*International Research Journal of Environmental Sciences*_____ ISSN 2319–1414
Vol. **6(4),** 1-5, April **(2017)** *Int. Res. J. Environmental Sci.*

# An assessment of antifeedant potential of azadirachtin, a plant product of *Azadirachta indica,* against *papilio demoleus* L. (lepidoptera: papilionidae) larvae

**Srinivasa Rao Vattikonda*** **and Sabita Raja Sangam**
Department of Zoology, Nizam College, Osmania University, Hyderabad, Telengana State, India
vattikonda18@gmail.com

**Available online at: www.isca.in, www.isca.me**
Received 25th August 2016, revised 21st February 2017, accepted 18th March 2017

## Abstract

*The lemon butterfly Papilio demoleus is the most serious pests whose larval forms causes the serious damage by cultivated species of citrus during their various stages of development. Presently scientists focus their research and efforts to develop eco-friendly pest control methods, one of the most accepted and suitable method is natural antifeedants. So controlling this larva plant products are introduced in this study the Azadirachtin a secondary metabolite isolated from Azadirachta indica was evaluated their antifeedant activity against fourth instar larvae of Papilio demoleus using no-choice leaf bioassay method, feeding activity is related to food utilization at different concentrations of 200, 150, 100 and 50ppm the antifeedant index calculated over 24 hrs. and 48 hrs. Results showed that the Azadirachtin treated larvae showed maximum antifeedant activity of 86.28% and 70.43% at 200ppm concentration. The antifeedant activity were increased with increasing concentration. Azadirachtin could also be considered for use in the management of pests.*

**Keywords:** Antifeedant activity, Plant Products, *Papilio demoleus*, Azadirachtin, *Azadirachta indica.*

## Introduction

Citrus varieties are widely used and commercially important fruit crops in the world and it is prominently cultivated in the subtropical and tropical regions. It is found in much of South Asia to Australia, New Guinea, and the Philippines. Recently it has been introduced into the Caribbean[1].

Citrus industry is one of the largest fruit industries in the world, occupying 3rd largest and 6% of the total area of different fruits cultivation. Sweet orange cultivated in an area of 204.1 lakh hectares with an annual production of 30.61 lakh tonnes of fruits and productivity of 15 MT/Ha in Andhra Pradesh and Telengana states. In India Citrus crops are attacked by nearly 250 or more insect species at various stages of growth from budlings and seedlings in fields and nurseries, in these insect species nearly 165 species are causing severe damage to citrus crops an estimated loss of 30 per cent in yields[2].

Among that different insect pest attacked on citrus, the citrus butterfly, *Pailio demoleus* L is one of common pest in nurseries, citrus gardens and fully grown up various citrus plants, they feed eagerly voraciously and made sever damage to nurseries and young seedlings leaving behind midribs only. Severe damage results in defoliation of the tree[3] and leads to hamper the plant growth and sever loss in fruit yield[4]. Finding the external features and internal biology of *Pailio demoleus* larvae will be useful to control effective management method, against citrus butterfly[5].

Presently synthetic pesticides play a key role in controlling the various insects and these are usually giving a strong protection against insect pests. All over the world, it was approximately 1.8 billion people depends in agriculture sector so maximum people utilize nearly 5.6 billion pounds of chemical pesticides are protected the various crops food grains, vegetables, fruits and profitable crops they yield[6]. Chemical pesticides and their products are highly preserved in water, land and agricultural products so finally influence on climate, animals and human strength being during arrangement, utilization and the application on crops. These signs of chemical pesticides to know the importance in the use the plant products for agriculture security in the agricultural fields and during storage[7].

Plant products are using ancient day onwards so it was well known as an attractive alternative choice to chemical pesticides for insect pest management, they effect either low or no harm to the ecosystems, environment, and to human health. Last few decades on wards people was used plant products for the control the various diseases in pest management, cure of diseases, as pesticides to supervise microbial growth, weeds and many more new approaches was introduced[8]. Therefore, various plants and their products were used for medicinal purposes in human health point of view and explain different potential insect control agents in agriculture. Generally, plant products is a combination of mixture of bioactive compounds these are varied benefits in related to short life span, efficacy and there was no resistance is developed in pests and pathogens[9]. The bio-pesticides are naturally species-specific, safe to non-target

animals in this human being and natural enemies of insect pests, and these are degrade rapidly in sunlight, environmentally safe and eco-friendly, no pollution to air and moisture, so they are less persistence in the environment, and giving a speed action on the insects, there was no effect on plant natural growth, seed growth and giving quality of the grains and are low cost and easily accessible in the farmers natural environment[10] . Now it is a time to look necessary for another pest management technique. So finally, the use of plant products has been recommended for a one of the best and as a suitable alternative of plant protection with low negative risks[11].

