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Effect of forskolin on the growth and differentiation of the ovary of *Papilio demoleus* L. (Lepidoptera: Papilionidae)

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Abstract

Insect growth regulators generally have a selective effect on the target insects and have practically no apparent side effect on non-target organisms especially vertebrates. Hence, insect growth regulators could be a suitable choice to control pests. Forskolin is a diterpene extracted from the roots of Coleus forskohlii affected normal growth and development of the ovaries of Papilio demoleus L (Lepidoptera: Papilionidae). The fourth instar, fifth instar and pupae were treated with 2, 4, 6, 8 and 10 µg / µl doses. The growth of ovaries was seriously affected in treated resultants, females number of follicles and weight of ovary were reduced significantly, Forskolin affected ovarioles showed disrupted structures of the germarium and vitellarium with either complete or partial damage of few or all oocytes These defects are possibly caused by the Forskolin it inhibits fat body and germinal part of the growing ovariole. Our results indicate that the inhibition of ovarian development, affecting the fertility and the reproductive potentiality of Papilio demoleus suggesting that usage of this plant product is safe anti-fertility agent for the control of this pest.

Keywords: Forskolin, *Papilio demoleus* L, Ovarian abnormalities, Resorption, Anti fertility agent.

Introduction

Insect control has been a challenging task from the beginning of organized agriculture in recent years several conventional chemical pesticides have been used to control the various insect populations. Over the last three decades, the intensive use of broad-spectrum insecticides against these pests has led to the damage to the environment, development of resistance against many registered pesticides, disrupts the ecological cycle, detrimental effects on the natural enemies, pollinators and all other non-target insects, and serious toxicological problems to humans and the environment[1]. In view of this effect of various chemical pesticides have forced the researchers to find out the new methods for the integrated pest management of insect pests[2-4.] Consequently searching for various alternative ways of insects controlling are very important. In this regard, Insect control using plant material is an ancient practice all over the world. All the efforts have been devoted to search for new classes of insecticides derived from plants with lower mammalian toxicity and a lesser persistence in the environment. Plant-derived extracts and phytochemicals was extensively studied from the last 30 years on wards, an attempt has been made to promote a alternatives to synthetic pesticides but with low negative health risks and environmental impacts[5,6.] However, increased insect resistance to pesticides has led to the finding of new molecules from botanicals as alternative pest control agents; it is a well established approach in control strategies for pests[7]. Plant derived products are best and widely accepted of the suitable alternatives for the harmful synthetic pesticides. Plant products could be promoting the various modes of deformities in pests, which could safely be used in pest control[8]. In this scenario research has been carried out around the world to assess the insecticidal properties in plants, it has been revealed that disturbance on growth and reproduction are the one of the important factors in the pest control rather than antifeedants and repellents[9].

Insect growth regulators (IGR) are considered as the possible alternative way of conventional insecticides for controlling pests[10] because they differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system[11]. IGRs mode of action as chitin synthesis inhibitors and substances that interfere with the action of insect hormone[12].

IGRs block embryogenesis of insects, reduce egg production in emerging adults and cause severe morphological disorders. Although the prime target site of IGRs are endocrine systems there are many physiological and biochemical changes in metabolic pathway caused by these compounds. For the consideration of these reasons, less harmful and those involved in behavioral alterations and IGR got the importance in the agricultural pest management system. Many plant products are great sources of IGR and reproductive abnormalities have been examined in this view[13].

The Lemon butterfly *Papilio demoleus* L. (Lepidoptera: Papilionidae), is a serious pest on citrus, chemical pesticides can

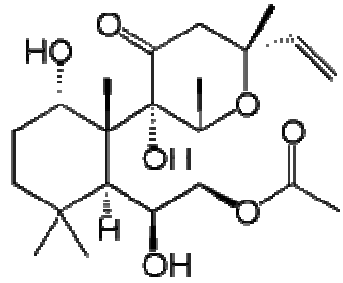
effect on it, but there are lot of adverse side effects which are more harmful to beneficial organisms. In this scenario, less poisonous options such as use of resistant cultivars, behavior changed IGR's have got the attention in agricultural management systems. Several plant sources of insect growth regulators tested against various insects in this respect[14,15]. Practice of various plant products leads to various morphological, physiological and reproductive abnormalities in pests[16]. Now a day's advance chemical pesticides, they play a key role to control population of pests in the succeeding generations thus forming an ideal component of the integrated pest management systems. In this line of exploration an attempt has been made to study the sterilant activity of Forskolin extracted from the roots of *Coleus forskohlii* on the Lemon butterfly *Papilio demoleus* L. (Lepidoptera: Papilionidae)

