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Biological Activity of Insect Growth Regulators, Pyriproxyfen, Lufenuron and Methoxyfenozide against *Tribolium castaneum* (Herbst)

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ABSTRACT

Three insect growth regulators (IGRs), juvenile hormone analogues (pyriproxyfen), ecdysone agonist (methoxyfenozide) and chitin synthesis inhibitor (lufenuron) were tested against egg, larva, pupa and adult stages of *Tribolium castaneum.* Keeping laboratory conditions constant at 30±2°C and 60±5% R.H, six doses (2.5, 5, 7.5, 10, 15 and 20ppm) of these IGRs were applied to observed their effects. The results showed that highest (100%) ovicidal action was observed in case of pyriproxyfen followed by methoxyfenozide and lufenuron, respectively. For their lethal effect against larvae, lufenuron (100%) was most effective, while pyriproxyfen (66.10%) was least effective. In case of pupae treatment, adult inhibition was highest (96.67%) in pyriproxyfen treatment and minimum (26.67%) in methoxyfenozide. The results regarding adult exposure to these IGRs treated diet showed that pyriproxyfen have no effect on adult mortality. While the adult mortality due to lufenuron and methoxyfenozide was 33.33 and 23.33%, respectively. Results concerning the efficacy of concentration showed that there was a direct dose dependent response. The present data suggest that the lufenuron (chitin synthesis inhibitors) was most effective than others for the management of *T. castaneum.*

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Authors' Contribution
MUH designed the study. QA conducted the experiments, recorded data and wrote the article. MUH, MS and LJM supervised the work and provided guidance for preparation of manuscript. NJ statistically analyzed the data.

Key words
***Tribolium castaneum*, insect growth regulators, pyriproxyfen, lufenuron, methoxifenozide, hatching, mortality, adult emergence**

INTRODUCTION

In spite of the wide use of chemical pesticides for pest management, insect population remains the major competitor of human beings for food (Udo, 2011). During storage the damage caused by insect pests may account for 10-40% (Raja *et al*., 2001; Papachristos and Stamopoulos, 2002). The attack of stored grain insect pests has a direct effect on quality and quantity of the stored cereals and their products (Burkholder and Faustini, 1991; Campbell and Arbogast, 2004). These losses resulted not only because of feeding, but also by frass accumulation (Mondal, 1994), webbing (Hill, 1990) and secretion of quinones (El-Mofty *et al*., 1989; Mondal, 1992). *Tribolium castaneum* (Herbst) is cosmopolitan pest having an extensive association with stored foodstuff (Danahaye *et al*., 2007). In case of heavy infestation, the flour becomes mouldy and greyish having unpleasant smell (Atwal and Dhaliwal, 2005). Economic loss caused by this pest, estimated to be of 40% in wheat flour (Ajayi and Rahman, 2006).

Current control strategies include the use of conventional synthetic insecticides and fumigants (Jackai and Asante, 2003). But their indiscriminate use has some inherent problems like destruction of beneficial insects, environmental hazards (Marx, 1977; Pimental, 1983) and development of resistance (Arthur, 1996). Consequently, there is dire need to introduce alternative control techniques which are more effective, less persistent, having low toxicity to non-target organisms, more pest specific and relatively safer to the environment. In the search of new control tactics, insect growth regulators (IGRs) have been receiving a great interest of stored product insect control (Fox, 1990). IGRs 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). They have shown lethal effect against eggs, larvae, pupae as well as F_1 adult progeny (Mian and Mulla, 1982).

Based on their mode of action IGRs 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; Oberlander and Silhacek, 2000). JHAs are responsible for the maintenance of shape and form 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*.,

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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 instars of insect (Schneiderman, 1972; Fox, 1990; Wing and Aller, 1990). They also have chemosterilant activity when females are exposed to them (Heller *et al*., 1992).

