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RESEARCH ARTICLE

ANDROGRAPHOLIDE - AN EFFECTIVE INSECT GROWTH REGULATOR OF PLANT ORIGIN AGAINST *Tribolium confusum* (Duval)

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ABSTRACT

Andrographolide, a terpenoid isolated from the leaves of *Andrographis paniculata* (Acanthaceae) exhibited growth regulating activity against the stored grain pest *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae). The fifth instar, sixth instar larvae and pupae were treated with 1µg/µl of Andrographolide. The larval and pupal development to adults was greatly reduced resulting in the formation of permanent larvae, larval-pupal intermediates, abnormal pupae, pupal-adult intermediates and abnormal adults due to the interference of Andrographolide with moulting process and adult eclosion. These resultant forms were ruled out from further development and reproduction. The strong effect of Andrographolide on larval-pupal and pupal-adult moults is interpreted as an interference with moulting hormone pools. Our results suggest that Andrographolide shows an effective insect growth regulating activity and exhibits great promise in inhibiting growth, development and morphogenesis of *Tribolium confusum.*

INTRODUCTION

Repeated use of synthetic pesticides for pest management has disrupted natural biological control systems and led to pest resistance, pest resurgence and secondary pest out breaks. More over they are highly persistent accumulating themselves at various concentrations in different levels of ecosystem and are carcinogenic (Kabeh and Jalingo, 2007). It is thus necessary to develop an alternative method of pest control and one of the promising ways to do this is by the use of insect growth regulators (Vaclav Nemec, 1993 and Fathpour *et al.,* 2007). Insect growth regulators are currently the fastest growing class of insect control agents (Husseyin cetin *et al.,* 2009). They are aimed mainly at covering the need for safer compounds and overcoming the development of resistance to "classical" insecticides (Graf, 1993). Natural compounds with insect growth regulating activity have been isolated from plants (Vaclavnemec, 1993). Much effort has been made to utilize a variety of biologically active analogues as IGRs (Insect Growth Regulators) (Graf, 1993; Vardhini *et al.,* 2001 and Ikbal *et al.,* 2006). On this back drop Andrographolide a terpenoid isolated from the leaves of the medicinal plant *Andrographis paniculata* is chosen. *Andrographis paniculata* is a herbaceous plant in the family Acanthaceae, native to India and Srilanka. It is widely cultivated in Southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as tribal medicine in India and some other countries for multiple clinical applications. Its therapeutic value is due to its mechanism of action which is perhaps by enzyme induction (Meenatchi Sundaram *et al.,* 2009). *Andrographis paniculata* plant extract is known to possess a variety of pharmacological activities. Andrographolide, the major constituent of the extract is implicated towards its pharmacological activity (Rajgopal *et al.,* 2003). The enormity of work conducted on Andrographolide is large but only a handful of data is available with regard to its bio-pesticidal control. Therefore in the present work an attempt has been made to investigate the insect growth regulating activity of Andrographolide on *Tribolium confusum* one of the most serious cosmopolitan pest in stored grain and related products.

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MATERIAL AND METHODS

Tribolium confusum were reared on mixed flour of wheat and jowar (1:1) and maintained at 27 ± 1^{0}C temperature and 60 ± 5% relative humidity. Freshly moulted fifth instar, sixth instar larvae and zero hour pupae were treated topically on the abdominal region with 1µg of Andrographolide dissolved in 1µl of acetone with the help of a Hamilton micro syringe. Thirty fifth instar, sixth instar larvae and zero hour pupae were treated each time and the experiments were replicated five times. Parallel controls treated with 1µl of acetone were maintained. After the treatment a suitable gap of time was

given for the total absorption of the extract and they were transferred into the diet. The treated larvae and pupae were observed daily to note the changes.

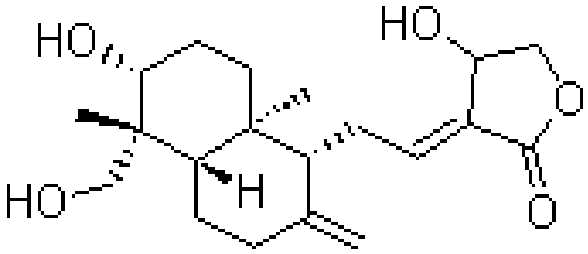

Fig. 1. Structure of Androgrpholide

RESULTS

1µg/µl of Andrographolide affected moulting, growth, development and induced a wide range of morphological abnormalities in *Tribolium confusum*.

