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TRANSGENERATIONAL EFFECT OF INSECT GROWTH REGULATORS ON THE *Trogoderma granarium* (EVERTS) (COLEOPTERA: DERMESTIDAE) UNDER DIFFERENT ABIOTIC FACTORS

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Stored cereals and food products are severely infested by different insect pests throughout the world but *Trogoderma granarium* is found most destructive insect pest of stored grains. Temperature and relative humidity affects the growth and development of these insect pests of stored grains. Present study was designed to investigate the effects of temperature (20, 25 and 30°C) and relative humidity levels (55, 65 and 75%) on the efficacy of three synthetic Insect Growth Regulators (IGRs) i.e., pyriproxyfen, lufenuron and buprofezin at concentrations of 1, 5 and 10 ppm. The study aimed to estimate the fecundity and adult emergence inhibition of *T. granarium* under controlled laboratory conditions. Counted numbers of grubs were exposed to IGRs at different levels of temperature and relative humidity. F_1 adult emergence results at 20°C; showed highest percent reduction in adult emergence (84.38, 70.65 and 79.94%) when exposed to lufenuron, buprofezin and pyriproxyfen treated diet, respectively. At 75% relative humidity lufenuron, buprofezin and pyriproxyfen caused 77.53, 80.00 and 80.32% reduction in adult emergence, respectively. Adults were exposed to IGRs at different temperature and relative humidity to evaluate the oviposition inhibition too. Percentage of reduction in fecundity (87.95, 80.45 and 70.55%) perceived after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively at 20°C was found highest. While 75% relative humidity reduced fecundity most effectively (86.73, 83.72 and 69.11%) when test insects were treated with buprofezin, pyriproxyfen and lufenuron, respectively. It is concluded that temperature and relative humidity significantly affected the activity of tested growth regulators which may lead to standardization of abiotic factors to gain maximum efficiency of IGRs in future.

Keywords: Insect pests, stored cereals, fecundity, insect growth regulators, *Trogoderma granarium.*

INTRODUCTION

The 9-20% post-harvest losses by stored grain pests were recorded worldwide (Phillips and Thorne, 2010). Insect pest causes alterations in the chemical structure of the products by destroying the quality and quantity of food commodities. Economy has been damaged by infestation of stored product insects. Among the insect pests of stored cereals, Khapra beetle *Trogoderma granarium* (Everts) (Dermestidae: Coleoptera) is a serious insect pest of stored grains and their products (Jood *et al*., 1996; Burges, 2008; Ali *et al*., 2012.). In case of severe infestation, quality and quantity of grains reduced by feeding and contaminating with sheded skin. While hairs of larvae are responsible for bad effects on human health (Hosseininaveh *et al*., 2007; Ahmedani *et al*., 2009).

Excessive use of conventional synthetic pesticide to protect stored cereals has resulted the development of insecticide resistant strains, handling hazards, insecticide residues on food, threat to human health and serious environmental issues (Bell, 2000; Benhalima *et al*., 2004; Desneux *et al*., 2007). There is a need to replace synthetic chemical insecticides with safe grain protectants. Insect Growth Regulators (IGRs) are one of the best alternatives to conventional synthetic pesticides that are highly effective against pests of stored grain commodities because they have low mammalian toxicity, environmental and health hazard effects (Kostyukovsky *et al*., 2000; Mondal and Parveen, 2001; Ishaaya *et al*., 2007). IGRs affect metamorphosis and molting by simulating juvenile hormone) or interfering JH activity or by disturbing the cuticle formation (Oberlander *et al.*, 1997). In contrast to traditional insecticides, IGRs are slightly toxic to higher animals. They inhibit the chitin synthesis of insects by causing abnormal endocuticular deposition and absorptive molting. IGRs are used to manage a wide range of insect species by interfering with their process of growth and development (Yu, 2008).

Lufenuron (CSI) is a new synthetic insect growth regulator. It is highly effective for controlling lepidopteron and

coleopteron larvae on maize, cotton and vegetables while rust mites and citrus whitefly on citrus fruits. Ecdysone agonists such as methoxyfenozide, and juvenile hormone mimics such as pyriproxyfen disturb the metamorphosis and molting process (Dhadialla *et al*., 1998). Buprofezin has become successful IGR to manage the insect pests in various countries (USA, Australia etc). The reproduction ability of adult females is reduced by feeding on buprofezin treated diet (Izawa *et al*., 1985; Konno, 1990). Pyriproxyfen is an IGR that strives for juvenile hormone binding position, juvenile hormone mimics and thus retaining an immature stage (Sullivan and Goh 2008). It is a safer compound for non-target organisms and used for management of public health pests (Miyamoto *et al*. 1993). Adult emergence and embryogenesis suppression is also ascribed to pyriproxyfen. (Ishaaya and Horowitz, 1995).

