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Effect of Pyriproxyfen on Orthaga Exvinacea Hampson (Lepidoptera: Pyralidae)

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Abstract
Pyriproxyfen is an important Insect Growth Regulator (IGR) mimicking Juvenile hormone. IGRs are usually used to control insect pests in Integrated Pest Management (IPM) Programme. In the present study topical application of Pyriproxyfen produced irregular moulting, dark cuticle, shrinkage of the body and mortality in the larvae of Orthaga exvinacea. When the larvae were fed with Pyriproxyfen treated mango leaves, they showed feeding inhibition and weight reduction.
Keywords: *Feeding Inhibition; Insect Growth Regulator; Integrated Pest Management Programme; Juvenile Hormone; Weight Reduction*

1. Introduction
Mango is a highly demanded fruit crop widely grown in India with much export value. A lot of insects, mites and nematodes are known to attack mangoes all over the world and of which more than 100 species have been reported from India (Tandon and Verghese, 1985; Srivastava, 1998). Orthaga exvinacea Hampson, (Lepidoptera: Pyralidae), known commonly as Mango leaf webber is a major insect pest responsible for low productivity (Sreenivasa Reddy, 2013) [1-3]. The larvae of this pest loosely web many leaves together and remain inside for feeding (Kannan & Rao,2006). The totally affected tree shows a burnt appearance. In the present investigation, effect of a few concentrations of Pyriproxyfen (PPN) on Orthaga exvinacea has been studied. Pyriproxyfen (4-phenoxy phenyl (RS)-2-(2)-pyridiloxy propyl ether) is an important Insect Growth Regulator (IGR) able to mimic Juvenile Hormone (JH). IGRs based on insect hormones have great significance as excellent environmentally friendly chemical probes to elucidate the role of hormones in the basic physiological processes of insects [5]. In insects endogenous JH is secreted by corpora allata (CA) which control many physiological processe metamorphosis, reproduction, behavior etc. (Gelman, et al., 2007). To carry out these natural activities a critical endogenous titer of JH is essential in the haemolymph. IGRs based on JH can produce an imbalance in the endogenous titer and thus an interference to the above mentioned activities which may lead to the disruptive metabolism and failure of successful reproduction [6-9]. Williams (1967) suggested that JH or its analogues/agonists could be used as specific control agents against insect pests. JH analogues/agonists are highly effective and environmentally safe. Palumbo (2001) has reported that PPN is slow acting with long residual effects and selective on target species. PPN is reported to have immense effect against Lepidopterans (Smagghe and Degheele, 1994).

2. Materials and Methods
Pyriproxyfen (PPN) was obtained from Dr. V.M. Kannan, Professor, Department of Zoology, University of Calicut, Kerala, India [10]. PPN was dissolved in acetone and measured quantities of this was used for treatments using a 2 µl &20 µl micro pipette. About two hundred larvae were collected from an infested mango tree from the Little Flower College campus and kept in large glass bottles filled with fresh mango leaves. The bottles were covered with a muslin cloth and were kept as the stock culture. Larvae of the stock culture were closely examined under Magnus Binocular Stereo Zoom dissection microscope and studied their morphological features. Length and width of the larvae were measured by placing each larva on a glass slide kept over a graph

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paper. Seventy developmentally synchronous larvae were isolated from the stock culture and were divided into four experimental sets of ten larvae each and three control sets comprising of ten larvae each [11-13]. Each set of larvae were reared and maintained in separate culture bottles containing fresh mango leaves The first three experimental sets of larvae were applied topically with 1µg/1µl, 10µg/10µl and 20µg/20µl of PPN respectively. Two sets of control larvae were maintained against these sets topically applied with 1 µl and 20µl of acetone respectively. The day of the treatment was considered as day 0 and successive days were counted as day 1 and so on. All sets of larvae were closely observed on each post treatment for mortality or any other morphogenetic changes. The fourth experimental set of larvae was kept in a beaker and fed with known amount of mango leaves applied with 20µg PPN. The control larvae were also fed with equal amount of normal leaves [14]. The weight of the larvae was taken in subsequent days to calculate the weight reduction of experimental larvae over control [15].