Effect of Andrographolide on fifth instar larvae of *Tribolium confusum*

Among the treated fifth instar larvae few of them died during moulting, few remained as permanent larvae, some of them developed into larval-pupal intermediates, abnormal pupae and remaining treated fifth instar larvae developed into morphologically normal adults. 30% of the treated fifth instar larvae died during moulting. They were unable to shed their exuviae, exhibiting complete ecdysial failure (Fig 6A).10% of the treated fifth instar larvae remained as "over aged" larvae for an extended period of time and eventually died. 33.33% of the treated fifth instar larvae moulted into mosaics exhibiting larval and pupal characters. These mosaics consisted of larval head, larval mouth parts and a pupal body. Intermediates with larval exuvium attached to the pupal body were also produced (Fig 6B). Such forms were inactive and their life cycle was terminated. 6.66% of the treated fifth instar larvae metamorphosed into abnormal nonviable pupae. 20% of the treated fifth instar larvae moulted normally into sixth instars and pupated which later eclosed into morphologically normal adults. (The percentages are depicted in Fig 2).

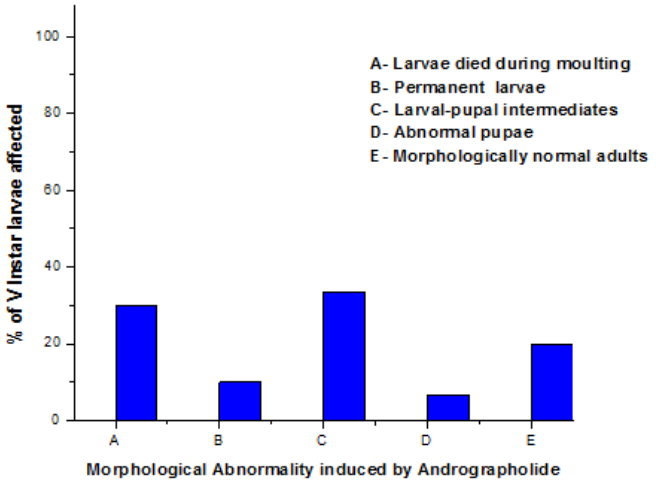

Fig. 2. Morphogenetic effects of Andrographolide against V instar larvae of *Tribolium confusum*

Effect of Andrographolide on sixth instar larvae of *Tribolium confusum*

Some of the treated sixth instar larvae failed to pupate while few of them developed into abnormal pupae, pupal-adult intermediates, abnormal adults and rest of them metamorphosed into morphologically normal adults. 23.33% of the treated sixth instar larvae were unable to pupate and they finally died. 30% of the treated sixth instar larvae emerged into larval-pupal intermediates with larval exuvium attached to the posterior end. These intermediates were inactive and died within a few days.13.33% of the treated sixth instar larvae metamorphosed into pupae with exuvium attached to the wing pads (Fig 6C). These abnormal pupae eclosed into deformed adults which failed to mate or oviposite. 10% of the sixth instar larvae moulted into pupal-adult intermediates. These forms failed to shed their pupal case. These intermediates did not undergo subsequent developmental changes and ultimately died. 6.66% of the treated sixth instar larvae metamorphosed into abnormal adults with deformed mouth parts, wings and appendages partially attached to the abdomen (Fig 6D). These deformities prevented the insects from feeding, flying and mating. 16.66% of the treated sixth instar larvae developed into normal pupae and morphologically normal adults eclosed from these pupae. (The percentages are depicted in Fig 3).

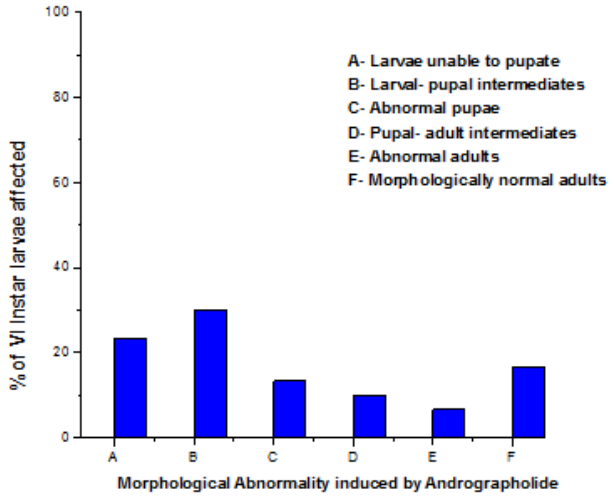

Fig. 3. Morphogenetic effects of Andrographolide against VI instar larvae of *Tribolium confusum*

Effect of Andrographolide on zero hour pupae of *Tribolium confusum*

Few of the treated pupae died during adult eclosion, few of them developed into pupal-adult intermediates and abnormal adults and the remaining emerged as morphologically normal adults. 20% of the treated pupae exhibited serious disturbances during adult eclosion. The adults died within the pupal cuticle. 33.33% of the treated pupae emerged into pupal-adult intermediates (Fig 6E). These intermediate forms were ruled out from further development and reproduction. 26.66% of the treated pupae eclosed into abnormal adults with exuvium attached to the posterior region (Fig 6F) preventing the insects from defaecation, mating and oviposition. 20% of the treated pupae developed into morphologically normal adults. (The percentages are depicted in Fig 4)

