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Effect of Different Applicable Conditions of the Insect Growth Regulator (Cyromazine) on the Southern Cowpea Weevils, *Callosobruchus maculatus* Reared on Peas

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Abstract.- This study investigates the effect of different concentrations of the growth regulator (Cyromazine), temperature and treatment methods on the reproductive rate, generation lifespan and weight loss of the Southern cowpea weevils *Callosobruchus maculatus* (Coleoptera: Bruchidae). Data showed that weevils failed to complete their life cycle on the host peas**,** *Pisum sativum***,** pre-dipped in a 5% cyromazine at 30°C. Cyromazine-dipped peas seeds were protected from insect attack comparing to cyromazine-sprayed seed. Moreover, increasing the cyromazine concentration increased the average period of insects generation since the longest generation period recorded from cyromazine-sprayed insects at 25°C was 42.33 day post-treatment. Increasing cyromazine concentration reduced the percentages of pupation and adult emergence down to19.83 and 27.08%, respectively compared to control treatment which were 87.33 and 88%, respectively. The results showed little overlapping effect on the three factors on the sex ratios and the mean weight of male and female.

Key words: Food consumption, generation lifespan, pea, Trigard, Cyromazine.

INTRODUCTION

Pea which has a widespread variety of use in different regions of world has the largest sowing area in the world after beans and chickpeas and has the largest production value after the bean (Hulse, 1994). Furthermore, pea is an important grain legume that has the highest unit area yield in the world and its farming is concentrated more in developed countries (Ozdemir, 2002). Throughout temperate regions both green and dried peas are an important garden and field crop. Pea is the predominant export crop in world trade and represents about 40% of the total trade in pulses (Oram and Agcaoili, 1988). Worldwide field pea plants are used for forage, hay, silage, and green manure (Purseglove, 1987).

Southern cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae), is considered among the important pests which attacks not only the pods in field, but also continue to produce several successive generations in the dried seeds (Abo-Hegazi, 1978). Southern cowpea weevils are agricultural pest insects of Africa and Asia that presently range throughout the tropical and subtropical world. Larvae of this species feed and develop exclusively on the seed of legumes. The adults do not require food or water and spend their limited lifespan (one - two weeks) mating and laying eggs on beans.

Insect growth regulators (IGRs) are a diverse group of insecticides, with a range of effects on insect specific phenomena, disrupting the growth and development of insects and other arthropods. They mainly affect the development of immature stages, and disrupt metamorphosis and reproduction (Retnakaran *et al.*, 1985; Graf, 1993) and are becoming more important in the management of insect pests (Grenier and Grenier, 1993). IGRs include various chemical categories including: juvenile hormones, chitin synthesis inhibitors, and

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triazine derivatives (Retnakaran *et al.,* 1985) with different modes of action. Cyromazine (CGA 72662, N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine) represents a new class of IGRs derived from aziodotriazine herbicides (Shen and Plapp, 1990). It was discovered by Ciba-Giegy Ltd. in the mid 1970s and originally developed under the trade name of 'Vetrazine', a blow-fly control agent. Cyromazine is also applied topically to control housefly larvae in manure ('Neporex'), as a feed–through in poultry ('Larvadex'), as well as in crop protection ('Trigard') (Graf, 1993; Moreno-Mari *et al.,* 1996).

The aims of the current study were to determine the impact of overlapping between different concentrations of cyromazine, temperature and treatment method on the biological activity of southern cowpea weevils reared on peas.

MATERIALS AND METHODS

Insects

The southern cowpea weevil, *C. maculatus* F. (Bruchidae: Coleoptera) were obtained from the Entomological Research Laboratory, College of Agriculture and Forestry, Mosul University, Iraq. Insects were added to glass jars (each containing 1/2 kg cowpea seeds, and covered with a piece of cloth and bond with rubber firmly. Jars were then, incubated at 30±2°C and 50±5% (Ishimoto *et al.,* 1996) throughout the experiment. Cultures were renewed after each generation by taking the newly emerged insects from pupae for construction of a new culture to conduct further studies.

