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Full Length Research Paper

Overlap effects of cyromazine concentration, treatment method and rearing temperature on the Southern cowpea weevil (*Callosobruchus maculatus* F.) reared on cowpea

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Overlap of insect growth regulator (cyromazine) concentration, treatment method and rearing temperature was studied on the southern cowpea weevil, *Callosobruchus maculatus* F. (Bruchidae: Coleoptera), under laboratory conditions. A concentration of 5% cyromazine and dipping treatment at 30°C showed a significant reduction in the reproductive rate by 0.5%. Moreover, increasing the cyromazine concentration led to a decrease in food consumption. Hence, treatment of cowpea seeds via dipping method provided more protection compared to spraying method. Also, the increase of cyromazine concentration led to an increase of generation's lifespan. The longest generation lifespan recorded was 33.33 days when treated with 5% cyromazine by dipping at 30°C. On the other hand, the shortest generation lifespan recorded was 25.33 days when treated with 1% cyromazine concentration by dipping at 30°C. This study showed that there is no effect of the overlap between studied factors, neither in the disparity of sex ratio nor in the disparity of males and females body weights.

Key words: IGR, Insect pest, reproductive rate, sex ratio, Trigard, cyromazine.

INTRODUCTION

Legumes are considered as important crops in the world, as they are used for feeding human and animals in the form of green crops or dry seeds. Legumes are attacked by many insect pests in the field and in stores. The southern cowpeas weevil, *Callosobruchus maculates*, is one of the most common pests, with more than 35 kinds (in the field and stores) of which the most important of them is the cowpea weevil. This insect inflicts heavy losses to seeds up to 62%, since a single larva consumes about 50% of single seed weight during its development (Elazawe and Mahadi, 1983). Saplina (1980) reported that this insect is spread over more than 30 countries around the world. Its ability to fly facilitates the operation of transferring from store to field and reverse easily. The damage is caused as a result of the larvae feeding on the growing seeds, and this damage increases as larvae continue to grow due to increasing consumption of seeds contents (Howe and Currie, 1964; Pajni, 1965; Elazawe et al., 1990). Bastos (1973) found out when testing 241 samples of cowpea seeds that the rate of hit with southern cowpea was 37.8% of the tested samples and after storage for 56 days the damage percentage reached 68.6%, which subsequently led to decrease of the trade value of cowpea seeds by 56%.

The presence of residues in food, resistance develop-

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ment by pest species, health risks (Arthur, 1996), increased cost (Hagstrum and Subramanyam, 2006) and toxicity to non-target organisms (Fields, 1992) has created strong concerns with the use of synthetic chemicals in controlling stored-product insects. Thus, insect growth regulators (IGR) are considered one of the most suitable alternative natural pesticides as they may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development. 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 (susceptibility to natural enemies, environmental conditions, etc) or from an abnormal termination of a developmental stage itself (Tunaz, 2004). Miller et al. (1981) pointed out that the use of insect growth regulator (cyromazine) 0.25, 0.5 and 0.1% concentration against the larvae of *Musca domestica* Linn caused 95% mortality, recording rate of success of 3 to 10 over the growth regulator diflubenzuron. In addition, Saito (1988) reported that adding 75% of cyromazine to water resulted in 88% mortality amongst larvae of *Liriomyza bryoniae* Kalt. In an experiment involving the use of cyromazine at the rate of 0.1, 0.5 and 1.1% g/ kg weight of animal in the field of milk cows, Miller et al. (1996) found that its residue in cows' waste discouraged the development of *M. domestica* larvae. Levot and Sates (1998) also found that the use of cyromazine and dezinion each individually against *M. domestica* by concentration 0.4 g/L led to a reduction in the number of *M. domestica* at rate of 69% after one day of treatment and 99.97% in three days of treatment. Vazirianzadeh et al. (2007) concluded that cyromazine (Trigard) should be used in a larvicidal program to control house-fly. More importantly, insect growth regulators were considered to be better used in an integrated pest management program, rather than being used alone (Oberlander et al., 1997).

