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Full Length Research Paper

Control of the seed beetle *Callosobruchus muculatus* reared on chickpeas by different applicable conditions of the insect growth regulator, Cyromazine

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Efficiency of most pesticides is monitored by many factors such as the method of application, the concentrations and the temperature. The study was aimed to investigate the optimal conditions for using Insect Growth Regulator (IGR), cyromazine to control the Southern cowpea weevil *Callosobruchus muculatus* Fab. (Bruchidae: Coleoptera). Interactions of the method of application and concentrations of cyromazine together with temperatures significantly affected the reproductive rate of *C. muculatus*. Accordingly, when seeds were completely embedded (dipping method) in 5% cyromazine at 30°C, the insect reproductive rate was significantly decreased to 1.37%. In addition, results revealed that the increase in cyromazine concentration simultaneously with the seed-dipping method clearly decreased the consumption of food storage by the insect population. However, the generation lifespan of the insect was increased. No significant change was observed in the percentage of the sexual ratio, while a clear significant difference was recorded in the weights of the males and females. In conclusion, the seed-embedding method with a high concentration (5%) of cyromazine at 30°C resulted in the most significant food consumption with a clear reduction in the generation reproductive rate.

Key words: Food consumption, IGR, increasing rate, sex ratio.

INTRODUCTION

The chickpea (*Cicer arietinum*) is an edible legume of the family Fabaceae, subfamily Faboideae. Chickpeas are high in protein and one of the earliest cultivated vegetables; 7,500-year-old remains have been found in the Middle East. This crop is prone to heavy damage by the Southern cowpea weevil *Callosobruchus muculatus* Fab. Initial infestation occurs in the field prior to harvest and from there the insects are carried to storehouses where the population can build up rapidly (Prevett, 1961; Huignard, 1985).

There are concerns with the use of chemicals to control insects; presence of residues in food, resistance development by pest species, health risks (Arthur, 1996) and increased cost (Hagstrum and Subramanyam, 2006). IGRs are compounds that interfere with insect specific physiological systems that are not shared by vertebrates, or differ from vertebrate systems, and they constitute targets to IGRs. Most IGRs used today in insect control either interfere with the formation of the new cuticle or disturb metamorphosis. IGRs deregulate rather than regulate insect development and therefore, the term “Insect Development Inhibitors” is more suitable (Pener, 2002). Some insects exposed to such compounds may die due to abnormal regulation of hormone-mediated cell or organ development. Other insects may die either from a prolonged exposure at the developmental stage to other mortality factors or from an abnormal termination of

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a developmental stage itself (Tunaz, 2004).

Miller et al. (1996) found the use of cyromazine at the rate of 0.1, 0.5 and 1.1% g/ kg of the animal body weight in the field of milk cows that it's remaining in cows' waste led to discourage the development of *Musca domestica* larvae. Levot and Sates (1998) found that the use of cyromazine and Dezinion each individually against *M. domestica* by concentration of 0.4 g/liter led to a reduction in the number of *M. domestica* at the rate of 69% after one day of treatment and 99.97 in three days later of treatment. More importantly, IGR were considered to be better used in an integrated pest management program, rather than being used alone (Oberlander et al., 1997). Vazirianzadeh et al. (2007) concluded that cyromazine should be used in a larvicidal program to control house-fly.

Environmentally, it was found that novaluron, an insect growth regulator, interferes with amphibian development if found as contaminant in the water bodies where amphibians live and reproduce (Pancharatna et al., 2010). The use of very low doses of insecticides that have strong sublethal effects represents an environmentally friendly option to improve existing integrated pest management (IPM) strategies (Sial and Brunner, 2010). Thus, the objective of this study was to determine the impact of overlapping between the different concentrations of cyromazine, method of treatment and the temperature in the biological activity of southern cowpea weevils to select the optimal conditions.

