

Toxicity of Insect Growth Regulator, Pyriproxyfen, on larvae of *Spodoptera mauritia* Boisduval (Lepidoptera: Noctuidae)

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Abstract – Insect growth regulators belong to a class of compounds which interfere with normal growth, development and reproduction in insects. Many insect growth regulators are analogues or mimics of insect hormone ecdysone or juvenile hormone. Pyriproxyfen is an insect growth regulator which mimics the action of juvenile hormone. The effects of insect growth regulators on many lepidopteran pests have been studied with respect to its toxicity and developmental alterations. But the toxicity of pyriproxyfen to larvae of *Spodoptera mauritia* is not explored. In this study we treated 3rd, 4th, 5th & 6th instar larvae of *Spodoptera mauritia* Boisduval with different concentrations of pyriproxyfen (Knack IGR). It was found that the LD₅₀ value of pyriproxyfen for the 3rd, 4th, 5th and 6th instar larvae were 14.13±2.67, 15.85±3.67, 39.81±2.61 and 316.20±2.64 µg/larvae respectively. Studies are ongoing to understand the effect of sub lethal concentrations of pyriproxyfen on the development of *S.mauritia*.

Keywords – JH Analogue, Insect Growth Regulators, Pyriproxyfen, *Spodoptera mauritia*, LD₅₀.

I. INTRODUCTION

Insects are the largest group in the animal kingdom. Some of them are pests and cause considerable economic loss. *Spodoptera mauritia* Boisduval (Lepidoptera: Noctuidae) or rice swarming caterpillar or paddy army worm is a sporadic pest of paddy distributed all over the world. *Spodoptera mauritia* has six larval instars before pupation. The larvae feed on leaves of paddy or alternate host plant such as *Ischaemum aristatum*. In India it is found in all the rice growing areas especially along the west coast and delta in Kerala and Tamil Nadu. [1]. It has attained the status of a major pest of rice in Eastern India, especially in Orissa, Chhattisgarh, Jharkhand and Bihar.

Pest management is an integral part of any successful agriculture. Application of conventional insecticides poses great threat to the human health and environment. Development of more eco-friendly pest management approaches is of prime importance for human health and environment. Use of Insect Growth Regulators (IGRs) for pest management is an alternative as they are more target-specific, non-persistent, biodegradable and environmentally benign substances, with less toxicity to non-target organisms. Insect Growth Regulators belong to a group of compounds which interfere with normal growth, development and reproduction in insects by disrupting hormonally regulated physiological processes

of insects. Several such compounds are known and their effects on metamorphosis and reproduction in a number of insect species have been extensively studied.[2,3,4] Advantages of Juvenile hormone analogues (JHAs) include their fast penetrance through the insect cuticle, needed only in low concentrations and get degraded to non toxic compounds in a short time period. Already more than 500 analogues with juvenile hormone (JH) activity have been discovered. Among the well known JHAs are, Epofenonane, Methoprene, Hydroprene, Kinoprene, and Fenoxycarb.[5] The earlier reported JHA of commercial success were Methoprene and Hydroprene.[6] Methoprene is active against dipteran insects and fleas and hydroprene is active against cockroach. These compounds however, were too unstable under field conditions to be used for agriculture.[7] Krysan, J. L. (1990) reported the photostable JH analogue, fenoxycarb was effective not only on household pests but also on agricultural pests such as leaf rollers and codling moth.

Pyriproxyfen is a JH analogue preventing the larvae from developing in to adulthood and thus rendering them unable to reproduce. Most common morphogenetic effect of JHA treatment is the production of extra larval, nymphal or pupal form. The formation of extra larval instar depends on stage and age of the larvae at the time of treatment. Pyriproxyfen has relatively low mammalian toxicity and was first registered in Japan in 1991 for controlling public health pests.[8] Pyriproxyfen is a commonly used insect growth regulator against whiteflies.[9,10] In 1996 it was introduced in US to protect cotton crops against whitefly attack. The utility of pyriproxyfen in whitefly management was demonstrated based on suppression of embryogenesis and adult formation in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae).[10] and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae).[11] In a leaf-disk bioassay using pyriproxyfen on the oblique banded leaf roller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), the LC₅₀ values for males and females were found to be 2.4 and 4.8 ppm, respectively.[12] Moadeli *et al* (2014)[13] reported that in a leaf dip bioassay with recommended field rate (1000 ppm) of pyriproxyfen, the 1st instar larvae of *Spodoptera exigua*, showed delay in larval development and in turn pyriproxyfen prolonged the feeding period and growth. In their study when compared

to control the mean generation time was higher in pyriproxyfen treated insects.

