



Effect of Costunolide a plant product of *Saussurea lappa* on feeding behaviour of *Papilio demoleus* L. (Lepidoptera: Papilionidae) Larvae

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Abstract

Insect pest management is facing the economic and ecological challenge worldwide due to the environmental hazards caused by the synthetic pesticides. A search for alternate techniques for the insect pests' management arises in recent years. Botanicals act not only as insecticides but also function as antifeedants, oviposition deterrents and ovicides. The present study was carried out to evaluate the antifeedant activity of Costunolide which was isolated from root extract of Saussurea lappa to citrus pest Papilio demoleus by non choice bioassay method. The test compound dissolved in acetone was separately tested at 200, 150, 100, 50ppm concentration continuously for 24 and 48 hours. The result showed that presence of antifeedant effect was maximum at 200ppm and least at 50ppm Costunolide influence on feeding activity of fourth instar larvae significantly reduced the food consumption, growth rate and utilization of the ingested food and reduced digestibility. Thus the feeding deterrence was markedly influenced when larvae fed on Costunolide sprayed leaves which caused more than 80% of antifeeding effect.

Keywords: Costunolide, feeding deterrence, *Papilio demoleus*, insect pest management, growth rate.

Introduction

One of the biggest challenges that humanity confronts is providing enough food to the fast growing population of the planet, which is calculated to reach approximately 8 billion by 2020¹. In order to materialize this, it is necessary to increase food production, which mainly depends on plants. These are often affected by many pests and diseases among which insects play a preponderant role. It is estimated that almost 30% of plant crops is lost due to attack of feeding insects, wastage, or disease². Therefore there is continuous drive to develop new compounds in order to maintain food and feed reserves. Commercially available synthetic pesticides are the most effective means of pest control for the last 60 years worldwide. Synthetic insecticides possess natural toxicities that affect the health of farmers, consumers and the environment. However, the continuous and indiscriminate use of pesticides substances have not only caused adverse effects on human health, but also affected other beneficial organism of the ecosystem³ and the environment⁴. They are also responsible for the development⁵ of resistance in pathogens⁶.

The recognition and documentation of many unwanted agro ecological, environmental, social and economic problems resulting from pesticide over use, has led scientists to look for alternatives among which botanical insecticides are very promising because of their low cost, easy availability broad-spectrum of pesticide activity and plants affecting the target pest in very small quantities^{7, 8}. Some plants having antifeedant^{9, 10}, insecticidal¹¹ and ovicidal properties have been reported earlier^{12, 13}. In this context, new ways of pest control must be

studied and established. There are many options, but the most promising one is usage of secondary metabolites produced by plants. Many of secondary metabolites are highly toxic to a wide spectrum of insects and microorganisms. Secondary metabolites can partly be considered as weapons to defend plants against pests and diseases that have competed with them since time immemorial¹⁴.

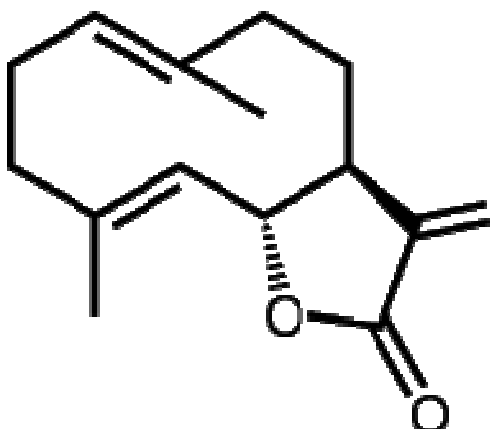
In the present work *Papilio demoleus* is serious pest of citrus plantation in Pakistan, India, Saudi Arabia, Iran, China and Africa¹⁵ has been selected for the present study. The pest is active almost throughout the year and the larvae are vigorous foliage feeders¹⁶. In case of severe infestation, citrus trees are seriously affected and the young seedlings become completely defoliated¹⁷. The objective of the present study was to evaluate the bioefficacy of Costunolide on feeding activity isolated from root extract of *Saussurea lappa* against of *Papilio demoleus*.

Material and Methods

Insect: Fresh eggs and newly hatched young *Papilio demoleus* larvae were collected along with the citrus leaves from the fields and reared in the laboratory conditions for several days. Larvae were maintained providing fresh citrus leaves daily. 10% honey solution was supplied for feeding to adults and fresh citrus twigs for oviposition.

Test product: Costunolide: (C₁₅H₂₀O₂): The roots of *Saussurea lappa* has been widely used as traditional medicine for the treatment of various kinds of disorders such as asthma, cough, diarrhea, indigestion, antiulcer, anti-cancer, antiarthritic,

anti-viral, skin and microbial infections¹⁸. Several of its activities are well-proved and established through in-vitro and in-vivo methods. Pharmacological test revealed that costunolide apart from exhibiting antibacterial and antiviral activities, could also dilate bronchus, depress blood pressure, and relieve the spasm of smooth muscle and it was having breast carcinoma activity and prominent juvenile hormone analogues^{19, 20, 21}.



Chemical Structure of Costunolide

For conducting the present experiments, Costunolide was supplied from the research scholars of Department of Chemistry, Natural Chemistry Division, Osmania University, Hyderabad.