The objectives of the current study were to determine the impact of overlapping between the different concentrations of growth regulator (cyromazine), treatment methods and the temperatures in the biological activity of southern cowpea weevils.

MATERIALS AND METHODS

Experimental insects

The southern cowpea weevil, *Callosobruchus maculatus* F. (Bruchidae: Coleoptera), was obtained from an entomological research laboratory in the College of Agriculture and Forestry, University of Mosul. All cowpeas (*Vigna unguiculata*) seeds were put in glass jar (1/2 kg each). The southern cowpea weevils were added (20 weevil/jar), covered with a piece of cloth, bond with rubber firmly and then incubated at 30 ± 2°C or 50 ± 5% (Ishimoto et al., 1996). Cultures were renewed after each generation by taking the newly emerged insects for construction of a new culture to conduct further studies.

Insect growth regulator (cyromazine)

This pesticide works as a growth regulator and is used to control larvae of Diptera and Coleoptera. It is being used as a powder in water containing 750 g /kg effective cyromazine (N-cyclopropyl 1, 3, 5-triazine-2, 4, 6-triamine) and sold under the names of various commercial (Larvadex, Premix) classifies toxically within the Class III according to the classification of the World Health Organization (WHO). Solutions of cyromazine were freshly prepared immediately prior to the experiments (Awad and Mulla, 1984).

Pesticide bioassays

Three concentrations of cyromazine were applied (1, 3 and 5%) in the treatment of chickpea seeds. Seeds (25 g per time) were treated twelve times and six times treated in a spraying manner using the Potter Tower at 5 lbs/inches pressure and 2.5 ml of cyromazine solution, ensuring coverage of the surface of seeds. The remaining six were treated by dipping in the cyromazine solution for 1 min. For the control, seeds were treated with water only. Drought seeds have been placed in plastic pots (7 × 7 cm) then five pairs (males and females) of newly emerged adults were transferred into each pot and covered with a piece of cloth sealed with a rubber bond. Pots were then incubated at 25 ± 1 or 30 ± 1°C and 50 ± 5% RH. Treatments were followed up to two successive generations to specify the overlap between different concentrations of cyromazine, treatment method and rearing temperature on the following: Reproductive rate of the southern cowpea weevils is calculated for two successive generations using the formula of Krebs (1978):

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

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

The rate of food consumption was measured by weighing the treated seeds after the end of experiment and deducted from the original weight (25 g). Generation lifespan was calculated from the new adult emergence (from the pupae) until the advent of insects in the second-generation. Sex ratio and weight of males and females were tested by taking a random group of full complete insects each in pot and calculating the number of males and females and their weight.

Data analysis

For conducting the test and analyzing its data, the factorial completely randomized design and Duncan's multiple range tests to change the averages of endurable level 5% (Daoud and Elyass, 1990) were used.

RESULTS AND DISCUSSION

The results presented in Table 1 indicate that increasing in the cyromazine concentration resulted in significant reduction in the reproductive rate of the treated southern cowpea weevils compared to control ones. Cyromazine-treated insects (with a concentration of 5%) showed reproductive rate of 2.58% compared to 36.1% in control ones. House et al. (1978) stated that when they used

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

		Concentration (%)				Treatment methods		Temperatures	
		1	3	5	Control	Dipping	Spraying	25°C	30°C
Reproductive rate		23.3^{C}	8.74^{B}	2.58^{A}	36.11^{D}	14.41^{A}	21^{B}	18.50^{B}	16.9^{A}
Food consumption		8.87^{C}	5.61^{B}	2.89^{A}	18.28^{D}	6.89^{A}	10.94^{B}	9.29^{B}	8.54^{A}
Generation lifespan		28.7^{C}	29.79^{B}	30.67^{A}	27.71^{D}	28.81^{B}	29.63^{A}	31.79^{A}	26.65^{B}
Sex ratio	M	1.05^{A}	1.18^{A}	1.19^{A}	1.11^{A}	1.16^{A}	1.11^{A}	1.10^{A}	1.17^{A}
	F	1.14^{A}	1.05^{A}	1.05^{A}	1.04^{A}	1.07^{A}	1.07^{A}	1.07^{A}	1.07^{A}
Average weights	M	0.96^{A}	1.07^{B}	1.05^{B}	0.95^{A}	0.99^{A}	1.02^{A}	1.02^{A}	0.99^{A}
	F	1.36^{A}	1.59^{B}	1.42^{A}	1.44^{A}	1.42^{A}	1.49^{A}	1.45^{A}	1.46^{A}