Materials and methods

Experimental insect: *Papilio demoleus* Linnaeus, the Lime Swallowtail, is one of the most wide spread members of the family Papilionidae and one of the most studied butterfly species partly due to the quick expansion of its range and potential danger to agriculture. The *Papilio demoleus* Linnaeus, is sometimes called the chequered or citrus swallowtail. It is a beautiful black yellow butterfly. There are large yellow marking and a series of yellow sub marginal spots on the upper side of both wings of this butterfly. Male and female do not differ in colour but can be distinguished by a red spot with an anterior blue lunule on the hind wing of the female. It was reported to be a pest throughout India, it is a major pest of *Citrus* sp. (Citrus) and *Murraya koenigii* (Curry Leaf) plantations[17,18]. The lemon butterfly *P. demoleus* most notorious and destructive pest of citrus plantation throughout the world, developmental stages of this insect cause a great loss to citrus family by prevalent and destructive pest in terms of its foliage damaging various stages of their growth period[19] the butterfly undergoes five instars as a caterpillar. The newly hatched young larvae are more active and starved, these larvae's are attacked on host plant and intensively feed on leaves and young seedlings cause to death also[20]. This pest attacked mainly on plants of genus Citrus[21,22] the larval stages of *P. demoleus* also attacked and cause a great loss and damage the plants like *Aegle marmelos, Psoralea corylifoli, Murraya koenigi* and *Chloroxylon sweiteni* and caused defoliation. Secondary hosts include various genera of Rutaceae, such as *Triphasia Glycosmis, Aegle, Murraya, Toddalia, Euodia, Zanthoxylum, Atalantia* and *Poncirus* in the forests of Malaysia[23]. In India it is commonly appeared in the plains but can be found on the hills of peninsular India and up to 7000 feet in the Himalayas. Several pesticides fast and efficiently control of the pest. Keeping in view their major damage is on the climate and other important beneficial organisms, an attempt is made to review the historical distribution, anatomy, and biological management technique which are best method to integrated pest management system[24].

Collection of Larvae and Maintenance: Different stages of larvae and Eggs of *Papilio demoleus* L were collected from Citrus gardens with no history of pesticide use, and reared in the laboratory at 28±2°C, 70±5% relative humidity and 12 hrs. light – 12 hrs. dark photoperiod. Larvae were kept in glass troughs and provided with fresh *Citrus* leaves daily. Adults were maintained in ventilated cages and provided with a 10% honey solution for feeding and fresh *Citrus* twigs for oviposition.

Test compound: Forskolin ($C_{22}H_{34}O_7$): Forskolin is a labdane diterpene it is extracted from the roots of the plant of *Coleus forskohlii.* Forskolin influences general morphological characters, growth and development of insects by associating with the various physiological processes. These plant extracts reduce oviposition rate and suppress the adult emergence[25]**.** Forskolin 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[26].



Forskolin is used as a test compound in this study. This phytochemical was provided by the Natural Products Lab, Dept. of Chemistry, Osmania University, Hyderabad, Telangana State, India.

Preparation of test solution: Forskolin and the desired doses were prepared by dissolving a known amount of Forskolin in 1 µl of acetone to obtain 2, 4, 6, 8 and 10 µg / µl doses.

Treatment with Forskolin: Thirty freshly moulted fourth instar, fifth instar larvae and thirty zero-hour pupae were treated topically on the abdominal region with 2, 4, 6, 8 and 10 µg/ µl of Forskolin with acetone as the carrier solvent with the help of Hamilton micro syringe. Thirty larvae and pupae were treated each time with Forskolin and the experiments were performed in triplicate. Controls were treated each time with an equivalent volume of carrier solvent acetone. After total absorption of Forskolin the larvae and pupae were transferred into the diet. The treated resultant females were observed for ovarian deformities and the results were compared with controls ovaries (Figure-1).