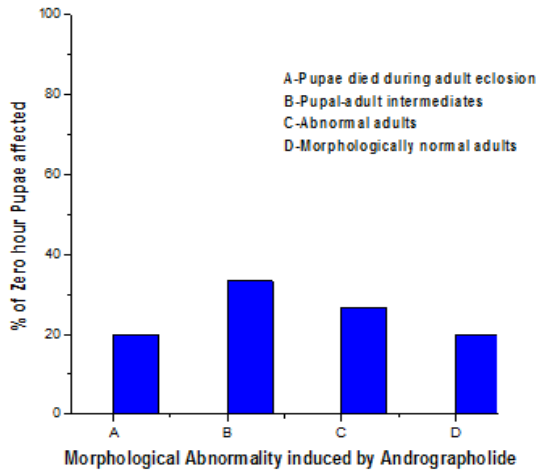

Fig. 4. Morphogenetic effects of Andrographolide against Zero hour pupae of *Tribolium confusum*

Andrographolide induced a decrease in the percentage of adult emergence compared to the controls. Only 18.88 ± 1.928 adult emergence was observed with Andrographolide while 100% adult emergence was seen in controls (Graph 5).

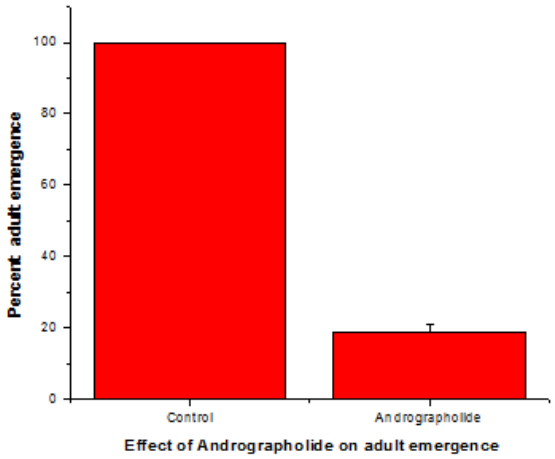

Fig. 5. Effect of Andrographolide on adult emergence of *Tribolium confusum*

DISCUSSION

The present study clearly indicates the fact that Andrographolide deranges the development of *Tribolium confusum*. Andrographolide interfered with the normal development and metamorphosis of *Tribolium confusum,* which was manifested at different stages of the life cycle. Andrographolide prevented normal development of the fifth instar larvae of *Tribolium confusum,* resulting in prolongation of final instar, permanent larvae, death of larvae during moulting and production of larval-pupal intermediates preventing their transformation into pupae. Those treated larvae which metamorphosed into pupae were also abnormal with larval exuvium attached to the wing pads and nonviable.

Most of the treated fifth instar larvae died during moulting exhibiting ecdysial failure. During a moult, ecdysteroid levels first rise to stimulate onset of apolysis and cuticle synthesis, but then must fall to facilitate release of eclosion hormone (EH) (Trumann *et al.,* 1983) and the ecdysis triggering hormone (ETH) (Zitnan *et al.,* 1996, 1999). These hormones act in concert to trigger insect ecdysis during the final stages of the moult (Carlos *et al.,* 2005). Andrographolide may disrupt ecdysteroid metabolism resulting in inhibition of emergence behavior or may alternatively act directly to inhibit the release of ecdysis-triggering hormone as also reported by Gaur and Kumar (2010) working with acetone leaf extract of *Withania somnifera* on *Spodoptera littura*. Permanent larvae have a greatly extended instar and survive for several weeks beyond the normal instar length. Such insects have not metamorphosed into pupae and thus have not achieved the imaginal competence for adult physiology and development as observed in *Locusta* (Pener *et al.,* 1989 and Vander Horst *et al.,* 1989). Most of the treated fifth instar larvae moulted into mosaics with larval and pupal characters while few metamorphosed into abnormal, nonviable pupae. Similar observations were seen in *Euproctis fraterna* treated with leaf extract of *Catharanthus roseus* (Nalina Sundari, 1997).

