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OVICIDAL EFFECT OF INSECT GROWTH REGULATORS AGAINST EGGS OF *Trogoderma granarium*(EVERTS) AND *Triboliumcastaneum*(HERBST)

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The herein reported studies were conducted to investigate the ovicidal effect of different insect growth regulators (IGRs), viz., lufenuron 50EC, flufenoxuron 10DC, pyriproxyfen 10.8EC, tebufenozide 20SC, methoxyfenozide 240SC, triflumuron 20SC, and buprofezin 25%WP against *Trogoderma granarium* (Everts) and *Triboliumcastaneum*(Herbst). All these IGRs were applied to the glass vials at four concentrations viz., 2.5, 5, 7.5 and 10 ppm, additionally, an untreated control using four replicates through Completely Randomized Design (CRD). Results showed that highest ovicidal action was exerted by pyriproxyfen where only 32.83% hatching was recorded in *T. castaneum* eggs followed by lufenuron at 10ppm while methoxyfenozide was least effective. Similarly, in *T. granarium*, the hatching suppression was highest (38.83% hatching) due to pyriproxyfen treatment and was lowest in methoxyfenozide treatment. The growth rate was highly effected by pyriproxyfen and least effected by methoxyfenozide against both insects. 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. Hence, these compounds particularly pyriproxyfen, an analog of the juvenile hormone (JH) followed by Chitin synthesis inhibitors (i.e., flufenoxuron, lufenuron and triflumeron) should be considered as potential components in managing immature stages of stored grains insect pests.

Keywords: Insect growth regulators, ovicidal effect, *trogodermagranarium, triboliumcastaneum.*

INTRODUCTION

Storage of cereals and their products is a vital part of post-harvest operations through which food commodities passes from farmer field (producer) 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). There is a continuous need to protect the stored grains and their products against deterioration, especially the loss of weight and quality during storage. Insect pests are the main factor responsible for these losses as they reduce both the quality and quantity of grains (Weaver and Subramanyam, 2000).Sabet and Sabr, 2015).The Khapra Beetle, *Trogoderma granarium*(Everts) is a serious pest of stored grains and their products (Burges, 2008; Mark *et al*., 2010). The khapra beetle generally restricts its activity to the top 12 inches of the stored grains. The beetle has a substantial economic impact due to its capability to cause huge losses to grains through its ravenous feeding (Rees, 1998; Ahmedani*et al*., 2007). Therefore, control of this pest is not only vital to confirm food safety and food security condition, but also a pre-requisite for the export of surplus cereal grains (Ahmedani*et al*., 2007).

Red flour beetle, *Triboliumcastaneum*(Herbst) is a malicious and cosmopolitan pest having an extensive association with stored food (Hulasare*et al*., 2003; Rees, 2004). Although, *T. castaneum* is considered a secondary pest (pest of flour and other milled cereal products) (Irshad and Talpur, 1993; Suresh *et al*., 2001), a single larva of this pest can attack and damage 88 grains during its life, which when compounded by multiple individual beetles, leads to considerable loss in quality, quantity and viability of grain (Atanasov, 1978). Strictly speaking, the overall loss in quantity and quality of the stored grains, and the health hazards attributed to the insect damage as waste products translate to a monetary concern that could amount to millions of rupees further emphasizing the need to prevent infestation (Ajayi and Rahman, 2006).

At present, there is an emphasis on the use of insecticides having biorational properties (Phillips and Throne, 2010) owing to many concerns about the use of neurotoxin insecticides. This concept also includes the use of reduced risk control tactics, such as insect growth regulators (IGRs) (Oberlander and Silhacek, 2000; Arthur, 2007). The IGRs include chemicals which mimic hormones that control molting and metamorphosis in insects and thus disrupt the process of normal insect development (Kostykovsky*et al.*, 2003). The use of IGRs as grain protectants has certain advantages over conventional neurotoxic pesticides. The IGRs are biodegradable in the environment (Staal, 1975) and possess low mammalian toxicity to non-target organisms (Staal, 1975; Oberlander *et al*., 1997).Silhacek and Oberlander (1975) reported that IGRs are quite effective against conventional insecticide resistant strains. According to Slama (1971) and Wright and Spades (1972), these IGRs could be used in 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 (Loschiavo, 1976; Nickle, 1979). All of these features make them effective alternatives to typical pesticides for insect pest control.

