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***Research Article***

# EFFECT OF BETULINIC ACID ON FEEDING DETERRENCE OF *PAPILIO DEMOLEUS* L. (LEPIDOPTERA: PAPILIONIDAE) LARVAE

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## ABSTRACT
Betulinic acid, a secondary metabolite isolated from *Ziziphus jujuba* was evaluated for its effect on the feeding deterrence in relation to food utilization at concentrations of 50, 100, 150, 200ppm against the fourth instar larvae of *Papilio demoleus* following a non choice leaf disk method. Significant reduction in food consumption and digestion was observed which reduced the growth of larvae. The efficiency of the larvae to convert digested and ingested food into body tissues was observed. The antifeedant activity of Betulinic acid proved to be the most potent against all developmental stages of *Papilio demoleus* with antifeedant activity percentage between 90-95 at 200ppm in first 24hrs and between 80-85% after 48hrs exposure. Significant antifeedant activity found in 200ppm concentration was 94.04%. This plant extract has the potential to serve as an alternate biopesticide in the management of *Papilio demoleus* larvae.

***Keywords:*** *Antifeedant Activity, Papilio Demoleus Larvae, Betulinic Acid, Plant Products*

## INTRODUCTION
Current world population is expected to reach 10.5 billion by 2050 (UN March, 2011), further adding to global food security concerns. According to Alexandratos and Bruinsma (2012), food supplies would need to increase by 60% in order to meet the food demand in 2050. Food availability and accessibility can be increased by increasing production, improving distribution, and reducing the losses. Food and Agriculture Organization of U.N. predicts that about 1.3 billion tons of food is globally wasted per year (Gustavasson *et al*., 2011). Reduction in these losses would increase the amount of food available for human consumption and enhance global food security, In addition, crop production contributes significant proportion of typical incomes in certain regions of the world and reducing food loss can directly increase the real incomes of the producers (World Bank, 2011).
The production in agriculture is reduced by losses as high as 45% before or after harvesting due to attack of a variety of pests, including insects, nematodes, virus and bacteria induced diseases and competition by weeds. An estimated one third of global agricultural production valued at several billion dollars is destroyed annually by over 20,000 species of insect pests in field and storage (Mariapackiam and Ignacimuthu, 2008). Insect pests play a major role in damaging the crops and hence there is a need to use efficacious control agents. Chemical pesticides play a significant role in increasing agricultural production by controlling the insect pests. However, synthetic insecticides possess natural toxicities that affects on elimination of existing natural enemies and pollution of soil, water, air, food, health of farmers, consumers and the environment. Fox *et al.,* (2007) observed that most of the chemical insecticides containing pentachlorophenol (PCP) caused the strongest inhibition for symbiotic nitrogen fixation resulting in the lowest plant yields and also seed germination. The injudicious use of synthetic pesticides can lead to secondary outbreaks of pests that are normally under natural control, resulting in their rapid proliferation. There have also been cases of pests becoming tolerant to insecticides, resulting in the use of double and triple application rates. The threats posed by chemical pesticides demand an urgent search for an environmentally safer alternative method of crop protection.
Several scientists are working to protect the crops from insect infestation by indigenous plant materials. Hence search for viable and sustainable alternatives to synthetic pesticides is given priority. More than 2,000 species of plants are known to possess insecticidal properties (Klocke, 1989). Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sadek, 2003). Numerous plant species have been identified as possessing pesticidal

properties and have shown potential as alternative to chemical pesticides(Kaushik and Kathuria, 2004). Plants are endowed with a potential to produce a range of secondary metabolites like alkaloids, terpenoids, flavonoids, phenols, glycosides, sitosterols and tannins. These phytochemicals are known to protect the plants from the attack of insect-pests (Ahmad, 2007**.** The action of the plant derived compounds on pest insects is exerted through many ways such as antifeedant (Raja *et al*., 2005), larvicidal (Kabaru and Gichia, 2001), ovicidal and oviposition deterrent (Pavunraj *et al.,* 2006), repellent (Schmutterer, 1995) and others. Some botanicals have an effect on juvenile hormone and ecdysone actions; they also have substances that disrupt insect growth by antagonizing juvenile hormone action (Williams *et al.,* 1986).

Citrus is an important tropical fruit, it has been traditionally cultivated in home gardens modern plantations (Saljoqi *et al.,* 2006) and is used in food industries, pharmaceuticals, perfumes, cosmetics and aromatherapy. A number of insect pests attack citrus plants both in the nurseries as well as in the orchards inflicting heavy economic losses. Some of the most serious pests of citrus includes citrus caterpillars (*P. demoleus* and *P. polytes*), citrus psylla (Saljoqi *et al.,* 2006). In the present study *Papilio demoleus* L. was selected as test species to evaluate the antifeedant activity by using the plant product Betulinic acid. The lemon butterfly is one of the economically important pests whose larval forms cause serious damage to foliage of Rutaceae during the later stages of their development. The genus *Papilio* is widely distributed all over the world, is the most prevalent species and was found in greater parts of Asia, Farmosa and Japan etc.