Averages of similar characters refer to the existence of significant differences at the 0.05% level of probability. M, Male; F, female.

diflubenzuron to control the boll weevils, *Anthonomus grandis* (Boheman), at a rate of 35, 70 and 140 g/ha, there was an effective reduction in the total percentage of adult emergence from pupae (37.7, 22.21 and 15.8%, respectively). The use of 4 growth regulator, hydroprene, methoprene, diflubenzuron, and MV-678 to control *Ephestia cautella* (Walker) on the peanut, with increased concentration resulted to a decrease of adult emergence (Nickle, 1979). These growth regulators led to down-regulation of the rate of the fertility among treated insects. In oblique-banded leafroller *Choristoneura rosaceana* (Harris), the pupation and adult emergence was significantly delayed at pyriproxyfen concentrations higher than 1 ppm (Sial and Brunner, 2010).

Considering the effect of treatment methods on the reproductive rate, Table 1 shows a decrease in the southern cowpea weevils' number when treated via dipping method (14.41%), compared with the spraying methods (21%). The production rate was monitored with temperature since it was 16.90 at 30°C, while at 25°C it was 18.50. Buholzer et al. (1992) also tested the efficiency of growth regulator (Match) at three different degrees of temperature 18, 24, 30°C in controlling cotton leaf worm *Spodoptera littoralis* (Boisd.) and reported a positive relation between temperature and the efficiency of the Match. The mortality rate increase accordingly with increase in temperature, perhaps that relevant to increasing of insect activity at high temperature leading to increase of growth regulator pick up.

The data from overlap experiment indicated that dipping-treated cowpea seeds (in 5% cyromazine solution at 30°C) showed significant decrease of the production rate of the southern cowpea weevil, recording 0.5, where it was 36.43 in spraying methods (Table 2). The obtained results as shown in Table 1 revealed that the rate of food consumption (8.87, 5.61, and 2.89 g) decreased when cyromazine concentration (1, 3, and 5%) increased compared to control (18.28 g). A significant influence of treatment methods, preferring the dipping method over spraying one where the dipping method resulted in decrease in the rate of food consumption (6.89 g) compared to that of the spraying methods (10.94 g). Temperature also had a significant impact, where at 25°C the rate of food consumption was high (2.29 g), compared to that at 30°C (3.30 g). Gabouri (2000) mentioned that Southern cowpea weevils at 25°C consumed 17.17 g of the food during a complete generation in contrast with that raised at 30-35°C which consumed 12.38 g, and 10.88 g, respectively. Data from overlap experiment indicated that cowpea seeds treated via dipping method in 5% cyromazine solution at 30°C led to significant decrease of the food consumption rate of the southern cowpea weevil, recording 0.5, whereas it was 4.04% in spraying methods (Table 2).