Therefore, the present investigation was designed to assess the ovicidal effect of different insect growth regulators against *T. granarium*and *T. castaneum.*

MATERIALS AND METHODS

***Insects Culture*:** Mixed age cultures of *Trogoderma granarium* (Everts) and*Triboliumcastaneum* (Herbst) were collected from farm houses as well as wheat stores at Punjab Food Departments located at various districts in Punjab province, Pakistan. The culture of *T. granarium* was reared on healthy sterilized wheat grains, while the culture of *T. castaneum*was reared on wheat flour, apparently free from insect infestations. 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. Khapra beetle and red flour beetle pupae were separated from the heterogeneous cultures obtained from the locations above 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. After three days, beetles were sieved out from the rearing medium and discarded. The resulting rearing medium, along with eggs of these insects, 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.

***Insect Growth Regulators Application Procedure*:** Commercial formulations of seven IGRs were used 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 10 mg of technical IGR/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 10 ppm 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.

***Bioassay*:** To study the ovicidal effect of IGRs against *T. granarium* and *T. castaneum* eggs, thirty eggs having age of one day, were obtained from adults that had been fed on an untreated diet. The eggs were separated with a 70-mesh sieve and transferred to IGR glass vials. Each glass vial was treated by dipping it for 5 seconds in each of the IGRs concentrations previously described. The number of eggs that hatched during the first week was recorded under a binocular microscope. Larvae were transferred to untreated diet and survival of larvae, pupae and adults were recorded. The time taken for adult emergence was recorded,and the survival rate during the post-embryonic development was calculated using the equation: [(Number of emerged adults/ number of eggs laid)]*100.

***Statistical Analysis*:** All the treatments were replicated four times using Completely Randomized Design (CRD). Data was collected for hatching inhibition, larval emergence, percent pupation, percent adult emergence. 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

***Ovicidal Effect*:** The ovicidal action of all the IGRs under study is shown in Fig. 1 against the 24 hours old eggs of *T. granarium* and *T. castaneum.* Results indicated that the highest ovicidal action was observed due to the application of pyriproxyfen where only 32.83% hatching was observed in *T. castaneum* eggs, followed by lufenuronwith 47.16% hatching.

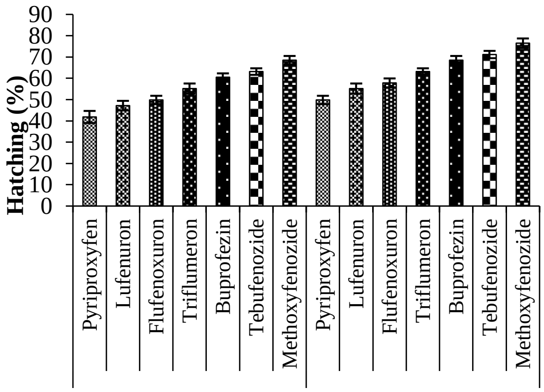

Figure 1. Comparative effect of different IGRs against egg hatchability percentage of *T.granarium* and *T. castaneum.*

The application of flufenoxuron, triflumeron and buprofezinresulted in 49.83, 55.16 and 60.50% hatching of eggs of *T. castaneum*. It was also obvious from the results that methoxyfenozide was the least effective where maximum hatching 68.50% was recorded. In *T. granarium* eggs treatment the hatching was 38.83% due to the effect of pyriproxyfen followed by lufenuron, flufenoxuron and triflumuron with 55.17, 57.83 and 63.16% hatchability were observed. Maximum hatching (76.50%) was noted where methoxyfenozide was applied.

Results concerning the effect of different concentrations on egg hatchability of *T. castaneum* and *T. granarium* were given in Fig. 2. In the case of *T. castaneum*eggs, the highest hatching 55.83% was observed at 2.5ppm that was the lowest concentration after control treatment (99.16%). At 5 and 7.5ppm concentrations, the hatching was 28.34 and 40.83%, respectively. The minimum hatching 31.67% was noted at 10ppm that was the highest concentration. In *T. granarium* at 10ppm the least hatching 41.67% occurred, followed by 7.5, 5 and 2.5ppm having 50.83, 58.34 and 65.83%, respectively. From these results, it can be concluded that the 10ppm was the most effective and 2.5ppm was the least effective concentration. It was also concluded that the ovicidal action of all the test IGRs was increased with the increase in dose rate.

