# The ecdysone mimic, methoxyfenozide, alters the level of major haemolymph proteins in the larvae of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae)

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## ABSTRACT

Insect growth regulators (IGRs) belong to a class of compounds which interfere with normal growth, development and reproduction of insects. Through greater selectivity of action IGRs have less undesirable effects on man, wild life and environment. Many of the IGRs mimic the action of insect hormones, ecdysone or juvenile hormone (JH). Methoxyfenozide is a potent non-steroidal ecdysone agonist developed as an insecticide and is effective against lepidopteran pests. When the protein profile of the haemolymph of the methoxyfenozide treated 5th instar larvae of *Spodoptera mauritia,* a pest of paddy, were analyzed by SDS-PAGE, there was an increase in intensity of the three major protein bands in the treated larvae compared to control. The polypeptides increased in intensity are 51 kDa , 78 kDa and 115 kDa in size. The total protein concentration of the treated larvae also increased in conformity with the observed protein profile changes. The major proteins in the haemolymph of lepidopteran insects are members of the storage protein family and as storage proteins are crucial for insect development they may be targeted for developing better insect control strategies.

**Keywords**: *Spodoptera mauritia*, haemolymph protein, ecdysone agonist, methoxyfenozide

## INTRODUCTION

Insects are the largest groups in the animal kingdom. Some of them are pests and cause considerable economic loss. Hormones influence every aspect of insect's life, especially molting and metamorphosis. Ecdysone and juvenile hormone (JH) play a crucial role in insect development. Juvanile hormones are a group of acyclic sesquiterpenoids that regulate many aspects of insect physiology. It has a wide range of functions in regulating development and physiological process such as metamorphosis, caste determination, ovarian maturation, diapause and migration in insects [1]. Ecdysone is a steroidal prohormone of the major insect molting hormone 20-hydroxyecdysone. Ecdysone is secreted from the prothoracic glands and it along with JH regulates the process of moulting and many other metabolic processes.

Insect Growth Regulators (IGR's) belong to a group of compounds which interfere with normal growth, development and reproduction in insects by disrupting hormonally regulated physiological processes in insects. Several such compounds are known and their effects on metamorphosis and reproduction in a number of insect species have been extensively studied [2,3]. These compounds

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are considered to be useful in pest control programs because they are target-specific, non-persistent, biodegradable and environmentally benign substances with less toxicity to non-target organisms. Many IGR's are ecdysone or juvenile hormone agonists. Diacylhydrazines (DAHs) are potent nonsteroidal ecdysone agonists, and four of them, tebufenozide, methoxyfenozide, chromafenozide, and halofenozide, have been developed as insecticides. Although these compounds are very toxic to insects, they are relatively safe for mammals and are environmentally benign. Their action on insects is also selective; the first three are effective against Lepidoptera but weakly active or inactive on Diptera and Coleoptera. On the other hand, halofenozide is effective on Coleoptera but mildly active on Lepidoptera [4]. Tebufenozide and methoxyfenozide possess strong ecdysone-like activity against last-instar larvae of the beet armyworm, *Spodoptera exigua*, and the cotton leafworm, *Spodoptera littoralis*, leading to precocious lethal moulting.

Ecdysone agonists exert their toxicity by binding to the ecdysone receptor as does the natural insect molting hormone, 20-hydroxyecdysone [5, 6, and 7]. Most common effect of ecdysone agonist treatment is precocious lethal moult [8].

Protein metabolism plays a key role in rebuilding adult structures during the transformation of larvae/pupae into adult. Hemolymph protein levels generally increase during each instar but decline during moulting. Several hemolymph proteins like insect hexamerins are thought to transport hormones, phenols /or some cuticular proteins to the hypodermis. Typically two to four physico-chemically distinct storage protein species occur. The last larval instar of holometabolous insects has been characterized by active synthesis of arylphorin (aromatic amino acids bearing storage proteins) and pupal storage proteins [9]. During metamorphosis larval plasma proteins were hydrolyzed to free aminoacids and major part being incorporate into new adult proteins. Thus haemolymph proteins are crucial for insect development. In this study we demonstrate the effect of methoxyfenozide, an ecdysone mimic, on larval protein profile of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae), or paddy army worm, a pest of paddy (*Oriza sativa)*.