Bioassays

Three concentrations (1, 3 and 5%) of cyromazine were applied to the seeds of *Pisum sativum* (L) according to Al-Mekhlafi *et al.* (2012). Seeds (25g per time) were first treated with six sprays, each with 2.5 ml of cyromazine solution using the Potter Tower at 5 lbs/inches pressure, followed by dipping in the cyromazine solution (test) or water (control) six times, each for one minute. Drought seeds were placed in plastic boxes (7 x 7cm) to which five pairs (male and female) of newly emerged of adults cowpea weevil were place. Boxes were then covered with a piece of cloth sealed with rubber bands and incubated at 25±1°C and 30±1°C, 50±5% RH for two successive generations.

Reproductive rate of southern cowpea weevils was calculated according to Krebs (1978).

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

where, r is the rate of reproduction; n is number of colony individuals; dn is change in the number of colony individual; and dt is change of time.

Rate of food consumption was measured by weighting the treated seeds after the end of the experiment and deducted from the original weight (25g) (Al-Mekhlafi *et al.,* 2012). 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 male and female were tested by taking a random group of full complete insects each in box and calculating the number of male and female and their weight.

Data analysis

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

RESULTS AND DISCUSSION

The results showed that there was a significant difference ($P<0.05\%$ n=6) in the reproductive rate between the concentrations used, as it reached 0.03% at the 5% concentration while it was 7.25% in the control (Table I). House *et al.* (1978) found that the percentage of full insects appearance reached 15.8, 22.21 and 37.7%, when they used the growth regulator, Diflubenzuron to control *Anthonomus grandis* with the rate 140, 70, 35 g/ha. Nickle (1979) used four orderly growth which are hydroprene, methoperene ,diflubenzuron, and MV-678 to control *Ephestia cautella* (Walker) on peanut, they found that increasing of concentration leads to decreasing adult emergence

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

	Concentration (%)			Control	Treatment methods		Temperatures	
	1	3	5		Dipping	Spraying	25°C	30°C
Reproductive rate	1.49 c	0.23 b	0.03 a	7.25 d	2 a	2.50 b	2.25 a	2.24 a
Food consumption (g)	2.66 b	1.83 b	1.52 a	7.69 d	1.95 a	3.30 a	3.55 a	4.91 b
Generation lifespan (day)	18.21 d	18.71 c	19.11 b	35.38 a	8.88 b	36.83 a	25.97 a	19.73 b
Sex ratio								
Male	0.57 a	0.54 a	0.52 a	1.04 b	0.25 a	1.10 b	0.68 a	0.67 a
Female	0.54 a	0.57 a	0.59 a	1.07 b	0.25 a	1.12 b	0.68 a	0.69 a
Average weights (mg)								
Male	0.46 a	0.47 a	0.48 a	1 b	0.28 a	0.93 b	0.55 a	0.65 b
Female	0.69 a	0.66 a	0.82 b	1.63 ac	0.41 a	1.51 b	1.90 a	1.01 b

*Averages of similar characters refer to the existence of significant differences at the (P<0.05, n=6) level of probability.

and that these regulators also leads to reduction of fertility. The emergence percentage of housefly adult from eggs treated with sublethal concentrations of Cyromazine was reduced where it reached 30% in comparison with 63.50% of the control (Al-Abadi, 2001). 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). Ali *et al.* (2011) study the influence of two temperatures (30°C and 35°C) and three humidities (40, 60 and 80% RH) on the adult emergence of the carpet beetle *Attagenus fasciatus* and they stated that, there was 100% emergence of adults from pupae under all test conditions except 40 and 60% RH at 35°C where it fell to about 90%.

