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SCREENING OF SEVEN INSECT GROWTH REGULATORS FOR THEIR ANTI-INSECT ACTIVITY AGAINST THE LARVAE OF *Trogoderma granarium* (EVERTS) AND *Tribolium castaneum* (HERBST)

Qurban Ali[1], Mansoor ul Hasan[2,*], Muhammad Sagheer[2], Shahzad Saleem[3], Muhammad Faisal,[2] Asma Naeem[4] and Jamshed Iqbal[5]

[1]Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan; [2]Department of Entomology, University of Agriculture, Faisalabad, Pakistan; [3]Department of Biosciences, COMSATS Institute of Information Technology, Sahiwal, Pakistan; [4]Biochemistry and Toxicology Research Lab., Department of Zoology, University of the Punjab Lahore Pakistan;[5]Department of Entomology, Gomal University, Dera Ismail Khan, Pakistan.
[*]Corresponding author's e-mail: mansoorsahi2000@yahoo.com

Seven insect growth regulators (IGRs) (One juvenile hormone analogues [Pyriproxyfen 10.8EC], two ecdysone agonist [Tebufenozide 20SC, Methoxyfenozide 240SC] and four chitin synthesis inhibitor [Lufenuron 50EC, Flufenoxuron 10DC, Triflumuron 20SC, and Buprofezin 25%WP]) were tested under laboratory conditions against the larvae of *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst). Four concentrations of these IGRs (2.5, 5, 7.5, and 10ppm) were applied to observe their effects at 30±2°C and 65±5% r.h. after 7 and 14 days of treatment application. The overall results showed that all the tested IGRs showed significant larvicidal activity against the larvae of both *T. granarium* and *T. castaneum*. Results revealed that the highest larval mortality of *T. castaneum* was 77.30 and 83.54% with flufenoxuron followed by methoxyfenozide (70.40, 76.65%), pyriproxyfen (56.61, 62.86%) and lufenuron (53.16, 59.41%) at 7 and 14 days after treatment (DAT). The lowest mortality 42.81 and 49.06% was resulted in case of triflumeron treatment after 7 and 14 days, respectively. Similarly, In case of *T. granarium* the highest mortality was 66.96 and 73.20% due to the effect of flufenoxuron, and was lowest 32.47 and 38.72% where triflumeron was applied after 7 and 14 days, respectively. Results regarding the comparative effect of different concentrations showed that highest mortality 65.23% in *T. castaneum* and 54.88% in *T. granarium* larvae was noted at 10 ppm and mortality was lowest at 2.5 ppm against both the test insects. Bioactivities of all the tested IGRs were found to be dose and exposure period dependent. The results regarding the relative potency of the entire tested IGRs indicated that they were more effective when applied on wheat as compared to rice and maize. From these results, it is concluded that the applications of IGRs have proved to be very effective against both species examined in the present study. These compounds particularly chitin synthesis inhibitors (CSIs) (flufenoxuron and lufenuron) should be considered as potential components in IPM of stored grains insect pests.
Keywords: Insect growth regulators, Chitin synthesis, larvicidal effect, stored grains, postharvest losses

INTRODUCTION

Storage of cereals and their products is a vital part of post-harvest operations, through which food commodities passes from its way from farmer field to the ultimate consumer. Annual post-harvest losses of stored cereals due to various biological factors in the storages range from 10-20% of overall production (Phillips and Throne, 2010). As in field crops, the stored foodstuffs are under attack of a wide range of insect pests such as, *Trogoderma granarium*, *Rhyzopertha dominica*, *Tribolium castaneum*, *Sitotroga cerealella* and *Sitophilus* spp. These are of economic importance because they feed on a wide range of stored cereals and their products (Arbogast, 1991; Khan *et al.,* 2010; Udo, 2011). The current study is focused on only two insects that are *Trogoderma granarium* and *Tribolium castaneum.*