Results and discussion

Different concentrations of the plant product Forskolin applied on fourth instar, fifth instar larvae and pupae. The phytochemical exhibits the ecdysis inhibition the inhibition rate is increasing as per concentration increased. The treated

resultants developed abnormalities in larvae, pupae and the adults. The adult survival rate is decreased as per the increase of concentration. The treatment with Forskolin, some of the treated larvae pupated normally but ovaries of these resultant adult were drastically affected. However the ovarioles of the affected ovaries showed variation in the number and the size of the oocytes there were a large number of these resultant adults where in the ovarioles had matured oocytes but remained unovulated (Figure-2). In few treated resultant abnormal adults ovarioles exhibited compound egg chambers following degeneration, in some of ovarioles showed totally resorption takes place but few in other ovarioles one or two oocytes developed, extreme condition of resorption was observed.

The present investigation shows that the effects of Forskolin on ovarian growth and differentiation are depend on the stage of development of *Papilio demoleus* at the time of treatment. Treatment of various concentrations of Forskolin on the IV and V instar larvae and zero hour pupae of *Papilio demoleus* induced various developmental aberrations in the ovary, hormones are internal secretions that regulate a wide variety of physiological processes especially those concerned with growth development and egg maturation[27]. The studies on normal ovarian growth and previtellogenic differentiation of follicles take place in the last instars and pupal period[28]. Treatment of Forskolin inhibited the ovarian growth, the ovarioles also showed various morphological and histological abnormalities. These observations suggest that Forskolin interferes with the cell division and differentiation of ovarian cells terminal oocytes abnormally large and unovulated (Figure-3).

The induced abnormalities seen to arise at a later stage in the differentiation of follicles. Possibly the plant product Forskolin causes this effect during the differentiation of follicular epithelial cells. In most of the abnormal follicles the follicular epithelial cells are either absent or irregularly arranged chorionated fully developed unovulated oocytes (Figure-4). An orderly arrangement of follicular epithelial cells is necessary for the ovaries to undertake their normal physiological functions during vitellogenesis[29]. According to Koeppe J. K., et al.[30] endocrine system causes the physical and biochemical alterations in the arrangement of follicle cells and also involved in the formation of yolk. Forskolin might be induce the interruption in the normal pattern of juvenile hormone, in this line the results in the atrophy of the follicle, the follicular cells then taking part in the degeneration.

In the treated resultant *Papilio demoleus* the ovaries of pupal adult intermediates and abnormal adults showed large fused oocytes blocking the oviducts, in certain cases wherein the ovarioles appeared as long continuous tubes, In some other studies reported that ovarioles formed loops, while in some oocytes were changed from their original position (Figure-5) retention of unovulated oocytes and marked decrease in the oocytes number. Similar results were also reported by various researchers[31,32] with JH analogues and plant extracts on various insects.

Finally present study proved that, Forskolin is eco-friendly protection against the insect pest *Papilio demoleus* might be possible for applying the plant product in controlling the insect.

Conclusion

According to the results of this study and other reports, the application of plant product on the *Papilio demoleus* not only induces morphological deformities, but also causes the sterility of adults. This may be due to the destructive effects of these compounds on reproductive organ development. The plant extract inhibited the growth and development suggesting its use as an insect growth regulator in controlling pests like *Papilio demoleus*.

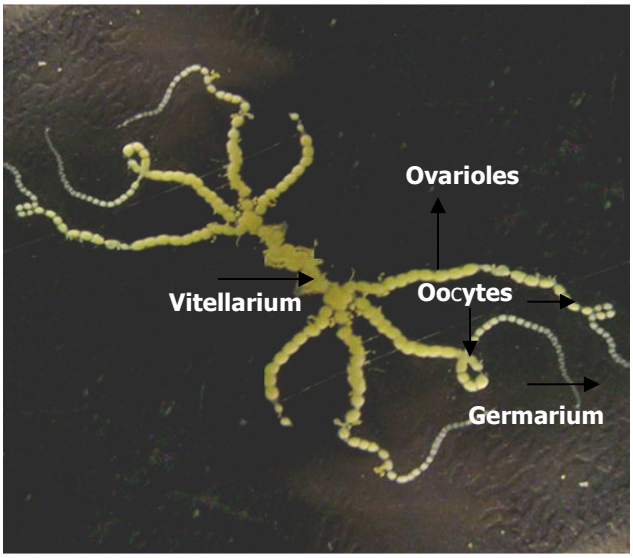


Figure-1: *Papilio demoleus* Ovarian deformities of Forskolin treated resultants.