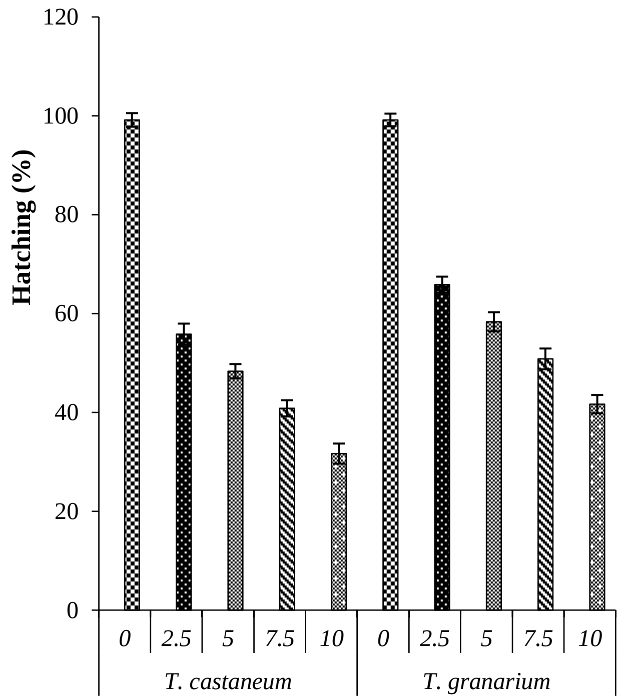

Figure 2. Comparative effect of different concentrations of test IGRs against egg hatchability percentage of *T. granarium* and *T. castaneum.*

The interaction effect of IGRs and concentrations against egg hatchability of *T. castaneum* and *T. granarium*is given in Table 1. The lowest hatching was 25.00 and 15.00% against the eggs of *T. granarium*and *T. castaneum*, respectively at 10ppm of pyriproxyfen. After control, the highest hatching 82.50% in *T. granarium* and 71.50% in *T. castaneum* at 2.5ppm of methoxyfenozidewas noted. On the basis of these results, it can be concluded that the pyriproxyfen was the most effective and methoxyfenozide was the least effective in term of their ovicidal action.

Table 1. Hatchability percentage of *T. granarium* and *T. castaneum* against IGRs applied at different concentrations

Insect growth regulators	Conc. (ppm)	Hatchability (%)	
		T. granarium	***T. castaneum***
Pyriproxyfen	0	94.17±1.83a	89.17±0.42a
	2.5	49.17±0.83j	39.17±1.65j
	5	41.67±0.96l	31.67±1.74l
	7.5	34.17±0.65m	24.17±1.56m
	10	25.00±0.73n	15.00±0.23n
Luferuron	0	92.17±1.23a	91.17±0.72a
	2.5	55.83±1.74hi	45.83±0.74hi
	5	48.33±1.83jk	38.33±0.73jk
	7.5	40.83±1.46l	30.83±1.90l
	10	31.67±1.85m	21.67±1.71m
Flufenoxuron	0	97.17±0.74a	97.17±1.22a
	2.5	59.17±1.54gh	49.17±0.62gh

	5	51.67±0.42ij	41.67±0.74ij
	7.5	44.17±0.86kl	34.17±1.34kl
	10	35.00±0.51m	25.00±1.43m
Triflumeron	0	91.17±1.45a	94.17±1.54a
	2.5	65.83±1.75e	55.83±0.65e
	5	58.33±1.09gh	48.33±1.76gh
	7.5	50.83±1.72j	40.83±1.45j
	10	41.67±1.27l	31.67±1.84l
Buprofezin	0	94.17±1.80a	82.17±0.74a
	2.5	72.50±1.73cd	62.50±0.74cd
	5	65.00±0.65ef	55.00±0.75ef
	7.5	57.50±0.72gh	47.50±0.53gh
	10	48.33±1.98jk	38.33±0.73jk
Tebufenozide	0	95.17±1.40a	98.17±1.65a
	2.5	75.83±1.54c	65.83±0.73c
	5	68.33±1.75de	58.33±0.52de
	7.5	60.83±1.44fg	50.83±1.74fg
	10	51.67±1.37ij	41.67±1.35ij
Methoxy-fenozide	0	90.17±0.74a	83.17±0.74a
	2.5	82.50±1.03b	71.50±0.86b
	5	75.00±1.65c	65.00±0.63c
	7.5	67.50±1.65e	57.50±0.64e
	10	58.33±0.82gh	48.33±0.74gh