Trogoderma granarium (Everts) is a serious pest of stored grains and their products (Burges, 2008; Mark *et al*., 2010). It has a great economic importance, due to its capability to cause huge loss through ravenous feeding and heating of grains and to withstand starvation in larval stage (Rees, 1998). Similarly, *Tribolium castaneum* (Herbst) is most malicious and cosmopolitan pest having an extensive association with human beings stored food (Via, 1999). Both larvae and adults cause damage. In case of heavy infestation, the flour becomes mouldy and greyish having an unpleasant smell. Due to this infestation, flour becomes unfit for human intake (Atwal and Dhaliwal, 2005). Economic loss caused by this pest is estimated to be of 34% in stored millet and 40% in wheat flour (Ajayi and Rahman, 2006).
The use of conventional insecticides and fumigants against insect pests of stored commodities has been resulted in

complications like development of resistance, resurgence and residual toxicity. These issues have argued to develop more effective and comparatively safer insecticides (Smet *et al*., 1990). Insect Growth Regulators (IGRs) are biodegradable in the environment (Staal, 1975; Zurfleuh, 1976) and possess low mammalian toxicity to non-target organisms (Staal, 1975; Oberlander *et al*., 1997). Chitin synthesis inhibitors belong to the IGRs group, prevent normal molting of larval insects by disrupting the normal process of cuticle formation. Silhacek and Oberlander (1975) reported that IGRs are quite effective against the insecticides resistant strains. According to Slama (1971), Wright and Spades (1972) these IGRs could be used insect pest management techniques, due to their high biological activity. Insect pests of stored commodities can also be controlled by the use of potentially available IGRs (i.e. methoprene and hydroprene) (Loschiavo, 1976; Nickle, 1979). All of these features make them potentially effective alternatives to typical pesticides for insect pest control.

Keeping in view the potential effectiveness of IGRs, present studies were conducted to investigate the insecticidal effect of seven insect growth regulators *viz.*, lufenuron, flufenoxuron, pyriproxyfen, tebufenozide, methoxyfenozide, triflumuron and buprofezin against the larvae of *T. granarium* and *T. castaneum.*

MATERIALS AND METHODS

Present studies were carried out at the Grain Research, Training and Storage Management Cell of the Department of Entomology at the University of Agriculture, Faisalabad Punjab, Pakistan.

***Handling and rearing of insects*:** Mixed age cultures of *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst) were collected from farm houses as well as wheat stores at Punjab Food Departments located at various districts in Punjab province, Pakistan. Culture of *T. granarium* and *T. castaneum* was reared on healthy sterilized wheat grains and wheat flour, respectively. The insects were reared in glass jars, each containing one kg of sterilized wheat grain/flour. The jars were covered with muslin cloth and placed in the laboratory at 30±2°C and 65±5% relative humidity with a photoperiod of 16:8 L:D. The *T. granarium* and *T. castaneum* pupae were separated from the heterogeneous cultures obtained from the aforementioned locations and kept in an incubator (Model MIR-254, SANYO) at optimum conditions until adult emergence. After 24 to 48 hours, one hundred adult beetles were released into the jars containing rearing medium (wheat grain/flour). After three days, beetles were sieved out from the rearing medium and discarded. The remaining rearing medium containing insect eggs were placed into jars and incubated at optimum growth conditions to get a homogenous population. Finally, the uniform sized progeny of these test insects were used for further bioassay studies.

***Treatments and their application*:** Commercial formulations of seven IGRs were used. These include; lufenuron 50EC (Match), flufenoxuron 10%DC (Cascade), pyriproxyfen 10.8%EC (Bruce), tebufenozide 20%SC (Top Gun), methoxyfenozide 240%SC (Runner), buprofezin 25%WP (Buprofezin) and triflumuron 20%SC (Capture).

An acetone stock solution containing 10mg of technical IGRs/ml was prepared for each chemical sample. Aliquots of each solution were then diluted to the concentration of IGR required which were 2.5, 5, 7.5 and 10ppm for each treatment. The insecticidal assays were conducted with four replicates. All chemical stocks and prepared solutions were stored at 1°C when not in use.