Table I shows that the reproductive rate dropped when pea seeds were treated by dipping comparing to spraying method, as the increasing rate reached 2.0 by dipping while it reaches 2.5 by spraying method. And this also could be due to the reproductive rate of insect's on peas was low as a result of the insect no-preference to peas or the increasing of span ion material. Mahmud (1989) referred the injury of some legumes seeds by southern cowpea weevil to the Spanion percentage in the legumes seeds, and as much as the percentage increased, the more the legumes seeds resisted the injury of that insect. He also found that cowpea seeds are sensitive to get hit by this insect as it contains 0.22% of Saponin while lentils seeds and soybeans are resistant to the injury of that insects as they contain 2.48-1.95% of Saponin. The results showed the existence of significant differences (P< 0.05%, n=6) in the reproductive rate depending on the treatment method used in the study (Table I). The insect on peas seeds which have been treated by dipping at all concentrations used and breeding temperature didn't complete its life cycle. While when it was treated by spraying method, the highest reproductive rate reached 3.20% at the concentration of 1% and temperature 25°C food consumption and the lowest reproductive rate was zero at the concentration of 5% and temperature 30°C (Table II). A dose-dependent relationship in the rate reproduction of the Southern cowpea weevils with cyromazine in the current study agrees with the results of Tomberlin *et al.* (2002) and Al-Mekhlafi *et al.* (2012) on *C. maculates* reared on mung bean. They obtained similar results using cyromazine in an oral application against black soldier fly, *Hermetia illucens* (L.). Cyromazine and triflumuron had a significant effect on larval mortality of the house fly compared with their controls among the concentrations including a dose-dependent relationship Vazirianzadeh *et al.* (2007).

Table II shows that increasing cyromazine concentrations lead to significant decrease in food consumption compared to control experiment and that the 5% concentration was the most effective one in decreasing food consumption as it reached 1.25g compared to 7.69g in the control experiment. Results also showed that the treatment method has a

Table II.- 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 (°C)	Reproductiverate (%)	Food consumption rate (g)	Generation lifespan (day)
1	Dipping	25	DAI a	DAI a	DAI i
3			DAI a	DAI a	DAI i
5			DAI a	DAI a	DAI i
Control			7.73±0.09 h	7.73±0.01 ih	40.67±0.33 d
1	Spraying		3.20±0.15 d	5.76±0.06 f	41.50±0.58 c
3			0.73 ±0.19 l	3.82 ±0.02 d	42.33±0.33 b
5			0.1±0.06 a	3.57±0.09 c	43.10±0.21 a
Control		30	6.27±0.15 e	7.25±0.10 h	40.17±0.17 f
1	Dipping		DAI	DAI a	DAI i
3			DAI	DAI a	DAI i
5			DAI	DAI a	DAI i
Control			8.28±0.09 i	7.85±0.09 i	30.33±0.33 c
1	Spraying		2.77±0.15 c	4.89±0.16 e	31.33±0.17 h
3			0.17±0.9 c	3.52±0.6 c	32.50±0.29 f
5			DAI	2.50±0.6 b	33.33±0.17 e
			6.73±0.15 f	7.67±0.18 ih	33.33±0.33 i

DAI, Death of all insects.
Averages of similar characters refer to the existence of significant differences at the (P<0.05, n=6) level of probability.

significant effect at the rate of food consumption as it decreased significantly by the dipping method compared to spraying method as it reached 1.95mg by the dipping method while it was 4.91mg by spraying method. Moreover, the insect could not complete its life cycle for the first generation by the dipping method while it could by the spraying one (Table I). The results confirmed the presence of significant differences (P< 0.05%) in the food consumption rate according to the method of treatment. Temperature also has an effect in the rate of food consumption since 25°C increased the rate of food consumption (3.55 mg/insect) compared to the 30°C temperature (3.30 mg/insect). This may indicate that 25°C is the optimal temperature for *C. maculatus* activity. Hallak (1993) mentioned that increased in breeding temperature for southern cowpea weevil from 25 to 30°C leads to decreased egg laying and egg hatching. Al-Mekhlafi *et al.* (2012) stated that the increase in cyromazine concentration leads to a significant reduction in the food consumption. There was a significant reduction in food consumption in case of dipping method as compared with spraying method. Also, temperature had an effect on the rate of food consumption. Ali *et al.* (2011) found that, at 35°C, eggs of the carpet beetle *Attagenus fasciatus* took less time to hatch than that at 30°C and there was a positive correlation between egg duration and relative humidity. Pyripoxyfen and fenoxycarb were also shown to suppress egg hatch in pear psylla, *Casopsylla pyricola* (Foerster) (Higbee *et al.*, 1995), and egg hatch and adult emergence in *Bemisia tabaci* (Ishaaya and Horowitz, 1992) and *Haematobia irritans* (L.) (Bull and Meola, 1993).

