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Overlap Effects of Cyromazine Concentration, Treatment Method and Rearing Temperature on Southern Cowpea Weevil *Callosobruchus maculatus* Reared on Mung Bean

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Abstract.- Insect pests of stored products constitute a worldwide problem because they reduce the quantity and quality of stored products. In this study we investigated the interactions between different factors that affect the growth of two successive generations of the southern cowpea weevils, *Callosobruchus muculatus* (Fab.) (Bruchidae: Coleoptera) reared on mung bean, to find a best conditions to control this pest. These factors include cyromazine concentration, treatment method and breading temperature. Our results showed a variable effect in the reproductive rate of *C. muculatus* treated with 5% cyromazine concentration using dipping method at 30°C. In addition, increasing cyromazine concentration resulted in decreasing the average of food consumption and increasing the average of generation lifespan. Moreover, seeds treated by dipping method are more protected in comparison with those treated by spraying. The overlap between the three factors had no influence on the sex ratio, but it had an impact on the average weights of males and females reared on a mung bean.

Keywords: Cyromazine, food consumption, IGR, mung bean, sex ratio.

INTRODUCTION

Pulses are important protein source for Asian people, many of whom largely depend upon cereals and pulses for their daily requirements. Mung bean seeds are sprouted for fresh use or canned for shipment to restaurants. Sprouts are high in protein (21%–28%), calcium, phosphorus and certain vitamins. Because they are easily digested they complement the scarce animal protein in human diets in tropical areas of the world (Cupka, 1987).

Pulses are attacked by many pests in the field and store. The Southern cowpea weevil, *C. maculatus* (F.) is one of the most common and dangerous pests among other types of the family of Bruchidae (Southgate, 1979). Crop damage is caused by feeding of insect larvae on the growing seeds and this damage increases when the larvae continue to grow and consume huge contents of seeds (Howe and Currie, 1964; Pajni, 1965; Al-Azzawi *et al.*, 1990). The insects attack the seeds of about 35 types of legumes in the field and store and causes 62% crop loss. During generation lifespan, the individual larva of this insect consumes about 5% from the seed weight (Al-Azzawi and Mahdi, 1983). Indeed *C. maculatus* infestation on stored legumes may reach 50% within 3-4 months of storage (Pascual-Villalobos and Ballesta-Acosta, 2003).

Several approaches were developed to control *C. muculatus*. These involve agricultural, pesticides and physical means. Although the use of pesticides is associated with several disadvantages. So, a new approach was developed that relies on the use of active compounds such as insect growth regulators (Williams, 1967). They are also characterized by their high biological activity at very low concentrations. Cyromazine was effective when used to control larvae of house flies *Musca domestica* Linn. The effect of cyromazine was more

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efficient by 30-10 times than diflubenzuron against the larvae of house flies (Miller *et al.*, 1981, 1996; Brake *et al.*, 1991; Keiding *et al.*, 1991). The use of cyromazine along with diazenon reduced the number of the house flies by 69 % after one day of treatment and 97-99% after three days of treatment. Whereas, the use of cyromazine and diazenon individually at concentration 0.4 g/liter reduced the number of house flies by 12% and 62%, respectively (Levot and Sates, 1998). Cyromazine was found to be effective at 0.3% against the onion fly *Hylemia antiqua* Meig (Szczepanska, 1987). Wettable powder of cyromazine (75%) was reported to be effective in killing larvae of the tomato leaves digger**,** *Liriomyza bryoniae***,** Kalt where the percentage of deaths reached 88% at 200 ppm (Saito, 1988) and 92.94% at 225 ppm (Al-Mashhadani, 1998). The use of 10 and 20% cyromazine inhibited the development of fly *Delia radicum* during its attack on the root system (Etienne *et al.*, 1993). Also, cyromazine was reported to persist for long lifespan in the environment which increases its effectiveness. The effect of cyromazine on the metabolic activity of tomato as well as its persistence in nature was stable with little loss within the body during the lifespan of 30 days (Root *et al.*, 1996).

The current study was aimed at establishing an effective and promising strategy to fight *C. maculatus* for the purpose of reducing the use of conventional pesticides and supporting the program of integrated management of these pests. The study was focused on the effect of different concentrations of cyromazine powder, method of treatment and rearing temperature on the biological activity of the southern cowpea weevils reared on mung bean under experimental lab conditions.