Though insecticides containing pyriproxyfen like 'Knack IGR' are used against a variety of pests, the toxicity of pyriproxyfen is not tested on the larvae of *Spodoptera mauritia*. More over determination of the toxicity of pyriproxyfen will be helpful in determining the sub lethal concentration for use in experiments to study effects on development. In the present study we used 'Knack IGR', a pesticide containing pyriproxyfen, as the active ingredient to test its toxicity on the different larval stages of *Spodoptera mauritia*.

II. MATERIALS AND METHODS

A. Collection, Rearing and Maintenance of the Larvae of *Spodoptera Mauritia* Boisid.

The moths were attracted by fluorescent lamps during night. They were collected using an insect sweeping net. The adults were kept in glass chimneys closed at both ends with muslin cloth and fed with 10% solution of honey. The adults lay eggs on the cloth covering the container. The caterpillars were fed with fresh, tender leaves of the grass *Ischaemum aristatum*. Larvae were maintained at room temperature (28°C) with relative humidity of 70-80%

B. Treatment to Test Toxicity of Pyriproxyfen

From the laboratory culture the 3rd, 4th, 5th and 6th instar larvae were sorted out on the basis of moulting marks. Different concentrations of pyriproxyfen in acetone was applied topically on the dorsal side of the 3rd, 4th, 5th & 6th instar day 0 larvae of *Spodoptera mauritia* using a Hamilton Micro-Syringe in a total volume of 2µL. An equal volume of acetone was applied in the same manner to the control larvae. At least 3 replicates were done for each experiment and the number of larvae per experiment varied from 10 to 15. Mortality was recorded after 24 hours and from the average percentage mortality for different concentrations of pyriproxyfen, LD₅₀ value for each instar was calculated from a plot of log concentration versus percentage mortality.

III. RESULTS

Table I: Percentage mortality of 3rd, 4th, 5th & 6th instar larvae of *Spodoptera mauritia* treated with different concentrations of pyriproxyfen

Amount of pyriproxyfen applied/ larva	Average percentage mortality ± SE			
	3 rd instar	4 th instar	5 th instar	6 th instar
Control	0	0	0	0
5µg	14.4±2.1	12.5±2.5
10µg	25±5.0	27.5±6.4
25µg	85±2.9	70.8±5.1	15.8±2.0
50µg	96.7±3.3	91.7±4.4	65.8±2.2
100µg	100±0.0	100±0.0	75±2.9	8.13±2.8
125µg	96.7±3.3	13.8±1.3
200µg	32.5±2.5
300µg	45±5.0
400µg	73.3±1.7

A. Toxicity of Pyriproxyfen to Larvae of *Spodoptera Mauritia*

The average percentage mortality for 3rd, 4th, 5th & 6th instar larvae of *Spodoptera mauritia* treated with different concentrations of pyriproxyfen was calculated (Table 1.) With increase in concentration of pyriproxyfen, the mortality increased in all the instars of larvae tested.

B. Calculation of LD₅₀ value

The LD₅₀ value (24 hours) of pyriproxyfen for the 3rd, 4th, 5th & 6th instar larvae of *Spodoptera mauritia* was found to be 14.13±2.67, 15.85±3.67, 39.81±2.61 and 316.20±2.64 µg/larvae respectively (Table 2)

Table 2: LD₅₀ value (24 hours) of pyriproxyfen for 3rd, 4th, 5th & 6th instar larvae of *Spodoptera mauritia*

SL. NO.	LARVAL NSTAR	LD ₅₀ VALUE (µg) (MEAN ±SE)
1	THIRD	14.13±2.67
2	FOURTH	15.85±3.67
3	FIFTH	39.81±2.61
4	SIXTH	316.20±2.64