The generation lifespan reached 28.71, 29.79, and 30.67 days at the concentration of 1, 3, and 5% respectively, in contrast with water treated control. The generation lifespan clearly decreased when the dipping method was applied at 28.81 days, while in spraying method was 29.63 days. Temperature plays important role; at 30°C the average of the generation lifespan was 26.65 days, in contrast with generation lifespan at 25°C recorded 31.79 days (Table 1). The highest generation lifespan recorded was 33.33 days in spraying treatment at 5% concentration and 25°C, and the lowest generation lifespan was 26.33 days in dipping treatment at 1% concentration and 30°C (Table 2). Furthermore, Tables 1 and 3 showed no significant differences observed for the sex ratio in any test concentration, treatment method and the temperature compared to the control. Similarly, in *Chironomus riparius,* no significant differences to solvent control were observed in any test concentration, although an exclusive production of males by Daphnia was observed with pyriproxyfen at 100 ng/L (Wang et al., 2005; Tatarazako et al., 2003). Other studies with *C. riparius* which reported sex-related effects at sensitive (molecular) level also exist (Hahn et al., 2001; Hahn and Schulz, 2002). For example Hahn and Schulz (2002)

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. maculates* reared on cowpeas.

Concentration (%)	Treatment method	Temperature	Reproductive rate	Food consumption rate	Generation lifespan
1	Dipping	25	20.60 ± 0.26^{F}	7.23 ± 0.16^{F}	31.17 ± 0.17^{C}
3			3.27 ± 0.15^{C}	4.93 ± 0.23^{E}	32.33 ± 0.17^{B}
5			1.53 ± 0.15^{B}	2.56 ± 0.09^{B}	32.5 ± 0.29^{AB}
Control			36.07 ± 0.23^{J}	12.72 ± 0.14^{K}	30.17 ± 0.17^{D}
1	Spraying		30 ± 0.29^{I}	11.18 ± 0.16^{G}	32 ± 0.50^{B}
3			15.47 ± 0.15^{E}	7.75 ± 0.25^{I}	32.67 ± 0.33^{AD}
5			4.9 ± 0.06^{D}	4.46 ± 0.07^{DE}	33.33 ± 0.33^{A}
Control			36.2 ± 0.12^{J}	23.45 ± 0.21^{N}	30.17 ± 0.17^{D}
1	Dipping	30	15.5 ± 0.29^{E}	7.02 ± 0.18^{FH}	25.33 ± 0.17^{HI}
3			1.4 ± 0.21^{B}	3.19 ± 0.04^{C}	26.67 ± 0.33^{H}
5			0.05 ± 0.06^{A}	0.50 ± 0.06^{A}	27.67 ± 0.17^{F}
Control			36.43 ± 35^{J}	16.94 ± 0.24^{L}	24.67 ± 0.17^{J}
1	Spraying		27.43 ± 0.07^{H}	10.04 ± 0.28^{J}	26.33 ± 0.17^{H}
3			14.83 ± 0.44^{E}	6.55 ± 0.16^{F}	27.5 ± 0.50^{F}
5			3.4 ± 0.21^{C}	4.04 ± 0.18^{D}	29.17 ± 0.17^{E}
Control			35.73 ± 0.15^{J}	20.02 ± 0.13^{M}	25.83 ± 0.17^{HI}

Averages of similar characters refer to the existence of significant differences at the 0.05% level of probability.

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

Concentration (%)	Treatment method	Temperature	Mean of the sex ratio ± S.E	
			Male	Female
1	Dipping	25	1.05 ± 0.05^{A}	1.12 ± 0.12^{AB}
3			1.13 ± 0.13^{A}	$1.O3 \pm 0.02^{AB}$
5			1.09 ± 09^{A}	1.06 ± 0.06^{AB}
Control			1.11 ± 0.08^{A}	1.06 ± 0.06^{AB}
1	Spraying		1.07 ± 0.07^{A}	1.04 ± 0.03^{AB}
3			1.15 ± 0.10^{A}	1.09 ± 0.09^{AB}
5			1.09 ± 0.09^{A}	1.12 ± 0.08^{AB}
Control			$1.O7 \pm 0.07^{A}$	1.04 ± 0.04^{AB}
1	Dipping	30	1 ± 0^{A}	1.32 ± 0.27^{B}
3			1.25 ± 0.09^{AB}	1 ± 0^{A}
5			1.47 ± 0.14^{B}	1 ± 0^{A}
Control			1.17 ± 0.17^{A}	1 ± 0^{A}
1	Spraying		1.07 ± 0.04^{A}	$1.O7 \pm 0.07^{AB}$
3			1.19 ± 0.10^{AB}	1.06 ± 0.06^{AB}
5			1.11 ± 0.08^{A}	1.04 ± 0.04^{AB}
Control			1.07 ± 0.07^{A}	1.07 ± 0.07^{AB}

Averages of similar characters refer to the existence of significant differences at the 0.05% level of probability.