MATERIALS AND METHODS

Insects

The southern cowpea weevil, *Callosobruchus maculatus* (Bruchidae: Coleoptera) obtained from an entomological research laboratory farm was reared on mung bean *Phaseolus aureus* Roxburgh. Adults of *C. maculatus* were placed in glass jar containing ½ kg seeds of each host and covered with a piece of cloth tightened firmly with a rubber band and then incubated at 30±2°C and relative humidity 50±5% (Ishimoto *et al.*, 1996). Cultures were renewed after each generation by taking the newly emerged insects from pupae for making new cultures.

Bioassays

Three concentrations (1, 3 and 5%) of cyromazine were applied to treat the mung bean seeds. Seeds (25g each time) were treated twelve times. Six times the insecticide was sprayed 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 treatments were by dipping the seed in the cyromazine solution for one minute. Control seeds were treated in the same way with water only. Dried seeds were placed in plastic pots (7 x 7cm). Five pairs (male and female) of newly emerged adults were transferred to each pot, which was then covered with a piece of cloth sealed with the rubber band. Pots were then moved into incubators (25±1°C and 30±1°C, 50±5% RH). Pots were observed for two successive generations to specify the overlap between different concentration of cyromazine, treatment method and rearing temperature as follows:

Reproductive rate of southern cowpea weevils is calculated for two successive generations according to Krebs (1978) using the following formula.

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

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

The rate of food consumption was measured by weighing the treated seeds at the end of the experiment and deducted from the original weight (25g). Generation lifespan was calculated from the newly emerged adult from the pupae until the advent of insects in the second-generation. For calculation of sex ratio and weight of male and female the adult insects were taken at random from each box.

Data analysis

The factorial complete randomized design and Duncan's multiple range tests were used to determine significance of change (Daoud and Elyass, 1990).

RESULTS AND DISCUSSION

As shown in Table I increased cyromazine concentration has led to a significant reduction in the reproductive rate of the southern cowpea weevil compared with the control. At a concentration of 5% it reached 6.25% compared with 40.21% of the control. The use of insect growth regulators Dofenapyn, Fenoxycarb, Mv-678 and R-20458 at concentrations 10-100 mg/kg of food for adult insects of the southern cowpea weevil has shown good efficacy in reducing the number of adult emergence from pupae (EL-Glia, 1992). In consistent with this finding, House *et al.* (1978) used Diflubenzuron to control the weevil cotton *Anthonomus grandis* (Bohe.). The adult emergence percentage was 37.7, 22.21 and 15.8 after Diflubenzuron administration at a dose of 35, 70 and 140 g ai/ha, respectively. In another study Hydroprene, Methoperane, Diflubenzuron and MV-678 were used to control *Ephestia cautella* (Walker) on peanuts. The increase in concentrations of these compounds resulted in decrease in fertility as well as adult emergence (Nickle, 1979).

The reproductive rate decreased significantly when seeds of mung bean were treated by dipping method in comparison with spraying method, where the reproductive rate reached 12.44 and 25.51 for dipping and spraying methods, respectively (Table I). This could be attributed to the lack of the ability of the insect from completing its life cycle on mung was bean treated by dipping at a temperature of 30°C, but they were able to complete their life cycle in spraying method. Also, results revealed significant differences in the reproductive rate with increasing temperature where the reproductive rate was 18% and 19.96% at a temperature of 30°C and 25°C, respectively (Table I). The efficacy of Match against *Spodoptera littoralis* (Boid) was tested at three different temperatures 18, 24 and 30°C. This may be due to increased activity of insect at high temperature, which leads to increased capture of the pesticide by the insect. Buholzer *et al.* (1992) found that the biological activity of Match increased with increasing temperature.

Treatment of seeds by dipping in cyromazine concentration of 5% at 30°C led to a significant reduction in the reproductive rate where it reached 0.40%. On the other hand, when reared on seeds treated by spraying method, 1% cyromazine spray at 25°C the reproductive rate was 32.07% (Table II).

Table I shows that the increase of cyromazine concentration leads to a significant reduction in the amount of food consumption in comparison with the control. A 5% concentration was the best in reducing the amount of food consumption to 3.30 g as against 17.08 g of control. There was a significant reduction (5.97 g) in food consumption in case of dipping method as compared with 10.60 g in spraying method. Also, temperature had an effect in the rate of food consumption, at 30°C there was an increase in the rate of food consumption. The southern cowpea weevil insects reared at 25°C consumed 17.17 g of food during the whole generation compared to 12.38, 10.88 g of those bred on temperatures at 30 and 35°C, respectively (Gabouri, 2000). Treatment of seeds by dipping in 5% cyromazine at 25°C and in concentrations of 1, 3 and 5% at 30°C led to a significant decrease in the rate of food consumption (Table II).

