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Evaluating the Antifeedant Effects of Andrographolide-based Formulations against *Lampides boeticus* L., under Laboratory Condition

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Authors' contributions

This work was carried out in collaboration between both authors. Author NGR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SKT managed the analyses of the study, managed the literature searches and approved the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antifeedant activity of *Lampides boeticus* (Linnaeus,1767), larvae exposed to various concentrations of Andrographolide-based formulations under laboratory conditions.
Study Design: Completely Randomized Design
Place and Duration of Study: Department of Entomology, College of Agriculture, Vellayani and NCESS, Kerala during 2019- 2023.
Methodology: The experiment was conducted with 9 treatments and 4 replications, treatments included various concentrations (1,3,5 and 7%) of formulations A (Andrographolide (70%) + Neem oil (20%) + Triton X-100 (10%)) and B (Andrographolide (70%) + Pungam oil (20%) + Triton X-100 (10%)). To assess the impact of formulations in the feeding behaviour of freshly moulted third instar larvae of *L.boeticus* L., no choice bioassay method was used. Each Petri plate lined with moist filter

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paper, confined a single larva with the treated pods of each specific concentration. The weight of the food consumed by the larvae was recorded at an interval of 24 hours after treatment and calculated percent feeding, per cent feeding inhibition, antifeedant activity (protection over control) and preference index of *L. boeticus* L., larvae against the formulations.

Results: The results revealed that 7% of formulation A exhibits the highest larval feeding inhibition (93.05%), followed by 7% of formulation B (87.81%). Both formulations at 5% concentrations also demonstrate significant antifeedant effects. Preference index values categorize 7% of formulations A and B as extremely antifeedant. The findings highlight the efficacy of these formulations in inhibiting larval feeding, with potential applications in pest management strategies.

Conclusion: The present findings have demonstrated the notable efficacy of formulations A and B, particularly at the 7% concentration, in significantly inhibiting larval feeding of *L.boeticus* L., and the reduction in larval feeding percentages, coupled with high antifeedant activity and feeding inhibition rates, highlights the potential of andrographolide based formulations as potent biopesticides.

Keywords: Andrographolide, neem oil; pungam oil; triton X-100; biopesticides; antifeedant activity; Lampides boeticus L.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an important legume crop native to central Africa and belongs to the family Fabaceae, holds significance due to its adaptability to various agro-climatic conditions and substantial contributions to global food security and nutrition due to its rich protein content. In Kerala, vegetable cowpea cultivated across an extensive area of 5450 hectares plays a pivotal role in overall vegetable cultivation, covering approximately 14% of the total vegetable cultivation area of 38,386 hectares [1]. Despite its importance, cowpea cultivation faces a major challenge, primarily related to insect pests. Various insect pests pose a threat to this crop at different growth stages, with pod borers being the most significant ones, causing severe damage and yield losses of up to 60 per cent [2]. Various borer pests pose a significant threat to crops, affecting them from the seedling stage through harvest, aligning with crucial phenological stages. The abundance and activity of this pest complex are heavily influenced by abiotic factors, manifesting in succession during various reproductive phases in the cowpea ecosystem. The pod borers pose a substantial risk to crops by directly targeting the economic part of the plant and are referred to as hidden and notorious pests due to their elusive nature [3]. Among the pod borers, the globally distributed Pea blue butterfly, *Lampides boeticus* L., infects several leguminous crops, including cowpea, beans, peas and soybeans [4]. It causes considerable damage to flower buds and pods, leading to a reduction in yields ranging from 60 – 90 per cent [5]. Chemical insecticides have been widely used for pest management, but their adverse impacts on the environment and human health have raised concerns. Thus, the search for eco-friendly and sustainable alternatives has intensified, focusing on botanical pesticides and plants with insecticidal activity emerges as a promising tool, offering not only direct toxic effects on insects but also environmentally friendly attributes, such as easy biodegradability and the significant potential of plant secondary metabolites, including alkaloids, saponins, phenols, and terpenes, for developing innovative and ecologically friendly methods to control a wide range of insect pests through specific plant species [6,7].

The medicinal plant, *Andrographis paniculata* (Burm.f.) Wall. ex Nees, commonly known as the 'King of Bitter,' has emerged as a promising candidate for eco-friendly insecticides. Recent studies highlighting its insecticidal activity against various insect pests, attributed to its active constituent, andrographolide (labdane diterpenoid). Bhavyasree [8] developed an oil-based ready-to-use formulation of *A. paniculata* for the management of the sucking pest complex in chilli. Hence, the present study is designed to evaluate the antifeedant activity of andrographolide-based formulations against *L. boeticus* L., aiming to provide valuable insights into the development of eco-friendly pest management strategies under laboratory conditions.

2. MATERIALS AND METHODS

This experiment was conducted at the Department of Entomology, College of Agriculture, Vellayani and National Centre for Earth Science Studies (NCESS),

Thiruvananthapuram, Kerala, India, during 2019- 2023.

