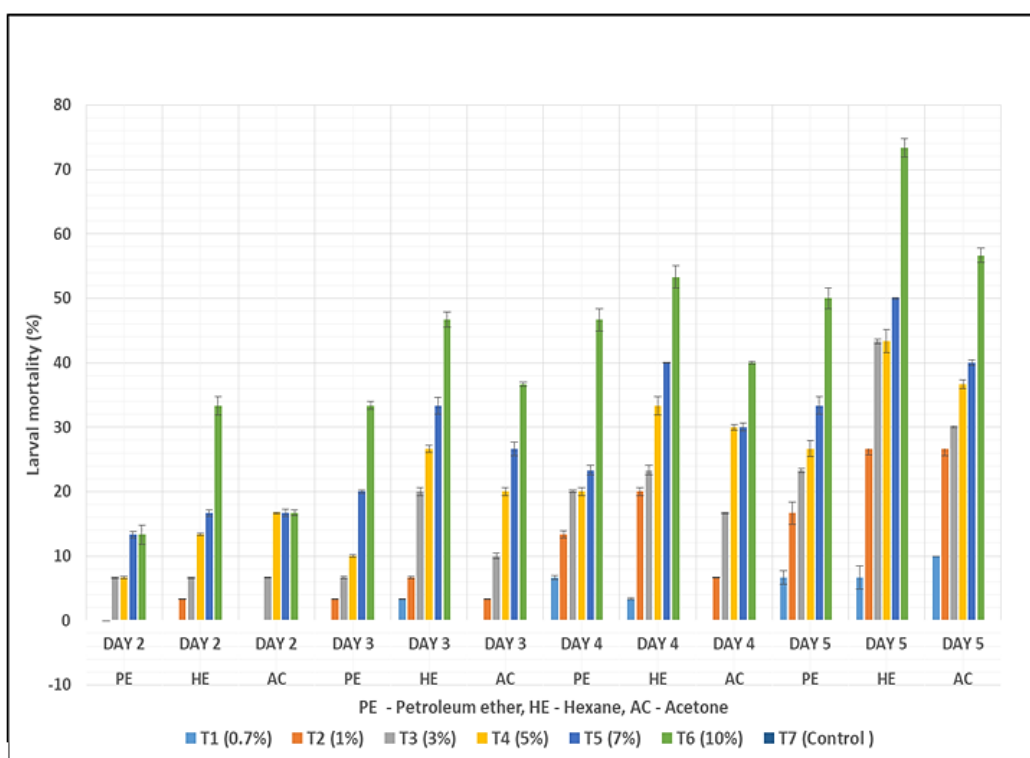


Fig. 2. Effect of different extracts of *C. inerme* on mortality of *A. merione* larvae

This indicates the possibility of these botanicals to be used as natural bio-pesticides instead of the synthetic insecticides and the hexane and acetone extracts of *C. inerme* can be used as eco-friendly insecticides to control *A. merione* in castor cultivation. The findings of the study are far reaching in pest control since it provides a natural method of controlling pests instead of using synthetic pesticides which are environmentally unfriendly and cause pest resistance. Nevertheless, more studies are required to determine the exact phytochemicals that are responsible for the observed effects and to assess the effectiveness of these compounds under real-world conditions [13].

4. CONCLUSION

In conclusion, the hexane and acetone extracts of *C. inerme* show promise as eco-friendly insecticides for controlling *A. merione* in castor cultivation. These findings contribute to the growing body of knowledge on botanical pesticides and their role in sustainable agriculture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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