Toxicity of an insecticide is affected by several factors including temperature, insect species, insecticide type and nature of the food on which insect develops (Kljajic and Peric, 2007; Liang *et al*., 2007). Integration of temperature with other control measures is a modern pest management program for stored grain insect pests (Fields *et al*., 1997; Dowdy, 1999). Similarly, temperature and relative humidity play a significant role in the efficacy of spinetoram, it becomes less effective at higher dose (Vassilakos and Athanassiou, 2013).

Keeping in view the above mentioned facts, the present study was designed to determine the effect of insect growth regulators on percent reduction in fecundity and adult emergence of *Trogoderma granarium* and to check the impact of relative humidity and temperature on the effectiveness of IGRs dosage against test insect.

MATERIALS AND METHODS

***Insect rearing*:** Mixed population of *T. granarium* was collected from grain market and godowns of Punjab Food Department, Faisalabad, Pakistan. Keeping optimum laboratory conditions (30±2°C and 65±5% RH) in Stored Grain Management Cell (SGMC), Department of Entomology, University of Agriculture Faisalabad, Pakistan. *T. granarium* was reared on whole wheat grains in an incubator (SANYO-MIR-254) according to the procedure used by Ali *et al*. (2012). Briefly the grains (200g) were sterilized at 70°C for 15 minutes in an oven and then placed in separate glass jars (250g capacity). Fifty adults were released in each jar. The mouth of the jars was tightly covered with muslin cloth using rubber band to prevent the escape of adult beetles. After three days these beetles were sieved out from culture. The wheat grains having freshly laid eggs were put into separate glass jars of 250g capacity and kept in incubator (SANYO-MIR-254) at optimum growth conditions 30±2°C and 65±5% relative humidity to get homogenous population. Five days old grubs were used in further series of experiments.

***Insect growth regulators* (IGRs):** Locally available three synthetic insect growth regulators, (1) pyriproxyfen (Peradigm^{R}) 10.8EC, (2) lufenuron (Lufenuron^{R}) 5EC (3) buprofezin (Buprofezin^{R}) 25WP were obtained from FMC United (Pvt) Limited and used in bioassays at the concentrations of 1, 5 and 10 ppm.

***Commodity and insecticide treatment*:** Untreated whole hard wheat (*Triticum aestivum* L.) with moisture contents 10%, (Dickey John moisture meter was used in the tests). Lots of 1.5 kg of grains were equally sprayed with IGRs at concentrations of 1, 5 and 10 ppm by using volume at the rate of 100 ml of formulated spray per kg (150 ml of formulated spray per 1.5 kg of commodity). Additionally 1.5 kg lots of grains were sprayed with distilled water and used as a control. After treatment application, the jars containing insecticide treated diet allowed to dry at room temperature for 30 minutes in order to evaporate the solvent.

***Effect of IGRs on adult emergence of T. granarium at different temperature and relative humidity*:** Five days old twenty larvae of tested insects placed into each plastic vial of 50ml capacity, with separate vials for three IGRs. Different combinations of three temperatures (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%) were maintained to evaluate the efficacy of three IGRs i.e., pyriproxyfen, lufenuron and buprofezin, at concentrations of 1, 5 and 10 ppm on adult emergence inhibition of *T. granarium.* The vials placed in separate incubators (SANYO-MIR-254) with saturated salt solutions at the bottom in order to maintain the relative humidity at the desirable level. After 42 days, adult emergence was observed for *T. granarium*. Percent reduction in adult emergence was calculated by using the following formula (Sagheer *et al*., 2012).