CSIs interfere 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 *T. castaneum.*

MATERIALS AND METHODS

Insects

The population of test insect, *T. castaneum* was obtained from the laboratory colonies of the Department of Entomology at Purdue University, West Lafayette, Indiana USA and they had no former history of pesticide exposure. A diet of wheat flour mixed with 10% (by wt) Brewer's yeast 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 (JHAs) (99% purity); lufenuron (CSI) (99.7% purity) and methoxyfenozide (ecdysone agonists) (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 age and sex of *T. castaneum* were placed in plastic containers (400ml) with perforated lid containing wheat flour and brewer's yeast 9:1 ratio as diet. The containers were held in incubator (Model MIR-254, SANYO) under optimum condition of growth and development for 24 h period of time. After this 24 h period, the eggs were sifted from the diet using a No. 80 sieve (Seedburo Equipment Company (Des Plaines, IL, USA)/ 0.18 mmhole size). Ten eggs were placed singly with the help of small paint brush into each small glass vials having capacity of 10ml (treated with 100µl solution of the desired concentrations in its base). After hatching in control treatments, the number of larvae in other treatments were also counted.

Larvicidal effect

In the larval exposure experiment, a group of twenty, 1st instar larvae of test insects were placed in 100ml plastic container containing 15g IGRs treated diet (wheat flour mixed with 10% brewer's yeast) for each concentration (*i.e.,* 2.5, 5, 7.5, 10, 15 and 20ppm) along with one control treatment (acetone only). The IGRs (40gm/4ml) 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 diet along with larvae were placed in the incubator at 30±2ºC and 60±5% r.h. Data regarding larval mortality was taken after 14 days of treatment.

Effect on pupae

For pupal bioassay, one to two days old pupae were used. Each pupa was treated with 0.5µl of each IGR containing the desired concentrations. There were four replications for each treatment with one un-treated check (acetone only). Direct topical application method was used to treat the pupae. After treatment the petri dishes containing these treated pupae (ten pupae per petri dish) were placed in the incubator at 30±2ºC and 60±5% r.h. The pupae were examined after one week. Data regarding percentage mortality was observed on the basis of the number of adults emerged in relation to the number of pupae per petri dish. Incomplete emergence of adult was also taken as dead.

Effect on adult mortality

In the adult exposure experiment, twenty mixed sex adults of test insects (aged 12-15 days after eclosion) were kept on food (wheat flour plus brewer's yeast) treated with a series of IGRs concentrations (*i.e.,* 2.5, 5, 7.5, 10, 15 and 20ppm) along with an untreated control, in three replicates for each treatment. Parental mortality was assessed after a period of 14 days exposure to IGRs treated or untreated diet.

All the collected data were analyzed statistically, and comparison of means were done by using Tuckey's HSD Test ($P = 0.05$).

RESULTS

Ovicidal effect

Results regarding ovicidal effect (Fig. 1) of IGRs showed that the main effects (IGRs and their concentrations) and their associated interactions were significant. Hatching was maximum (about 100%) in control treatment, followed by lufenuron (93.33%) at 2.5ppm, while at highest concentration (20ppm) of lufenuron the hatching was 33.33%. Hatching inhibition was highest (100%) in the 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 *T. castaneum.* 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

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 2.5ppm 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. Adult emergence in the 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 the case of pyriproxyfen and maximum in the case of methoxyfenozide treatment. In the case of pyriproxyfen and lufenuron treatment, a lot of pupal adult intermediate was also observed.

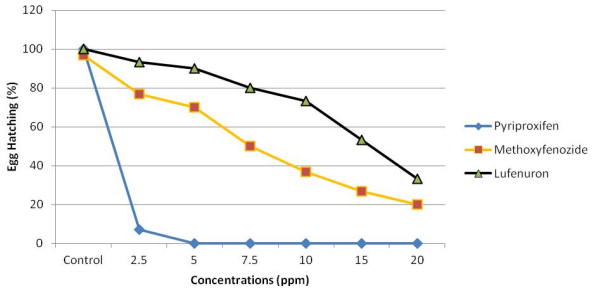

Fig. 1. Ovicidal effect against the eggs of *T. castaneum* due to the direct application of pyriproxyfen, methoxyfenozide and lufenuron at different concentrations.