IV. DISCUSSION

When the 3rd instar day 0 larvae were treated with 5, 10, 25, 50 and 100 µg per larva of pyriproxyfen, the average percentage mortality after 24 hours was found to be 14.38±2.13, 25±5.0, 85±2.88, 96.67±3.33 and 100±0.0 percentage respectively. The LD₅₀ 24 hours, calculated for the 3rd instar larvae is 14.13±2.67µg (Table: 2). In the case of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) 3rd instar larvae, the reported LC₅₀ value based on a leaf dip bioassay is 1.223 g L⁻¹. [14]. When pyriproxifen was incorporated in the artificial diet of ten-day-old larvae of Indian meal moth *Plodia interpunctella* (Lepidoptera: Pyralidae) at 0.02, 0.04, 0.08, 0.16, and 0.3 ppm concentrations, it resulted in increased duration of the larval period till the emergence of adults, decreased adult longevity and significant reduction in mean number of eggs laid by adults compared to the controls[15]

In the case of 4th instar day 0 larvae treated with pyriproxyfen, 5, 10, 25, 50 and 100µg per larva, the average percentage mortality after 24 hour was found to be 12.5±2.50, 27.5±6.37, 70.83±5.07, 91.67±4.41 and 100±0.0 percentages respectively. The LD₅₀ 24 hour for the 4th instar larvae is 15.85±3.67µg/larva. In the freshly moulted fourth instar larvae of citrus swallowtail *Papilio demoleus* (Lepidoptera: Papilionidae) topical administration of pyriproxyfen (7.5, 15, 30 and 60 µg/1µl/larva) induced a delay in larval– larval ecdysis and subsequent larval–pupal ecdysis. It is also reported that the treatment reduced the frequency of pupation, increased mortality and ecdysial failure, and inhibited adult emergence. [16]

For the 5th instar day 0 larvae there was no mortality for 5µg and 10µg pyriproxyfen per larva. Thus higher amount of pyriproxyfen was applied. The average percentage mortality after 24 hours for 25, 50, 100 and 125µg of pyriproxyfen per larva were 15.83±2.01, 65.83±2.21, 75±2.89 and 96.67±3.33 percentage respectively. The

LD₅₀ calculated based on this percentage mortality was found to be 39.81±2.61µg/larva.

Sixth instar day 0 larvae when treated with 100, 200, 300 & 400µg of pyriproxyfen per larvae, the mortality was found to be 8.13±2.77, 13.75±1.25, 32.5±2.5, 45±5.0 and 73±1.67 percentage respectively. On calculation the LD₅₀ value was found to be 316.20±2.64µg/larvae. Singh et al (2015)[17] topically administrated sub-lethal doses (0.5, 1.0, 2.5 & 5µg/ µl/larvae) of pyriproxyfen and difenolan on the 6th instar larvae of *Spodoptera litura* (Lepidoptera: Noctuidae) and reported that these JHAs severely hampered the metamorphosis and development with prolonged larval duration, mortality, ecdysial failure, formation of larval - pupal mosaics, reduced pupation and formation of abnormal pupae, complete suppression of adult emergence and production of adultoids. Significant differences in number and hatchability of eggs, wing abnormalities and morphological ovarian abnormalities were observed when pyriproxyfen was topically applied to *Spodoptera litura* (Lepidoptera: Noctuidae). Application of 0.1 ng of pyriproxyfen to day-1 female pupae and 0.125 µg to day-0 6th stadium larvae reduced the total number of eggs oviposited and hatchability of eggs. Day-1 pupal stage treated with 0.3 ng of pyriproxyfen showed wing abnormalities and about 40% of female adults showed morphological ovarian abnormalities. [18]

As pyriproxyfen is effective at lower concentrations to disrupt the larval development leading to failure in healthy adult emergence, most of the studies are concentrated on sublethal effects. In this study we showed that pyriproxyfen at relatively higher concentration causes death of the larvae in 24 hours after application. The results can be extrapolated to find sub-lethal concentration such as LD₁₀ or other sub lethal concentrations for *S. mauritia* for experiments to study its effect on development. Studies are ongoing to find effect of sub lethal concentration of pyriproxifen on development of *Spodoptera mauritia*.

V. CONCLUSIONS

At higher concentrations, pyriproxyfen caused the death of the larvae of *S.mauritia* after 24 hours and with increase in concentration of pyriproxyfen, the mortality also increased in 3rd 4th 5th and 6th instar larvae. The LD₅₀ value of pyriproxyfen for the 3rd, 4th, 5th and 6th instar larvae of *Spodoptera mauritia* was found to be 14.13±2.67, 15.85±3.67, 39.81±2.61 and 316.20±2.64 µg/larvae respectively.

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