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Antifeedant activity of forskolin, an extract of *Coleus forskohlii,* against *Papilio demoleus* L. (Lepidoptera: Papilionidae) larvae

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ABSTRACT

The anxiety to control pests has resulted in indiscriminate use of pesticides in the field causing environmental pollution and posing threat to human safety and public health. Amongst the alternatives, uses of bio pesticides have been found effective, eco friendly and could possibly provide a viable option. In the present study Forskolin was used to assess feeding intensity of Papilio demoleus larvae against citrus leaves for its antifeedant activity. Papilio demoleus is commonly known as citrus swallowtail. The caterpillars feed on leaves, prefer blossoms and young ones. Attack by larvae on young trees result in a great loss of foliage causing general weakness and sometimes death also. Forskolin is a plant extract, which was isolated from the Coleus forskohlii. A large leaf disc 36.5 sq.cm of lemon was used after spraying with different concentrations such as 50, 100, 150, 200ppm uniformly on either side by using an atomizer and placed in a petridish. Single Papilio demoleus fourth instar larva was introduced into each petridish and observations were noted at 24hrs and 48hrs duration. Mean average of the 10 sets were taken for the activity and the consumed area was measured graphically. The % of leaf area protection was calculated and control sets were maintained simultaneously. A significant antifeedant activity was observed against fourth instar larvae of Papilio demoleus at 200ppm.

Key words: Antifeedant activity, *Papilo demoleus*, Forskolin, Plant products, Pest control.

INTRODUCTION

It is estimated that almost 30% of planted crops are lost due to attack of feeding insects; wastage, or disease [1], Synthetic organic insecticides have played a major role in pest control. However, their increasing use has created a range of ecological problems such as bio-magnification, resurgence and the development of insecticide tolerant strains of pest species. It has been reported in India, that there has been a 164-300 fold increase in insect resistance to some insecticides because of indiscriminate use in cotton fields [2]. Due to many problems associated with the use of acute toxic synthetic chemicals as insecticides, a search for an alternative technique for the management of insect pests arises [3]. Therefore it is necessary to identify simple and environment friendly alternative methods to manage pests. Use of nontoxic antifeedant plant products which kill the pests indirectly is an alternative method [4]. Plant derivatives are highly toxic to many insect species without phytotoxic properties [5]. Plant materials comprise rich source of phytochemicals which are widely used in the place of synthetic insecticides [6]. Binder et al [7] and Kathirvelu et al [8] reported that a lot of physiologically active substances isolated from plants affect behavior, development and reproduction of insects. *Papilio demoleus L.* (Papilionidae: Lepidoptera) is a major pest of citrus

all over the world. According to the UN Conference on Trade and Development [9], globally 140 countries are involved in citrus production. Of the world's total citrus production, approximately 70 have been reported from the northern hemisphere, with Brazil being the top citrus producing country following by the USA. India ranks eight in world citrus production [10].

MATERIALS AND METHODS

Papilio demoleus, the citrus butterfly is an important cosmopolitan pest of citrus plantation [11]. The caterpillars feed on leaves and prefer blossoms and nurseries. Attack by larvae on young nurseries result in a great loss of foliage causing general weakness and sometimes death of the trees. The larvae of later stages are vigorous foliage feeders and in severe infestation young seedling may completely be defoliated [12]. A single larva consumes nine complete leaves in the course of its development. In the present work an attempt has been made to study the antifeedant activity of Forskolin, a diterpene obtained from the roots of a medicinal plant *Coleus forskohlii* against *Papilio demoleus* larvae.

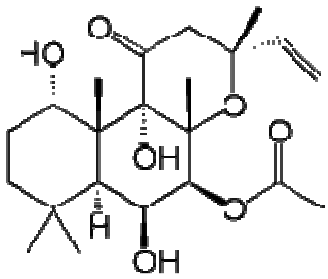
Insect Rearing

For experimental purpose the *Papilio demoleus* larvae were reared in the laboratory under controlled conditions of temperature 27° ± 2°C and 65 ± 5% relative humidity.

Forskolin: ($C_{22}H_{34}O_7$)

Forskolin is a labdane diterpene that is produced by roots of the plant *Coleus forskohlii*. It is commonly used in the study of cell physiology. Labdane-type diterpenes constitute a significant class of natural products. They occur in several plants such as conifers and genus cistus, and have been reported to exhibit a variety of biological activities such as anti algal, anti bacterial, anti fungal, anti protozoal, enzyme inducing, anti-inflammatory, modulation of immune cell functions, as well as cytotoxic effects against leukemic and human tumor cell line. Forskolin resensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing the intracellular levels of cyclic Adenosine Mono Phosphate (cyclic AMP). Cyclic AMP is an important signal carrier that is necessary for the proper biological response of cells to hormones and other extracellular signals. For conducting the experiments Forskolin was supplied by Department of Chemistry, Natural Products Lab, Osmania University, and Hyderabad.

Chemical structure of Forskolin



Antifeedant activity evaluation method

A large leaf disc 36.5 sq.cm of lemon was sprayed with test compound uniformly on either side by using an atomizer and placed on moist filter paper in a petridish. Single *Papilio demoleus* fourth instar larva was introduced into the petridish and parallel controls were maintained with similar leaf discs. Ten such petridishes were taken for each experiment. The setup was kept for observation at 24hrs and 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 of leaf area protection was calculated as suggested by earlier authors [13].