Data presented in Table I indicate that the increase in cyromazine concentration and the treatment method led to a prolonged lifespan compared with the control. The generation lifespan was also affected by temperature. It was significantly reduced at 30°C where the average generation lifespan was 26.71 days compared to 32.56 days in control at 25°C. The highest average generation lifespan was 33.50 days when treated by spraying with 5% cyromazine concentration at 25°C. The lowest average generation lifespan at cyromazine concentration of 1% was 27 days at 30°C (Table II).

It is clear from the Tables I and III 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. As indicated in Table I no significant difference in the average weights of males reared on mung bean, where it reached 1.01, 0.98 in case of

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

	Concentrations (%)				Treatment methods		Temperatures	
	1	3	5	Control	Dipping	Spraying	25°C	30°C
Reproductive rate %	19.29 C	10.16 B	6.25 A	40.21 D	12.44 A	25.51 B	17.83 B	18 A
Food consumption (g)	7.5 C	5.25 B	3.3 A	17.08 D	5.97 A	10.97 B	7.84 A	8.72 B
Generation lifespan (day)	28.88 B	30.25 A	30.75 A	28.67 B	29.21 B	30.06 A	32.56 A	26.71
Sex ratio								
Male	1.04 A	1.26 A	1.25 A	1.07 A	1.22 A	1.1 A	1.05 A	1.26 B
Female	1.11 A	1.17 A	1.17 A	1 A	1.13 A	1.09 A	1.12 A	1.11 A
Average weights (mg)								
Male	0.95 A	0.97 A	0.98 A	1.05 A	1.01 A	0.89 A	0.91 B	1.07 A
Female	1.68 C	1.65 C	1.52 B	1.44 A	1.59 A	1.56 A	1.42 A	1.73 B

Averages of similar characters refer to the existence of significant differences at the $P<0.05$ % level of probability

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)	Reproductive rate (%)	Food consumption rate (g)	Generation lifespan (day)
1	Dipping	25	11.97±0.26 C	4.94±0.14 C	32.17±0.27 ABC
3			3.57±0.35 B	3.13±0.37 B	33.33±0.44 AB
5			1.33±0.09 A	1.34±0.08 A	33.50±0.17 A
Control			40.5±0.12 F	14.08±0.06 G	32±0.29 BC
1	Spraying		32.07±0.23 E	11.27±0.33 F	31.67±0.33 C
3			17.63 ±0.32 D	7.57 ±0.26 D	32.83 ±0.17ABC
5			11.77±0.91 C	4.97±0.42 C	33.50±0 A
Control			40.83±0.17 F	15.44±0.32 H	31.50±0.29 C
1	Dipping	30	1.77±0.15 A	1.62±0.08 A	24.67±0.33 F
3			0.50±0.06 A	1.51±0.11 A	26.33±0.88 E
5			0.40±0.06 A	1.44±0.03 A	27±0.58 E
Control			39.5±0 F	19.70±0.12 I	24.67±0.33 F
1	Spraying		31.37±0.47 E	12.17±0.30 F	27±0.29 E
3			18.93±1.7 D	8.79±0.51 E	28±0.58 D
5			11.50±1.06 C	5.45±0.76 C	29±0.29 EF
Control			40±0 F	19.10±0.10 I	26.5±0 E

Averages of similar characters refer to the existence of significant differences at the $P<0.05$% level of probability

dipping and spraying method, respectively. The overlap between the cyromazine concentrations, method of treatment and temperature had an impact on the average weights of males reared on a mung bean (Table IV). Results showed that there were significant differences in the average weights of males, reaching the highest average weight 1.25±0.05 mg at a concentration of 5% cyromazine in insects reared on a mung bean treated by dipping method at 30°C, as well as at a concentration of 3% in insects reared on a mung bean treated by spraying method at temperature 30°C. The lowest average

weight had been 0.75 mg at a concentration of 3% in the mung bean treated by dipping method at 25°C (Table IV). Sublethal doses of methoprene can cause changes in sex ratios of horn fly parasites, *Spalangia cameroni*, were largely unaffected when exposed to methoprene (Roth, 1989). Pyriproxyfen could cause disruption of sex ratio when used against the sunn pest, E*urygaster integriceps* Puton (Mojaver and Bandani, 2010).