***Effect on Growth Rate*:** The effect of all tested IGRs under investigation is shown in Fig. 3 against *T. granarium* and *T. castaneum.* Results indicated that the lowest growth rate 24.33% was observed due to the application of pyriproxyfen in *T. castaneum*, which means that pyriproxyfen was the most effective in term of its toxic effect, followed by lufenuron (28.00%). The growth rate in case of flufenoxuron, triflumeron, buprofezin and tabufenozide was 30.67, 36.00, 41.33 and 44.00%, respectively. While methoxyfenozide was the least effective where the highest growth rate 49.34% was recorded against *T. castaneum*. In *T. granarium*, the growth rate was lowest 30.83% due to the effect of pyriproxyfen followed by lufenuron, flufenoxuron and triflumuron with 36.16, 38.83 and 44.16%. In the case of *T. granarium* highest growth rate of 57.50% was also observed where methoxyfenozide was applied.

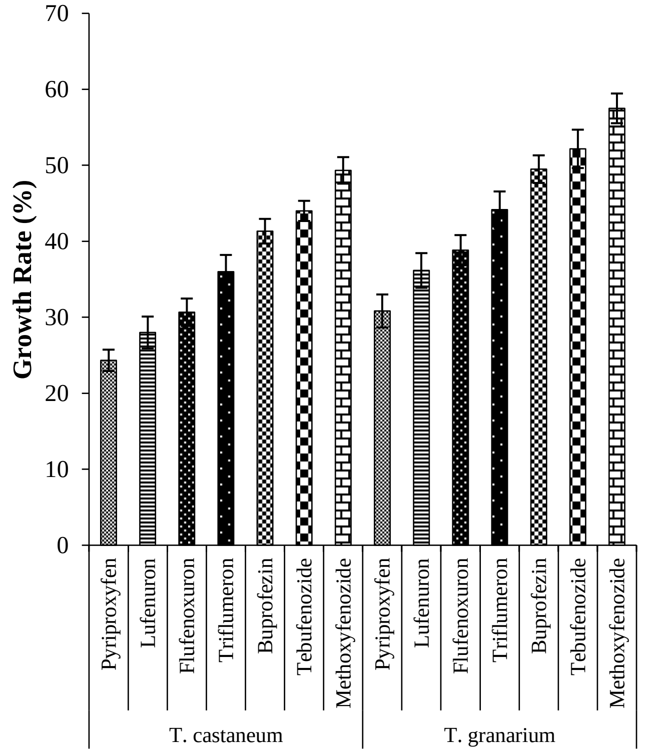

Figure 3. Comparative effect of different IGRs against growth rate of *T. granarium* and *T. castaneum.*

The effect of different concentrations (2.5, 5, 7.5 and 10ppm) on the growth rate of *T. castaneum* and *T. granarium*ispresented in Fig. 4. In *T. castaneum* the highest growth rate 32.50% was observed at 2.5ppm that was the minimum concentration after control treatment (96.67%). The growth rate was 25.00 and 17.50% at 5 and 7.5ppm concentrations. The minimum growth rate 9.52% was noted at a 10ppm concentration which was the highest concentration under study. In *T. granarium* at 10ppm, the lowest growth rate 18.33% was observed, followed by 7.5, 5 and 2.5ppm having 27.5, 35.00 and 42.5% growth rate, respectively. The growth rate was decreased with the increase in concentration.