## MATERIAL AND METHODS

### *Test Insect*

*Papilio demoleus* L., commonly known as the lime or citrus swallowtail. It is a beautiful black yellow butterfly and has a successful dispersal and becoming a major pest of citrus plantations throughout Asia (Eastwood *et al.,* 2006) causing significant economic losses. The insect has been reported to feed on every citrus cultivars and varieties thus it has the potential to become a major pest in area where it has been reported.

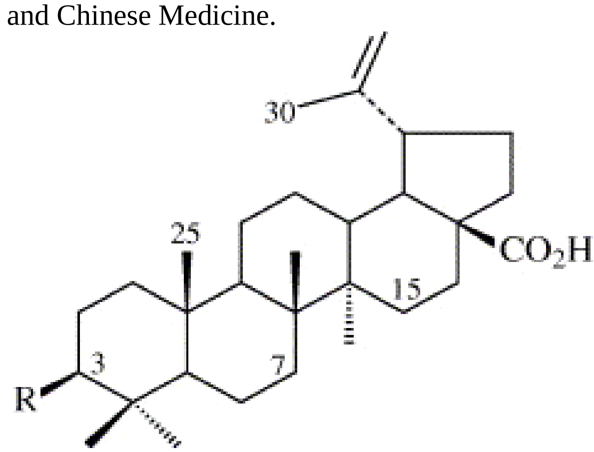
### *Collection of Larvae and Maintenance*

Eggs and the larvae of *Papilio demoleus* were initially collected from the Garden of Citrus species, Larvae were reared in the Petri plates and kept in a wooden box (20” x 20” x 20”) having wire-netted sides and top. Temperature was maintained at 27°c ± 2°c and relative humidity was 70% ± 5%. The larvae were fed on fresh leaves of citrus. The completely grown larvae, about to pupate were sorted out and placed in a separate glass dish at room temperature.

### *Plant Material*

### *Betulinic Acid ($C_{30}H_{48}O_3$)*

Betulinic acid was an important compound isolated from bark of *Ziziphus Jujuba*. It’s medicinal benefits are mentioned in Ayurveda and Chinese Medicine.




**Chemical Formula of Betulinic acid: ($C_{30}H_{48}O_3$)**

Betulinic acid, a triterpenoid found in many plant species, has attracted attention due to its important pharmacological properties, such as anti-cancer (Kumar *et al*., 2010) and anti-HIV activities (Theo *et al*., 2009), anthelmintic activity (Enwerem *et al*., 2001), antifeedant activity(Chandramu *et al*., 2003). It also exhibits antibacterial (Woldemichael *et al*., 2003), anti- inflammatory (Recio *et al*., 1995) and anti-malarial (Bringmann *et al*., 1997) properties.
For conducting the present experiments Betulinic acid was supplied from Department of Chemistry, Natural Chemistry Lab, Osmania University, Hyderabad.

***Preparation of Test Concentration***

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 200ppm, 150ppm, 100ppm; 50ppm concentrations were prepared from the stock by dilution method

***Antifeedant Activity***

Antifeedant activity of the Betulinic acid was studied using leaf disc no-choice method. Different concentrations of plant product were prepared by dissolving in acetone and tested against *Papilio demoleus* larvae. Fresh citrus leaf discs of 36.5sq.cm diameter were punched using cork borer and dipped in 50ppm, 100ppm, 150ppm, and 200ppm concentrations of Betulinic acid individually of isolated compound. Betulinic acid was used as positive control, leaf discs treated with acetone and without solvent were considered as negative control. After air-drying, each leaf disc was placed in petridish containing wet filter paper to avoid early drying of the leaf disc and single 2 hrs pre-starved fourth instar larva of *Papilio demoleus* was introduced into petridishes containing the respective leaf discs. For each concentration 10 replicates were maintained. Progressive consumption of leaf area by the larva after 24 hrs and 48hrs of feeding was recorded in control and treated discs using Leaf area meter. Leaf area consumed in plant extract treatment was corrected from the control. The percent antifeedant index was calculated using the formula of Ben *et al*., (2000).

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

***Statistical Analysis***

Antifeedant activity was analyzed using one way ANOVA. Significant differences between treated and control groups were determined. The results are expressed as Mean ± SD and data was statistically analyzed with the level of significance set at $p<0.05$ using SPSS software.