Figure-2: Treated-Ovarioles with abnormal Oocytes

Figure-3: Treated-Terminal oocytes abnormally large and unovulated

Figure-4: Magnified view showing chorionated fully developed unovulated oocytes

Figure-5: The Oocytes of abnormal adult varing in size and length showing fused oocytes and mature unovulated oocytes blocking the oviduct

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References

1. Malarvannan S., Senthil Kumar S., Prabavathy V.R and Sudha Nair. (2008). Individual and Synergistic Effects of Leaf Powder of Few Medicinal Plants against American Bollworm. *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). *Asian J. Exp. Sci.,* 22(1), 79-88.

2. Abudulai M., Shepard B.M. and Mitchell P.L. (2001). Parasitism and predation on eggs of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) in cowpea: impact of endosulfan sprays. *J. Agr. Urban Entomol*., 18(2), 105-115.

3. Isman M.B. (1995). Leads and Prospects for the Development of New Botanical Insecticides. *Reviews of Pesticide Toxicology.*, 3, 1-20.

4. Jeyasankar A., Raja N. and Ignacimuthu S. (2010). Antifeedant and Growth Inhibitory Activities of *Syzygium lineare* Wall (Myrtaceae) Against *Spodoptera litura* Fab (Lepidoptera: Noctuidae). *Current Research Journal of Biological Sciences,* 2(3), 173-177.

5. Isman M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.,*51, 45–66.

6. Rita de C. S., Shikano I., Akhtar Y. and Isman M.B. (2010). Effects of crude seed extracts of *Annona atemoya* and *Annona squamosa* L. against the cabbage looper, *Trichoplusia ni* in the laboratory and greenhouse. *Crop Protection.,* 29(1), 20–24.

7. Roger C.R., Hamraoui A., Holeman M., Theron E. and Pinel R. (1993). Insecticidal effect of essential oils from Mediterranean plants upon *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae), a pest of kidney bean (*Phaseolus vulgaris* L.). *Journal of chemical ecology*., 19(6), 1233-1244.

8. Sreelatha K.B., Krishna R., Aswathi V.S., Nair V.V., Chikku G.R., Vipin V. and Mohan A. (2011). Laboratory evaluation of insecticidal activity of *Adathoda vasica* (Acanthaceae) and *Glyricidia maculate* (Leguminosae) on the third instar larvae of *Oryctes rhinoceros* L. (Coleoptera: Scarabaridae). *Journal of biopesticides*., 4 (2), 144-149.

9. Mordue Luntz A.J. and Nisbet A.J., (2000). Azadirachtin from the neem tree *Azadirachta indica* its action against insects. *Anais da Sociedade Entomologica do Brazil*., 29(4), 615-632.

10. Raslan S.A.A. (2002). Preliminary report on initial and residual mortality of the natural product, Spinosad, for

controlling cotton leaf worm egg masses in Egypt. *Plant Prot. Res. Inst*., 1, 635-637.

11. Oberlander H., Silhacek D. L., Shaaya E. and Ishaaya I. (1997). Current status and future perspectives of the use of insect growth regulators for the control of stored product insects. *J. Stored Prod. Res*., 33(1), 1-6.

12. Tunaz H. and Uygun N. (2004). Insect growth regulators for insect pest control. *Turkish J. Agric. Forestry.,* 28, 377-387.

13. Hebsy Bai and George Koshy (2004). Juvenomimetic activity of extracts of *Thevetia neriifolia* Juss. to *Dysdercus cingulatus* F. (Hemiptera: Pyrrhocoreidae). *Journal of Tropical Agriculture* 42 (1-2), 45-47.

14. Prabhu V.K.K., Johm M. and Ambikamma B. (1973). Juvenile hormone activity in some South Indian plants. *Curr. Sci*., 42, 725-726.

