Morphological Abnormalities of Betulinic Acid from *Ziziphus Jujuba* against the Callasobruchus Chinensis (Coleoptera: Bruchidae)

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Betulinic acid is a compound isolated from bark of *Ziziphus jujuba.* Betulinic acid,a triterpenoid. Betulinic acid exhibits a broad range of biological activities. It is an insect growth regulator, we observed the effect of different concentrations (10, 8, 6, 4and 2 ìg / ìl doses) Betulinic acid on *Callosobruchus chinensis* growth and development, in our observations we observed various morphological abnormalities like degeneration, deformation in larval, pupal stages. Untreated *Callosobruchus chinensis* showed normal in the developmental stages with the larval instar stages and henceforth developimg into pupa without any deformities. However Betulinic acid affected larval instars showed disrupted structures of the cuticle like tanning of cuticle and abnormal larvae over-aged larva with either complete or partial damage of pupa. The results demonstrated that Betulinic acid causes rapid cessation of growth due to disruption of larval structure and inhibition of growth following topical treatment on 4th5th instar and pupae of *Callosobruchus chinensis.*

Keywords: Betulinic acid,*Callosobruchus chinensis*,Larvae, pupa.

The modern insecticide researches started 65 years before with the chlorinated hydrocarbons, organophosphates, methyl carbamates and botanicals, these organic insecticides are used to control insect pests[9]. In the past few years, for pest management use of synthetic pesticides has become controversial. The pesticides are known to cause environmental threat as the pesticides accumulate in different concentrations in various levels of ecosystem. Another reason of controversy is to pest developing resistance to pest. Even though insects are exposed to insecticide for a prolonged period of time they manifest gradually, insect will not only develop resistance against particular insecticides to which they are exposed to, but also a group of insecticides will also develop resistance by a method of cross-resistance. To control these problems, the use of cultural practices, biological control, use of antifeedants, hormonal insecticides (IGRs) plant extracts are the other methods developed for pest control[2, 31]

The search for safe insecticide approaches, interest on products of plant acting like an insecticides has grown more, such that pesticides are removed from use because of environmental and food safety problems[14] Diverse biological effects on the insects are provided by the plant kingdom which is the store house of chemicals.

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Several plants with insecticidal properties have been recognized in current years. Environmental safe pesticides have been particularly toxic, and are not bio-accumulate, and show short persistence in the environment. More particular ways of work and decreased risks for non-target organisms and the environment they are more advisable in modern integrated pest management programs, in previous two decades with expansion of natural and synthetic compounds efficient of interfering with process of growth, development, metamorphosis of selected insects. Plant products (insecticides) seem to be eco-friendly and mixtures of biologically active substances, because they have been found to be selective[24] and cause low negative effects to the ecosystems apart from conventional insecticides[27].

Use of plant products to insect pest control that untimely affect insect growth and development without causing environmental hazards. These substances were categorized as "insect growth regulators" (IGRs) or "Insect hormone mimics". They were considered for having less risk as reported by the[29] to being soft to beneficial insects and target specific for juvenile stages. The insect growth regulators are - hormonal, enzymatic and chitin synthesis inhibitors. Both juvenile hormone analogues and ecdysone inhibitors disrupt the ratio of hormones in the young insect. The correct ratio of juvenile hormone and ecdysone must be present for an insect to be moult in the next stage. Primary moulting hormone that is Ecdysone is necessary for insects to change from the larval to pupal stage. If the ratio of one hormone to other is not correct, the insect fail to become adult, decreasing reproduction and subsequently population increase. With some IGRs, adults even fail to produce usable eggs.

Plants are potential producer of novel chemical compounds which cannot yet be synthesized. Estimations suggest that over 2000 plant species have the potential to identify and develop new chemistries to reduce bacteria, fungal, and insect/arthropod pests[13]. The complexity in chemical compounds in biorational products can also make development of resistance by insect pests is more difficult[21]. These environmental issues have driven agricultural researchers to search for better ecofriendly based pesticides[30]. *Callosobruchus chinensis* was believed to be of eastern origin but became a cosmopolitan species spreading throughout the world with the transport of food stuffs. Its absence from apparently suitable habitats ex. Flour mills in Iraq, Iran and areas of Pakistan was believed to be due to low moisture contents of stored food in such places.*Callosobruchus chinensis* attacked stored products like Bengal grams, cotton, wheat and wheat bran, Sorghum, rice and rice bran, maize, groundnut seed and cakes, cereals, pulses, oil cakes, nuts, dried fruit and various processed foods, redgram, greengram, cowpea, and gingelly cakes in descending order based on the severity of infestation and damage.