***Commodities*:** Different genotypes of wheat, maize and rice were used. The seeds of these cereal genotypes were obtained from Punjab Seed Corporation and were cleaned of straw and dust, prior to use.

Individual lots of 500g of wheat, rice, and maize were divided into sub-lots of 100g each (five sub-lots for each commodity). Separate insecticide solutions of 2.5, 5, 7.5 and 10 ppm of the IGRs were used to treat each commodity. In addition to the four insecticide dose rates, a fifth treatment acting as a control was untreated and sprayed only with acetone. The desired relative humidity was maintained by using a saturated sodium chloride solution (Greenspan, 1977). The grains were treated in plastic jars into which the serial dilutions of insecticides were pipetted. Care was taken that the acetone had evaporated from the treated and control samples by mixing and ventilating the culture medium for 24 h before insects were added to the treated food.

***Bioassay*:** In this experiment, a group of twenty 3rd instar larvae of *T. castaneum* and *T. granarium* were placed into 250ml glass jars containing 50g IGRs treated diet (wheat, maize, rice), with four replicates for each IGRs concentration (2.5, 5, 7.5 and 10ppm) along with an untreated control. These larvae were exposed to IGRs treated diet until pupation and remaining stages were transferred to untreated diet. The jars were kept in 30±2°C and 65±5% r.h. Data regarding larval mortality was taken after 7 and 14 days of treatment application. The quality of the IGR's as an insecticide was calculated into a percentage by dividing the number of F_I progeny in the manipulated larvae by the untreated control.

***Statistical analysis*:** All the treatments were replicated four times using Completely Randomized Design (CRD). The collected data was analyzed statistically by using the statistical software (Stat Soft, 8.0) and the means of the treatments were compared by using a Tukey HSD test ($p \leq 0.05$).

RESULTS

***Effect on Larval Mortality of T. castaneum*:** The overall effect of all tested IGRs under study after 7 and 14 days of treatment on the larvae of *T. castaneum* is shown in Figure 1. All the insecticides have shown significant effect against larval mortality. After 7 days, the highest larval mortality (77.30%) was observed due to the application of flufenoxuron followed by methoxyfenozide, pyriproxyfen and lufenuron with 70.40, 56.61 and 53.16% mean mortality values, respectively. The lowest mortality 42.81% was observed in triflumeron treatment followed by buprofezin (46.26%). Similarly, flufenoxuron again was the most effective after 14 days and counted for 83.54% larval mortality and triflumeron were the least effective with mean mortality of 49.06%.

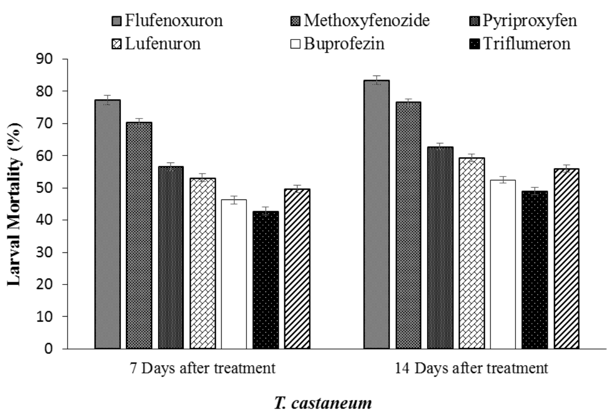

Figure 1. Mortality effect of insect growth regulators against the larvae of *T. castaneum* after 7 and 14 days of treatment application.

The comparative effect of different concentrations against the larvae of *T. castaneum* is shown in Figure 2. The results revealed that the highest mortality (65.23%) was noted at 10ppm concentration followed by 60.05% at 7.5ppm concentration and was lowest 47.12% at 2.5ppm against larvae of *T. castaneum* after 7 days of treatment application. After 14 days of treatment, the maximum mortality (71.26%) was at 10ppm and minimum (54.88%) at 2.5ppm. It is also obvious from these results that there is dose dependent response of larval mortality.