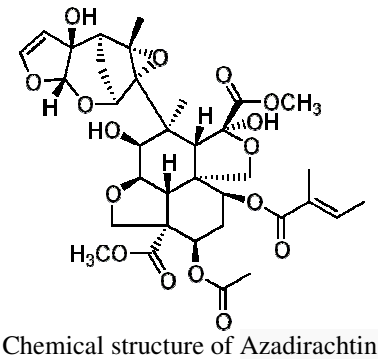
In the present investigation *Papilio demoleus* L. was selected as a test species to evaluate the antifeedant activity by using the plant product azadirachtin from *Azadirachta indica.*

## Materials and methods

**Test insect:** The lemon butterfly, *Papilio demoleus* is a key insect pest on citrus, usually five larval instars in their life cycle so the larval forms causes huge affect to citrus family, these larval stages in their development they feeds voraciously on vegetative growth of citrus plants throughout the year. It is most destructive to citrus seedlings as well as new flushes[12]. The *Papilio demoleus*, is also called as the citrus Swallowtail and it belongs to the family Papilionidae.

**Collection of larvae and maintenance:** Larvae of different stages *Papilio demoleus* were collected from Citrus fields in Nalgonda (District) of Telangana State, India were reared in the laboratory at 27 ± 2°C temperature and 70% ± 5% R.H., in the glass petri plates, kept in a wooden box (20cmx20cmx20cm), with wire-netted sides and top. The larvae of the species were daily fed on fresh leaves of lemon. The fully grown fourth instar larvae were separated and kept in a separate petri dish at room temperature for the experiment.

**Plant material:** *Azadirachta indica* is a plant of Meliaceae family, in this plant many of the active compounds are identified in that azadirachtin is more active compound, which is identified in the leaves and seeds and various parts of plant. It is a bitter and complex chemical in nature which comes under the limonoid group, molecular formula is $C_{35}H_{44}O_{16}$ and it was proved strong biological activities on various insects[13]. Azadirachtin showed its power on nearly about 200 species of various insect pests by assisting as a feeding deterrents and insect growth regulators. Azadirachtin has toxicity and huge effects on insects[14] but it was a low toxicity to mammals and also showed it was a non mutagenic[15]. Azadirachtin was degraded rapidly in different conditions such as ecological factors as UV radiation in sunlight, air moisture, heat, acidity and presence of enzymes. Therefore, it was a good alternative, the advantage of azadirachtin as ecologically safe, plant products with selective toxicity to targeted pests, low toxic to plants and mammals and eco-friendly in nature.



Chemical structure of Azadirachtin

**Preparation of Test Compound:** Preparation of the test solutions Acetone was used as a solvent; the test compounds solubility was very high in acetone. 1% stock solution was prepared using acetone and 200 ppm, 150 ppm, 100 ppm and 50ppm concentrations of azadirachtin were prepared from the stock by dilution method.

**Experimental Procedure: Antifeedant activity evaluation method:** Antifeedant activity of the Azadirachtin a pure compound isolated from *Azadirachta indica* was assayed against fourth instar larvae of *Papilio demoleus* using a leaf disc in no-choice bioassay method. The experiment was done in Petri dishes. A moist paper towel was kept at the bottom of each container in order to maintain relative humidity and to keep the lemon leaves fresh. The observations were recorded after 24hrs and 48hrs after treatment. Fresh, tender leaf discs (36.5 sq.cm) of lemon were used as food. Concentrations of the pure compounds such as 50ppm, 100ppm, 150ppm and 200ppm were prepared.

The leaf discs treated with acetone were used as a negative control, and leaf discs treated with azadirachtin were used as a positive control. Single *Papilio demoleus* fourth instar larva was introduced into the Petri dish. Parallel controls of *Papilio demoleus* maintained with similar leaf discs. Ten such Petri dishes were taken for each experiment. The experiment was finished in the laboratory conditions of 28 ± 1°C, 14:10 L:D photoperiod, and 75 ± 5% relative humidity. Progressive consumption of leaf area by the larvae was recorded after 24 hrs. 48hrs. using a leaf area meter.