Different concentrations, temperature and treatment methods have significant effect on the rate of food consumption. There was no loss in peas seeds that have been treated by dipping at all concentrations and temperatures used in the study, while there was a loss when it was treated by spraying as the highest rate of consumption was 5.76 mg/insect at the concentration of 1% and temperature 25°C and the lowest one was 2.5 mg/ insect at the concentration 5% and temperature 30°C (Table II). Gabouri (2000) referred that the southern cowpea weevil insect bred on a temperature 25°C consumed 17.17 mg /insect of food during the period of a whole generation compared to the ones bred on a temperature of 30 and 35°C that consumed 12.38 and 10.88 mg/insect, respectively.

The increasing concentration of cyromazine reduced the generation lifespan down to 18.21, 18.71, and 19.11 days at the concentrations 1, 3, 5%

respectively, compared to 35.38 days in the control experiment. That variation may be attributed to the disability of insects to complete the lifespan of the first generation on peas treated by dipping method. The generation's lifespan has been shown to be reduced by dipping methods compared to spraying one. For the insects raised on peas treated by dipping, the generation's lifespan reached 8.88 days and for those treated by spraying it reached 36.83 days. This may be attributed to the disability of insects to complete their lives till the end of the first generation on peas treated by dipping method while it was able to complete it on the one treated by spraying. As it shown by Table I, the temperature also has an effect on the generation's lifespan as it reduced clearly at the temperature of 30°C, also the average length of generation reached 19.73 days at a temperature 30°C compared to 25.97 days at a temperature 25°C. Table II shows that insects bred on peas that has been treated by dipping didn't complete their first generation period at all concentrations and temperatures used in the study while its life cycle was completed on the one treated by spraying as the highest average of generation's duration reached 43.10 days at the concentration of 5% and a temperature of 25°C, while the lowest one was 31.33 day at the concentration 1% and a temperature 30°C.

Increasing cyromazine concentration has no effect in the average of the sex ratio of the southern cowpea weevil bred on peas (Table I) and that this ratio was often tending to the favor of females and again the results showed the existence of significant differences ($P < 0.05\%$, $n=6$) in the average values of sexual ratio between the cyromazine concentrations used in the study and the treatment of control experiment to the insects bred on peas as it reaches (0.54,0.57), (0.57,0.54) and (0.59,0.52) (female, male) at the concentrations 1, 3, 5% respectively compared to control experiment method of treatment (1.04, 1.07) (male, female). Table I showed that the average sexual ratios has been reduced by the dipping method compared it to spraying one as the value reached 0.25:0.25 (male, female) by the dipping method while it reached 1.12:1.10 (male, female) by spraying and this goes back to the non-appearance of full insects bred on peas treated by dipping. The overlap between cyromazine concentrations, method of treatment and temperature had an impact on the sex ratios of southern cowpea weevil reared on peas (Table III). On the contrary, Al-Mekhlafi *et al.* (2012) indicated that the cyromazine concentration, method of treatment and the temperature had no influence on the sex ratio of the southern cowpea weevil reared on mung bean. Roth (1989) stated that sublethal doses of methoprene can cause changes in sex ratios of horn fly parasites, *Spalangia cameroni*, were largely unaffected by methoprene but exposure did change the sex ratio in their progeny. Pyriproxyfen could cause disruption of sex ratio when used against the sunn pest, E*urygaster integriceps* puton (Mojaver and Bandani, 2010)**.**