MATERIALS AND METHODS

Insects

The Southern cowpea weevil *C. maculatus* F. (Bruchidae: Coleoptera) obtained from an entomological research laboratory farm which has already been bred insects in the College of Agriculture and Forestry, Mosul, Iraq. All chickpea seeds were put in glass jar with a capacity of each 1/2 kg and then added to them the Southern cowpea weevil and covered with a piece of cloth and bond with rubber firmly. Then incubated at 30 ± 2°C and 50 ± 5% as previously described by Ishimoto et al. (1996). Cultures renewed after each generation by taking the newly emerged insects from pupae for construction of a new culture to conduct further studies.

Insect growth regulator (Cyromazine)

This pesticide, working as a regulator of growth, is used to control larvae of Diptera and Coleoptera. It is used as a powder containing 750g /kg effective cyromazine (N-cyclopropyl 1, 3, 5-triazine-2, 4, 6-triamine) and sold under the names of the various commercial (Larvadex, Premix) classifies toxically within the Class III according to the classification of the World Health Organization (WHO).

Cyromazine is not a cholinesterase inhibitor. Cyromazine exerts its toxic action by affecting the nervous system. Solutions were prepared immediately prior to the experiments (Awad and Mulla, 1984).

Application method of the IGR

Three concentrations of cyromazine were applied (1, 3 and 5% weight of IGR/Volume of distilled water) in the treatment of chickpea seeds. Seeds (25 g per each treatment) were treated twelve times (replicates), six times were treated in a spraying manner using the Potter Tower at 5 lbs/inches. The remaining six were treated by dipping in the cyromazine solution for one minute. For the control, seeds were treated with water only. Drought seeds have been placed in plastic pots (7 x 7 cm) then five pairs (male and female) of newly emerged adults were transferred for each pot and covered the pots with a piece of cloth sealed with a rubber bond and incubated at 25 ± 1°C and 30 ± 1°C, 50 ± 5% relative humidity.

Rate of reproduction

Treatments were follow-up to two successive generations to specify the overlap between different concentration of IGR, treatment method and rearing temperature on the following: The rate of reproduction of the southern cowpea weevils is calculated for two successive generations via formula (Krebs, 1978):

$$r = \frac{dn/dt}{n}$$

Where; r= the reproductive rate; n= number of colony individuals; dn = change in the number of colony individual; and dt= change of time.

Rate of food consumption

It was measured by weighting the treated seeds after the end of the experiment and deducted from the original weight (25 g).

Generation lifespan

It was calculated from the new adult emergence from the pupae until the advent of insects in the second-generation.

Sex ratio and weight of male and female

Sex ratio and weight of male and female were tested by taking a random group of full complete insects (10 insects) each in box and calculating the number of male and female and their weight.

Data analysis

Data were analyzed using the factorial complete randomized design and Duncan's multiple range tests to change the averages of endurable level 5% (Daoud and Elyass, 1990) were used.

RESULTS

Effect of the individual parameters and overlap on the reproductive rate

High concentration of cyromazine led to a significant reduction in the reproductive rate of the southern cowpea weevils compared with water-treated controls (Table 1). Dipping method was found to decrease the number of the southern cowpea weevil (Table 1). Temperature (30°C) was found to significantly decline the reproductive rate while it was elevated at 25°C. Overlap study indicated that, when chickpea seeds treated via dipping method in

Table 1. Summarized results of studied parameters on the southern cowpea weevil, *C. maculatus* with the different concentrations of cyromazine, treatment methods and temperatures.