The leaf area consumption was recorded by the leaf area meter before treatment and after the 24 hrs and 48 hrs, of the treatment. The percentage of antifeedant activity was calculated using the formula[16]

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

**Statistical analysis:** One way ANOVA was used to analyze the antifeedant activity. Significant differences were observed between treated and control groups. 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

Botanical insecticides are the best alternative to the chemical pesticides to very less negative impacts on environment and human health. One of the better ways to reduce the high consumption of chemical insecticides is the application of botanical insecticides, which are promoted to be environmentally and medically safe[17].

According to our results various concentrations was used for present experiment and they were low to the mortality concentration, so that an efficient dose without killing the insect but had an antifeedant activity.

Plant kingdom is a huge source of biologically more active natural chemicals; nearly 10,000 secondary metabolites have been chemically identified from the plant kingdom. Neem products are widely used such as neem leaf extract, neem seed extract, neem cake and neem oil acting as an insect repellents and insecticides against a more number of pests insects[18]. Azadirachtin, is the most potent natural insect antifeedant. According to various studies higher antifeedant index normally indicates decreased rate of feeding. In the present study, the extract azadirachtin was applied on the leaf surface using different concentrations such as 200ppm, 150ppm, 100ppm and 50ppm for 24 hrs and 48 hrs duration. The results of present study showed 86.28 % and 70.43% antifeedant activity at 200ppm concentration after 24 hrs and 48 hrs exposure, respectively. The protected leaf area at 200 ppm was 33.37 ± 1.04* sq.cm and 27.15 ± 0.74* sq.cm after 24 hrs and 48 hrs, respectively. The antifeedant activity varied significantly based on the concentration and type of plant product used for the formulations. Antifeedant is a chemical that inhibits the feeding without killing the insect directly, while the insect remains near the treated foliage and dies through starvation[19]. Similar results were observed in *Spodoptera mauritia*, *Ephestia kuehniella Zell* and *Manduca sexta* when subjected to azadirachtin[20].

In our study, an additive effect in the antifeedant activity was recorded in the azadirachtin treatment, which was significantly high when treated with 150ppm concentration the antifeedant activity was 73.67% and 62.13% for 24 hrs and 48 hrs exposure and the protected leaf area was 28.24 ± 0.78* sq.cm and 24.60 ± 0.80* sq.cm after 24 hrs and 48 hrs, respectively. In our experimental result matched with the findings of Kumar[21] who reported that methanolic extracts of karanj and neem were exhibited well in the antifeedant activity against *Tetranychus* species in other experiment the karanj and neem mixture showed synergistic activity against aphid *Macrosiphoniella sanborni.* Schoonhoven[22] were also in agreement with the present work on feeding deterrency and repellency of neem antifeedant property of neem leaf extract on various agricultural pests and found the highest antifeedant effect showen by neem therefore their work also supported present investigation.

This study indicated that the *Papilio demoleus* larvae is determined not only by feeding inhibition, as indicated by reduced food uptake at 100ppm concentration 65.83% and 52.08% antifeedent activity was observed for 24 hrs and 48 hrs exposure and protected leaf area was 25.17 ± 0.76* sq.cm and 19.75 ± 0.93* sq.cm after 24 hrs and 48 hrs respectively, digestibility as indicated by reduced efficiency of *Papilio demoleus* larvae in converting ingested food to growth. Similar results were found the Dancewicz[23] also was in agreement that *Allium sativum* and *Oscimum sanctum* suppress the feeding behavior of *Myzus persicae.* Feeding behavior is governed by both neural input from the insect's chemical senses and central nervous integration of this sensory input. The primary antifeedant effects might be attributable to a direct action of neem-based biopesticides on the centers that control feeding and metabolism. Azadirachtin stimulates specific deterrent cells in chemoreceptors on insect mouthparts and blocks the receptor cells that normally stimulate feeding. A secondary antifeedant effect of azadirachtin results from the disturbance of hormonal and other physiological systems.