$$\text{Percent reduction in adult emergence} = 100 \times (1\text{-}t/c)$$

Where, t = Number of adults in treated diet, c = Number of adults in control

***Effect of IGRs on fecundity of T. granarium at different temperature and relative humidity*:** Three plastic cylindrical vials (3 cm in diameter, 8 cm in height) were used as replicates. Each vial was filled with 20 g of treated grain and twenty adults of *T. granarium* were placed in each vial. These vials were placed in separate incubators (SANYO-MIR-254) with saturated salt solutions at the bottom in order to maintain three temperatures (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%). The relative humidity in the plastic containers was continuously monitored by digital Hygrometer. Fecundity of exposed individuals was assessed after 4 days. Percent reduction in fecundity was calculated by using the formula (Sagheer *et al*., 2012).

$$\text{Percent reduction in fecundity} = 100 \times (1\text{-}t/c)$$

Where, t = Number of eggs in treated diet, c = Number of eggs in control

***Statistical analysis*:** Data subjected to statistical software Statistix 8.1 for analysis of variance Completely Randomized Design (CRD). The means of significant treatment were

compared using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance.

RESULTS

In current study the effects of three different levels of temperature (20, 25 and 30ºC) and relative humidity (55, 65 and 75%) on the efficacy of three synthetic IGRs i.e., pyriproxyfen, lufenuron and buprofezin at concentrations of 1, 5 and 10ppm were assessed against the fecundity and adult emergence inhibition of *T. granarium.*

Significant variation was observed in adult emergence inhibition of *T. granarium* at different temperature (F=3.26; P<0.05), relative humidity (F=14.63; P<0.001) and concentration (F=20.01; P<0.001) after buprofezin treatment. Adult emergence inhibition varied with alterations in temperature (F=8.35; P<0.001), relative humidity (F=6.81; P<0.05) and concentration (F=21.08; P<0.001) after exposure to pyriproxyfen treated diet. Effect of temperature (F=16.82; P<0.001), relative humidity (F=21.23; P<0.001) and lufenuron concentrations (F=14.16; P<0.001) caused significant variations in adult emergence reduction of *T. granarium.*

Maximum percent reduction in adult emergence, (70.65, 79.94 and 84.38%) was observed at 20°C after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively (Table 1). At 75% relative humidity, highest inhibition of adult emergence (80.00, 80.32 and 77.53%) was perceived on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 1). While maximum reduction in adult emergence (81.46, 86.39 and 82.45%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Fig. 1).

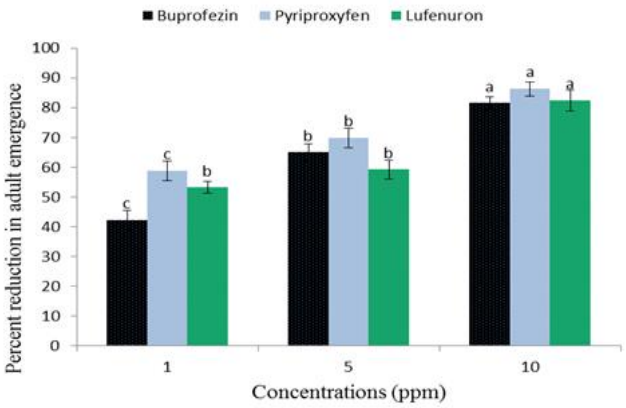

Figure 1. Impact of various concentrations of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium.*

Interaction effect of temperature and relative humidity caused maximum reduction in adult emergence (86.77%) of *T. granarium* at 25°C temperature and 75% relative humidity after buprofezin treatment (Table 2); while highest adult

Table 1. Impact of temperature and relative humidity on effectiveness of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium*.

Insect Growth Regulators	Temperature (°C)			Relative Humidity (%)		
	20	25	30	55	65	75
	Mean ± MSE			Mean ± MSE		
Buprofezin	70.65±2.04a	54.75±1.09b	63.15±2.55ab	46.31±2.50c	62.24±1.91b	80.00±2.23a
Pyriproxyfen	79.94±1.57a	72.60±2.48ab	62.57±1.30b	64.88±3.65b	69.91±2.36b	80.32±1.39a
Lufenuron	84.38±2.23a	56.01±1.66b	54.42±2.50b	43.16±2.99b	74.12±3.92a	77.53±3.90a

*Percent reduction in adult emergence is calculated by formula=100×(1-t/c), where "t" is the number of adults in treated diet, and "c" is the number of adults in control treatment.

Table 2. Interaction effect of temperature and relative humidity on activity of Insect Growth Regulators against percent reduction in adult emergence of *Trogoderma granarium*.