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

Conc. (%)	Treatment method	Temp. (°C)	Mean±SEM	
			Male	Female
1	Dipping	25	1.06±0.05A	1.04±0.04 A
3			1.12±0.12A	1.09±0.07 A
5			1.25±0.25	1.01±0.01 A
Control			1.±0 A	1.08±0.07 A
1	Spraying		1.07±0.07A	1.04±0.02 A
3			1.06±0.06A	1.07±0.07 A
5			1.43±0.31A	1.07±0.07 A
Control			1 ±0 A	1.02±0.02 A
1	Dipping	30	1.24±0.14A	1.07±0.07 A
3			1.34±0.34A	1.66±0.33 B
5			1±0 A	1.65±0.38 B
Control			1±0 A	1.17±0.17 A
1	Spraying		1.09±0.07A	1.01±0.01 A
3			1.18±0.18A	1.22±0.16AB
5			1 ±0 A	1.26±0.08AB
Control			1 ±0 A	1±0 A

Means with similar letter show significant differences at the $P<0.05\%$

Data presented in Table I shows that increased cyromazine concentrations resuled in reduced average weights of females reared on mung bean. The weights increased to 1.68, 1.65 and 1.53 mg at 1, 3 and 5% Cyromazine, respectively. The average weight of females was 1.44 mg in the control. The average weight reached 1.73 and 1.42 mg at 30 and 25°C, respectively, while there was no significant difference in the average weights of females between dipping and spraying methods. Results showed that the highest average weight of female reached 1.90 mg at a concentration of 5% on mung bean treated by dipping at 30°C. The lowest average of female weight was 1.20 mg at a concentration of 5% on mung bean treated by spraying method at 25°C (Table IV). Mojaver and Bandani (2010) reported that adult weights in Sunn pest were strongly affected by the insect growth regulator, pyriproxyfen.

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

Conc. (%)	Treatment method	Temp. (°C)	Mean ±SEM	
			Male	Female
1	Dipping	25	0.87±0.06AB	1.67±0.04BCD
3			0.75±0.06 A	1.62±0.02BCD
5			0.83±0.03AB	1.22±0.04 A
Control			1.15±0.03DE	1.25±0.05 A
1	Spraying		0.95±0ABCD	1.60±0.08 BC
3			0.77±0.02EFG	1.53±0.03 B
5			0.88±0.06ABC	1.2±0.03 A
Control			1.10±0.03CDE	1.25±0.03 A
1	Dipping	30	0.97±0.09ABCD	1.63±0.09BCD
3			1.17±0.03 DE	1.89±0.1 E
5			1.20±0.06 E	1.9±0.01 E
Control			1.10±0.10 CDE	1.52±0.02 B
1	Spraying		1.03±0.03 CDE	1.80±0.06 DE
3			1.20 ±0.06 E	1.57±0.O3 BC
5			1±0.06 BCDE	1.8±0.06 DE
Control			0.87±0.18 AB	1.73±0.13CDE

Means with similar letter show significant differences at the $P<0.05\%$

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 reproductive rate, on the rate of food consumption and on the average of generation lifespan. There is no effect neither in the disparity of sex ratio nor in the disparity in the 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, in the present study, is to provide the best way to control these insects.

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REFERENCES

AL-AZZAWI, A.F. AND MAHDI, M.T., 1983. *Insects stores*, University Press, Mosul, Iraq.

AL-AZZAWI, A.F., QUADO, I.Q. AND AL HAYDARY, H.S., 1990. *Economic insects*. Dar Al-Hekma Press, University of Baghdad, Iraq

AL-MASHHADANI, S.A., 1998. *Environmental and biological studies on the tomato leafminer* Liromyza bryoniae *Kaltenbach* (*Ayromyzidae: Diptera) and some means of control*. Master thesis, Faculty of Agriculture, University of Baghdad, Iraq.

BRAKE, J., AXTELL, R.C. AND CAMPBELL, W.R., 1991. Retention of larvicidal activity after feeding cyromazine (Larvadex) for the initial 20 weeks of life single comb, white leghorn layers. *Poult. Sci.*, **70**: 1873-1875.

BUHOLZER, F., DRABER, J., BOURGEOIS, F. AND GUYER, W., 1992. CGA, 184; 699. A new acylurea insecticide. *Med. Fac. Landbouw. Univ. Gent.*, **57**: 790.

CUPKA, T., 1987. Mung bean in grain legumes as alternative crops. A symposium sponsored by Center for Alternative Crops & Crop Products. Bloomington, MN. July 23–24, pp. 89–96.