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

| Conc. in ppm | No of Insects | Mean ± SD after 24 hrs. | Mean ± SD after 48 hrs. | Antifeedant activity (%) after 24 hrs. | Antifeedant activity (%) after 48 hrs. |
|---|---|---|---|---|---|
| 200 | 10 | 33.37 ± 1.04* | 27.15 ± 0.74* | 86.28 | 70.43 |
| 150 | 10 | 28.24 ± 0.78* | 24.60 ± 0.80* | 73.67 | 62.13 |
| 100 | 10 | 25.17± 0.76* | 19.75± 0.93* | 65.83 | 52.08 |
| 50 | 10 | 21.61 ± 0.91* | 15.51± 0.99* | 58.73 | 43.28 |
| Control | 10 | 14.05 ± 0.92* | 09.73 ± 1.14* | --- | --- |

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

Antifeedant activity was mainly depends on concentration, lower dose of 50ppm concentration reduced the larval feeding up to a large extent that was 58.73% after 24 hrs and 43.28% after 48 hrs and the protected leaf area was 19.05 ± 0.92* sq. cm and 10.73 ± 1.14* sq.cm after 24 hrs and 48 hrs, respectively. Similar observations were also noticed in *Papilio demoleus* treated with plant products such as andrographolide and Costunolide[24,25]. Plant–based products are most efficient antifeedant, they contribute great value in the protecting crops from insect attack and pest infestation and may be replace the chemical insecticides in coming days. Antifeedants gave the signal to the pests insects to stop feeding and perhaps starve or to move from the treated crop to other plant. Plant products consists mixtures of bioactive constituents, these metabolites may gave toxic effects if ingested leading to rejection of the host plant.

The plant products and active compounds acting as a repellents, antifeedants, insect growth regulators, regulation and control of oviposition. Azadirachtin had efficient antifeedant activity at 200ppm and less quantity of the food consumption takes place, at 150 and 100ppm were identified to have a moderate antifeedant activity was observed. Similar type of experiments were reported earlier studies by the main active plant product in the *C. odorata* wood, gedunin, had previously shown moderate antifeedant activity against various insect species[26] depends on the various treated concentrations, antifeedants are showed some physiological or toxic actions on insects.

## Conclusion

The present findings suggest that plant extracts of Azadirachtin, contain biologically active compound, which were efficiently and vital antifeedant effect on *Papilio demoleus.* Thus, these plant extracts contribute significant promise for fighting the threats posed by Citrus farmers. The aim of this work is its adaptability for use of small scale farmers plagued by the challenge of not being able to afford conventional pesticides on the market.

## Acknowledgement

The authors are very much thankful to Prof. Naidu Ashok, former Principal and Prof. V. Vanita Das, former Head, Department of Zoology, Nizam College (Autonomous) Osmania University for extending lab facilities and for their constant encouragement.

## References

**1.** Eastwood R., Boyce S.L and Farrell B.D. (2006). The provenance of Old World swallowtail butterflies, Papilio demoleus (Lepidoptera: Papilionidae), recently discovered in the New World. *Ann. Entomol. Soc. Am*., 99(1), 164-168.

**2.** Butani D.K. (1979). Insect pests of citrus and their control. *Pesticides,* 13(4), 15-21.

**3.** Butani D.K. and Jotwani M.G. (1975). Trends in the control of insect pests of fruit crops in India. *Pesticides,* 9(4), 139-149.

**4.** Pruthi H. (1969). Text book on Agriculturla Entomology. *Ind. Council of Agril. Res*, New Delhi, 634.

**5.** Rao Ramakrishna A. (2015). Studies on Biology and Morphometrics of Citrus Butterfly *Papilio demoleus* (Linnaeus) (Lepidoptera: Papilionidae) on Sathgudi Sweet Orange *Citrus sinensis* Swingle. *International Journal of Current Research in Life Sciences,* 4(3), 168-171.

***6.*** Alavanja M.C. (2009). Pesticides Use and Exposure, Extensive Worldwide. *Reviews on Environmental Health*, 24(4), 303-309.

**7.** Sola P., Mvumi B., Ogendo J., Mponda O., Kamanula J., Nyirenda S., Belmain S. and Stevenson P. (2014). Botanical Pesticide Production, Trade and Regulatory Mechanisms in Sub-Saharan Africa: Making a Case for Plant-Based Pesticidal Products. *Food Security*, 6(3), 369-384.