Callosobruchus chinensis is a pest of stored grain. The quality of the grain will deteriorate unless protected from pests. The larvae of *Callosobruchus chinensis* damage the grains of rice and maize by feeding under silken webs. In case, of whole grain Kernels are bound into lumps. When infestation increases entire stock of grains have a possibility to be connected into a webbed mass. Eventually a typical bad smell develops and the grain is rendered unfit for human consumption. Larvae are serious economic pests that cause quantative and qualitative losses in tropical and sub-tropical regions. In the present study Betulinic acid which was isolated from *Ziziphus jujuba.*We investigated the developmental activity effects of Betulinic acid on the fourth and fifth instars and pupae of *Callosobruchus chinensis,* in laboratory assays.

METHODS AND MATERIALS

Betulinic Acid isolated from *Z. jujube*

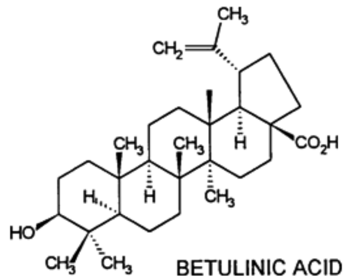
At room temperature the pulverized dried roots of *Z. jujuba* were macerated with MeOH (2×60 L) for each one week. To give a crude extract the MeOH extract was concentrated in vacuo. The extract which was concentrated is suspended in H2O and acidified with 1N HCl to pH 3. To yield EtOAc fraction the acidic solution was extracted with EtOAc. The aqueous residue with NaOH was basified to pH 9 and extracted with CHCl3 for an alkaloid fraction which was not applied in the present study. The fraction of EtOAc was subjected to a normal silica gel CC with mixture of CHCl3 and MeOH (100:1 to 1:1) and 10 subfractions (EA-1-10) are given. The EA-3 fraction was fractioned by normal silica gel CC with a mixture of MeOH

(100:1 to 5:1) and CHCl3, giving seven fractions. By re-crystallization with 100% MeOH, Fraction 3 and 4 yielded Betulinic acid.

Test Product: Betulinic acid

Betulinic Acid ***(C30H48O3)*** Betulinic acid is a compound isolated from bark of *Ziziphus jujuba*. Its medicinal benefits are indicated in Ayurveda and Chinese Medicine. Betulinic acid, a triterpenoid present in many plant species, has captivated the attention due to its important pharmacological properties, such as anti-cancer and anti-HIV activities, anthelmintic activity, antifeedant activity. It also exhibits antibacterial, anti- inflammatory and anti-malarial properties.

Chemical Formula :C30H48O3



Preparation of test solution

By dissolving a known amount of Betulinic acid in 1 ìl of acetone the different concentrations of Betulinic acid doses were prepared 10, 8, 6, 4 and 2 µg / µl doses.

Test Insect

The larvae of *Callosobruchus chinensis* caused damage to stored products firstly by feeding and secondly by leaving silken threads wherever they move, thus forming a dense webbing leading to the formation of silken galleries. In case of heavy infestation, food materials become tightly matted together with webbing, larval galleries, cocoons and excreta. Severe pest attack resulted in entire destruction of the stored productsand leads to damage of the foodstuffs. Various insecticides provide effective and quick control of pest. With the aspect of their adverse effects on environment and other different non-targeted organisms now there is an attempt made to review biology, biological management methods, historical distribution which are most prominently suited to the programme of integrated pest management.

