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Bio-efficacy of Different Botanical Extracts on *Aphis craccivora* in Black Gram

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The studies on "Bio-efficacy of botanical extracts against *Aphis craccivora* in black gram was carried out in the Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu. The black gram aphid, *Aphis craccivora* Koch. (Hemiptera: Aphididae), is one of the major devastating and cosmopolitan-sucking pests in Blackgram. Bio-efficacy of *Pongamia pinnata* leaf extract 5 % and 10 %, *Annona squamosa* leaf extract 5% and 10%, *Andrographis panniculata* leaf extract 5% and 10% and *Catharanthus roseus* leaf extract 5% and 10% with *Azadirachtin* as a standard check it was evaluated on 3rd instar nymphs of *Aphis craccivora* on topical bioassay and feeding bioassay. In which *C. roseus* leaf extracts of 5% and 10% has shown the highest mortality rate on par with *Azadirachtin* 5% which is kept as a standard check.

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Keywords: Aphids; blackgram; botanicals; contaminated food bioassay; periwinkle; topical bioassay.

1. INTRODUCTION

Aphis craccivora Koch (Hemiptera: Aphididae), is a widespread pest that infests various host plants, with a particular preference for the Fabaceae family, of all the insect pests affecting legume crops, *A. craccivora* stands out as the most destructive and globally distributed pest [1]. Both the nymphs and adults of the aphids feed on the sap from plant cells, leading to leaf distortion and stunted growth, ultimately resulting in the production of poor-quality pods. Furthermore, they secrete substantial amounts of honeydew, which encourages the growth of sooty mold and disrupts the plant's photosynthetic capabilities. Additionally, the bean aphid serves as a vector for various non-persistent viruses, contributing to the spread of diseases among multiple crops [2].

Aphids are highly prolific breeders and insecticides are often applied randomly to achieve quick control. However, the use of chemical protection methods comes with numerous significant drawbacks. Their extensive and indiscriminate application leads to ecological imbalances, the development of pest resistance, pest resurgence, outbreaks of secondary pests, as well as phytotoxicity. It also results in residues in foods and feeds and can harm beneficial organisms within the ecosystem. Consequently, researchers and scientists worldwide are actively exploring alternative approaches to safeguard crops from insect pests. One promising avenue involves the utilization of natural products as an effective means of pest control [3]. Many natural botanicals have been considered research interest because of the fewer hazards to non-target organisms, less environmental residue, the inability of pests to develop resistance, no adverse effect on plant growth, seed viability, and cooking quality of grains and it is less expensive, and easily available because of their natural occurrence [4].

The primary objective of this study was to assess the efficacy of various botanical extracts against third-instar nymphs and adults of *A. craccivora* in a controlled laboratory environment. This investigation aimed to gather data regarding the direct mortality of insect pests. The findings may pave the way for safer alternatives to chemical pesticides, thus reducing the ecological impact of agricultural practices.

2. MATERIALS AND METHODS

The experiments were carried out to evaluate the bio-efficacy of various botanical extracts at the Department of Entomology, Faculty of agriculture, Annamalai University, Chidambaram during Dec 2020 – April 2021.

2.1 Collection and Rearing of Test Insect

The black gram aphid (*A. craccivora*) was mass cultured on blackgram seedlings raised on the plastic cups in a screen house. Aphids was collected from the infested blackgram plants in the campus of Annamalai University (Department of Entomology) were released on the blackgram seedlings in the screen house and were allowed to multiply. Once the blackgram seedlings became stunted and their leaves dried due to aphids feeding, the aphids were transferred to new seedlings. To maintain the aphid culture, black gram seedlings were raised at weekly intervals in the screenhouse. Aphids maintained in the screenhouse were used for various stuides. The rearing technique followed the method described by [5]. All the studies were conducted during 2020- 21 at the Department of Entomology, Faculty of agriculture, Annamalai University, Chidambaram.

2.2 Collection, Processing, and Preparation of Test Plant Solution

Leaf extracts of *Catharanthus roseus* (Periwinkle), *Pongamia pinnata* (Pongam), *Annona squamosa* (Custard apple), *Andrographis paniculata* (Siriyanangai) were prepared using the following method.

Fresh leaves were collected from mature plants in and around Annamalai Nagar. The leaves were washed thoroughly under tap water and dried in shade, after drying, the leaves were cut into small pieces and macerated. The macerated plant material was filtered using muslin cloth to obtain crude extract. To prepare the extracts, for a 5 % extract, 5 ml of leaf extract was mixed with 95 ml of distilled water and for a 10 % extract, 10 ml of leaf extract was mixed with 90 ml of distilled water.