2.1 Insect Culture

Adults of *Lampides boeticus* L. were collected from the cowpea field in the College of Agriculture, Vellayani, India and kept in plastic containers to facilitate oviposition under room temperature. To sustain the adult, cotton swabs dipped in 10% honey solution were suspended in a hanging position within the containers, serving as a nutritional food source. Fresh flower buds were strategically placed to stimulate egg laying, and a piece of black muslin cloth was provided for egg deposition and ease of identification. After egg laying the eggs were gently collected using a fine hair brush and then transferred to separate plastic containers for further development. The hatching larvae were individually reared to ensure uniform growth. Later. the second instar larvae were selected for subsequent laboratory studies.

2.2 Isolation of Plant Compound Andrographolide

The compound andrographolide was isolated from the plant, Andrographis paniculata using the modified version of the method reported by Pundarikakshudu et al. [9]. 50g of A. paniculata plant powder underwent maceration in 200 ml of methanol (MeOH) for 24 hours, followed by refluxing for one hour. The resulting mixture was filtrated and treated with animal charcoal while heating and filtered through Whatman No. 1 filter paper. MeOH was evaporated at 70ºC in a water bath. The resulting solid crystalline mass was washed with 2 x 10 mL of cold MeOH and filtered to get white crystals.

2.3 Development of Formulations

Two emulsifiable formulations, designated as formulation A (comprising Andrographolide (70%) + Neem oil (20%) + Triton X-100 (10%)) and formulation B (comprising Andrographolide (70%) + Pungam oil (20%) + Triton X-100 (10%)), were synthesized according to the following procedure. The Andrographolide solution was prepared by dissolving the compound in methanol and diluting it with distilled water. Concurrently, the emulsifier Triton X-100 was dissolved in carrier oils (neem and pungam oil) under continuous agitation using a mechanical stirrer for 15 minutes. Subsequently, this emulsifiable mixture was introduced into the andrographolide solution and thoroughly blended in a mechanical shaker for 30 minutes to ensure complete homogeneity. The resulting formulations were then stored in airtight glass bottles for subsequent studies.

2.4 Antifeedant Activity Bioassay

The experiment was conducted in a completely randomized block design with nine treatments and four replications. The treatments include various concentrations of formulations A and B as described below;

List 1. List of treatments used for the experiment

Sl. No	Treatments
T1	1% of formulation A
T2	3% of formulation A
T3	5% of formulation A
T4	7% of formulation A
T5	1% of formulation B
T6	3% of formulation B
T7	5% of formulation B
T8	7% of formulation B
T9	Untreated control

To assess the impact of formulations in the feeding behaviour of freshly moulted third instar larvae of *L.boeticus* L., no choice bioassay method was used as suggested by Jackai et al. [10]. For the bioassay study, third instar larvae of *L. boeticus* L. were subjected to a 6-hour starvation period before use. The fresh cowpea pods collected from the field were meticulously washed, dried and separately treated with different concentrations of formulations A and B, separately for one minute, followed by air drying. In the untreated control, the pods were dipped in distilled water and air-dried before feeding to the larvae. Each Petri plate lined with moist filter paper, confined a single larva with the treated pods of each specific concentration. To maintain the moisture conditions, the filter paper lining the bottom of the Petri plates was drenched with water. The weight of the food consumed by the larvae was recorded at an interval of 24 hours after treatment. Following are the formulas used for calculating percent feeding, per cent feeding inhibition, antifeedant activity (protection over control) and preference index.

Per cent feeding in each treatment over control was calculated using the formula:

Percent feeding =[(Initial food given for feeding-food left after feeding)/Initial food given for feeding)] x 100^

Per cent feeding inhibition (FI)was calculated using the formula [11]

> FI = (consumption of food in untreated control-consumption of treated food) / (consumption of food in untreated control+consumption of treated food) x 100

Antifeedant activity (Protection over control):

> (Per cent feeding in untreated control-Per cent feeding on treated food)/Per cent feeding in untreated control

Preference index (C) was calculated [12]:

> C=(2(Consumption of treated food)) / (Consumption of untreated food+Consumption of treated food)

The antifeedant category of 1, 3, 5 and 7% of formulations A and B was worked out on the basis of preference indices (C values) according to the following scale [13]:

List 2. Antifeedant category with C-Value

C-Value	Antifeedant Category
0.1 - 0.25	extremely antifeedant
0.26 - O.50	strong antifeedant
0.51 - 0.75	moderately antifeedant
0.76 - 0.99	slightly antifeedant
>1	preferred plant extract

The data obtained from laboratory studies was subjected to analysis of variance (ANOVA) using GRAPES software and the treatment differences were compared.

3. RESULTS AND DISCUSSION

The detailed results of the comparative antifeedant activity of different concentrations of formulations A and B against *L. boeticus* at 24 hours after treatment under no-choice test are presented in Table 1. Among the treatments of formulations, the percentage of larval feeding ranged from 4.77 to 53.79 %, in comparison to 68.80 per cent in the untreated control. The lowest percentage of larval feeding was observed in 7% of formulation A (4.77%) and formulation B (8.42%), which were found to be statistically on par with each other. Following this, 5% of formulations A (17.52%), 3% of formulation A (20.77%) and 5% of formulation B (22.08%) were on par with each other, while 3% of formulations B recorded the feeding percentage of 29.57 per cent, followed by 1% of formulations A (53.67%) and B (53.79%) showed similar levels of larval feeding.