## MATERIALS AND METHODS

### Chemicals

Bovine albumin fraction V, Acrylamide and bisacrylamide was from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Protein Molecular Weight Markers from Genei Pvt. Ltd., Bangalore. Other reagents used were of analytical grade.

### Collection, Rearing and Maintenance of the Larvae of *Spodoptera mauritia*

The moths were attracted by fluorescent lamps during night. They were collected using an insect sweep net. The adults were kept in glass chimneys closed at both ends with muslin cloth and fed with 10% solution of honey. The adults were allowed to lay eggs on the cloth. The caterpillars were fed with fresh, tender leaves of grass *Ischaemum aristatum*. Larvae were maintained at room temperature (28$^{o}$C.).

### Treatments

Sublethal concentration of methoxyfenozide (0.1µg/larvae) in 10µl of acetone was applied topically along the dorsal midline of meso and metathorax and to the abdomen of fifth instar larvae (day 0) using a Hamilton Micro-Syringe. Control larvae received an equal volume of acetone. Fourth instar larvae with moulting marks were separated on the previous day to get fifth instar larvae (day 0).

## Collection of Haemolymph

Larvae were anesthetized in a specimen tube using diethyl ether. One of the prolegs of larvae excised with a pair of sterilized scissors and the exuded haemolymph from each larva were drawn into separate centrifuge tubes stored at -20$^0$C.

## Estimation of Haemolymph Total Proteins

Concentration of total protein in the haemolymph was estimated by modified Lowry's method [10] using bovine serum albumin (BSA) as standard.

## Electrophoretic Analysis of Haemolymph proteins

The protein samples were subjected to SDS-PAGE under reducing conditions using 10% acrylamide in a mini slab gel according to the method described by the Laemmli [11].

# RESULTS AND DISCUSSION

## Quantitative changes in haemolymph protein

Total haemolymph protein concentration of fifth instar (day 0) larvae of *Spodoptera mauritia* treated with methoxyfenozide increased significantly (p value is 0.005) compared to control (Table 1). The increase in haemolymph protein concentration may be due to the effect of methoxyfenozide on synthesis or degradation of proteins/peptides in the haemolymph.

Table 1. Quantitative changes in haemolymph total protein of methoxyfenozide treated and untreated *S.mauritia* fifth instar larvae (day 0).

| Treatment | Haemolymph protein concentration µg/µl (mean ± SE) | |
|---|---|---|
| | Control | Test |
| Methoxyfenozide 0.1 µg/larvae | 8.43 ± 0.12 | 9.32 ± 0.09 |

Effect of methoxyfenozide on haemolymph protein profile

When protein profile of the haemolymph of the methoxyfenozide treated larvae were analyzed by SDS-PAGE, there was an increase in intensity of three major protein bands (51.29 kDa, 77.62 kDa and 114.8 kDa ) in the treated compared to control after 24 hour of exposure to methoxyfenozide (0.1µg/larvae) (Figure 1).

These protein bands are the major polypeptides in the haemolymph of 5$^{th}$ instar larvae. Storage proteins represent the predominant proteins in the haemolymph of insect larvae. The most abundant storage proteins that accumulate in the hemolymph or fat body are composed of six subunits and thus are also called hexamerins. Hexamerins are mainly synthesized by the fat body during larval development, stored in the hemolymph, and serve as sources of nitrogen and amino acids for pupae and adults during metamorphosis and reproduction.

