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BIO EFFICACY OF β-ASARONE ON FEEDING DETERRENCE OF *Papilio demoleus* L. FOURTH INSTAR LARVAE (Lepidoptera: Papilionidae)

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Abstract: Antifeedant activity of the β-asarone was evaluated through non-choice method since its design closely approaches a practical application. Fresh lemon leaf discs of 36.5 sq.cm were dipped in the corresponding test solutions for 1 min initially. Control leaf discs were immersed in the acetone kept for dry at room temperature. Leaf discs and the fourth instar larvae were introduced into each petridish. The larvae were deprived of food for 4 hrs before being placed individually in petridishes. Progressive consumption of leaf area by the treated and control larvae was recorded after 24 and 48hrs by laying the leaf on a graph paper and counting the number of 1 mm squares consumed. The feeding deterrence of β-asarone was evaluated at 200, 150, 100, 50 ppm concentrations for 24hrs and 48 hrs exposure, all the concentrations showed significant antifeedant activity in a dose dependent manner. Significant antifeedant activity was found at 200 ppm for 24hrs and 48hrs exposure and it was 75.85% and 72.48% respectively. β-asarone has the potential to serve as an alternate biopesticide in the pest management.

Keywords: Antifeedant activity, *Papilio demoleus*, *Acorus calamus* L, β-asarone.

INTRODUCTION

Agriculture is an important sector of the Indian economy, accounting for 14% of the nation's GDP, and more than 80% of the population depends on agriculture both directly and indirectly (Ashokraj *et al.*, 2013). Pathogenic organisms and insect pests cause crop loss of 120 billion US dollars worldwide and reduce the yield by 20–40% (Zhou, 2001). In India, nearly 18% of food grains are lost due to pathogens. In the servey of Asochai and S Bank (2014), fifty thousand crores of crops lost in cultivation of various crops due to disease and pests. To control the pests and reduce the loss different chemical pesticides are used. Many workers reported that the indiscriminate use of chemical pesticides over a long period has not only been proved to be harmful to micro organisms but also contributed to a number of side effects such as pest resistance, pests resurgence, outbreak of new pests, toxicity to non-target organism and presence of pesticide residues in seeds, vegetables, fruits, (Kannaiyan, 2002).

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Due to higher dose and repeated frequency of application, every year one million people suffer from pesticide poisoning (Bami, 1997).

In this context harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives. Therefore researchers world over are engaged in a mission to hunt for novel phytochemicals that could potentially be used in the management of insect pests. Plant derived pesticides are eco-friendly, non-toxic to non target organisms, non persistent in nature, besides they do not promote drug resistance (Ashokaraj *et al*., 2013; Liu *et a*l., 2000).

According to Jayaraj (2005) more than 650 species of insects and mites have developed resistance to insecticides. Antifeedants and insecticidal toxicants can play a significant role as part of integrated pest management (Isman, 2002). Usually antifeedant, insecticidal and growth inhibitory activities are ascertained by screening and analyzing the bioefficacy of plant extracts or compounds. Plant extracts interfere with the normal embryonic development of the eggs by suppressing hormonal and biochemical process (Fagoonee and Lauge, 1981). Plant products also control the pests at various stages, which kill or extend the life stages (larvae, pupae, eggs) of *Spodoptera litura*, *Helicoverpa armigera* and protect the crops (Raja *et al*., 2005; Ignacimuthu *et al*., 2006; Baskar *et al*., 2012).

Citrus industry is the third largest in the world after mango and banana. In India citrus crop occupies a prominent place covering an area of about 8.5 L ha with an annual production of 74.64 L tonnes with a productivity of 8.8 t/ha (Gopali *et al*., 2011). The state of Andhra Pradesh is one of the major citrus producing states in India and ranks first in sweet orange (*C. sinenesis*) and acid lime (*C. aurentifolia*) area and production. Citrus crop is being infested by around 165 species of economically important insect pests in India causing up to 30 per cent yield loss. About 55 insect and mite species were reported from Rayalaseema region of Andrapradesh (Ramasubbareddy *et al*., 1989) and a dozen of them attack this crop regularly right from nursery stage to the harvest with cognizable damage. Four species of citrus butterflies viz., *P. demoleus L., P. polytes L., P. polymnestor and P. helenus* were recorded to be damaging citrus in south India (Shivankar, 1999).