3. Results & Discussion

3.1.Results

3.1.1.Effects of Topical Application of 1µg PPN PPN

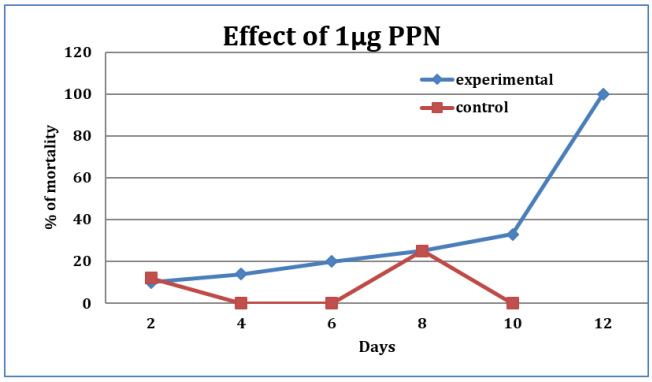

Figure 1 Effects of 1µg PPN on % Mortality

On day 1 of treatment no mortality was observed among the treated larvae but 10% of the larvae were less active and another 10% were shrunken with discoloration. 80% of the larvae showed moulting. On subsequent post treatment days moultings were observed in treated larvae. 100 % mortality was occurred by day 12. The colour of the skin changed to dark brown in all the dead larvae. Mortality was less in control larvae compared to the experimental. They showed pupation and adult emergence [16-19].

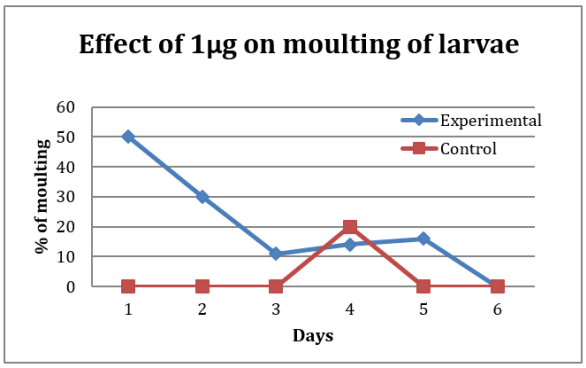

Figure 2 Effects of 1µg PPN on % Moulting

3.1.2.Effects of Topical Application of 10µg PPN

33.3% mortality was observed among the treated larvae on day 1 [20]. The remaining larvae appeared less active and their body were shrunken. The control larvae appeared normal and no mortality was observed among them. 33% mortality was observed among the treated larvae on day 2. Another notable feature was the darkening of the cuticle on the mid dorsal region of some larvae [21]. The remaining larvae appeared less active. 16. 5% larvae showed moulting. 50% mortality was observed among the treated larvae on day 3. The remaining larvae were less active. The control larvae appeared normal and no mortality was observed among them. On day 5 of treatment 100% mortality was observed among treated larvae [22-24]. The control larvae appeared normal except 10% mortality on day 4.

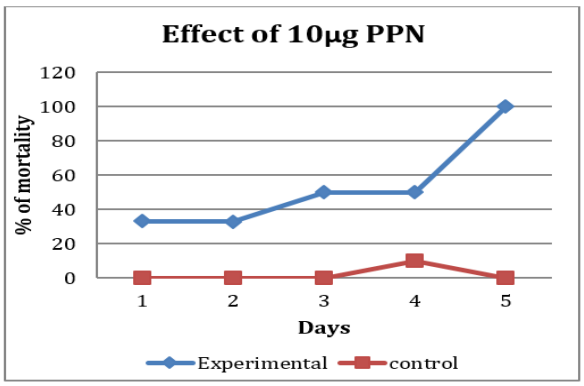

Figure 3 Effects of 10µg PPN on % Mortality

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Figure 4 Larvae with Dark Cuticle and Shrunken Body Treated with 10µg PPN

Figure 5 Control Larva

3.1.3.Effects of Topical Application of 20µg PPN

On day 1, 83% mortality was observed among treated larvae and 12.3% mortality among control larvae. The remaining treated larvae became less active [25].

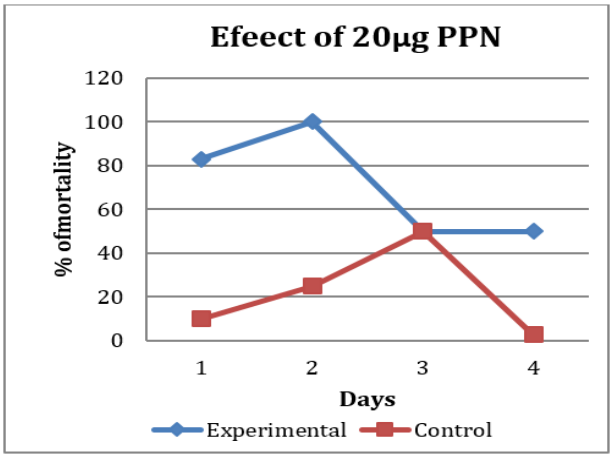

Figure 6 Effects of 20µg PPN on % mortality

3.1.4.Effect of 20µg PPN Applied to The Leaves

On day 1 of treatment no mortality was observed among the treated larvae except they were less active [26]. But the colour of cuticle became darker in subsequent days. They treated larvae showed weight reduction (Table 1) and feeding inhibition over control [27].