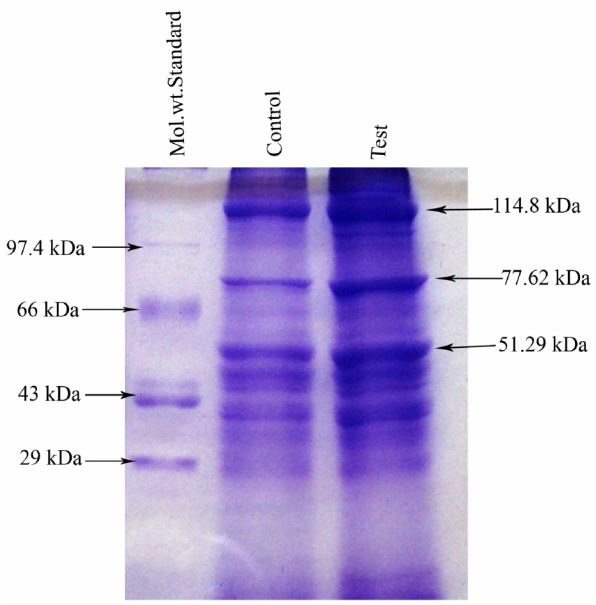


Figure 1. Polyacrylamide gel (10%) electrophoresis of haemolymph of 5th instar larvae of *S.mauritia* treated with methoxyfenozide and control. Haemolymph was collected after 24 hours of treatment and equal volume of haemolymph (10 µl/well) from test and control loaded.

Three storage proteins named SL-1, SL-2 and SL-3, the former two being synthesized only in the last larval instar, were purified from haemolymph of the common cutworm, *Spodoptera litura* [12]. All these three storage proteins have molecular sizes between 400 and 450 kDa, and are composed of subunit(s) which range in size from 70 to 80 kDa. Two storage hexamerins have been cloned and characterized from *Spodoptera exigua* [13]. Alteration in protein profile is also reported from methoxyfenozide treated *Bomboxi mori* larvae [14].The major polypeptides affected by methoxyfenozide in *S.mauritia* may probably represent the subunits of the storage proteins. Methoxyfenozide may be affecting the synthesis/degradation of the major haemolymph proteins but whether the increased concentration is offering any protection or resistance to the insecticide remains to be investigated. As storage proteins are crucial for insect development they may be targeted for developing better insect control strategies.

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## REFERENCES

[1] Riddiford LM. Adv. Insect Physiol. 1994, 24:213–274.
[2] Retnakaran A, Granett J, Ennis T,.In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, Kerkut GA, Gilbert LI(Eds.), Pergamon Press, Oxford, 1985,Vol.12: 529-601.
[3] Nair VSK. In: Chemical Ecology of Phytophagous Insects, Ananthakrishnan, TN, Raman A,(Eds), Oxford-IBH Publ. Co, New Delhi, 1993, 89-104.
[4] Nakagawa Y, Vitam Horm. 2005, 73:131-73.
[5] Wing K.D, Slawecki R., Carlson GR. Science, 1988, 241: 470–472.
[6] Darvas B, Polgar L, El-Din, HT, Eröss K, Wing KD. J. Econ. Entomol.1 992, 2107–2112.
[7] Smagghe G, Degheele D. Pestic. Sci. 1994, 42: 85–92.
[8] Dhadialla TS, Carlson GR, Le D. Annu. Rev. Entomol. 1998 ,43:545–69

[9] Wyatt, GR, Pan, ML. A rev. biochem. 1978, 47: 779-817.
[10] Sandermann, H,Jr, Stromiger JL. J. Biol. Chem, 1972, 247:5123-5131.
[11] Laemalli UK, Nature. 1970, 227:680–685.
[12] Tojo S, Yoshiga T, Insect Biochemistry and Molecular Biology. 1994, 24: 729-738.
[13] Tang B, Wang S, Zhang F. BMC Molecular Biology. 2010, 11:65.
[14] Rajathi A, Pandiarajan J, Krishnan M. Biologia. 2010, 65: 903-913.