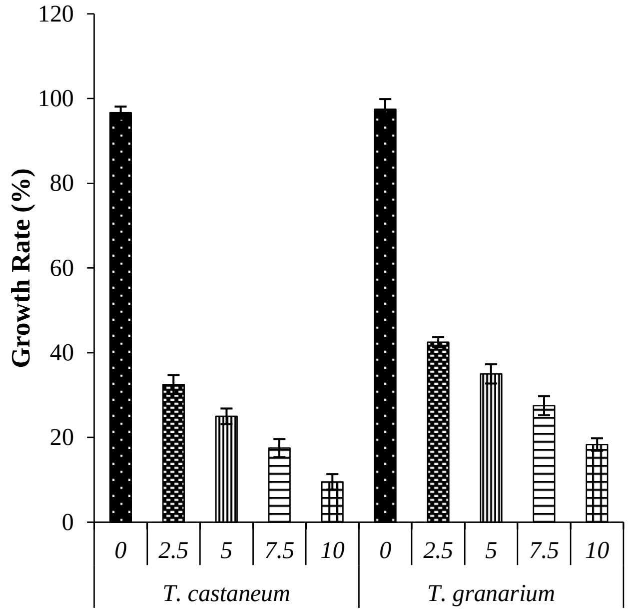

Figure 4. Comparative effect of different concentrations of test IGRs against growth rate of *T. granarium* and *T. castaneum.*

The interaction effect of IGRs and concentrations against the growth rate of *T. castaneum* and *T. granarium* isshown in Table 2. The lowest growth rate was 1.67 and 0.00% against *T. granarium*and *T. castaneum,* respectively at 10ppm of pyriproxyfen. After control, the highest growth rate of 59.17% in *T. granarium* and 49.17% in *T. castaneum* at 2.5ppm of methoxyfenozide was noted. Lufenuron ranked second in term of its toxic effect on the growth rate of both the test insects after pyriproxyfen. The application of flufenoxuron against *T.granarium* and *T. castaneum* has resulted in 11.67 and 1.67% at 10ppm concentration whereas at 2.5ppm the growth rate was 35.83 and 25.83%, respectively. Overall results revealed that the lowest growth rate was observed in pyriproxyfen treatment application and was highest where methoxyfenozide was applied.

Table 2. Growth rate percentage of *T. granarium* and *T. castaneum* against insecticides applied at different concentrations

Insect growth regulators	Conc. (ppm)	Growth Rate (%) *T. granarium*	Growth Rate (%) *T. castaneum*
Pyriproxyfen	0	97.50±0.56a	90.67±0.74a
	2.5	25.83±1.65j	15.83±0.73ij
	5	18.33±1.74l	8.33±0.96k
	7.5	10.83±0.23m	0.83±1.84l
	10	1.67±1.83n	0.00±0.22l
Luferuron	0	97.50±1.83a	96.67±0.86a
	2.5	32.50±0.73hi	22.50±0.84gh
	5	25.00±0.45jk	15.00±0.72ij
	7.5	17.50±1.74l	7.50±0.72k
	10	8.33±1.73m	1.67±0.67l
Flufenoxuron	0	87.50±0.73a	96.67±1.82a
	2.5	35.83±1.73gh	25.83±0.72g
	5	28.33±1.45ij	18.33±0.81hi
	7.5	20.83±1.83kl	10.83±0.76jk
	10	11.67±0.56m	1.67±1.61l
Triflumeron	0	37.50±1.64a	96.67±1.34a
	2.5	42.50±0.74e	32.50±1.72ef
	5	35.00±1.74gh	25.00±1.45g
	7.5	27.50±1.73j	17.50±1.36hi
	10	18.33±1.43l	8.33±1.74k
Buprofezin	0	97.50±0.35a	96.67±0.76a
	2.5	49.17±1.53cd	39.17±1.86cd
	5	41.67±1.63ef	31.67±1.85ef
	7.5	34.17±1.72gh	24.17±0.65g
	10	36.00±0.76jk	15.00±0.98ij
Tebufenozide	0	99.50±0.73a	96.67±1.45a
	2.5	52.50±0.23c	42.50±0.34c
	5	45.00±0.61de	35.00±0.37de
	7.5	37.50±0.73fg	27.50±1.03fg
	10	28.33±0.83ij	18.33±0.34hi
Methoxyfeno zide	0	94.50±0.64a	96.67±1.34a
	2.5	59.17±1.54b	49.17±1.72b
	5	51.67±1.64c	41.67±1.56c
	7.5	44.17±1.74e	34.17±1.73de
	10	35.00±1.79gh	25.00±1.94g