Collection of Larvae , their Maintenance Larvae

The larvae start feeding immediately after hatching. They grow in 25-35 days. There are five instars. The larvae alone cause damage to the grains. Pre-pupal stage: It is the non feeding stage when the larvae make silken cocoons among the grains for pupation. The duration of this stage is 4-5 days. Pupal Stage: This stage spreads over 7-8 days and the insect is in a quiescent state. This arrangement has helped the larvae and adults to evolve in different directions, the larvae specializing in food gathering and the adults developing advanced means of reproduction and dispersal .The adult moths of *Callosobruchus chinensis*survive for 8-10 days and are of great economic importance. The adults have a siphoning type of mouth parts, so adults do not feed on the grains. The larva has a well-developed chewing type of mouth parts. It is the larva that feeds on the grains and forms infesting stage .Based on the body weight and head capsule size the larval stages were classified for the current study. IV Instar, chosen for the present study was 45- 55mg on an average in weight. The head capsule size was 0.72-0.78mm.

V Instar larvae weighed 76-85mg and the head capsule size was 103-108mm .The completely grown fourth instar and fifth instar larvae were classified and kept in a separate glass dish at room temperature for the experiment For each experiment 3-5 replicates were done and each experiment was repeated at least 5 times.

Treatment with Betulinic acid

With the help of Hamilton micro syringe freshly thirty moulted fourth instar, fifth instar larvae and thirty zero-hour pupae on the abdominal region were treated topically with 2, 4, 6, 8 and 10 ìg/ ìl of Betulinic acid by acetone as carrier solvent .Each time by Betulinic acid thirty larvae and pupae were treated and in triplicate the experiments were performed. The acetone controls were treated each time with an equivalent volume of carrier solvent. After total absorption of Betulinic acid.

Into the diet the larvae and pupae were transferred. The treated resultant females were observed for morphological deformities and compared with controls for results.

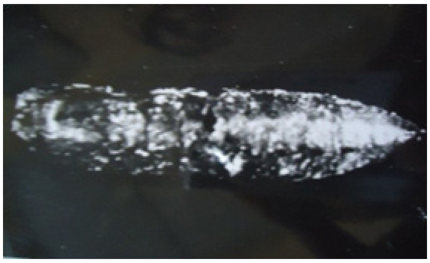
DISCUSSION AND RESULTS

A plant product which causes the morphogenetic and physiologic abnormalities

Resultant larva with pupal cuticle patch

Resultant larval-pupal intermediates with exuvia attached

Control Pupa & Pupa with deformed posterion region



Overaged larvae

Larval-Pupal intermediate retaining the larval head capsule

Fig. 1. *Callosobruchus chinensis* treated with Betulinic acid: Morphological deformities

may result in insects reproductive failure can be applied to control the *Callosobruchus chinensis*. It is one of the relatively new method to control the *Callosobruchus chinensis*[11]. During this stage, to initiate the development ecdysone is released and reproductive organs differentiation, leading for metamorphosis, the internal reproductive organs morphological abnormality and testis and ovaries histological disruption were similar to findings reported by other researchers[4,19]. On fifth instar, fourth instar larvae and pupae the plant product Betulinic acid of different concentrations is applied. the inhibition rate is increasing as per concentration increased because of the phytochemical exhibiting the ecdysis inhibition . Abnormalities in larvae, pupae, adults were developed in treated resultants (Figure-1). As per the increase of concentration the survival rate of adult is decreased. With treatment by Betulinic acid some of the treated larvae pupated abnormally and henceforth drastically affected.

The incompletely development of larvae abnormalities such as deteriorated and defective pupae and partially developed adults and overaged larvae are formed in the presence of Betulinic acid. With results of this study and other reports, application by Betulinic acid on the *Callosobruchus chinensis* will not only produces morphological deformities, but will also cause the sterility in adults. We found the Betulinic acid application it had a direct role on fecundity, fertility of treated insects, so it can be concluded that this compound had an effect on growth of larvae, pupae. Decrease in fecundity similar observations had been reported by[22] on *Aphis glycines* and[4] on *Rhyzoperthadominica.*

CONCLUSIONS

On the basis of overall findings, it can be concluded that Betulinic acid is toxic to *Callosobruchus chinensis*, as it mimics the action of JH and maintains insect in an immature state. Betulinic acid caused mortality in larvae and produced abnormal adults and it also affected sterility in adults. This can be because of the destructive effects of these compounds on reproductive organ development. Growth and development and fecundity of the *Callosobruchus chinensis* are inhibited by the plant extract. Thus Betulinic acid may be considered as a leading target compound having the potential to control *Callosobruchus chinensis* and can therefore form an important component of various Integrated Pest Management (IPM) programs.

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