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EFFECT OF INSECT GROWTH REGULATORS; LUFENURON, PYRIPROXYFEN AND METHOXYFENOZIDE FOR THE CONTROL OF *Trogoderma granarium* (EVERTS) (COLEOPTERA: DERMESTIDAE)

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Technical grade material of three insect growth regulators viz., lufenuron, pyriproxyfen and methoxyfenozide were evaluated against eggs, larvae and pupa of *Trogoderma granarium* Everts under laboratory conditions. The tested IGRs were applied at four concentrations (i.e., 5, 10, 15 and 20ppm) at optimum conditions of 30 ± 2°C and 60 ± 5% r.h. The results revealed that pyriproxyfen showed highest (100%) ovicidal effect followed by methoxyfenozide and lufenuron, respectively. While against larvae, lufenuron was the most effective (74.86%) while pyriproxyfen was least effective. Adult suppression was minimum (23.41%) in case of methoxyfenozide treatment while highest (89.74%) in pyriproxyfen treated pupae. The results about the F_1 adult progeny emerge from adult exposed to the IGRs treated diet showed that pyriproxyfen gave 100% adult suppression and resulted into the formation of supper larvae followed by lufenuron and methoxyfenozide. Results regarding the effect of concentration showed that a direct dose dependent response was observed in all the bioassays. The current investigations suggest that the lufenuron would be a better option for the management of *T. granarium.*
Keywords: Insect growth regulators, lufenuron, pyriproxygen, methoxifenozide, *Trogoderma ranarium*, ovicidal, mortality, F_1 adult progeny.

INTRODUCTION

In spite the wide use of chemical pesticides for the pest management, insect populations remain the major competitors of human beings for food. During storage the damage caused by insects may accounts for 10-40% (Raja *et al*., 2001; Papachristos and Stamopoulos, 2002). The attack of stored grain insects has a direct effect on quality and quantity of the stored cereals and their products (Burkholder and Faustini, 1991; Campbell and Arbogast, 2004). It resulted not only by feeding, but also by frass accumulation (Mondal, 1994), webbing (Hill, 1990) and secretion of quinones (El-Mofty *et al*., 1989; Mondal, 1992).

Current control strategies include the use of conventional synthetic insecticides and fumigants. But their indiscriminate uses have some inherent problems associated with them like destruction of beneficial insects, environmental hazards (Marx, 1977; Pimental, 1983), development of resistance (Arthur, 1996). Consequently, there is dire need to introduce alternative control techniques which are more effective, less persistent, with low toxicity to non-target organisms, more pests specific and relatively safer to the environment. In the search of new control tactics, insect growth regulators have been receiving a great interest of stored product insect control entomologists (Fox, 1990). They possess a novel mode of action, affecting the molting and metamorphosis process in insects (William, 1956; Oberlander *et al*., 1997; Mondal and Parween, 2000; Oberlander and Silhacek, 2000). The have ovicidal, larvicidal and lethal affect to pupae as well as F_1 adult progeny (Mian and Mulla, 1982).

Based on their mode of action Insect growth regulators have been divided into three groups: (i) juvenile hormone analogues (JHAs), (ii) ecdysteroid agonists and (iii) chitin synthesis inhibitors (CSIs) (Wing and Aller, 1990; Oberlander *et al*., 1997; berlander and Silhacek, 2000). JHAs are responsible for the maintenance of larval stage of insect (Edwards and Menn, 1981; Mamatha *et al*., 2008). Larvae treated with JHAs resulted into abnormal pupae. They also inhibit the embryonic development (Oberlander *et al*., 1997), influence the mating performance (Segura *et al*., 2009) and may (Chanbang *et al*., 2008) or may not (Wijayaratne *et al*., 2012) affect the fecundity of adults.

The use of edcysone agonists leads to premature synthesis of insect cuticle and also causes feeding inhibition regardless of the age or instar of insect (Schneiderman, 1972; Fox, 1990; Wing and Aller, 1990). They also have chemosterilant activity when female have exposed to them (Heller *et al*., 1992).