Increasing cyromazine concentration has lead to the reduction in male's weight of southern cowpea weevil bred on peas compared to control ones as the average weight of males reached 0.46, 0.47, 0.48 mg at the concentration 1, 3, 5% respectively compared to the control method as it reached 1 mg (Table I). Temperature also has a significant effect on the average of male's weight as it reached 0.65, 0.55 mg at both temperatures of 25, 30°C, respectively, and the method of treatment has significant affect in the average of male's weight as it decreased in the seeds treated by dipping compared to the ones treated by spraying as it reached 0.28, 0.93 mg, respectively (Table I). As for the affect of the overlap between cyromazine concentrations, temperature and method of treatment on the average of male's weight, the results confirmed the existence of a very slight difference between methods of treatment in the average of male's weight as the highest average was 1.2 mg at the concentration of 3%, treated by spraying and a temperature of 30°C while the insects treated by dipping, at the temperature of 25, 30°C couldn't survive. From Table I, it is clear that the increasing cyromazine concentration leads to increase in the average female weight of southern cowpea weevil as it reached 0.69, 0.66, 0.82 mg at the concentrations 1, 3, 5%. But generally compared to the control. The female's weight reached 1.86 mg. Temperature also has significant affect on the average female's weight as it reached 1.01 mg at 30°C, and 0.91 mg at 25°C (Table I). The weights of adults were significantly increased when Sial and

Table III.- 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 (°C)	Mean of the sex ratio ± S.E.	
			Male	Female
1	Dipping	25	DAI a	DAI a
3			DAI i	DAI a
5			DAI a	DAI a
Control			1±0 b	1.±0 b
1	Spraying		1.04±0.04 bc	1.19±0.10 b
3			1.22±0.11 d	1±0
5			1.15±0.07 bcd	1.07±0.07 b
Control			1.07±0.07 bcd	1.17±0.17 b
1	Dipping	30	DAI a	DAI a
3			DAI i	DAI a
5			DAI a	DAI a
Control			1±0 b	1.±0 b
1	Spraying		1.14±0.08 bcd	1.10±0.10b
3			1.08±0.04 bcd	1.15±0.15b
5			1.20±0.07 cd	1±0 b
Control			DAI a	DAI a

DAI, Death of all insects.
Averages of similar characters refer to the existence of significant differences at the (P<0.05, n=6) level of probability.

Table IV.- Overlap effect of different concentrations of cyromazine, treatment methods and temperatures on the average weights of the southern cowpea weevil, *C. maculates*

Concentration (%)	Treatment method	Temperature (°C)	Mean of the weight ratio ± S.E.	
			Male	Female
1	Dipping	25	DAI a	DAI a
3			DAI a	DAI a
5			DAI a	DAI a
Control			1.10±0.06 fe	1.5±0.06 de
1	Spraying		0.98±0.06 de	1.3±0.1 bc
3			0.68±0.03 b	1.42±0.09 cd
5			0.90±0.08 cde	1.63±0.06 ef
Control			0.75±0.03 bc	1.47±0.09 cde
1	Dipping	30	DAI a	DAI a
3			DAI a	DAI a
5			DAI a	DAI a
Control			1.10±0 ef	1.8±0.06 fh
1	Spraying		0.85±0.05 bcd	1.47±0.07 cde
3			1.20±0.18 f	1.23±0.06 b
5			1.02±0.04 def	1.63±0.06 ef
Control			1.03±0.03 fed	1.93±0.09 h

DAI, Death of all insects.
Averages of similar characters refer to the existence of significant differences at the (P<0.05, n=6) level of probability.

Brunner (2010) studied the effect of Pyriproxyfen, on Oblique banded Leafroller *Choristoneura rosaceana* (Harris).

The highest average female's weight was reached by spraying method at the concentration of 5% and that average was 1.63 mg at both

temperatures 25, 30°C, while the insects fed on seeds treated by dipping at all the concentrations used in the study and at both temperatures couldn't complete its life cycle (Table IV). These findings are supported by Al-Mekhlafi *et al.* (2012) who showed that the cyromazine concentration, method of treatment and the temperature had no influence on the average of female's and male's weight of the same insect reared on mung bean.

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