Insect Growth Regulators	Temperature (°C)	Relative Humidity (%)		
		55	65	75
		Mean ± MSE	Mean ± MSE	Mean ± MSE
Buprofezin	20	64.26±3.33 ab	67.02±1.76 ab	80.66±2.21 ab
	25	27.19±2.35 c	50.28±2.51 bc	86.77±1.22 a
	30	47.48±2.18 bc	69.43±3.43 ab	72.55±3.01 ab
Pyriproxyfen	20	31.33±3.56 b	35.07±2.38 b	76.07±3.96 a
	25	63.92±1.85 ab	77.72±2.43 a	80.55±2.32 a
	30	68.81±2.57 a	70.51±3.01 a	90.74±3.89 a
Lufenuron	20	27.11±1.84 b	26.29±2.82 b	76.07±1.96 a
	25	63.88±1.84 a	77.94±3.46 a	80.55±3.32 a
	30	68.51±2.48 a	73.33±2.95 a	90.74±1.89 a

Table 3. Impact of temperature and relative humidity on effectiveness of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*.

Insect Growth Regulators	Temperature (°C)			Relative Humidity (%)		
	20	25	30	55	65	75
	Mean ± MSE			Mean ± MSE		
Buprofezin	87.95±1.24a	83.30±1.02b	86.61±1.42ab	86.29±1.22a	84.87±1.46a	86.73±1.00a
Pyriproxyfen	80.45±2.10a	75.65±2.34ab	72.66±3.49b	67.32±3.65b	77.72±1.52a	83.72±1.54a
Lufenuron	70.55±0.43a	54.40±3.35b	67.27±2.86a	55.75±2.01b	67.37±1.29a	69.11±1.44a

*Percent reduction in fecundity is calculated by formula=100×(1-t/c), where "t" is the number of eggs in treated diet, and "c" is the number of eggs in control treatment.

Table 4. Interaction effect of temperature and relative humidity on activity of Insect Growth Regulators against percent reduction in fecundity of *Trogoderma granarium*

Insect Growth Regulators	Temperature (°C)	Relative Humidity (%)		
		55	65	75
		Mean ± MSE	Mean ± MSE	Mean ± MSE
Buprofezin	20	84.68±2.41 abc	88.61±2.11 ab	90.5±1.41 a
	25	82.26±1.07 bc	79.86±1.08 c	87.8±1.90 ab
	30	91.92±1.01 a	86.15±3.12 abc	81.8±1.41 bc
Pyriproxyfen	20	71.66±0.68 a	70.22±0.75 a	70.55±1.02 a
	25	32.11±3.15 b	64.72±2.15 a	68.61±2.53 a
	30	68.66±3.49 a	67.16±3.02 a	68.16±3.51 a
Lufenuron	20	70.88±0.38 a	70.52±0.75 a	70.55±1.02 a
	25	29.88±2.75 b	64.72±2.15 a	67.61±2.53 a
	30	66.50±2.62 a	64.16±3.02 a	68.16±3.51 a

emergence inhibition (90.74%) was perceived in case of both pyriproxyfen and lufenuron treated at 30°C temperature and 75% relative humidity (Table 2).

A significant variation in oviposition inhibition of *T. granarium* was observed at different temperature (F=6.01; P<0.05), relative humidity (F=8.49; P<0.001) and concentration (F=12.06; P<0.001) after buprofezin treatment. Reduction in fecundity varied with alterations in temperature (F=3.72; P<0.05), relative humidity (F=16.57; P<0.001) and concentration (F=12.06; P<0.001) after exposure to pyriproxyfen treated diet. Effect of temperature (F =15.94; P<0.001), relative humidity (F=11.53; P<0.001) and lufenuron concentrations (F=6.38; P<0.001) caused significant variations in fecundity reduction of *T. granarium.* Maximum percent reduction in fecundity, (87.95, 80.45 and 70.55%) was observed at 20°C after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively (Table 3). At 75% relative humidity, highest inhibition in oviposition (86.73, 83.72 and 69.11%) was perceived on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 3). Maximum reduction in fecundity (74.78, 84.43 and 72.85%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Fig. 2).

After the application of buprofezin the temperature and relative humidity caused maximum reduction in fecundity (91.92%) of *T. granarium* at 30°C temperature and 55% relative humidity (Table 4); while at 20°C temperature and 55% relative humidity showed the highest oviposition inhibition (71.66 and 70.88%) was perceived in case of pyriproxyfen and lufenuron treated diet, respectively (Table 4).