Variable		Concentrations				Treatment methods		Temperatures	
		1	3	5	Control	Dipping	Spraying	25°C	30°C
Reproductive rate		22.21C	10.69 B	2.78 A	33.83 D	15.21 A	19.54 B	17.83 B	16.93 A
Food consumption		6.80 C	3.69 B	2.21 A	15.50 D	6.18 A	7.92B	7.23 A	6.87 A
Generation lifespan		29.13C	30.21 B	31.21 A	27.87 D	29.50 A	29.71 A	32.27 A	26.94 B
	Male	1.19 B	1.16 AB	1.17 AB	1.03 A	1.13 A	1.13 A	1.12 A	1.15 A
Sex ratio	Female	1.01 A	1.01 A	1.03 A	1.02 A	1.02 A	1.02 A	1.03 A	1 A
	Male	0.81 A	1.09 B	1.10 B	1.26 C	1 A	1.13 B	1.09 A	1.05 A
Average weights	Female	1.68 B	1.5 A	1.39 B	1.97 C	1.72 B	1.55 A	1.66 A	1.62 A

Averages of similar characters refer to the existence of significant differences at the 5% level of probability.

Table 2. Overlap effect of different concentrations of cyromazine, treatment methods and temperatures on the reproductive rate, food consumption rate and generation lifespan of the southern cowpea weevil, *C. maculatus*.

Concentration %	Treatment method	Temperature	Reproductive rate	Food consumption rate	Generation lifespan
1	Dipping	25	18.33±0.73^{E}	6.72±0.08^{C}	31.33±0.44^{C}
3			7.83±0.13^{C}	2.71±0.16^{AB}	32.5±0.58^{B}
5			2.70±0.12^{AB}	2.05±0.11^{AB}	34±0.29^{A}
Control			33.27±0.62^{H}	14.03±0DE	30±0^{D}
1	Spraying		28.13±0.19^{G}	6.31±0.15^{C}	32.5±0^{B}
3			14.40 ±0.21^{D}	4.50 ±0.06^{BC}	33.5 ±0^{AB}
5			4.30±0.29^{B}	2.92±0.06^{AB}	34.17±0.33^{A}
Control			33.67±0.17^{H}	18.85±0.08^{F}	30.17±0.17^{D}
1	Dipping	30	17.03±0.49^{E}	7.03±0.40^{C}	26.33±0.33G^{H}
3			6.43±0.23^{C}	2.50±0.26^{AB}	27.5±0.29^{EF}
5			1.37±0.15^{A}	1.45±0.05^{A}	28.5±0.05^{E}
Control			34.73±2.62^{H}	12.98±0.58^{D}	25.83±0.44^{H}
1	Spraying		25.33±0.20^{F}	7.14±0.17^{C}	26.33±0.33^{GH}
3			14.10±0.31^{D}	5.70±0.09^{BC}	27.33±0.44^{FG}
5			2.73±0.15^{AB}	2.43±0.08^{AB}	28.17±0.6^{EF}
Control			33.67±0.17^{H}	16.38±0.12^{EF}	25.5±0.19^{H}

Averages of similar characters refer to the existence of significant differences at the 5% level of probability.

5% cyromazine solution at 30°C led to the most significant decrease in the reproductive rate (Table 2).

Reverse correlation was found for tested parameters with food consumption

High concentration of IGR was found to significantly decrease the rate of food consumption (Table 1). Dipping method led to a significant decrease of this rate (Tables 1 and 2). In addition, increase of temperature had the same impact on the food consumption since; the lowest consumption rate was obtained at 30°C.

Effect of individual parameters and overlap on the lifespan of generations

The generation lifespan was significantly increased with increasing the concentration of IGR in contrast with water treated control. The generation lifespan was significantly decreased by the dipping method recording 28.81 days;

Table 3. Overlap effect of different concentrations of cyromazine, treatment methods and temperatures on the sex ratio of the southern cowpea weevil, *C. maculatus*.