According to Jagannadh and Nair (1992) the production of larval-pupal intermediates and abnormal, nonviable pupae may be attributed to the absence of necessary titer of ecdysteroids needed for achieving the larval-pupal transformation normally. Some of the sixth instar larvae after treatment continued feeding, increased in size but failed to pupate and finally perished showing the high juvenilizing effect while few of them moulted into larval-pupal intermediates with exuvium attached to the body. These intermediates remained inactive and died within few days. Few of the treated sixth instar larvae metamorphosed into abnormal pupae which eclosed into deformed adults, while some of the resultant larvae were intermediates with pupal and adult characters. These intermediates did not undergo subsequent developmental changes and ultimately died. These effects are similar to that of the interference of juvenoid with the production, release and action of moulting hormone to varying degrees (Srivatasava and Gilbert, 1969). Treatment of *Trichopulsia ni* and *Spodoptera exigua* larvae with neem (*Azadirachta indica*) extracts also results in moult disturbances and formation of intermediates (Prabhakher *et al.,* 1986). The treatment of last instar larvae of *Tribolium confusum* with Andrgrapholide resulted in disruption of adult morphogenesis. The adult deformities varied between a failure to completely get rid of last larval exuvia and deformed mouth parts, deformed wings and appendages partially or completely attached to the abdomen preventing the insects from feeding, flying and mating. Disturbed adult morphogenesis is also seen in *Schistocerca gregaria* treated with methanolic and n-Butanolic extract of *Fagonia bruguieri* (Samira *et al.,* 2010).

The treatment of pupae of *Tribolium confusum* with Andrographolide resulted in partial or complete blockage of adult emergence. Some of the adults exhibited inability to extricate from the pupal case, dying within it. The resultant adults too, exhibited certain physiological disturbances during ecdysis and were unable to escape from the old exuvia. Exuviae was often seen attached to the posterior region of the body preventing the insects from defaecation, mating and oviposition. Similarly topical application of ethanolic extract from *Cyprus rotendus* on *Schistocerca gregaria* resulted in the formation of abnormal adults (Bakr *et al.,* 2008). The eclosion hormone, a blood born factor arising from the central nervous system (Truman and Riddiford, 1970) triggers eclosion. Andrographolide probably prevented this hormone from being

released at the appropriate time. Complete or partial blockage of adult emergence was reported for different insects by various botanicals such as the blocked emergence of *Rhynophorus ferrugineus* with azadirachtin (Abdel- Ghaffar *et al.,* 2008) and of *Tribolium castaneum* by the methanolic extracts from *Centauriaum erythreae* and *Pteridium aquilinum* (Jbilou *et al.,* 2008). Andrographolide disrupted moulting, affected larval growth and development, influenced larval-pupal and pupal-adult transformation, disturbed adult morphogenesis and inhibited adult emergence. A very few treated larvae and pupae developed in to morphologically normal adults. In the long run Andrographolide can reduce *Tribolium confusum* population through disrupted metamorphosis. The inhibition of metamorphosis would prevent subsequent generations from propogation. These results clearly indicate that Andrographolide shows an effective insect growth regulating activity and exhibits great promise in inhibiting growth, development and morphogenesis of *Tribolium confusum*.

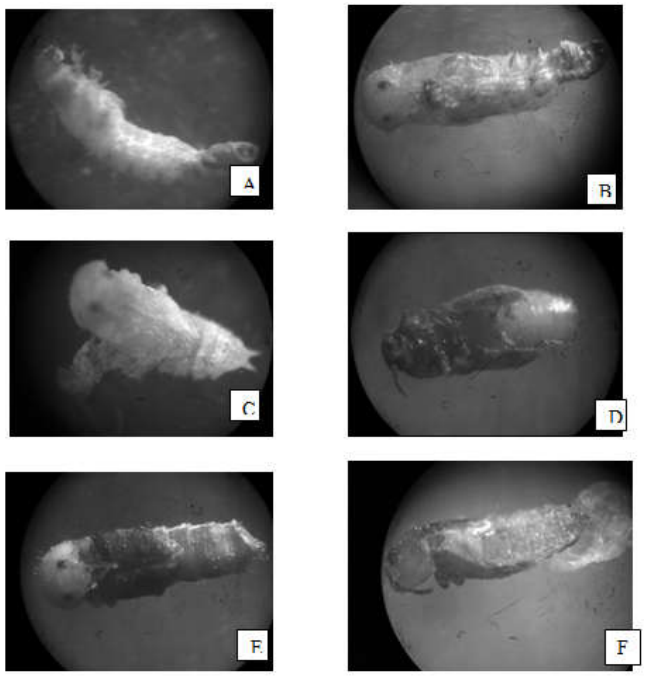

Fig. 6A Larva died during moulting
6B Larval-pupal intermediate with larval exuvium attached to the posterior region
6C Abnormal pupa with exuvium attached to the wing pads
6D Abnormal adult with appendages and wings attached to the abdomen
6E Pupal-adult intermediate
6F Abnormal adult with exuvium attached to the posterior region

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