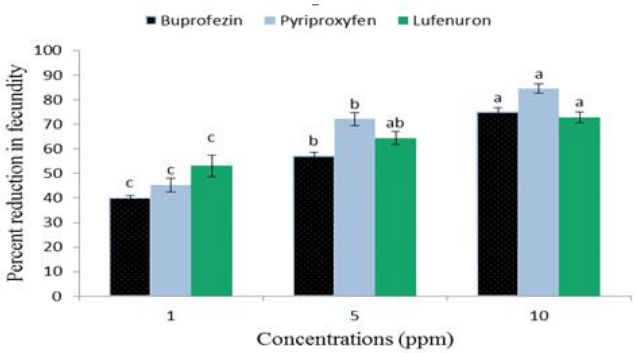

Figure 2. Impact of various concentrations of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*.

DISCUSSION

In this series of experiments larvae and adults of *T. granarium* were exposed to different concentration of IGRs at various levels of temperature and relative humidity. At 20°C temperature and 75% relative humidity highest reduction in

adult emergence was observed. Subsequent pupal development and adult emergence was completely prohibited. These results are similar to the findings of Sagheer *et al*. (2012). It has been reported that IGRs reduced body weight of insects (Smagghe *et al*., 1996; Parveen, 2000). Meola *et al*. (1999) reported that due to lufenuron treatment in fleas, larval hatching was prevented by raptures in the cuticle, which opened during eclosion resulting in the loss of hemolymph and desiccation of the larva.

It has been found that short term exposure to different levels of temperature had positive effects on toxicity of insect growth regulators. Lowest mortality was recorded at higher level of temperature due to decomposition of active ingredients of insecticides. These results confirm the findings on impact of high temperatures on efficacy of hydroprene applied to control *T. castaneum* (Arthur and Dowdy, 2003). Among the abiotic factors, temperature, grain moisture contents and gas compositions play a vital role in insect growth and development (Hagstrum and Milliken, 1988; Muir, 2000). The interaction temperature and relative humidity has been studied extensively with often inconsistent results (Arthur, 1999; Fields and Korunic, 2000; Fang and Subramanyam, 2003).

All the tested insect growth regulators showed reduction in fecundity of *T. granarium.* It was observed that different levels of temperature and relative humidity showed a significant variation in effectiveness of IGRs against egg laying capacity of test insects. Significant positive variations were found between temperatures at different levels of relative humidity. These results confirm the findings on effect of temperature and relative humidity on the efficacy of spinetoram for the control of three stored product beetle species (Vassilakos and Athanassiou, 2013). Temperature had a noticeable influence on tolerance of insecticide against the both test insects. These results are consistent with the published findings on legume type and temperature effects on the toxicity of insecticide (Gbaye *et al*., 2011).

Adults of *T. granarium* were released to oviposit on untreated and treated diet; fecundity was reduced significantly on treated diet as compared to control treatments. These results showed resemblance to transovarial activity of CSIs that caused reduction in fecundity in treated diets. Similar results have been reported that adults reared on treated diet lay fewer eggs as compared to untreated adults (Nickle, 1979; Parveen *et al*., 2001). It has been reported that insect growth regulators affect the embryogenesis partially or fully (Mian and mulla, 1982). Insect growth and development affected due to exposure of insect growth regulators (Soltani-Mazouni, 1994).

Furthermore, in this study IGRs did not kill the adults of *T. granarium* but induced suppression in egg laying capacity of treated insects as compared to untreated insects. These results are similar with other findings (Carter, 1975; Faragalla *et al*., 1985; Ammar, 1988; Elek and Longstaff, 1994; Kostyukovsky and Trostanetsky, 2006). It has been found that chitin synthesis inhibitors showed a strong insecticidal activity by foliar application against Colorado potato beetle and reduced oviposition (Cutler *et al*., 2005).

***Conclusion*:** Our study reveals that temperature and relative humidity play a significant role in insect growth regulators efficacy. Maximum control of stored grain insect pests was observed at lower temperature (20°C) and higher relative humidity (75%), because at high temperature insecticides start to degrade. Overall control of stored grain insect pests depends on biological and physical factors such as insect species, temperature, relative humidity, dose rate and time period for which insects were exposed to insecticides. In addition, some other factors that may affect the effectiveness of insecticides are grain type, grain moisture contents and methods of insecticide application.

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