CSIs interferes with the biosynthesis of chitin in insects and thus prevent moulting and formation of new cuticle (Ishaaya

and Casida, 1974; Hammock and Quistad, 1981; Mondal and Parween, 2000). They can also suppress the entire life cycle quite effectively (Verloop and Ferrell, 1977). By affecting the hormonal balance, they disrupt several physiological processes in insect body (Salama *et al*., 1976; Yu and Terriere, 1977; Mondal and Parween, 2000).

The objective of the current studies was to test the efficacy of pyriproxyfen (JHAs), lufenuron (CSI) and methoxyfenozide (Ecdysone agonist) for their possible biological activity as grain protectant against *Trogoderma granarium.*

MATERIALS AND METHODS

Insects: The population of *Trogoderma granarium* was obtained from the laboratory colonies of the Department of Entomology at University of Agriculture Faisalabad, Pakistan and they had no former history of pesticide disclosure. A diet of sterilized wheat grains was used for the colony maintenance at temperature 30 ± 2°C and 60 ± 5% r.h.

***Insect Growth Regulators*:** The IGRs used in these studies were: pyriproxyfen (99% Purity); lufenuron (99.7% purity); and methoxyfenozide (99.8% Purity) provided by Sigma-Aldrich Inc. USA. All compounds were technical grade materials. Stock solutions of 200ppm of technical grade materials were prepared by using acetone as solvent, giving a concentration of 0.202mg of active ingredient per ml. This stock solution was further diluted to get the desired concentrations of 2.5, 5, 7.5, 10, 15 and 20ppm. All the treatments were replicated three times with one untreated check (acetone only).

***Ovicidal Effect*:** To study the ovicidal activity of these test IGRs, the 24 h old eggs were used. To collect eggs, 100 adults of mixed ages and sexes of *Trogoderma granarium* were placed in plastic containers (400ml) with perforated lid containing wheat flour. The containers were held in incubator under optimum condition of growth and development for 24 h period of time. One-day-old eggs were sifted from the diet using a No. 80 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/ 0.18mm hole size). The eggs were placed singly with the help of small paint brush into small glass vials (each treated with 100µl solution of the desired concentrations in its base). After hatching in control treatments, the numbers of larvae in other treatments were also counted.

***Larvicidal Effect*:** In the larval exposure experiment, a group of twenty 1st instar larvae of test insect were placed into 100ml glass container containing 15g IGRs treated diet for each concentration (i.e. 2.5, 5, 7.5, 10, 15 and 20ppm) along with one untreated control treatments. The IGRs were applied to the diet with the help of fingertip sprayer. These 1st instar larvae were obtained from newly hatched eggs. After releasing the larvae into the IGRs treated diet, the plastic containers containing treated di*et* along with larvae were place into the incubator at 30 ± 2°C and 60 ± 5% r.h. Data regarding larval mortality will be taken after 14 days of treatment application.

***Effect on Pupae Treatment*:** For pupal bioassay, one to two days old pupae were used. Each pupa was treated with 0.5µl volume containing the desired concentrations. After treatment the petri dishes containing these treated pupae were placed into the incubator set at 30 ± 2°C and 60 ± 5% r.h. The pupae were examined after one week. Data regarding percentage mortality was observed on the base of the number of adult emerged in relation to the number of pupae per petri dish. Uncompleted emerging adults were counted as dead.

RESULTS

***Ovicidal Effect*:** Results regarding ovicidal effect (Fig 1) of IGRs under study showed that the main effects (IGRs and their concentrations) and their associated interaction were significant. Hatching was maximum (about 100%) in control treatment, followed by lufenuron at 2.5ppm, while at highest concentration (20ppm) of lufenuron the hatching was 33.33%. Hatching inhibition was highest (100%) in case of pyriproxyfen treatment even at 5ppm. In case of methoxyfenozide application the highest hatching inhibition was 80% at 20ppm concentration followed by 15ppm (74.35%) while inhibition was minimum (23.35%) at 5ppm. From this experiment it is concluded that pyriproxyfen was most effective in term of its ovicidal action followed by methoxyfenozide and lufernuron, respectively.

