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Influence of Abiotic Factors on the Efficacy of Insect Growth Regulators Against *Trogoderma Granarium* (Everts)(Coleoptera: Dermestidae)

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ABSTRACT

Present study was designed to investigate the effects of different combinations of three temperatures (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%) on the efficacy of three synthetic IGRs i.e., pyriproxyfen, lufenuron and buprofezin at concentrations of 1, 5 and 10ppm on fecundity and adult emergence inhibition of *T. granarium* under controlled laboratory conditions. This study was conducted at Grain Research Training and Storage management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. All the treatments were replicated three times using Completely Randomized Design. Larvae of *T. granarium*were exposed to IGRs at different levels of temperature and relative humidity. F_1 adult emergence results showed that at temperature 20ºC, the highest percent reduction in adult emergence (84.38, 70.65 and 79.94%) was recorded after exposure 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. The results revealed that at temperature 20°C, maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was recorded after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively. At 75% relative humidity buprofezin, pyriproxyfen and lufenuron caused 86.73, 83.72 and 69.11% reduction in fecundity, respectively. It is concluded that temperature and relative humidity play an important role in the effectiveness of insect growth regulators.

Key words: Temperature, Relative Humidity, *Trogoderma granarium,* Insect Growth Regulators, Efficacy

Introduction

About 9-20% post-harvest losses by stored grain pests had been reported in the developed and developing countries (Phillips and Thorne, 2010). Insect pests cause alterations in the chemical structure of the products by destroying the quality and quantity of food commodities. Among the insect pests of stored cereals, the Khapra beetle *Trogoderma granarium* (Everts) (Dermestidae: Coleoptera) is a serious pest of stored grains and their products (Burges, 2008; Ali *et al*., 2012). In case of severe infestation, quality and quantity of grains are reduced by feeding and contamination with shed skin. Hairs of larvae may adversely affect human health (Hosseininaveh *et al*., 2007; Ahmedani *et al*., 2009).

Excessive use of conventional synthetic pesticides (Organophosphates, Pyrethroids) to protect stored cereals has resulted in 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 the need to replace synthetic chemical insecticides with safe grain protectants (Silver, 1994). 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, little 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 (JH, juvenile hormone agonists) or interfering JH activity (ecdysteroid agonists) or by disturbing the cuticle formation (chitin synthesis inhibitors) (Oberlander *et al.,* 1997). In contrast to traditional insecticides, IGRs are less toxic to higher animals. They inhibit the chitin synthesis of insects by causing abnormal endocuticular deposition and absorptive molting (Post and Vincent, 1973). 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, vegetables, rust mites and citrus whitefly on citrus fruits. Buprofezin has become successful IGR to manage insect pests in various countries.The reproduction ability of adult females is reduced by feeding on buprofezin treated diet. (Uchida *et al*., 1985; 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 are 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 strategy for stored grain insect pests (Dowdy, 1999). Similarly, temperature and relative humidity play a significant role in the efficacy of spinetoram, which becomes less effective at higher dose (Vassilakos and Athanassiou, 2013).

Keeping in view the above mentioned facts, the study sought to determine the effect of insect growth regulators on percent reduction in fecundity and adult emergence of *Trogoderma granarium*; the impact of relative humidity and temperature on the effectiveness of IGRs against test insects and the influence of relative humidity and temperature on dose and response.

Materials and Methods

Insect Rearing

Mixed population of *Trogoderma granarium* was collected from grain market and godowns of Faisalabad district, Punjab Food Department of Pakistan. Under laboratory conditions in the Stored Grain Management Cell (SGMC), department of Entomology, University of Agriculture, Faisalabad,*T.*

granarium was reared on whole wheat grains in an incubator (SANYO-MIR-254) at 30±2°C and 65±5% relative humidity 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 put in separate glass jars (250g capacity). Fifty adults of mixed sex were released into the jars. The mouth of the jars was tightly covered with muslin cloth using rubber band to prevent the escape of adult beetles. After three days the parent 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 cooled 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 and (3) buprofezin (Buprofezin^{R}) 25WP were obtained from FMC United (Pvt) Limited and used in the bioassays at the concentrations of 1, 5 and 10 ppm.