DISCUSSION

The use of insect growth regulators (IGRs) has been shown to exert a profound ovicidal effect against the eggs of stored grains insect pests. In current study, the exposures of eggs to the different concentrations of IGRs were resulted in significant of egg hatchability of both, *T. granarium* and *T. castaneum.* Results revealed that the highest ovicidal action was observed due to the application of pyriproxyfen where only 32.83% hatching was recorded in *T. castaneum* eggs at highest concentration (10ppm). Lufenuron was at second number in term of its ovicidal action while methoxyfenozide was least effective.

Similarly, in *T. granarium* the hatching suppression was highest (38.83% hatching) due to pyriproxyfen treatment and was lowest in methoxyfenozide treatment to eggs. There was a direct relationship between ovicidal action and concentration of IGRs. These results are in accordance with those of Salokhe*et al*. (2003). Sub-lethal effects of IGRs persist for a long time due to their delayed metabolism in insect body after ingestion (Clarke and Jewess, 1990). Our results agree with those of Trostanetsky and Kostykovsky (2008) who also found that when adults of *T. castaneum*are treated with novaluron, it caused reduction in egg hatching. The JHAs (pyriproxyfen) function by preventing the embryonic development that inhibit the differentiation of embryos during blastokinesis (Edward and Menn, 1981), or effect yolk deposition in treated eggs (Slama, 1971). Mian and Mulla (1982), and Parween (1996) reported that chitin synthesis inhibitors (lufenuron, flufenoxuron, triflumeron and buprofezin) disrupt the embryogenesis fully or partially. Slama and Williams (1966) reported that reduced hatching in pyriproxyfen (JHA) treated eggs may be due to failure in differentiation, failure to close the dorsal suture and muscle atrophy and lethal effect on embryo. In some case IGRs completely prevented embryonic development (ovicidal effect) when newly laid eggs (El-Tantawi *et al.*, 1976; Mkhiz, 1993). Similar results have been reported by Silhacek*et al*. (1994) who treated newly laid eggs of *P. interpunctella*with fenoxycarb and pyriproxyfen. Our findings are in accordance with Mondal (1994) and Yasir *et al*. (2012) who studied the effect of triflumeron against the eggs of *T. castaneum.*

A significant reduction in growth rate was noted due to the effect of all the tested IGRs. Results indicated that pyriproxyfen was the most effective where minimum growth rate 24.33 and 30.83% was observed against *T. castaneum* and *T. granarium*, respectively while methoxyfenozide was the least effective. The growth rate was also concentration dependent against both the targeted insects. Similar results were reported by Howard and Wall (1996) and Yasir*et al*. (2012) and they also observed that IGRs treated eggs took longer time to hatch. Even at low concentrations the IGRs have substantial impact on fecundity and fertility, growth rate and emergence of progeny (Mian and Mulla, 1982; Mondal and Parween, 2001). Diflubenzuron also decreased the growth rate and reproductive potential of *T. castaneum*, *S. oryzae* and *T. confusum*(Faragalla*et al*.,1985). Similarly, teflubenzuron, chlorfluazuron and flufenoxuron significant effect the growth and population of stored grains insect (Eisa and Ammar, 1992; Kavallieratos *et al*., 2012). Our results are also in consistency with the findings of various previous researchers such as Samson *et al*. (1990), Ahire*et al*. (2008), Amani (2009) and Wijayaratne*et al*. (2012). According to Dales (1994) the ability of these compounds to decrease the fertility of female, or avert egg development and hatching, would limit the damage caused by the larval feeding stages.

***Conclusion*:** The herein reported studies were conducted to investigate the ovicidal effect of different insect growth regulators against *Trogoderma granarium* (Everts) and *Triboliumcastaneum*(Herbst). Bioactivities of all the tested IGRs were found to be dose and exposure period dependent. From this study, it is concluded that the applications of IGRs have proved to be very effective against both species examined. These compounds particularly pyriproxyfen, an analog of the juvenile hormone (JH) followed by Chitin synthesis inhibitors (i.e., flufenoxuron, lufenuron and triflumeron) should be considered as potential components in managing immature stages of stored grains insects pests.

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