***8.*** Offor C., Okaka A. and Aja P. (2014). Comparative Analyses on the Effects of Ethanol Leaf-Extracts of *Vernonia amygdalina* and *Azadirachta indica* on Lipid Profile in Albino Rats. *World Applied Sciences Journal*, 32(1), 61-65.

**9.** Kareru P., Maina E.W. and Rotich Z.K. (2013). Use of Botanicals and Safer Insecticides Designed in Controlling Insects: The African Case. INTECH Open Access Publisher, Winchester, 297-309.

**10.** Bhatt P., Thodsare N. and Srivastava R. (2014). Toxicity of Some Bioactive Medicinal Plant Extracts to Asian Army Worm, Spodoptera litura. *Journal of Applied and Natural Science*, 6(1), 139-143.

**11.** Mpumi Nelson, Mtei Kelvin, Machunda Revocatus and Ndakidemi Patrick A. (2016). The Toxicity, Persistence and Mode of Actions of Selected Botanical Pesticides in Africa against Insect Pests in Common Beans, P. vulgaris. *American Journal of Plant Sciences*, 7(1), 138-151.

**12.** Butani D.K. and Jotwani M.G. (1975). Trends in the control of insect pests of fruit crops in India. *Pesticides.* 9(4), 139-149

**13.** Khalil M.S. (2013). Abamectin and Azadirachtin as Eco-Friendly Promising Biorational Tools in Integrated Nematodes Management Programs. *Journal of Plant Pathology and Microbiology*, 4(4), 2-3.

**14.** Khater H.F. (2012).Prospects of Botanical Biopesticides in Insect Pest Management. *Pharmacologia*, 3(12), 641-656.

**15.** Aerts R.J. and Mordue A.J. (1997). Feeding Deterrence and Toxicity of Neem Triterpenoids. *Journal of Chemical Ecology*, 23(9), 2117-2132.

**16.** Tsman M.B., Koul O. and Luczynski A. (1990). Insecticidal and antifeedant bioactivities of neem oils and

their relationship to Azadirachtin content. *J. Agric. Food Chem,* 38(6), 1406-1411.

**17.** Mohan M., Haider Z., Andola H. and Purohit V.K. (2011). Essential oils as green pesticides: for sustainable agriculture. *Research Journal of Pharmaceutical, Biology and Chemical Sciences*, 2(4), 100-106.

**18.** Koul O., Tsman M.B. and Ketkar C.M. (1990).Properties and uses of neem-Azadirachta indica. A. Juss. *Can. J. Bot*, 68(1), 1-11.

**19.** Yasui H., Kato A. and Yazawa M. (1998). Antifeedants to armyworm, Spodoptera litura and Pseudaletia separata, from bitter gourd leaves, Momordica charantia. *J Chem Ecol*. 24(5), 803-813.

**20.** Jagannadh V. and Nair V. (1992). Azadirachtin-induced effects on larval-pupal transformation of Spodoptera mauritia*. Physiological Entomology*, 17(1), 56-61.

**21.** Kumar V., Chandrashekar K. and Sidhu O. (2007). Synergistic action of neem and karanj to aphids and mites. *J. Ent .Res*. 31(2), 121-124.

**22.**

**23.** Schoonhoven L.M. (1982). Biological aspects of antifeedants. *Entomologia Experimentalis et Applicata,* 31(1), 57-69.

**24.** Dancewicz K., Gabrys B. and Przybylska M. (2011). Effect of garlic (Allium sativum L.) and tansy (Tanaceum vulgare L.) extracts and potassic horticultural soap on the probing and feeding behaviour of Myzus persicae . *Aphids and other hemipterous insects* 17, 129-136.

**25.** Rao Srinivasa V. (2015). Effect of andrographolide on feeding behavior of *Papilio demoleus* L., (Lepidoptera: Papilionidae) larvae. *Asian Journal of Bio Science* 10 (1), 65-70.

**26.** Rao Srinivasa V., Rao Nageswara A. and Sangam Sabita Raja (2015). Effect of Costunolide a plant product of Saussurea lappa on feeding behaviour of Papilio demoleus L. (Lepidoptera: Papilionidae) Larvae. *Research Journal of Recent Sciences,* 4(7), 55-58.

**27.** Champagne D., Koul O., Isman M., Scudder G.G.E and Towers G.H.N. (1992). Biological activity of limonoids from Rutales*. Phytochemistry,* 31(2), 377-394.