2.3 Laboratory Bioassay of Botanical Against Black Gram Aphids

The biocidal efficacy of the prepared botanical extracts against *A. craccivora* was evaluated

using two techniques: topical bioassay and feeding bioassay (contaminated food technique - no-choice test).

2.3.1 Topical bioassay

Third-instar nymphs of *A. craccivora* were topically sprayed with 2 mL/replication of 5% and 10% extract concentrations using a sprayer. After 30 minutes, 10 nymphs were released onto separate untreated host plants using a soft camel hair brush. Mortality was monitored for 24, 48, and 72 hours. Percent mortality was then calculated.

2.3.2 Contaminated food bioassay

Black gram seedlings were sown in cups for each treatment (8 treatments with 3 replicates each). Ten pre-starved (3 hours) third-instar nymphs were released onto leaves treated with the extracts in petri dishes under laboratory conditions. Nymph establishment on treated leaves and mortality were observed at 24, 48, and 72 hours.

2.4 Statistical Analysis

The data recorded in the experiments were subjected to analysis of variance (ANOVA) under randomized block design (RBD) by adopting the procedures described by Gomez (1984). Necessary data transformation was made before analysis and OPSTAT and WASP 2.0 was used for the calculation.

3. RESULTS AND DISCUSSION

3.1 Nymphal Mortality of *A. craccivora* on Topical Bioassay Technique

Four plant extracts (Periwinkle, Andrographis, Annona, and Pungam) were tested at two concentrations (5% and 10%) against *A. craccivora* nymphs with *Azadirachtin* as a standard check. The effects were evaluated at 24, 48, and 72 hours after treatment.

At 24 hours, Periwinkle extract at 10% concentration caused the highest nymphal mortality (30.00%) which is comparable to the mortality rate of *Azadirachtin* (Std. check) which is 36.67%, followed by Periwinkle extract at 5% (23.33%). The lowest mortality (3.33%) was observed in Pungam extract at 5% concentration. There was no mortality in the untreated control group.

At 48 hours, *Azadirachtin* mortality rate recorded was 63.33% and it was comparable with Periwinkle extract at 10% concentration again showed the highest nymphal mortality (56.67%), followed by Periwinkle extract at 5% (50.00%). The lowest mortality (16.67%) was still observed in Pungam extract at 5% concentration. The untreated control group was recorded 10.00% mortality rate.

Finally, at 72 hours, Periwinkle extract at 10% concentration caused the maximum nymphal mortality (86.67%), followed by Periwinkle extract at 5% (80.00%). Pungam extract at 5% concentration showed the least effectiveness (23.33%). There was 16.67% of mortality rate was recorded in Control (Table 1).

3.2 Nymphal Mortality of A. *craccivora* on Contaminated Food Bioassay Technique

Periwinkle leaf extract proved to be the most effective botanical against *A. craccivora* nymphs in the contaminated food bioassay.

At 24 hours after treatment, Periwinkle extract at 10% concentration caused the highest nymphal mortality (33.33%) which is comparable to 40.00% of mortality rate by *Azadirachtin*(Std. check) followed by Periwinkle extract at 5% (30.00%). The least mortality (6.67%) was observed in Pungam extract at 5% concentration. There was no mortality in the untreated control group.

Table 1. Mortality rate of *A. craccivora* nymphs against certain botanicals (Topical bioassay)

T. No.	Treatment	Concent-ration (%)	*Nymphal mortality rate (Treated plants)		
			After 24 hours	After 48 hours	After 72 hours
T_1	Pungam leaf extract	5	3.33 (6.14)	16.67 (23.86)	23.33 (28.78)
T_2	Pungam leaf extract	10	6.67 (12.29)	20.00 (26.57)	26.67 (30.99)
T_3	Annona leaf extract	5	10.00 (18.43)	26.67 (30.99)	43.33 (41.15)

T. No.	Treatment	Concent-ration (%)	*Nymphal mortality rate (Treated plants)		
			After 24 hours	After 48 hours	After 72 hours
T_4	Annona leaf extract	10	13.33 (21.14)	30.00 (33.00)	46.67 (43.08)
T_5	Periwinkle leaf extract	5	23.33 (28.77)	50.00 (45.00)	80.00 (63.44)
T_6	Periwinkle leaf extract	10	30.00 (33.20)	56.67 (48.85)	86.67 (68.86)
T_7	Andrographis leaf extract	5	16.67 (23.85)	36.67 (37.23)	63.33 (52.78)
T_8	Andrographis leaf extract	10	20.00 (26.55)	46.67 (43.08)	73.33 (59.00)
T_9	Azadirachtin (Std. check)		36.67 (37.21)	63.33 (52.78)	93.33 (77.62)
T_{10}	Control (Untreated)		0 (0.29)	10.00 (18.44)	16.67 (23.86)
	C.D. (0.05%)		9.350	6.019	8.333
	S.E(d)		4.45	2.87	3.97

**Values are means of three replications*
Values in parentheses are arc sin transformed

This trend continued at 48 hours. Periwinkle extract at 10% concentration again showed the highest nymphal mortality (66.67%), followed by Periwinkle extract at 5% (60.00%). The lowest mortality (26.67%) was still observed in Pungam extract at 5% concentration. The untreated control group had 13.33% mortality rate.