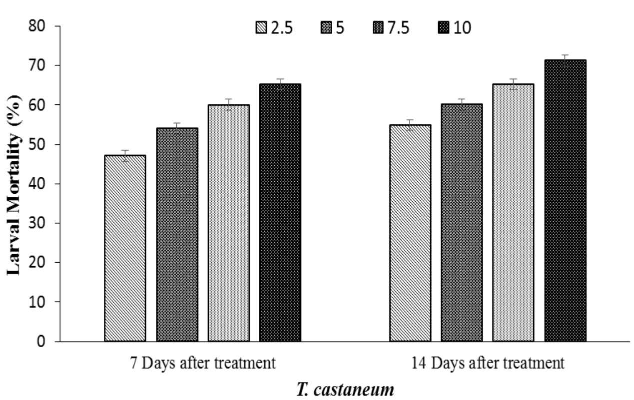

Figure 2. Effect of different concentrations of tested IGRs against the larval mortality of *T. castaneum* after 7 and 14 days of treatment application.

The results regarding the potency of the tested IGRs indicated that they were more effective when applied on wheat as compared to rice and maize (Fig. 3). After 7 days of exposure, the highest mortality 61.70% was on wheat followed by rice (57.26%) and maize (50.86%). After 14 days the lowest mortality 57.11% was on maize and highest (67.95%) on wheat.

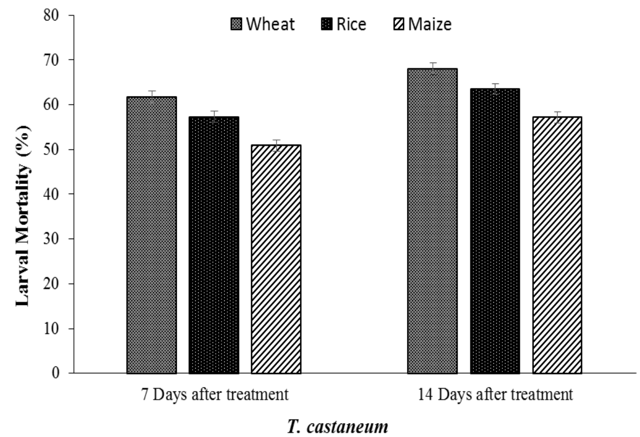

Figure 3. Response of larvae of *T. castaneum* exposed to IGRs treated diet of wheat, rice and maize after 7 and 14 days of exposure period.

The result in Table 1 shows the effect of wheat, rice and maize genotypes with the tested IGRs against the larval mortality of *T. castaneum* after 7 days of treatment. It is observed that all the IGRs have significant effect with respect to the tested commodities. The highest mortality was 85.35% which was observed on flufenoxuron treated wheat and least mortality (37.07%) was noted on maize treated with triflumuron. On rice the maximum mortality was 78.45% due to the effect of flufenoxuron followed by methoxyfenozide (68.10%), pyriproxyfen (57.76%) and lufenuron (54.31%). While in rice the lowest mortality was 43.97% due to the effect of triflumuron. On maize the highest mortality was 68.10% and it was also due to the application of flufenoxuron and it was at par with methoxyfenozide (68.10%). Lowest mortality on maize was 37.07% due to the effect of triflumuron followed by 40.52% due to the effect of buprofezin. The mortality effect after 14 days due to the interaction effect of test IGRs and wheat, rice and maize genotypes against the larvae of *T. castaneum* was shown in Table 1. The data indicated that the flufenoxuron was the most effective with mortality value 91.60% on wheat, 84.70% on rice and 74.35% on maize. The lowest mortality was due to the effect of triflumuron on maize with 43.32% mortality followed by 50.22% on rice and 53.67% on wheat. Methoxyfenozide was on 2nd number in term of its lethal effect against *T. castaneum* larvae having 81.25% on wheat, 74.35% on rice and that was on par with mortality on maize.

Table 1. Mean larval mortality of *T. castaneum* on wheat, rice and maize treated with different IGRs after 7 and 14 days of exposure.

