

Andrographolide: An effective anti-fertility agent for the control of *Tribolium confusum*

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ABSTRACT

*Andrographolide, a terpenoid isolated from the leaves of *Andrographis paniculata* [Acanthaceae] affected normal growth and development of the ovaries of *Tribolium confusum* [Duval] [Coleoptera: Tenebrionidae]. The fifth instar, sixth instar larvae and pupae were treated with 1µg/µl of Andrographolide. The treated resultant females exhibited ovarian deformities. The ovaries of the treated resultant *Tribolium confusum* showed variation in the length and size of the ovarioles, ovariole degeneration, oocyte degeneration, resorption and inability of the mature oocytes to oviposit. Our results show that the topical application of Andrographolide inhibits ovarian development, affecting the fertility and the reproductive potentiality of *Tribolium confusum* suggesting its use for the development of safe and specific anti-fertility agent for the control of this stored grain pest.*

Key words: Andrographolide, *Tribolium confusum*, Ovarian abnormalities, Resorption, Anti fertility agent.

INTRODUCTION

Botanicals have been identified as attractive alternatives to synthetic chemical insecticides for pest management. Many of them act as potent sterilants causing reproductive abnormalities including ovarian regression, abnormal or arrested oocyte development and vitellogenesis [1].

In recent past some botanicals have been reported to exhibit sterilant activity against insect pests. Application of *Pachypodanthium staudtii* [Annonaceae] on the beetles *Acanthoscelides obtectus* and *Callosobruchus maculatus*; triterpenes from *Dysoxylum malabaricum* Bedd. [Meliaceae] on *Anopheles stephensi* Liston and *Clausena dentata* [Rutaceae] [2] on *Helicoverpa armigera* Hubner resulted in reproductive abnormalities like reduced reproductive potential, inhibition of egg production, abnormal vitellogenesis and oocyte maturation and disturbance of ovarian protein synthesis [3]. The essential oil of *Aegle marmelos* [L.] at different doses significantly reduced oviposition of *Callosobruchus chinensis* in treated cowpea seeds [4].

On this back drop Andrographolide a labdane diterpenoid isolated from the leaves of the medicinal plant *Andrographis paniculata* is chosen [Fig 1]. There is growing interest in natural terpenoids because of their wide spectrum of activities against insect pests [5].

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 [6].

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 [7]. In the present work an attempt has been made to screen the anti fertility activity of Andrographolide on the stored grain pest *Tribolium confusum*.

MATERIALS AND METHODS

The *Tribolium confusum* was reared on jowar flour at a temperature of $27 \pm 1^{\circ}\text{C}$ and RH (relative humidity) $60 \pm 5\%$. Freshly ecdysed fifth instar, sixth instar larvae and zero-hour pupae were treated topically on the abdominal region with $1\mu\text{g}/\mu\text{l}$ of Andrographolide with acetone as the carrier solvent with the help of Hamilton micro syringe. Thirty larvae and pupae were treated each time with Andrographolide and the experiments were replicated five times. Controls were treated each time with an equivalent volume of carrier solvent acetone. After total absorption of Andrographolide 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.

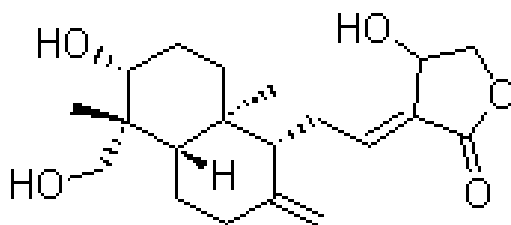


Fig1. Structure of Andrographolide

RESULTS

The control females displayed no ovarian abnormalities at any stage of the development where as the Andrographolide treated resultant insects showed severe disorders in the development of the ovaries. The ovaries of pupal-adult intermediates were undeveloped. They were small with underdeveloped vitellarium [Fig 2]. The resultant treated abnormal adults and few morphologically normal adults of fifth instar, sixth instar larvae and pupae showed severe abnormalities in the ovaries. In most of the cases the length of the ovarioles varied. In some ovarioles mature terminal oocytes remained unovulated resulting in resorption of the distal oocytes. [Fig 3]. Extreme condition of resorption was observed in certain cases wherein the ovarioles appeared as long continuous tubes [Fig 4]. In the ovaries of few treated resultant abnormal adults and morphologically normal adults few ovarioles exhibited degeneration while in the rest of the ovarioles mature terminal oocytes failed to oviposit blocking the oviduct [Fig 5].