Finally, at 72 hours, Periwinkle extract at 10% concentration caused the maximum nymphal mortality (90.00%), followed by Periwinkle extract at 5% (83.33%). Pungam extract at 5% concentration showed the least effectiveness (36.67%). There was 20.00% mortality rate recorded in Untreated control may be due to other factors (Table 2).

Our finding was supported by Ramya, [6] reported that the *H. armigera* was controlled by the extracts of *C. roseus* leaf extract.

This is in consistent with the report of [7] who found that the *Allium tuberosum* leaf, *Caesalpinia ferrea* leaf, *Piper aduncum* leaf, *Carica papaya* seed, *Dieffenbachia picta* leaf, *Cucurbita moschata* seed, and *Annona squamosa* seed had the maximum efficacy (50%) against *A. craccivora* nymphs, among these highest mortality rate was recorded from the extracts of *Dieffenbachia picta* leaf and Cucurbita moschata seed.

These findings are on par with the work of Sammour *et al*., [8] in which they reported that neemix and neem oil exhibited the antifeedant and aphicidal effect on the nymphal stage, since they caused prolongation of the nymphal duration, high percentage of mortality and only a few young ones could be succeeded to reach to the adult stage.

Table 2. Mortality rate of *A. craccivora* nymphs against certain botanicals (Contaminated bioassay)

T.No.	Treatment and Concentration	Concent-ration (%)	*Nymphal mortality rate treated plants		
			After 24 hours	After 48 hours	After 72 hours
T_1	Pungam leaf extract	5	6.67 (12.29)	26.67 (30.98)	36.67 (37.21)
T_2	Pungam leaf extract	10	10.00 (18.43)	30.00 (33.20)	40.00 (39.22)
T_3	Annona leaf extract	5	13.33 (21.14)	40.00 (39.22)	46.67 (43.06)
T_4	Annona leaf extract	10	16.67 (23.85)	43.33 (41.14)	50.00 (44.98)
T_5	Periwinkle leaf extract	5	30.00	60.00	83.33

T.No.	Treatment and Concentration	Concent-ration (%)	*Nymphal mortality rate treated plants		
			After 24 hours	After 48 hours	After 72 hours
			(33.20)	(50.75)	(66.12)
T_6	Periwinkle leaf extract	10	33.33 (35.20)	66.67 (54.76)	90.00 (71.54)
T_7	Andrographis leaf extract	5	20.00 (26.56)	46.67 (43.06)	66.67 (54.76)
T_8	Andrographis leaf extract	10	23.33 (28.77)	56.67 (48.83)	76.67 (61.20)
T_9	Azadirachtin (Std. check)	2	40.00 (39.22)	76.67 (61.20)	93.33 (77.69)
T_{10}	Control (Untreated)		0 (0.29)	13.33 (21.14)	20.00 (20.06)
	C.D. (0.05 %)		7.357	5.335	8.971
	S.E(d)		3.502	2.540	4.271

**Values are means of three replications*
Values in parentheses are arc sin transformed

Our findings are consistent with those of [9] who found that neem seed kernel extract (97.50%) and neem leaf extract 10% (61.88%) had shown the highest number of nymphal deaths.

This was partially in line with the findings of [10], who discovered that the hot water extract of *Polygonum hydropiper* (94.5 percent - nymphal mortality rate) was more effective than *A. indica* (80.47–80.96 percent -nymphal mortality rate). Other hot and cold extracts also appeared to be more useful as pesticides for *A. craccivora.*

4. CONCLUSION

Among the four botanical extracts periwinkle leaf extract has shown the highest mortality rate in both topical bioassay and contaminated food bioassay techniques at every 24, 48 and 72 hrs. These finding suggest that periwinkle leaf extract has promising potential as a botanical insecticide for controlling *A. craccivora* nymphs. Further investigations are needed to explore the specific bioactive compounds responsible for periwinkle's leaf extract's insecticidal properties and to optimize its application for field use.

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 writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exists.

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