Concentration %	Treatment method	Temperature	Mean of the sex ratio ± S.E	
			Male	Female
1	Dipping	25	1.14±0.08 A	1 ±0 A
3			1.14±0.5 A	1 ±0 A
5			1.21±0.12 A	1.04±0.0 4 A
Control			1.04±0.04 A	1.01±0.01 A
1	Spraying		1.23±0.12 A	1.05±0.05 A
3			1.03±0.02 A	1.01±0.01 A
5			1.11±0.08 A	1.07±0.07 A
Control			1.04±0.04 A	1 ±0 A
1	Dipping	30	1.17±0.17 A	1 ±0 A
3			1.27±0.14 A	1.01 ±0.1 A
5			1.10±0.05 A	1.01 ±0.01 A
Control			1.±0 A	1±0 A
1	Spraying		1.26±0.09 A	1.01±0.01 A
3			1.17±0.09 A	1±0 A
5			1.24±0.13 A	1 ±0 A
Control			1.04±0.02 A	1 ±0 A

Averages of similar characters refer to the existence of significant differences at the 5% level of probability.

while in spraying method was 29.63 days (Table 1). At 30°C, the generation lifespan was 26.65 days, in contrast to 25°C which recorded 31.79 days for generation lifespan. The highest generation lifespan was 33.33 days, in spraying treatment, at 5% concentration and 25°C, and the lowest generation lifespan was 26.3 days in dipping method at 1% concentration and 30°C (Table 2).

Unlike the sex ratio, the weights were significantly affected

Non significant differences were observed for the sex ratio in any test concentration, treatment method, and the temperature (Tables 1 and 3). Increase of IGR concentration led to an increase of male weight in contrast with control (Table 4). Accordingly, the lowest male weight was recorded at a low IGR concentration with the dipping method and 30°C temperature. Different results were obtained in the case of female average weight since; a significant decrease in weights was recorded with high IGR concentration (Table 1). The dipping method of treatment accompanied with an increase in the female weight comparing to spraying method. However, no change in the female weights with changing the temperature.

DISCUSSION AND CONCLUSION

Control of different insects requires changes in the management practices to reduce the spread of infection to different stored seeds. In southern cowpea weevils management programs, chemicals are extensively used. Overlapping use of IGR concentration, different method of application and different temperatures would yield a better selection to the best way to reduce the insect density. Thus, the aim of this study was to investigate the optimal conditions for using IGR to control the southern cowpea weevil *C. muculatus* Fab. Our results confirmed that the dipping method with a IGR concentration of 5% at 30°C resulted in the most significant reduction in both the food consumption the reproductive rate.

Here, we found that the high concentration of IGR significantly reduced the reproductive rate of the insect. Similarly, in Obliquebanded Leafroller *Choristoneura rosaceana* (Harris), the pupation and adult emergence was significantly delayed at Pyriproxyfen concentrations higher than 1 ppm (Sial and Brunner, 2010).

The reproductive rate was also greatly monitored by temperature since it was declined at 30°C while it was elevated at 25°C. Buholzer et al. (1992) tested the efficiency of growth regulator (Match) at three different temperatures 18, 24, 30°C in controlling cotton leaf worm *Spodopetra littoralis* (Boid) they noticed a positive relation between temperature and the efficiency of the match. The mortality rate was elevated with the increasing of temperature, perhaps that relevant to increasing of insect activity at high temperature leading to increase of growth regulator uptake. Therefore, overlap study indicated that, when chickpea seeds treated via dipping method in 5% IGR solution at 30°C led to the most significant decrease in the reproductive rate.

Reverse correlation was separately found for each of IGR concentration, treatment method and temperature with the rate of food consumption. In accordance, Gabouri (2000) found that the Southern cowpea weevils south consumed larger amount of food during a complete generation at 25°C than at 30 to 35°C.

Differences in the sex ratio were not significant in any test of concentration, treatment method or temperature. Similarly, in *Chironomus riparius*, no significant differences to solvent control were observed in any test concentration (Wang et al., 2005), although an exclusive production of males by Daphnia was observed with pyriproxyfen at 100 ng/L (Tatarazako et al., 2003). Other studies with *C. riparius* reported sex-related effects at sensitive (molecular) level also exist (Hahn et al., 2001; Hahn and Schulz, 2002). When kinoprene was applied, Rothwangl et al. (2004) found that the sex ratio was equivalent in the Petri dish experiment, whereas in the cage experiment the sex ratio was biased toward males.