Highlighting the protective impact over the control, the antifeedant activity was most pronounced in 7% of formulation A (93.05%), closely followed by 7% of formulation B (87.81%). Furthermore, 5% of formulations A (74.49%) and B (67.81%), as well as 3% of formulations A (69.78) and B (57.05%) exhibited notable antifeedant efficacy. A significant difference in larval feeding inhibition was observed across the treatments when compared to the untreated control. The highest percentage of feeding inhibition occurred in 7% of formulations A (87.14%) and B (77.63), while the minimum percentage was observed in 1% of formulations A (13.42%) and B (10.69%). In contrast, the feeding inhibition percentages among the other treatments were 59.79, 51.32, 56.35 and 39.06 per cent in 5% of formulations A and B and 3% of formulations A and B, respectively. The determination of preference index values categorized 7% formulations of A and B under the extremely antifeedant category, signifying their potent deterrent effect against larval feeding.

The current findings are in accordance with Hermawan et al. [14], who investigated the antifeedant activity of andrographolide against *Plutella xylostella* (Linnaeus, 1758), reporting its significant antifeedant activity. This correlation further supports the evidence of andrographolide's antifeedant properties. Furthermore, the results are consistent with Vattikonda [15], demonstrating a substantial reduction in the feeding ability of *Papilio demoleus* (Linnaeus, 1758) after treatment with andrographolide and recording 83.60% antifeedant activity after 24 hours. This underscores the efficacy of andrographolide, highlighting its potential as a deterrent for feeding larvae. Comparisons with neem oil and pungam oil provide a broader context for assessing the effectiveness of formulations A and B. Neem oil, recognized for its antifeedant effects on Lepidoptera insects [16], serves as a crucial benchmark for evaluating the observed antifeedant activity in the study. Additionally, the study resonates with Vattikonda and Sangam [17], reporting that azadirachtin, the main component of neem oil, exhibited 86.28 and 70.43 per cent antifeedant activity after 24 and 48 hours of treatment, respectively. This further validates the antifeedant efficacy of neem oil

Table 1. Comparative antifeedant activity of different concentrations of formulation A and B against *Lampides boeticus* L., after 24 hours

Treatments	*Feeding (%)	*Antifeedant activity (%)	*Feeding inhibition (%)	Preference index	Antifeedant category
1% of formulation A	53.67 (47.11)[d]	21.81	13.42	0.87	slightly antifeedant
3% of formulation A	20.77 (27.01)[b]	69.78	56.35	0.44	strong antifeedant
5% of formulation A	17.52 (24.44)[b]	74.49	59.79	0.40	strong antifeedant
7% of formulation A	4.77 (12.45)[a]	93.05	87.14	0.13	extremely antifeedant
1% of formulation B	53.79 (47.18)[d]	21.76	10.69	0.89	slightly antifeedant
3% of formulation B	29.57 (32.93)[c]	57.05	39.06	0.61	moderately antifeedant
5% of formulation B	22.08 (27.89)[b]	67.81	51.32	0.49	strong antifeedant
7% of formulation B	8.42 (16.36)[a]	87.81	77.63	0.22	extremely antifeedant
Control	68.80 (56.06)[e]	0.00	0.00	1.00	No antifeedant effect
SE(m)	1.65				
CD (0.05)	4.77				

**Mean of four replications, Figures in parentheses are values after arc sine transformation,*

observed in formulation A. Similarly, pungam oil is a valuable comparison, as demonstrated by Lakshmanan et al. [18], who showcased its effectiveness as a potent antifeedant against *Helicoverpa armigera* Hübner, [19] further substantiate the superiority of pungam oil, establishing it as a more effective antifeedant than neem oil and sesame oil against *Spodoptera litura* (Fabricius, 1775). This comparative analysis strengthens the argument for the efficacy of pungam oil in formulation B.

4. CONCLUSION

The study highlights the significant protective impact of formulations A and B, particularly in terms of their antifeedant activity against pod borer pests. The results underscore the efficacy of these formulations, with 7% concentrations exhibiting the most pronounced deterrent effect. Additionally, the utilization of botanical formulations aligns with environmentally sustainable practices, offering a promising alternative to chemical pesticides emphasising the potential for integrated pest management strategies. Moreover, comparisons with neem oil and pungam oil provide a valuable context, establishing the competitive edge of formulations A and B in terms of antifeedant activity. Overall, this research emphasizes the role of botanical formulations as viable alternatives for promoting sustainable and environmentally friendly approaches to crop protection. By mitigating the reliance on chemical pesticides, these formulations contribute to the preservation of environmental health and biodiversity, aligning with the goals of addressing environmental and climate change challenges.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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