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

Preparation of test solution

Acetone was used as the solvent in preparing the test solutions, since the solubility of the test compounds was very high in acetone. 1% stock solution was prepared using acetone and 200, 150, 100, 50ppm concentrations were prepared from the stock solution using dilution method.

Statistical analysis

The results were expressed as Mean ± SD and data was statistically analyzed by one-way ANOVA, with the level of significance set at $p<0.05$. SPSS software was used.

RESULTS

Antifeedant property of the Forskolin was assessed by comparing the averages of the leaf area consumed in the treated leaves with that of control. Efficacy of Forskolin was assayed against the fourth instar larvae of *Papilio demoleus* for its antifeedant activity.

Table 1: Mean and SD values of undamaged leaf area (sq.cm) and % of antifeedant activity with different concentration treatments of Forskolin

Conc. in ppm	No of Insects	Mean ± SD After 24 hrs	Mean ± SD After 48 hrs	Antifeedatnt activity after 24hrs	Antifeedatnt activity after 48hrs
200	10	26.08 ± 0.78*	19.52 ± 0.79*	66.01	60.89
150	10	23.54 ± 0.88*	17.52 ± 0.94*	59.41	58.03
100	10	20.59 ± 0.75*	14.51± 0.92*	52.37	52.08
50	10	18.53 ± 1.21*	11.55 ± 1.01*	48.08	47.24
Control	10	17.92 ± 1.69*	11.00 ± 0.67*	---	---

Mean and SD values are significant at p< 0.05.

Antifeedant activity of Forskolin was assessed based on antifeedant index. Higher antifeedant index normally indicate decreased rate of feeding. In the present study different concentrations such as 50, 100, 150, 200ppm of Forskolin were used. The antifeedant activity was evaluated based on leaf area consumed by larvae of *Papilio demoleus*. Table 1 showed undamaged leaf area of citrus plant species tested, the mean and SD values found to be significant against the fourth instar larvae of *Papilio demoleus*. Maximum antifeedant activity was recorded at 200ppm concentration and least antifeedant activity was observed at 50ppm of forskolin treatment, they are 66.01% and 48.08% respectively for 24hrs exposure. Undamaged leaf area was also observed after 48hrs treatment with forskolin with different concentrations. At 200ppm treatment the undamaged leaf area after 24 and 48hrs were 26.08 ± 0.78 and 19.52 ± 0.79 sq.cm and undamaged leaf area after 24 and 48hrs with 50ppm treatment was observed as 17.92 ± 1.69 and 11.00 ± 0.67 sq.cm respectively.

DISCUSSION

The plant extract forskolin of *Coleus forskohlii* was found to have significant antifeedant effect on the fourth instar larvae of *Papilio demoleus*. The feeding behavior of larvae was directly observed. The experiments showed that the test larvae frequently sampled the treated food, suggesting that the reduced feeding on treated food was a consequence either of rejection without substantial ingestion or of rejection following short periods of feeding. Antifeedant substances are classified into repellents, suppressants or deterrents basically. The present plant extract of forskolin had both suppressant and deterrent properties.

In the present investigation, forskolin showed 66.01% and 60.89% antifeedant activity at 200ppm concentration against *Papilio demoleus*, at 24hrs and 48hrs respectively. Earlier, Jeyasankar [14] reported that ethyl acetate extract of *Syzygium lineare* showed antifeedant activity of 79.4% against *S. litura* at 5% concentration. Ethyl acetate extract of *Couroupita guianensis* showed promising antifeedant activity of 66.68% and 69.70% against *H. armigera* at 2.5% and 5% concentrations respectively Baskar [15]. Bagavan [16] studied the larvicidal activity of hexane, chloroform and ethyl acetate extracts of *P. daemia* against tick, fluke and mosquitoes. They reported that ethyl acetate extract showed 100% mortality against *Anopheles subpictus* and *Culex tritaeniorhynchus* at 1000ppm concentration and 76% and 71% mortality against *Haemaphysalis bispinosa* and *Paramphistomum cervi*, respectively, at 2500ppm concentrations. Earlier, Muthu [17] reported that ethyl extract of *Atalantia monophylla* exhibited 78.67% antifeedant activity against *Earias vittella*.

The food consumption of fourth instar larvae of *Papilio demoleus* treatment was reduced by the extract of forskolin. Antifeedant activity was recorded using different concentrations such as 200, 150, 100, 50ppm of forskolin. Antifeedants can be found amongst all the major classes of secondary metabolites *viz*., alkaloids, phenolics and terpenoids which are the most probable toxic substances against insects [18]. The active principles present in the plants inhibit larval feeding behavior or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence. Previously several investigators have already reported that botanicals offer antifeedant activity against *Spodoptera litura* [19, 20, 21]. Mikolajczak and Reed [22] stated that the seed extracts of *Trichilia prieureana*, *Trichilia roka* and *Trichilia connaraides* exhibited high levels of antifeedant activity in leaf disc method against *Spodoptera frugiperda*. Many studies proved sesamin, a major lignan of *Piper mullesua* of Manipur origin, exhibited significant antifeedant activity and moderate growth inhibition towards 4th instar larvae of *Spilarctia oblique* [23].

CONCLUSION

Based on the present results, we are suggesting that forskolin can be used as an antifeedant substance against larvae of *Papilio demoleus* at 200ppm concentration effectively. Further it is concluded that instead of using synthetic insecticides for pest control on citrus, forskolin can be recommended. The present study using of forskolin clearly suggest the usage of plant extracts for effective control of pests at larval stages. Further investigation is needed to understand mechanism is involved at molecular level to find out the exact mechanism both in plant extract and larvae also.

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