Male and female weights were significantly affected by the studied interaction between the three parameters. The lowest male weight was recorded at a low IGR concentration with the dipping method and 30°C temperature. A significant decrease in weights was recorded with the increase of IGR. The dipping method of treatment accompanied with an increase in the female weight comparing to spraying method. However, no change in the female weights with changing the temperature. The weights of adults were significantly increased when Sial and Brunner (2010) studied the effect of Pyriproxyfen, on Oblique banded Leafroller *Choristoneura rosaceana* (Harris).

In conclusion, studying the overlap between cyromazine concentration, treatment method and temperature on the southern cowpea weevil indicated the existence of a difference in the rate of reproduction, on the rate of food consumption and on the average of generation lifespan. There is no effect neither in the disparity of sex ratio or weight of males and females. IGR must be applied in a potential and effective way to eradicate insects caused very serious commercial problems. Our aim then is to provide some warning signs for recognized but dangerous obstacles. On the other hand, what is currently accepted as the best way to control these insects in the present study, has to be extensively tested from the human health of view since, many studies recorded dangerous contaminations (Abel and Haarmann, 2010; Zhang et al., 2011; Seidler et al., 2011; Lehotay et al., 2011). This is necessary as this study recommend an increasing of the IGR dose. The literature is replete with evidence of human health risks associated with cleaning products, sterilants, disinfectants, antimicrobials, and a wide range of pesticides such as insecticides, fungicides, and rodenticides (Gilden, 2010). Ecological risk assessments of pesticides usually focus on risk at the level of individuals, and are carried out by comparing exposure and toxicological endpoints. However, in most cases the protection goal is populations rather than individuals (Schmolke et al., 2010).

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REFERENCES

Abel J, Haarmann-Stemmann T (2010). An introduction to the molecular basics of aryl hydrocarbon receptor biology. Biol. Chem., 391(11):1235-48.

Awad TI, Mulla MS (1984). Morphogenetic and histo pathological effects induced by the insect growth regulator cyromazine in *Musca domistica* L Diptera: Muscidae. J. Med. Entomol., 21(4): 419-426.

Buholzer F, Draber J, Bourgeois F, Guyer W (1992). a new acylurea insecticide. Med. Fac. Landbouw. Univ. Gent. 57(3):790.

Daoud KM, Elyass ZA (1990). Statistical methods for agricultural research, National Library of Printing and Publishing, University of Mosul, Iraq.

Gabouri IAH (2000). Food preference of the Southern cowpea weevil, *Callosobruchus maculatus* (F.) (Bruchidae: Coleoptera) and the effect of different temperatures on the biology, Master thesis, Faculty of Agriculture and Forestry, University of Mosul, Iraq.

Gilden RC (2010). Pesticides use in hospitals: health protection, health hazards, and viable alternatives. Nurs. Adm. Q., 34(4): 320-326.

Hagstrum DW, Subramanyam BH (2006). Fundamentals of Stored-Product Entomology. AACC International, St Paul

Hahn T, Liess M, Schulz R (2001). Effects of the hormone mimetic insecticide tebufenozide on *Chironomus riparius* larvae in two different exposure setups. Ecotoxicol. and Environ. Saf., 49(2): 171-178.

Hahn T, Schulz R (2002). Ecdysteroid synthesis and imaginal disc development in the midge *Chironomus riparius* as biomarkers for endocrine effects of tributyltin. Environ. Toxicol. and Chem., 21(5): 1052-1057.

Huignard J (1985). Importance of losses due to insect pests of seeds: problems of preservation of food legumes, vegetable protein sources. CUH Nutr Diet XX, 3: 193-199.