Insect Growth Regulators	Larval Mortality (%) ± SEM					
	After 7 days			After 14 days		
	Wheat	Rice	Maize	Wheat	Rice	Maize
Flufenoxuron	85.35±1.82a	78.45±1.42b	68.10±1.74d	91.60±1.66a	84.70±1.68b	74.35±1.74d
Methoxyfenozide	75.00±1.34c	68.10±1.65d	68.10±1.56d	81.25±1.46c	74.35±1.40d	74.35±1.67d
Pyriproxyfen	61.21±1.42e	57.76±1.69f	50.86±1.81h	67.46±1.83e	64.01±1.37f	57.11±1.61h
Lufenuron	57.76±1.61f	54.31±1.32g	47.42±1.68i	64.01±1.39f	60.56±1.84g	53.67±1.84i
Buprofezin	50.86±1.94h	47.42±1.49i	40.52±1.38k	57.11±1.56h	53.67±1.28i	46.77±1.21k
Triflumuron	47.42±1.51i	43.97±1.60j	37.07±1.62l	53.67±1.92i	50.22±1.94j	43.32±1.25l
Tebufenozide	54.31±1.98g	50.86±1.53h	43.97±1.83j	60.56±1.12g	57.11±1.93h	50.22±1.68j

***Effect on larval mortality of T. granarium*:** The overall effect after 7 and 14 days of treatment of all tested IGRs against the larvae of *T. granarium* is shown in Figure 4. All the insecticides have shown significant effect against larval mortality. After 7 days, the highest larval mortality (66.96%) was observed due to the application of flufenoxuron followed by methoxyfenozide, pyriproxyfen and lufenuron with values 60.06, 46.27 and 42.82%, respectively. The lowest mortality (32.47%) was observed in triflumeron treatment followed by buprofezin (35.92%). Similarly, flufenoxuron again was the most effective after 14 days and counted for 73.20% larval mortality and triflumeron were the least effective with mean mortality of 38.72%.

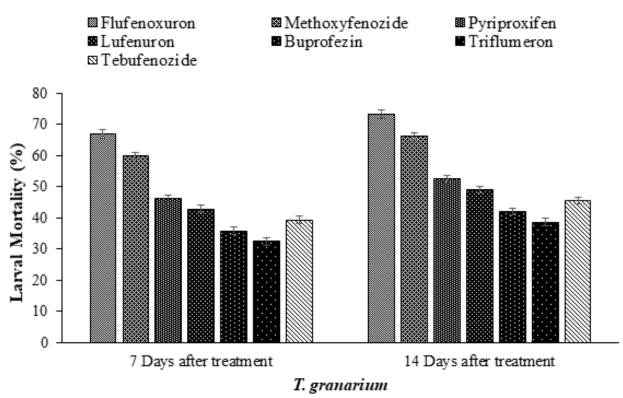

Figure 4. Mortality effect of insect growth regulators against the larvae of *T. granarium* after 7 and 14 days of treatment application

The relative effect of different concentrations against the larvae of *T. granarium* is shown in Figure 5. The results revealed that the highest mortality 54.88% was noted at 10ppm concentration followed by 49.71% at 7.5ppm concentration and mortality was lowest 36.78% at 2.5ppm against larvae of *T. granarium* after 7 days of treatment application. After 14 days of treatment the highest mortality was 60.92% at 10ppm and mortality was minimum 44.54% at 2.5ppm. It was also obvious from these results that there is dose dependent response of larval mortality.