Fig 2. Ovary of pupal- adult intermediate with underdeveloped vitellarium



Fig 3. The ovary of abnormal adult showing ovarioles of varying length. The enlarged terminal oocytes unable to ovulate resulting in resorption of distal oocytes

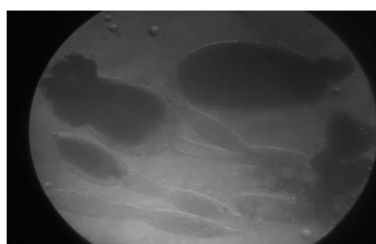


Fig 4. The ovarioles of abnormal adult exhibiting resorption of oocytes and appearing as continuous long tubes



Fig 5. The ovarioles of abnormal adult varying in length and size showing degeneration and unovulated terminal oocytes blocking the oviduct

DISCUSSION

The interruption of insect reproduction is an important and potent effect. Andrographolide prevented normal growth and development of the ovaries of *Tribolium confusum*. This prevention of ovarian development can lead to sterility in *Tribolium confusum*.

The ovaries of pupal-adult intermediates were undeveloped. They were small with underdeveloped vitellarium. No vitellogenesis was observed and remained immature. The ovaries of *Dysdercus similis* treated with methanol leaf extract of *Chrysanthemum indicum* also remained immature and failed to differentiate into germarium and vitellarium [8].

Ovarian development was drastically affected in Andrographolide treated resultant adults of *Tribolium confusum*. The ovaries of treated resultant abnormal adults and few treated resultant morphologically normal adults showed variation in the length and size of the ovarioles, ovariole degeneration, oocyte degeneration, resorption and inhibition of oviposition. Similar results were observed in *Dysdercus similis* [9].

In many cases yolk deposition was observed only in the terminal oocytes of the ovarioles. The distal oocytes degenerated due to the failure of the terminal oocytes to oviposit. Similarly application of extract of

Pachypodanthium staudtii [Annonaceae] on the beetles *Acanthoscelides obtectus* and *Callosobruchus maculatus* resulted in abnormal vitellogenesis and oocyte development [10].

According to Kaur *et al.* [11] any alteration in the juvenile hormone production results in resorption of the oocytes and suppression of oocyte growth. In *Tribolium confusum* application of Andrographolide may have disturbed the titre of juvenile hormone leading to resorption of the oocytes.

The inhibition of oviposition observed in most cases may be due to the interference of Andrographolide with the feedback mechanism which brings about oviposition as also reported by Slama *et al.* [12] with juvenoid. However Nayar [13] has reported that the fully grown eggs liberate an ovarian hormone which causes the neurosecretory cells to release a principle which in turn stimulates the mobility of the oviduct, thus inducing ovulation. The lack of this ovarian hormone may also be the cause for inhibition of oviposition in *Tribolium confusum*. Inhibition of oviposition was also observed in *Tribolium castaneum* and *Sitophilus oryzae* treated with essential oils from *Myristica fragrans* and *Illium verum* [14].

In certain cases vitellogenesis was not uniform. In abnormal adults the development of the oocytes was disorganized. In most of the cases ovarioles varied in length and exhibited degeneration. Extreme condition of resorption was observed in certain cases wherein the ovarioles appeared as long continuous tubes. Sometimes fully mature eggs were unable to oviposit. According to Kaur *et al.* [9] all these abnormalities show that mostly the prefollicular cells which later differentiate into follicle cells and perform either the function of yolk deposition or resorption are greatly affected.

The ovarian abnormalities observed in the present investigation were similar to those reported by Revathi *et al.* [15], Raghunath rao *et al.* [16] and Kaur *et al.* [17, 18].

The Andrographolide might have disturbed the uptake of proteins by the ovaries of *Tribolium confusum*. Hence the treated resultant adults exhibited disturbed vitellogenesis and reduced fecundity. Our results are in conformity with Chellayan and Karnaver [19] in *Trogoderma granarium*, Stark *et al.* [20] in *Ceratitis capitata*, Parkman and Pirenkowski [21] in *Liriomyza erifolu* and Rembold [22] in *Locusta migratoria*.

CONCLUSION

It can be concluded that Andrographolide inhibits ovarian development, affecting the fertility and the reproductive potentiality of *Tribolium confusum* suggesting its use for the development of safe and specific anti-fertility agent for the control of this stored grain pest.

Acknowledgements

The authors are thankful to Natural Products Lab, Department of Chemistry, Osmania University, Hyderabad, Andhra Pradesh for providing Andrographolide and to Prof. Naidu Ashok, Principal, Nizam College [A] Osmania University for extending lab facilities and for his encouragement.

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