## RESULTS AND DISCUSSION

**Table 1: Mean and SD of undamaged leaf area (sq.cm) and antifeedant activity with different concentration treatments of Betulinic acid**

| **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 | 35.09 ± 0.66* | 31.54 ± 0.87* | 94.04 | 86.78 |
| 150 | 10 | 32.68 ± 0.93* | 28.45 ± 0.65* | 84.63 | 79.40 |
| 100 | 10 | 28.84 ± 0.80* | 25.56 ± 0.86* | 71.40 | 73 |
| 50 | 10 | 25.32 ±1.35* | 19.99 ± 1.41* | 60.83 | 56.88 |
| Control | 10 | 19.74 ± 0.89* | 11.16 ± 0.50* | ------ | ------- |

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Antifeedants offer first line of crop protection against notorious insects. Any substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent. In general, antifeedants have profound adverse effects on insect feeding behavior and do not kill the insect pests directly, but rather limit its developmental potential considerably and act as a phagodeterrent or phagorepellent. However, the most effective insect feeding inhibitors come from terpenoids, alkaloids, saponins and polyphenols (Koul, 2005). The present study findings were presented in the Table 1. Among all the tested concentrations, the 200 ppm exhibited significant antifeedant activity. In the first 24hrs the antifeedant activity recorded was 94.04% and after 48hrs 86.78%. In 200ppm exposure the average protected leaf area was 35.09 ± 0.66 sq.cm in first 24hrs and after 48hrs it was 31.54 ± 0.87sq.cm. The result showed that when the concentration of Betulinic acid increased the food consumption was decreased. The antifeedent activity of the plant extract might be due to the presence triterpenoid and alkaloids. The similar results were also observed by Sahayaraj *et al.*, (2003).

Most potent insect's antifeedants are sesquiterpene lactones, diterpinoids, triterpinoids, quinoline and indole alkaloids, they enhance the feeding deterrent activity against pests (Baskar *et al.*, 2009). In the present study Betulinic acid exhibited 84.63% and 79.40% antifeedant activity after 24hrs and 48hrs respectively against the fourth instar larvae of *Papilio demoleus* at 150ppm concentration. Undamaged leaf area was measured at 150ppm and it was 32.68 ± 0.93 sq.cm and 28.45 ± 0.65 sq.cm after 24hrs and 48hrs respectively. The present investigation showed that Betulinic acid was found to be an effective antifeedant agent against *Papilio demoleus* larvae. It may have made food unpalatable or the substances directly acted on the chemosensilla of the larvae, resulting feeding deterrence due to synergistic effect. Feeding deterrent activity against *S. litura* was reported by Lingathurai *et al.*, (2011).

Betulinic acid as a class of pre ingestive compounds affecting gustatory receptors and evoke rejection of plant material. At 100ppm concentration the test compound showed moderate antifeedant activity in the first 24hrs where it was 71.40% and after 48hrs 73% respectively. The protected leaf area also measured after 24hrs and 48 hrs was 28.84 ± 0.80 sq.cm and 25.56 ± 0.86 sq.cm respectively. Messchendorp (1998) described antifeedants as compounds that inhibit feeding by sensory perception i.e. giving plant material an unpalatable taste, and may also reduce feeding by toxic, post ingestive effects. The reduced feeding intensity of *L. dispar* larvae on leaves treated with the extracts of *Hippocastanum* reported by Gvozdenac *et al.*, (2011). Betulinic acid is an active ingredient isolated from bark of *Ziziphus Jujuba*. Previously it has been showed to produce an antifeedant effect on *Spodoptera litura (F)* by Samba *et al.*, (1998). In the present study even in a low concentration of 50ppm, the antifeedant activity was 60.83 % and 56.88% after 24hrs and 48hrs respectively and protected leaf area also measured, in the first 24hrs it was 25.32 ± 1.35 sq.cm and after 48hrs 19.99 ± 1.41 sq.cm. At a low concentration of Betulinic acid stimulation of a specialized deterrent receptor disrupted the normal function of chemoreceptors responsible for perceiving glucosinolates, a strong phagostimulant for this lepidopteran pests. These findings concur with past studies in which *T. vogelii* leaves and seeds have been reported as a source of rotenoids, including rotenone, tephrosin, and deguelin, known to possess strong feeding deterrent activity (Ogendo, 2008).

***Concusion***

This present study reveals that Betulinic acid can potentially be used as eco friendly biopesticide to control the damage caused by larva of *Papilio demoleus.* The Betulinic acid also replaces the synthetic pesticides since it is safer to the environment and biodegradable in nature. It can also be used for the development of new insecticidal formulation for the management of field pests.

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