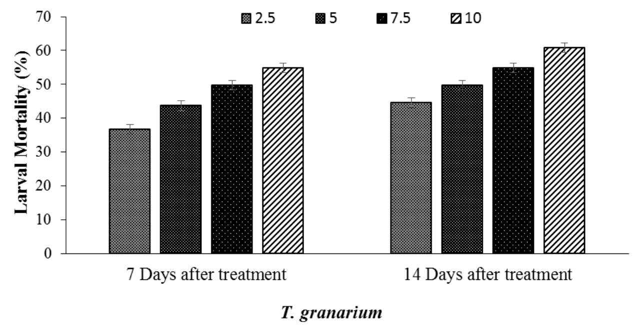

Figure 5. Effect of different concentrations of IGRs against the larval mortality of *T. granarium* after 7 and 14 days of treatment application

The results (Fig. 6) regarding the effectiveness of the entire test IGRs indicated that they were more effective when applied on wheat as compared to rice and maize. After 7 days of exposure, the highest mortality (51.35%) was on wheat followed by rice (46.92%) and maize (40.51%). After 14 days, the lowest mortality (46.76%) was on maize and the highest (57.60%) on wheat.

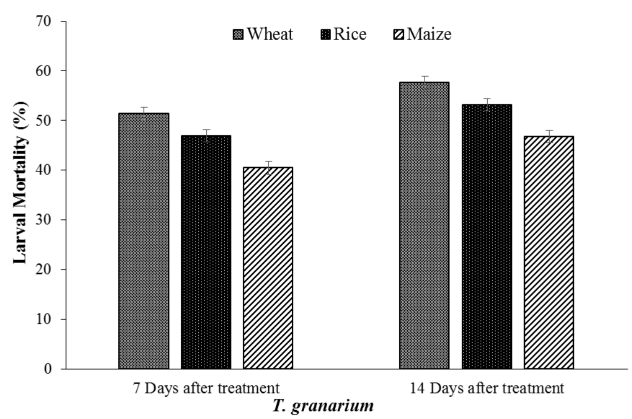

Figure 6. Response of larvae of *T. castaneum* exposed to IGRs treated diet of wheat, rice and maize after 7 and 14 days of exposure period

Table 2. Mean larval mortality of *T. granarium* on wheat, rice and maize treated with different IGRs after 7 and 14 days of treatment application.

Insect Growth Regulators	Larval Mortality (%) ± SEM					
	After 7 days			After 14 days		
	Wheat	Rice	Maize	Wheat	Rice	Maize
Flufenoxuron	75.00±1.17a	68.10±1.56b	57.76±1.76d	81.25±1.60a	74.35±1.27b	64.01±1.84d
Methoxyfenozide	64.66±1.84c	57.76±1.89d	57.76±1.41d	70.91±1.74c	64.01±1.35d	64.01±1.27d
Pyriproxyfen	50.86±1.56e	47.42±1.23f	40.52±1.34h	57.11±1.72e	53.67±1.64f	46.77±1.30h
Lufenuron	47.42±1.87f	43.97±1.35g	37.07±1.75i	53.67±1.35f	50.22±1.60g	43.32±1.68i
Buprofezin	40.52±1.78h	37.07±1.65i	30.17±1.82k	46.77±1.12h	43.32±1.68i	36.42±1.25k
Triflumuron	37.07±1.36i	33.62±1.45j	26.73±1.89l	43.32±1.64i	39.87±1.29j	32.98±1.64l
Tebufenozide	43.97±1.83g	40.52±1.83h	33.62±1.72j	50.22±1.62g	46.77±1.90h	39.87±1.61j

Table 2 showed the interaction effect of wheat, rice and maize genotypes with the IGRs under study against the larval mortality of *T. granarium* after 7 days of treatment. Overall this table shows that all the IGRs have significant effect on mortality with respect to test commodities. Flufenoxuron treated wheat showed the highest mortality of *T. granarium* (75.00%) whereas least mortality (26.73%) was noted on maize treated with triflumuron.

On rice, the maximum mortality was 68.10% with flufenoxuron followed by methoxyfenozide (57.76%), pyriproxyfen (47.42%), lufenuron (43.97%) and least mortality (33.62%) with triflumuron. On maize the highest mortality was 57.76% due to the application of flufenoxuron followed by methoxyfenozide (57.76%).