DAOUD, K.M. AND ELYASS, Z.A., 1990. *Statistical methods for agricultural research*, National Library of Printing and Publishing, University of Mosul, Iraq.

EL-GLIA, G.E.S.A., 1992. Effects of insect growth regulators with Juvenile hormone activity against *C. maculatus* (Fab) (Coleoptera Bruchidae). *Anz. Schadlings kunde-pflanzenschutz, Umwelt Schutz.*, **65**: 137-140.

ETIENNE, B., YVES, B. AND LAURENCE, R., 1993. Effect of Cyromazine insect growth regulator for cabbage root fly *Delia radicum* control on rape. *Bull. OILB Crop*, **16**: 210-215.

GABOURI, I.A.H., 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.

HOUSE, V.S., ABLES, J.R., JONES, S.L. AND BULL, D.L., 1978. Diflubenzuron for control of the Boll weevil in unsalted isolated cotton fields. *J. econ. Ent*., **71:**: 797-800.

HOWE, R.W. AND CURRIE, J.E., 1964. Some laboratory observations on the rates of development mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bull. ent. Res.*, **55:** 437-477.

ISHIMOTO, M., SATO, T., CHRISPEELS, M.J. AND KITAMURA, K., 1996. Bruchid resistance of transgenic azuki bean expressing seed amylase inhibitor of common bean. *J. Ent. Exp. Appl.*, **79**: 309-315.

KEIDING, J., JESPERSEN, J.B. AND EL-KHODARY, A.S., 1991. Diflubenzuron and Cyromazine For control of the house fly *M. domestica* L. *Pestic. Sci.*, **32**: 187-206.

KREBS, J., 1978. *The experimental analysis of distribution and abundance*. Harper and Row Publishers, New York, U.S.A.

LEVOT, G.W. AND SASTES, N., 1998. Effectiveness of amixture of Cyromazine and Diazinon for controlling fly strike on sheep. *Aust. Vet. J.*, **76**: 343-344.

MILLER, R.W., CORLEX, C. AND COHEN, C.F., 1981. (GA-19255 and CGA-72662: Efficacy against flies and possible mode of action and metabolism. *South W. Entomol.*, **6**: 272-278.

MILLER, R.W., SCHMIDTMANN, E.T., WAUCHOPE, R.D., CLEGG, C.M., HERNER, A.E. AND WEBER, H., 1996. Urine delivery of Cyromazine for suppressing house and stable flies in outdoor dairy calf hutches. *J. econ. Ent.*, **89**: 689-694.

MOJAVER, M. AND BANDANI, A.R., 2010. Effects of the insect growth regulator Pyriproxyfen on immature stages of sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). *Mun. Ent. Zool.*, **5**: 187-197

NICKLE, D.A., 1979. Insect growth regulators: New protectants against the almond moth in stored in shell peanuts. *J. econ. Ent.*, **72**: 816-819.

PAJNI, H.R., 1965. Bioassay of insecticides relative toxicity of films of different insecticides against the adults of *C. maculatus* (F.). *Res. Bull. Punjab Univ*., **16**: 559-341.

PASCUAL-VILLALOBOS, M.J. AND BALLESTA-ACOSTA, M.C., 2003. Chemical variation in an Ocimun basilicum germplasm collection and activity of the essential oils on *Callosobruchus maculatus*. *Biochem. Syst. Ecol*., **31**: 673– 679.

ROOT, D.S., HONGTRAKUL. T. AND DAUTERMAN, W., 1996. Studies on the absorption, residues and metabolism of Cyromazine in Tomatoes. *Pestic. Sci.*, **48**: 25-30.

ROTH, J.P., 1989. Some effects of methoprene on *Spalangia cameroni*, a parasitoid of horn fly pupae. *Southw. Entomol.*, **14**: 91-96.

SAITO, T., 1988. Insecticide activity of several chemicals to the bryony Leafminer *Liriomyza bryoniae* and their application methods. *Plant Prot.*, **35:** 168-171.

SOUTHGATE, B.J., 1979. Biology of Bruchidae. *Ann. Rev. Ent.*, **24**: 449-473.

SZCZEPANSKA, K., 1987. Effectiveness of new soil insecticides against the onion fly *Hylemia antigua* Meig Prace-Noukowe-Instytutu. *Ochrony-Roslin*, **28**: 125-137.

WILLIAMS, M.C., 1967. Third generation of pesticide. *Scient. Am.*, **217**: 13-17.

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