In the present study *Papilio demoleus* was used as test insect to assess the bio efficacy of β-asarone, which is one of the serious pests on Citrus. It attacks all species and varieties of citrus, including lime, orange and lemon. Its larvae are voracious feeders, with older instars being the most damaging. The larvae prefer young foliage, and heavy infestation can lead to complete defoliation of orchards and nurseries (Lewis, 2009).

MATERIALS AND METHODS

Test Insect:

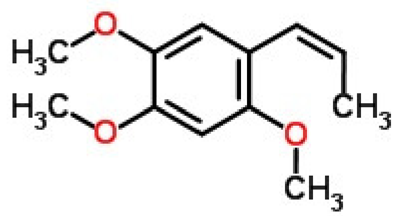
The lemon butterfly, *Papilio demoleus* is one of the economically important pests whose larval forms cause serious damage to citrus family by devouring large quantity of foliage during the later stages of their development (Singh, 1993). The caterpillars are voracious feeders of young seedlings and cause death of the seedling within no time (Resham *et al.,* 1986). Its principal host plants are the members of the genus Citrus (Guerrero *et al.,* 2004).

Collection of larvae and maintenance:

Eggs and early larval instars of *Papilio demoleus* were collected from Citrus fields. The eggs were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching, the neonate larvae were reared on leaves of Citrus. The room temperature (25± 2C) with 15–11 hour light dark photoperiod and 75±5% relative humidity were maintained and the laboratory reared larvae were used for bioassay

Plant material:

The sweet flag *Acorus calamus* L (vernacular: Bach) is a perennial herb belonging to the family Araceae. The main constituents of *Acorus calamus* L, is β-asarone and it has been successfully employed for the preparation of perfume, flavors and medicine. Traditionally it was used to treat the diseases such as dyspepsia, flatulence, cough, fever piles and asthma (Asha Devi *et al.,* 2005). The leaves and rhizome of *Acorus calamus* L is reported to posses antimicrobial and antifungal activity (Raja *et al.,* 2009). Other virtues of this plant include its mature leaves, which act as an insect repellent when cut up and stored in dry foods (Gerhard *et al.,* 1994) and antigonadal activity in insects (Saxena *et al.,* 1977).



β-asarone ($C_{12} H_{16} O_3$)

Preparation of test extracts

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 freshly from the stock by dilution method.

Antifeedant Activity:

Antifeedant activity of the β-Asarone was studied using leaf disc non choice method. Fresh citrus leaf discs of 36.5sq.cm diameter were punched using cork borer and it were dipped in 50, 100, 150, and 200ppm concentrations of β-asarone individually. The leaf discs dipped in acetone were used as negative control. In each petridish wet filter paper was placed to avoid early drying of the leaf discs and single fourth instar larva was introduced into each petridish. Progressive consumption of leaf by the treated and control larva in 24hrs and 48hrs was recorded using Leaf Area Meter. Leaf area eaten by larva in treatment was corrected from the negative control. Five replicates were maintained for each treatment with 10 larvae per replicate (n = 50). The antifeedant activity was calculated using the following formula of Isman *et al.,* (1990).

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control } - \text{Leaf area consumed in treatment}}{\text{Leaf area consumed in control } + \text{ Leaf area consumed in treatment}} * 100$$

Statistic Analysis

The results were subjected to one way analysis of variance (ANOVA). The results were expressed as Mean ± SD. The level of significance set at $p<0.05$. SPSS software was used for calculation.

RESULTS AND DISCUSSION

Table 1. Mean and SD of undamaged leaf area (sq.cm) and antifeedant activity with different concentration treatments of β-asarone

Conc. in ppm	No of Insects	Mean ± SD After 24 hrs	Mean ± SD After 48 hrs	Antifeedant activity % after 24hrs	Antifeedant activity % after 48hrs
200	**10**	29.80 ± 1.38*	25.32 ± 0.99*	75.85	72.48
150	**10**	27.07 ± 0.97*	22.31 ± 1.01*	67.60	60.32
100	**10**	24.80 ± 0.85*	17.42 ± 0.86*	61.31	57.20
50	**10**	21.34 ± 1.26*	14.67 ± 1*	52.58	42.49
Control	**10**	19.67 ± 0.87*	10.73 ± 0.90*	**------**	**------**

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

Quantification of antifeedant effect of botanicals is of great importance in the field of insect pest management. Antifeedant is a chemical that inhibits the feeding without killing the insect pests directly, while it remains near the treated foliage and dies through starvation (Pavunraj *et al.,* 2012) From an ecological point of view, antifeedants are very important since they never kill the target insects directly and allow them to be available to their natural enemies and help in the maintenance of natural balance. Higher antifeedant index normally indicate decreased rate of feeding.