The mortality effect after 14 days due to the interaction effect of test IGRs and wheat, rice and maize genotypes against the larvae of *T. granarium* was shown in Table 2. The data represents that the flufenoxuron was the most effective with mortality value 81.25% on wheat, 74.35% on rice and 64.01% on maize. The lowest mortality 32.98% on maize, 39.87% on rice and 43.32% on wheat was due to the application of triflumuron. Methoxyfenozide was on 2nd place in term of its lethal effect against *T. granarium* larvae having 70.91% on wheat, 64.01% on rice and that was at par with mortality on maize.

DISCUSSION

The current study revealed that all the tested IGRs exhibited larvicidal activity against both *T. granarium* and *T. castaneum* which is in consistent with the already published findings on the effects of different IGRs against stored grain insect pests (Parween, 2003; Salokhe *et al*., 2003; Kostyukovsky and Trostanetsky, 2006). In present experiment, the highest larval mortality (77.30%) was caused by flufenoxuron that belongs to chitin synthesis inhibitors (CSI) group. Similar results have been reported previously (Mian and Mulla, 1982; Yasir *et al*., 2012). The effectiveness of flufenoxuron is also reported due to its contact and stomach mode of action against the larvae of coleopterous insects of stored grains (Hammann and Sirrenberg, 1980; Fox, 1990; Wang *et al*., 1994, Rees, 2004). The application of methoxyfenozide, pyriproxyfen and lufenuron has been resulted in 70.40, 56.61 and 53.16% larval mortality, respectively. Methoxyfenozide belongs to ecdysone agonists group of IGRs, they pose their lethal effect by feeding inhibition (Locke, 1964; Willis, 1974; Tunas and Uygun, 2004), paralysis and then death (Heller *et al*., 1992). These compounds (ecdysone agonists) have both contact and stomach action (Fox, 1990), and fewer have systemic action (Heller *et al*., 1992). Pyriproxyfen is a Juvenile hormone analogue; they affect the larval stages by suppressing the metamorphic changes during moulting (Edwards and Menn, 1981; Retnakaran *et al*., 1985; Fox, 1990; Sagheer *et al*., 2011). Our findings about the effect of pyriproxyfen are in consistency with Kostyukovsky *et al*. (2000) who exposed the larvae to the diet treated with insect growth regulators (methoprene and pyriproxyfen). Lufenuron was a chitin synthesis inhibitor, has significant effect against the larvae of coleopterous and lepidopterous insect pests (Mishra *et al*., 2013). Results revealed that triflumeron was less effective against larvae as compared to other CSIs (flufenoxuron, lufenuron and buprofezin) under investigation. These results are supported by the findings of Kavallieratos *et al*., (2012) who investigated the efficacy of seven insect growth regulators as grain protectant on wheat, rice and maize against *Prostephanus truncates* and *Rhyzopertha dominica*.

In the present study, the results about the effect of different concentrations showed that the mortality increased with the increase in dose rate. Maximum mortality (65.23%) was observed at highest concentration (10ppm) and the lowest mortality (47.12%) was noted at lowest concentration (2.5ppm). Similar results regarding the dose mortality response have been reported by several other researchers (Smagghe *et al*., 1996; Parveen, 2000; Kavallieratos *et al*., 2012). Similarly, there was also increase in mortality with increased exposure to the IGR treated diet as reported by Oberlander *et al*. (1975), Mondal *et al*. (1999), Sagheer *et al*. (2012) and Yasir *et al*. (2012). Results about the relative response of IGRs treated wheat, rice and maize indicated that IGRs are more effective when applied on wheat as compared to rice and maize. This may be due to more intake of IGRs treated wheat because wheat is the most factious food as compared to the rice and maize for both the targeted insect pests (Samson *et al*., 1990; Sagheer *et al.*, 2011; Kavallieratos *et al*., 2012).

***Conclusion*:** the tested IGRs have strong larvicidal effect against larvae of *T. castaneum* and *T. granarium* and may be considered as potential candidates in the IPM of stored grain insect pests. Since, these bioassays revealed the dose dependent efficacy of IGRs against targeted insects. Therefore, additional detailed studies may be carried out to examine the compatibility of these IGRs with other low risk control tactics directing to provide long term protection to stored grains products.

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