Ishimoto M, Sato T, Chrispeels MJ, kitamura K (1996). Bruchid resistance of transgenic azuki bean expressing seed amylase inhibitor of common bean. Entomol. Experiment et Appl., 79: 309-315.

Krebs J (1978). The experimental analysis of distribution and abundance. Harper and Row Publishers. New York, U.S.A.

Lehotay SJ, Koesukwiwat U, van der Kamp H, Mol HG, Leepipatpiboon N (2011). Qualitative Aspects in the Analysis of Pesticide Residues in Fruits and Vegetables using Fast, Low-Pressure Gas Chromatography - Time-of-Flight Mass Spectrometry. J. Agric. Food Chem., 1518-1520.

Levot GW, Sastes N (1998). Effectiveness of amixture of cyromazine and Diazinon for controlling fly strike on sheep, Aus. Vet. J., 76(5):343-344.

Miller RW, Schmidtmann ET, Wauchope RD, Clegg CM, Herner AE, Weber H (1996). Urine delivery of Cyromazine for suppressing house and stable flies in outdoor dairy calf hutches. J. Economic Entomol., 89(3):689-694.

Oberlander H, Silhacek DL, Shaaya E, Ishaaya I (1997). Current status and future perspectives of the use of insect growth regulators for the control of stored product insects. J. Stored Prod. Res., 33: 1-6.

Pancharatna K, Ghodageri M, Deshpande PA, Javalkar R (2010).

Developmental disruptions induced by insect growth regulator (Novaluron) in Bufo melanostictus tadpoles. J. Appl. Toxicol., 30(5):431-436.
Pener MP (2002). Insect growth regulators, in Encyclopedia of Pest Management (PIMENTEL, D., Ed.). Marcel Dekker, New York, pp. 398-401.
Prevett PF (1961). Field infestation of cowpea (*Vigna unguiculuta*) pods by beetles of the families Bruchidae and Curculionidae in Northern Nigeria. Bull. Ent. Res., 52: 635-646.
Rothwangl KB, Cloyd RA, Wiedenmann RN (2004). Effects of insect growth regulators on citrus mealybug parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae). J. Economic Entomol., 97(4): 1239-1244.
Schmolke A, Thorbek P, Chapman P, Grimm V (2010). Ecological models and pesticide risk assessment: current modeling practice. Environ. Toxicol. Chem., 29(4): 1006-1012.
Seidler FJ, Slotkin TA (2011). Developmental Neurotoxicity Targeting Hepatic and Cardiac Sympathetic Innervation: Effects of Organophosphates are Distinct from those of Glucocorticoids. Brain Res Bull March 29
Sial AA, Brunner JF (2010). Lethal and sublethal effects of an insect growth regulator, pyriproxyfen, on obliquebanded leafroller (Lepidoptera: Tortricidae). J. Econ. Entomol., 103(2): 340-347.
Tatarazako N, Oda S, Watanabe H, Morita M, Iguchi T (2003). Juvenile hormone agonists affect the occurrence of male Daphnia. Chemosphere, 53(8): 827-833.
Tunaz H (2004) Insect Growth Regulators for Insect Pest Control. Turk. J. Agric. and Forest., 28: 377-387.
Vazirianzadeh B, Jervis MA, Kidd NAC (2007). The Effects of Oral Application of Cyromazine and Triflumuron on House-Fly Larvae. Iran J. Arthropod-Borne Dis., 1(2): 7-13.
Wang HY, Olmstead AW, Li H, LeBlanc GA (2005). The screening of chemicals for juvenoid-related endocrine activity using the water flea Daphnia magna. Aquat. Toxicol., 74(3): 193-204.
Zhang J, Qi S, Xing X, Tan L, Gong X, Zhang Y, Zhang J (2011). Organochlorine pesticides (OCPs) in soils and sediments, southeast China: A case study in Xinghua Bay. Mar Pollut Bull.