***Larvicidal Effect*:** In larvicidal bioassay, it is evident from the results (Fig 2) that all the three IGRs and their doses showed significant effect against 1st instar larvae of *Trogoderma granarium.* The effect of their interaction (IGR x Concentration) was also significant. The application of pyriproxyfen resulted in 66.10% larval mortality at 20ppm followed by 52.55% at 15ppm dose rates. Minimum mortality was 22.03% at 5ppm in pyriproxyfen treatment. Highest mortality (100%) was observed in lufenuron treatment application to the larvae even at 10ppm followed by 7.5, 5 and 2.5ppm with mortality values 98.30, 91.52 and 83.05%, respectively. In case of methoxyfenozide, highest mortality was 78.33% at 20ppm, followed by 15ppm (63.33%) and 10ppm (48.33%), while minimum mortality was 28.33% at 2.5ppm. From these results it was obvious that there was a direct dose response relationship. The overall results of this larvicidal bioassay showed that the lufenuron was the most effective while pyriproxyfen was the least effective for their mortality effect against 1st instar larvae.

***Effect on Pupae Treatment*:** In pupal treatment bioassay, results (Fig 3) revealed that the effect of IGRs, concentrations and their interaction was statistically significant. The adult emergence was highest (73.33%) at 1.5ppm concentration in methoxyfenozide treated pupae after control treatment. In pyriproxyfen treatment, the maximum adult emergence was

46.67% at 2.5ppm and, was minimum (3.33%) at 20ppm dose rate. Adult emergence in case of lufenuron treatment was 63.33, 50.00, 43.33, 36.67, 30.00 and 13.33% at 2.5, 5, 7.5, 10, 15 and 20ppm, respectively. Adult emergence was minimum in case of pyriproxyfen and maximum in case of methoxyfenozide treatment application. In case of pyriproxyfen and lufenuron treatment, a lot of pupal adult intermediate was also observed.

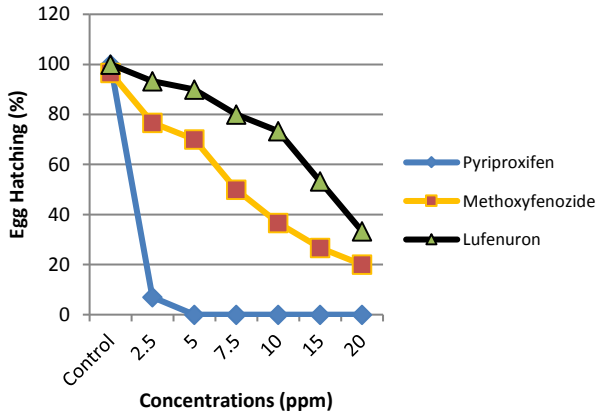

Figure 1: Ovicidal effect against the eggs of *T. granarium* due to the direct application of pyriproxyfen, methoxyfenozide and lufenuron at different concentrations.

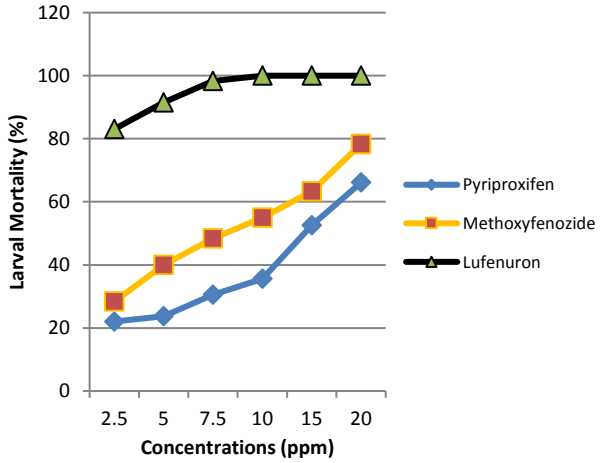

Figure 2: Mortality effect on larvae of *T. granarium* after 14 days of exposure in pyriproxyfen, methoxyfenozide and lufenuron treated diet.