15. Saradamma K. (1989). Biological activity of different plant extracts with particular reference to their insecticidal, hormonal and antifeeding actions. PhD Thesis, Kerala Agricultural University, Thrissur.

16. Jaiswal A.K. and Srivastava U.S. (1992). Mating disruption in *Dysdercus cingulatus* caused by *Blumea laciniata* extracts. *J. Appl. Zool. Res*., 3, 151-153.

17. Malik J. M. (1970). Notes on the butterflies of Pakistan in the collection of Zoological Karachi, Part I. Rec. Zool. Surv. Pakistan 2(2), 25-54.

18. Badawi A. (1981). Studies on some aspects of the biology and ecology of the citrus butterfly *Papilio demoleus* L. in Saudi Arabia (Papilionidae, Lepidoptera). *Zeit. Angew. Entomol*., 91(1-5), 286-292.

19. Singh, S.P. (1993). Species composition and diapause in citrus butterflies. *J. Insect Sci*., 6(1), 48–52.

20. Resham B.T., Fanindra P.N. and Butani D. K. (1986). Insect pests of citrus in Nepal and their control. *Pestology*. 10(4), 24-27.

21. Guerrero K.V., Veloz D., Boyce S.L. and Farrell B.D. (2004). First New World documentation of an Old World Citrus pest, the lime swallowtail *Papilio demoleus* (Lepidoptera: Papilionidae), in the Dominican Republic (Hispaniola). *American Entomol*., 50(4), 227-229.

22. Thakare K.R. and Borle M.N. (1969). Survey report of damage caused by lemon butterfly pest in Vidharbha region. Official communication vide D.O. No. 782/69 dt.26/08/1969.

23. Elliot J.N. (1992). The butterflies of the Malay Peninsula. 4th edition. Malayan Nature Society. Kuala Lumpur.

24. Sarada G., Gopal K., Venkata Ramana K.T., Mukunda Lakshmi L. and Nagalakshmi T. (2014). Citrus butterfly (*Papilio demoleus* Linnaeus) Bioiogy and Management: A Review. *Journal of Agriculture and Allied Sciences.,* 3 (1), 17-25.

25. Anand k. and Sabita raja S. (2014). Morphogenetic Effects and IGR activity of a botanical Forskolin against last Instar Larvae of *Callosobruchus chinensis* (Linn.). *IOSR Journal of Agriculture and Veterinary Science*., 7(5), 50-53.

26. Srinivasa Rao V., Nageswara Rao A. and Sabita Raja S. (2014). Antifeedant activity of forskolin, an extract of *Coleus forskohlii*, against *Papilio demoleus* L. (Lepidoptera: Papilionidae) larvae. *European Journal of Experimental Biology*., 4(1), 237-241.

27. Madhavi M. and Sabita Raja S. (2012). Effect of *Vitex negundo* on the growth and differentiation of the ovary of *Corcyra cephalonica* (Lepidoptera: Papilionidae). *Journal of Pharmacognosy*., 3(2), 73-74.

28. Mathai S. (1987). Effects of hormones and hormones analogues on the ovarian development and vitellogenesis in the paddy pest *Spodoptera mauritia* Boisd(Lepidoptera:Noctuidae). Ph.D Thesis, Calicut University

29. Koeppe J. K., Fuchs M., Chen T. T,, Hunt L.M,, Kovalick G. E. and Briers T. (1985). The role of Juvenile hormone in reproduction in comprehensive insect physiology. *Biochemistry and Pharmacology,* 8, 165 – 203.

30. Koeppe J. K., Hobson K. and Wellman S. E. (1980). Juvenile hormone regulation of structural changes and DNA synthesis in the follicular epithelium of *Leucophaea maderae. J Insect Physiol*., 26(4), 229-240.

31. Slama K., Romanuk M. and Sorm, F. (1974). Insect Hormones and Bioanalogues. Springer Verlag Wien, New York, 476.

32. Raghunatha Rao G., Raghavaiah G. and Nagalingam B. (1993). Effect of botanicals on certain behavioral responses and on the growth inhibition of *Spodoptera litura* F. *Botanical pesticides in integrated pest management,* 12, 175-182.