Experimental Procedure: Antifeedant activity evaluation

method: A large leaf disc 36.5 sq.cm of lemon was used. It was sprayed with test compound uniformly on either side by using an atomizer and placed on moist filter paper in a Petri dish. Single *Papilio demoleus* fourth instar larva was introduced into the Petri dish. Parallel controls of *Papilio demoleus* were maintained with similar leaf discs. Ten such Petri dishes were taken for each experiment. The setup was kept for observation at 24hrs; 48hrs and mean average of the ten sets were taken for the activity. The consumed area was measured graphically and also with Planimeter. The percentage leaf area protection was calculated by Isman M.B. et.al.²².

Leaf area consumed in control
– treated leaf

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{treated leaf}}{\text{Leaf area consumed in control}} \times 100$$

Preparation of test solution: Acetone was used as a solvent in preparing the test solutions, since the solubility of the test compounds was very high in acetone. 200, 150, 100, 50ppm concentrations were prepared from the stock solution.

Statistical analysis: The results are expressed as Mean \pm SD and data was statistically analyzed by one-way ANOVA, with the level of significance set at $p < 0.05$.

Results and Discussion

Plant material of Costunolide and its bioactivities were tested at different concentrations against *Papilio demoleus*. The bioactivity data were collected and subjected to one-way analysis of variance (ANOVA). Antifeedant activity of Costunolide and the results are presented in table 1. Antifeedant activity was assessed based on antifeedant index. Higher antifeedant index normally indicates decreased rate of feeding. In the present study the antifeedant activity varied significantly with increase in concentration.

Efficacy of plant products as an alternative to synthetic insecticides for insect pest management was studied by earlier others successfully and results were appreciable²³⁻²⁶. In the present study results showed that the Costunolide isolated from roots of *Saussurea lappa* significantly inhibited the feeding activity and adult survival and suppressing progeny emergence. The high antifeedant activity was showed at 200ppm concentration that was 80.33% and 79.86% respectively at 24hrs and 48hrs exposure. Protected leaf area also measured with planimeter after 24hrs and 48hrs that was 31.47 ± 0.89 sq.cm and 28.46 ± 0.83 sq.cm. The mode of action of *Saussurea lappa* root extract could be pungent odors prevented the insect from natural feeding and higher doses may have blocked the insect spiracles resulting to suffocation. Similar results were reported using *D. tripetala* seed extract against banana weevil²⁷.

Table-1

Mean and SD of undamaged leaf area (sq.cm) and antifeedant activity with different concentration treatments of Costunolide

Conc in ppm	No of Insects	Mean \pm SD After 24 hrs	Mean \pm SD After 48 hrs	Antifeedant activity after 24hrs	Antifeedant activity after 48hrs
200	10	$31.47 \pm 0.89^*$	$28.46 \pm 0.83^*$	80.33	79.86
150	10	$29.53 \pm 0.67^*$	$24.85 \pm 1.14^*$	73.74	72.09
100	10	$26.67 \pm 0.74^*$	$22.83 \pm 0.90^*$	64.86	68.03
50	10	$24.51 \pm 1.09^*$	$18.36 \pm 1.78^*$	58.74	61.23
Control	10	$19.66 \pm 1.16^*$	$10.27 \pm 0.82^*$	-----	-----

Mean and SD values are significant at $P < 0.05$.

In the present study at 150ppm concentration Costunolide that in the first 24hrs showed the antifeedant activity it was 73.74% and after 48hrs the antifeedant activity was 72.09%. The protected leaf area it was in first 24 hrs 29.53 ± 0.67 sq.cm and after 48hrs 24.85 ± 1.14 sq.cm. Our own previous results showed that antifeedant activity caused a reduction in food consumption and chronic toxicity leading to delayed growth, development and increased mortality. Many members in the families of Meliaceae, Solanaceae and Asteraceae have potential for development as commercial insecticides with broad-spectrum activity²⁸. Costunolide could affect biochemical processes, disrupt the endocrine balance leading to immobilization and toxicity to larvae. Our results are agreement with previous reports produced by organophosphates and carbamates^{29, 30, 31}.

The test compound Costunolide showed moderate antifeedant activity even a low concentration (50ppm), after 24 hrs 58.74% and after 48 hrs 61.23%. At 50ppm concentration the undamaged leaf area measured 24.51 ± 1.09 sq.cm after 24 hrs and after 48 hrs the protected leaf area was 18.36 ± 1.78 sq.cm. Antifeedants can prevent or suspend the feeding behavior of an insect when they are detected and insects will be able to detect antifeedants through contact chemoreceptors and sensory neurons. Sensory neurons can be phagostimulatory or deterrent cells and activity of these neurons to appropriate stimuli would enhance or reduce the feeding accordingly^{32, 33}.

Conclusion

It is concluded that Costunolide has a significant antifeedant activity against *Papilio demoleus*, and it could be used as a potential insecticide. It also concluded that Costunolide may be useful for effective control of pests at larval stages because of its larvicidal activities. Present study could be used for the development of new pesticidal formulations and future studies will be dedicated towards the characterization of the compound as well as biodegradation to examine its advantages over chemical pesticides.

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