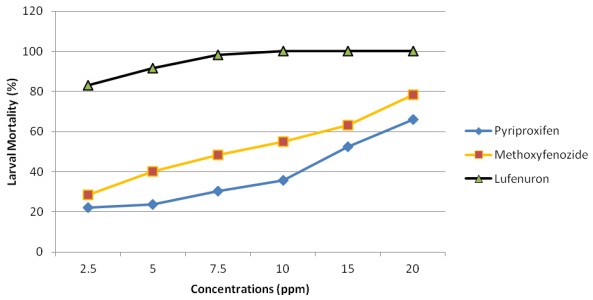

Fig. 2. Mortality effect on larvae of *T. castaneum* after 14 days of exposure in pyriproxyfen, methoxyfenozide and lufenuron treated diet.

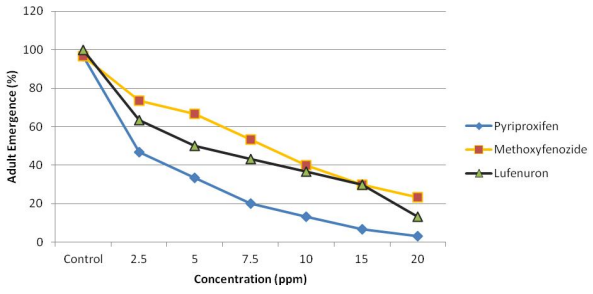

Fig. 3. Adult emergence (%) from the pupae treated with direct application of pyriproxyfen, methoxyfenozide and lufenuron.

Effect on adult mortality

In this experiment, results (Fig. 4) showed that the adult exposure to pyriproxyfen treated diet have no effect on adult mortality. Similarly, adult exposure to

methoxyfenozide and lufenuron also showed very little effect on adult mortality of *T. castaneum.* In case of lufenuron treatment to diet, highest adult mortality was 33.33% followed by 21.67% at 20 and 15ppm, respectively. While in methoxyfenozide treated diet maximum mortality was 23.33% at 20ppm concentration.

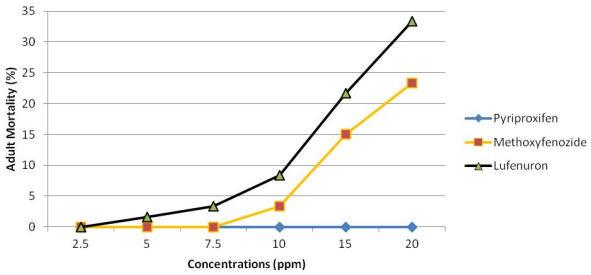

Fig. 4. Mortality of *T. castaneum* adults after 14 days of exposure in pyriproxyfen, methoxyfenozide and lufenuron treated diet.

DISCUSSION

The current study showed that the application of pyriproxyfen (JHAs) 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. castaneum* 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. Our results were also supported by the findings of the Saleem and Shakoori (2000) who observed the effect of diflubenzuron and permethrin against the larval of *T. castaneum*. 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 fed through diet to the two week old adults. Our results are in agreement with Arthur (2001) who reported that the application of hydroprene against *T. confusum* and *T. castaneum* can results greater that 75% reduction in population development.

The use of methoxyfenozide also has significant effect on egg hatching inhibition and larval mortality. Similar kind of results has been reported against *Plodia interpunctella* due to the application of RH-5849, 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 and adult mortality.

Our findings regarding the effect of lufenuron against all the four stages (egg, larva, pupae and adult) of *T. castaneum* 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. castaneum* and found similar developmental abnormalities. Our findings are also supported by the work of Arora *et al*. (2012) who reported the effect 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 *Rhyzopertha dominica* due to the application of *s*-methoprene.

In conclusion, application of tested IGRs has significant effect against various life stages of *T. castaneum.* Therefore, these compounds should be considered as potential candidates in the integrated management of stored product pests. These results also revealed that the 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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Statement of conflict of interest

Authors have declared no conflict of interest.

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