Grain treatment with IGRs

Untreated whole hard wheat (*Triticum aestivum* L.) with moisture contents 10 %, (as determined by 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 using volume at the rate of 100 ml of formulated spray per kg (150 ml of formulated spray per 1.5 kg of wheat grains). Additionally 1.5 kg lots of grains were sprayed with distilled water and used as the control treatment. After treatment application, the jars containing IGR treated diet were 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 temperatures and relative humidity levels

Five-days old larvae of *T. granarium* were placed into each plastic vial of 50ml capacity, with separate vials for the three IGRs. Different combinations of three temperatures regimes (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%) were maintained to evaluate the efficacy of pyriproxyfen, lufenuron and buprofezin, at concentrations of 1, 5 and 10 ppm on the inhibition of adult emergence of *T. granarium*.The vials were placed in separate incubators(SANYO-MIR-254) with saturatedsalt 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 using the following formula (Sagheer *et al*., 2012).

Percent reduction in adult emergence = 100 × (1-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 temperatures and relative humidity levels

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 20 adults of *T. granarium* were placed in each vial. The vials were placed in separate incubators (SANYO-MIR-254) with saturatedsalt 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 continuouslymonitored by digital Hygrometer. Fecundity (the number of eggs laid) of exposed beetles was assessed after 4 days. It was calculated using the formula (Sagheer *et al*., 2012).

Percent reduction in fecundity = 100 × (1-t/c)

Where

t = Number of eggs in treated diet

c = Number of eggs in control

Statistical analysis

Data were subjected to statistical software Statistix 8.1 for analysis of variance. The means of significant treatment were compared using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance.

Results

A significant variation in the inhibition of adult emergenceof *T. granarium* was observed at different temperature regimes (F=3.26; P<0.05), relative humidity levels (F=14.63; P<0.001) and concentrations (F=20.01; P<0.001) after buprofezin treatment. The inhibition of adult emergence varied with different temperature regimes (F=8.35; P<0.001), relative humidity levels (F=6.81; P<0.05) and concentrations (F=21.08; P<0.001) after exposure to pyriproxyfen treated diet. Similarly, 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 the reduction in adult emergence of *T.granarium.*

At temperature 20ºC, the maximum percent reduction in adult emergence, (70.65, 79.94 and 84.38%) was observed after exposure to buprofezin, pyriproxyfen and lufenuron treated diets, respectively (Table 1). At 75% relative humidity, the highest inhibition of adult emergence (80.00, 80.32 and 77.53%) was recorded on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 1). Maximum reduction in adult emergence (81.46, 86.39 and 82.45%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Figure-1).

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

IGRs	Temperature (ºC)			Relative Humidity (%)		
	20	25	30	55	65	75
		Mean±S.E			Mean±S.E	
Buprofezin	70.65±2.04a	54.75±1.09b	63.15±2.55 ab	46.31±2.50c	62.24±1.91b	80.00±2.23a
Pyriproxyfen	79.94±1.57a	72.60±2.48ab	62.57±1.30 b	64.88±3.65b	69.91±2.36b	80.32±1.39a
Lufenuron	84.38±2.23a	56.01±1.66b	54.42±2.50 b	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.

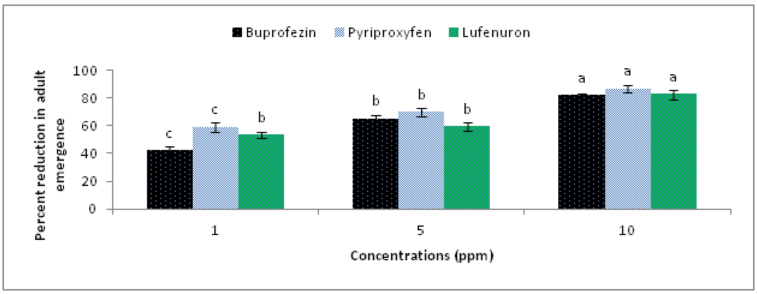

Fig. 1 Impact of various concentrations of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium*

The interaction effect of temperature and relative humidity caused maximum reduction in adult emergence (86.77%) of *T. granarium* at 25ºC temperature and 75% relative humidity after buprofezin treatment (Table 2); while highest adult emergence inhibition (90.74%) was recorded in both pyriproxyfen and lufenuron treated diet at 30ºC temperature and 75% relative humidity (Table 2).