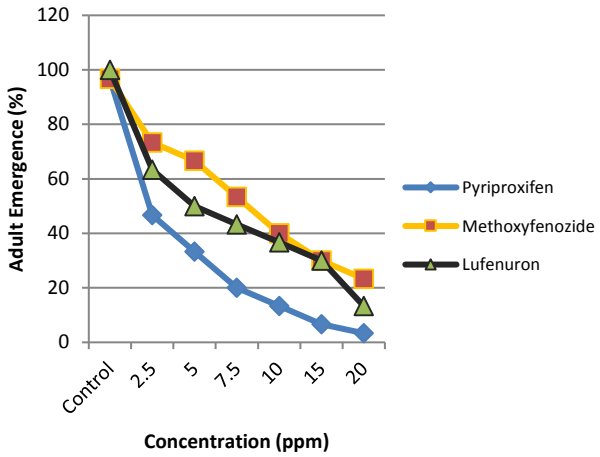

Figure 3: Adult emergence (%) from the pupae treated with direct application of pyriproxyfen, methoxyfenozide and lufenuron.

DISCUSSION

The current study showed that the application of pyriproxyfen to eggs resulted in 100% hatching inhibition even at 5ppm concentration. Similar results have been reported when pyriproxyfen was tested against eggs of *Eurygaster integriceps* (Mojaver and Bandani, 2010). Bhargava and Urs (1993) testified ovicidal effect of hydroprene against eggs of *Corcyra cephalonica.* The application of pyriproxyfen to 1st instar larvae of *T. granarium* have also been resulted in significant larval mortality. Dhadialla *et al.* (1998) reported that JHAs are quite effective at the early stages embryogenesis and metamorphosis in insects. In our study the direct application of pyriproxyfen to pupae have resulted in pupal adult intermediate formation and reduced adult emergence. But pyriproxyfen showed no effect when it was feed through diet to the two week old adults. Our results are in agreement with Arthur (2001) who studied the effect of hydroprene against *T. confusum* and *T. castaneum.*

The use of methoxyfenozide also has significant effect on egg hatching inhibition and larval mortality. Similar kind of results have been reported against *Plodia interpunctella* due to the application of RH-5849, methoxyfenozide tebufenozide and methoxyfenozide (Subramanyam *et al*., 2000). The work of Silhacek *et al.* (1990) and Oberlander *et al.* (1998) have also been reported similar results due to the effect of ecdysteroid. In our findings methoxyfenozide showed less activity against pupal inhibition.

Our findings regarding the effect of lufenuron against all the four stages (egg, larva and pupae) of *T. granarium* showed that it have some effect on egg hatching but have a strong effect (100% mortality) on 1st instar larvae after fourteen days exposure to treated diet. Its direct application to pupae has also been resulted to pupal mortality and pupal adult

intermediate formation. Our results of lufenuron are in accordance with that of Salokhe *et al.* (2003) who reported the effect of flufenoxuron in *T. casteneum* and found similar developmental abnormalities. Our findings are also supported by the work of Arora *et al*. (2012) who reported the effcct of sub-lethal concentrations of lufenuron against different developmental stages of *T.casteneum.* Lufenuron have very little effect against adults when feed through diet. Similar results have been reported by Arthur (2004) against the adults of *R. dominica* due to the application of *s*-methoprene.

In conclusion, application of tested IGRs has significant effect against various stages of *T. granarium*. Therefore, these compounds should be considered as potential candidates in the integrated management of stored product pests due to their ovicidal and larvicidal action. These bioassays also revealed that in most case efficacy was dose dependent. So, additional studies should be carried on to confirm the above findings and to examine the compatibility of these IGRs (particularly chitin synthesis inhibitors) with other low risk control tactics directing to provide long term protection in stored grains and their products.

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