Table 4. Overlap effect of different concentrations of cyromazine, treatment methods and temperatures on the average weights of the southern cowpea weevil, C. maculates reared on cowpeas.

Concentration (%)	Treatment method	Temperature	Mean of the average weights ± S.E	
			Male	Female
1	Dipping	25	1.07 ± 0.7^{CDE}	1.37 ± 0.02^{AB}
3			1.05 ± 0.05^{BCDE}	1.52 ± 0.02^{BC}
5			1 ± 0.03^{BCDE}	1.53 ± 0.15^{BC}
Control			1 ± 0.03^{BCDE}	1.45 ± 0.1^{AB}
1	Spraying		0.95 ± 0.03^{ABCD}	1.27 ± 0.07^{A}
3			1.08 ± 0.04^{CDE}	1.83 ± 0.14^{D}
5			1.02 ± 0.11^{BCDE}	1.30 ± 0.03^{AB}
Control			1.02 ± 0.02^{BCDE}	1.32 ± 0.03^{AB}
1	Dipping	30	0.82 ± 0.04^{A}	1.30 ± 0.09^{AB}
3			1.02 ± 0.03^{BCDE}	1.32 ± 0.06^{AB}
5			1.07 ± 0.04^{CDE}	1.37 ± 0.03^{AB}
Control			0.92 ± 0.04^{ABC}	$1.5 \pm 0.06A^{BC}$
1	Spraying		1 ± 0.05^{BCDE}	1.52 ± 0.06^{BC}
3			1.12 ± 0.02^{DE}	1.70 ± 0.06^{CD}
5			1.13 ± 0.0^{7E}	1.48 ± 0.02^{ABC}
Control			0.88 ± 0.05^{AB}	1.50 ± 0.05^{ABC}

Averages of similar characters refer to the existence of significant differences at the 0.05% level of probability.

observed a sex-related effect of tributyltin on the ecdysteroid synthesis and the imaginal disk development by *C. riparius*. In *Leptomastix dactylopii* (Howard), 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.

Regarding the rate of male weight, the increase of cyromazine concentration led to increase of male weight recording 0.96, 1.5 and 1.7 mg at concentrations of 1, 3 and 5, respectively (Table 1), in contrast with control treatment (0.95 mg). Also the study concluded a non significant effect of concentration, temperature and treatment method in the average of male weight recording 1.13 mg at 5% concentration using the spraying method at 30°C as the highest weight. However the lowest male weight recorded was 0.82 mg at 1% concentration via dipping method and at 30°C. For the female average weight, the weight recorded was 1.59 mg at concentration 3% and recorded 1.36, 1.42 mg at 1 and 5% concentration, respectively. Taking together, these results showed no significant differences in the average weight of female between the two concentrations (1 and 5%) and the control, but there was a little difference in female weight at 3% concentration. Also there were no differences in the female weight at 25 to 30°C and the two different methods of treatment (Table 1). The highest female average weight recorded was 1.83 mg at 3% concentration via spraying method at 25°C and the lowest weight recorded was 1.27 mg at 1% concentration via spraying method at 25°C (Table 4). Sial and Brunner (2010) studied the effect of pyriproxyfen, on oblique-banded leafroller *C. rosaceana* (Harris) and found that the weights of adults were significantly increased.

In conclusion, the study of the overlap between cyromazine concentration, treatment method and temperature on the southern cowpea weevil for two successive generations indicated the existence of a difference in the reproductive rate, the rate of food consumption and average of generation lifespan. However, there was no effect neither in the disparity of sex ratio nor in the disparity in the weight of males and females.

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