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

IGRs	Temperature (ºC)	Relative Humidity (%) 55 Mean±S.E	65 Mean±S.E	75 Mean±S.E
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

A significant variation in oviposition inhibition of *T. granarium* was observed at different temperature regimes (F=6.01; P<0.05), relative humidity levels (F=8.49; P<0.001) and concentrations (F=12.06; P<0.001) of buprofezin treated diet. Reduction in fecundity varied with different temperature regimes (F=3.72; P<0.05), relative humidity levels (F=16.57; P<0.001) and concentrations (F=12.06; P<0.001) of pyriproxyfen treated diet. 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*.

At temperature 20ºC, the maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was observed after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively (Table 3). At 75% relative humidity, the highest inhibition in oviposition (86.73, 83.72 and 69.11%) was recorded on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 3). The maximum reduction in fecundity (74.78, 84.43 and 72.85%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Figure 2).

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

IGRs	Temperature (ºC) 20	25 Mean±S.E	30	Relative Humidity (%) 55	65 Mean±S.E	75
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.

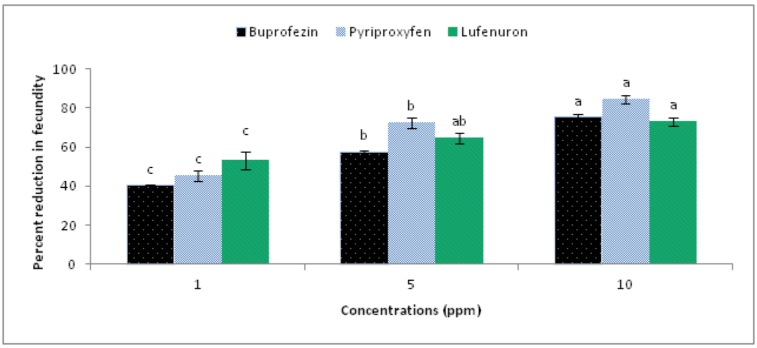

Fig. 2 Impact of various concentrations of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*

The interaction effect of temperature and relative humidity caused maximum reduction in fecundity (91.92%) of *T. granarium* at 30ºC temperature and 55% relative humidity after buprofezin treatment (Table 4); while at 20ºC temperature and 55% relative humidity highest oviposition inhibition (71.66 and 70.88%) was observed in case of pyriproxyfen and lufenuron treated diet, respectively (Table 4).

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

IGRs	Temperature (ºC)	Relative Humidity (%) 55 Mean±S.E	65 Mean±S.E	75 Mean±S.E
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

Discussion

In this series of experiments,the larvae and adults of *T. granarium* were exposed to different concentration of IGRs treated diet at various levels of temperature and relative humidity. IGRs significantly prolonged the larval duration of *T. granarium*at different temperature and relative humidity levels. At 20ºC temperature and 75% relative humidity the 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. The 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 of temperature and relative humidity has been studied extensively with often inconsistent results (Arthur, 1999; Fields and Korunic, 2000; Fang and Subramanyam, 2003).

All the three 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 *T. granarium*.Significant variations in response of insect were found between temperatures at different levels of relative humidity. At 20ºC temperature insect growth regulators were highly effective in percent reduction in fecundity of *T. granarium*compared to other levels of temperature, while at 75% relative humidity the highest reduction in fecundity was observed. These results confirm the findings ofthe effect of temperature and relative humidity on the efficacy of spinetoram for the control of three stored product beetle species (Vassilakos and Athanassiou, 2013).

When the adults of *T. granarium* were released to oviposit on untreated and treated diet; fecundity was reduced significantly on treated diet compared to control treatments. These results showed resemblance to transovarial activity of CSIs that caused reduction in fecundity in treated diets. Similar results that the adults of insects reared on treated diet lay fewer eggs compared to untreated adults have been reported by several workers (McGregor and Kramer, 1976; Nickle, 1979; Saxena and Mathur, 1981; Elek, 1998a; Parveen *et al*., 2001). It has been reported that insect growth regulators affect the embryogenesis partially or fully (Mian and mulla, 1982).

Furthermore, in this study IGRs did not kill the adults of *T. granarium* but induced suppression in egg laying capacity of treated insects 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).

Our study reveals that temperature and relative humidity have significant effecton the efficacy of the three insect growth regulators tested. Maximum control of stored grain insect pests was observed at lower temperature (20ºC) 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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Efficacy of pheromones for managing of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, in food and feed processing facilities

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