Table 1 Weight Reduction in Treated Larvae

Days	Control larvae	Treated larvae
	Average Weight in gms	
0	0.99	1.38
1	3.56	1.9
2	3.62	1.86
3	3.80	1.81
4	3.81	1.77
5	3. 87	1.61
6	4.25	0.79
7	4.49	0.48
8	4.59	0.35

4. Discussion

In the present study PPN treatments caused mortality in Orthaga exvinacea [28]. Early studies show that PPN cause direct mortality as well as strong sublethal effects on treated nymphs of Sunn pest Eurygaster integriceps Puton (Mojaver and Bandani, 2010). Similar results were reported {Lee (2001), Nayar et al (2002), Yapabandara & Curtis (2004), Nishiura et al (2005), Darriet & Corbel (2006), Thanuja & Nair (2008), Nampelah et al (2022)}. The notable features in the present investigation was the extra moultings in the treated larvae and the melanization of cuticle. Application of PPN to the last instar larvae of Spodoptera litura, Manduca sexta (Hatakoshi, et al., 1988) and the German cockroach, Blattella germanica (Reid, et al., 1994) induced moulting of larvae into supernumerary larvae. Richardson (2007) has reported extra moults on Aphis glycines Matsamura; Ouchi (2004) on Plutella xylostella. The status quo action of JH has been well reported in several insect orders, particularly in Coleoptera, Orthoptera and Lepidoptera, in which JH treatment caused supernumerary larval moulting. It is assumed that the brain of the larvae were presumed to be activated to secrete Ecdysteroids when a high dosage

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of PPN is introduced. Tunaz, and Uygun (2004) have reported in PPN induced larval moults, Ecdysteroid titer peaked in the penultimate larval instar. Melanization of cuticle was reported in Apis mellifera due to PPN treatments (Zufelato, et al., 2000). In the present investigation feeding inhibition and larval weight reduction were high in treated larvae. This might be due to the effect of PPN on the midgut tissues which could have negatively affected the feeding of the larvae. Studies show that JH analog, acts on Aedes aegypti by interfering with the expression of genes involved in 20E action resulting in a block in midgut remodeling and death during their development (Wu, et al; 2006). In the present investigation pupation did not take place in treated larvae. In control larvae pupation took place and adults emerged too. Studies of Sial and Brunner (2010) show that the pupation and adult emergence was significantly delayed by PPN treatments in Choristoneura rosaceana. Studies show that PPN affects the hormonal balance and results in a strong suppression of metamorphosis and adult formation. In normal conditions ecdysteroid titer drop to a negligible level towards the end of pupal phase for the eclosion hormone to be released for adult emergence (Truman, 1971; Riddiford, 1985). Application of PPN might have produced abnormally high endogenous ecdysteroid levels or might have delayed ecdysteroid peak and might have blocked the release of the eclosion hormone in Orthaga exvinacea.

Conclusion

In the 1µg PPN treatment, no mortality was observed in initial days and 80% of the larvae showed moulting but 100 % mortality was occurred by day 12. The colour of the skin changed to dark brown in all the dead larvae. In the 10µg PPN treatment 33.3% mortality was observed on day 1. The remaining larvae appeared less active and their body were shrunken. The control larvae appeared normal and no mortality was observed among them. Another notable feature was the darkening of the cuticle on the mid dorsal region of some larvae. 16. 5% larvae showed moulting. 50% mortality was observed among the treated larvae on day 3. On day 5, 100% mortality was observed among treated larvae. The control larvae showed 10% mortality on day 4. In 20µg PPN treatmentso On day 1, 83% mortality was observed and 12.3% mortality among control larvae. In the experiment of 20µg PPN applied to the leaves on day 1 no mortality was observed but the colour of cuticle became darker in subsequent days. They treated larvae showed weight reduction (Table 1) and feeding inhibition over control.

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