As presented in Table 1, the results revealed that irrespective of the test concentration β-asarone showed more than 50% feeding deterrent activity. The antifeedant nature of the β-asarone is produced a significant effect on *Papilio demoleus* at 200ppm concentration. The antifeedant activity of 200ppm for 24hrs and 48hrs exposure was 75.85% and 72.48%. Protected leaf area measured and it was 29.80 ± 1.38 at first 24 hours and after 48 hrs 25.32 ± 0.99. The concept of using insect antifeedants the modes of action are directed at the taste cells. A typical gustatory sensillum in an insect contains receptors for deterrents and others for stimulants. Although most antifeedants likely act by stimulating a deterrent receptor, that in turn sends a signal ("do not feed") to the feeding center in the insect's central nervous system, some antifeedants are thought to block interfere with the perception of feeding stimulants, whilst others may cause erratic bursts of electrical impulses in the nervous system preventing the insect from acquiring appropriate taste information on which it may choose an appropriate feeding behavior.

Several authors have reported that plant extracts possess similar type of antifeedant activity against lepidopteran pests (Morimoto *et al.,* 2002; Jeyasankar *et al.*, 2012). These results are similar with the findings of Susurluk *et al.,* (2007) who reported that the phagodeterrency of fraction of the methanol extract of *Tanacetum cadmeum* was higher than the crude extract on *Spodoptera littoralis*. Many authors reported that antifeedant activity against *H. armigera* (Raja *et al*., 2005), *Spodoptera litura* (Ignacimuthu *et al.,* 2006) and *H. zea* (Georges *et al.,* 2008) using different plant extracts.

The antifeedant activity of β-asarone at 150ppm was 67.60% for first 24hrs and 60.32% for 48hrs duration, similarly protected leaf area was also measured at 150ppm and it was 27.07 ± 0.97 and 22.31 ± 1.01 for 24 hrs and 48 hrs treatment respectively. This is in agreement with the findings of Srinivasa Rao *et al.,* (2014) where Forskolin has a significant antifeedant effect on the fourth instar larvae of *Papilio demoleus* at 200ppm where it showed 66.01 %

and 60.89% effect after 24hrs and 48hrs exposure respectively. Baskar *et al*,, (2008) observed that the hexane extract of *A. monophylla* had higher antifeedant activity on *S. litura*. The present results also corroborate with the findings of Tewary *et al.,* (2005) who reported that low polar solvent extract had higher activity than the high polar solvent extracts and Dolui *et al*., (2010) reported that *Heliotropium indicum* and *Spilanthes calva* have more antifeedant effects on the insects of *Helopeltis theivora*.

Food consumption was higher in control and lower in β-asarone treated cirtus leaves fed by *Papilio demoleus* larvae. The growth rate was also higher in control larvae. When *Papilio demoleus* grew older, irrespective of the life stages, food consumption index was higher in control followed by β-asarone. The approximate digestability was also higher in third instar followed by fourth and fifth instar. This parameter is an indirect indication of the amount of food converted into body biomass (Ananthakrishnan, 2002). According to this hypothesis our study revealed that body weight of *Papilio demoleus* larvae significantly reduced. β-asarone highly reduces the food consumption index, approximate digestibility, growth rate, and efficiency of conversion of ingested food. Hence β-asarone root extract can be explored in *Papilio demoleus* management.

The test compound β-asarone exhibited a moderate antifeedant activity at low concentration. At 50ppm the undamaged leaf area was 21.34 ± 1 and 14.67 ± 1 after 24hrs and 48hrs respectively. Antifeedant activity in first 24hrs recorded was 52.58% and 42.49% after 48hrs respectively. Similar results was observed by Srinivasa Rao *et al.,* (2014) using solasodine a pure compound isolated from *Solanum aviculare* against *Papilio demoleus.* At 50ppm concentration antifeedant activity was 55.77% and 53.27% for 24hrs and 48 hrs respectively. Ortego *et al*., (1995) reported that teucvin a compound isolated from *Teucrium chamaedrys* L at 100 ppm caused a significant reduction in feeding in *Leptinotarsa decemlineata* larvae.

CONCLUSIONS

In the present studies it was observed that β-asarone can be a good source to control *Papilio demoleus* and showed potent antifeedant activity against fourth instar larvae. Such studies are important for exploration of new biomolecules to be used by pharmaceutical and agrochemical industry to synthesize more potent molecules.

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