

**BIOLOGICAL PARAMETERS FOR EVALUATING THE EFFECT
OF INSECT GROWTH REGULATORS AGAINST
GRASSHOPPER, *EUPREPOCNEMIS PLOTRANS PLOTRANS*
CHARP. AND COTTON LEAFWORM, *SPODOPTERA*
LITTORALIS (BOISD.)**

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Abstract

Plainly the ideal approach would lie in finding agents that are highly specific in their effects on the metamorphosis, metabolic rate and reproduction potentiality in order to determine the consequence of hormone deficiency and the role of this compounds involved in the inactivation of prothoracic gland. Beside the known action of BAY-SIR compound as anti-moulting agents and long persistence in the nature which exhibited strong inhibited effect on the metabolism during vitellogenesis and hence on reproduction potentiality which may be more appropriate when using as pesticide.

On the other hand, pyriproxyfen compound induced pronounced sterility that induced over 99 % reduction in population and revealed sharply decrease for haemolymph total cholesterol. Therefore, it may have possible role as insecticide and /or chemosterilant.

INTRODUCTION

The development of certain chemical insecticides that are cholinesterase inhibitors to populations of defoliating forest insects, demonstrates obvious damage to the agro-ecosystem (Berrill *et al.* 1994). Therefore, it seems prudent to develop alternative biorational strategies that could be drawn upon to counter periodic population outbreaks. Retnakaran *et al.* (1995) suggested that juvenile hormone analogues [JHAs] and antimoulting might offer progressing control potential against variety of insect pest species by interrupting moulting and causing abnormal morphogenesis. Hence, it is important to gain a better understanding of sub-lethal effects on life-table parameters of grasshoppers and cotton leafworm. The objective of the study was to carry out a small scale evaluation on the efficacy of certain commercial formulations, using droplet sizes and densities comparable to those customarily used in forestry spraying.

MATERIALS AND METHODS

The tested chemical insecticides were BAY-SIR 8514 [2-chloro - N - 4 [trifluoromethoxy] phenyl amino carbonyl benzamide] formulated as 25%WP and pyriproxfen a Juvenyl Hormone Mimic [JHM] = 4- Phenoxy phenyl [RS]- 2- [2-pyridoxy] propyl ether, EC. 10 %, a water - based commercial formulation or with water [control].

Insects: Laboratory colonies grasshoppers were reared according to the method described by Hunter-ones (1966), and cotton leafworm are given in Abdel Hafez *et al.* (1988).

Application of Insecticides: glass petri-dishes [9 cm diameter] were sprayed with 2 ml. of an aqueous suspension of each insecticide treatment at 8 in. bar using potter tower equipment; which was housed in self-constructed spray chamber. Control treatment was sprayed with water. This resulted in a homogenous spray coverage of $0.93 \pm 0.08 \mu\text{l}$ (mean \pm SE) fluid per square centimeter.

The quantities (ppm) of BAY-SIR and pyriproxfen deposited were 10, 20,40, and 20,40,50,150, respectively for cotton leafworm treatment at 5th instar. But, with grasshoppers as the recommended field coverage. At last, 20 petri-dishes were sprayed per treatment. After the spray deposit had dried, 5 larvae were transferred to each contaminated petri-dish and kept until adult emergence.

Employed procedure: duration and development were obtained by using Dempster's equation (1957). Mortality as soon as abnormalities data were corrected by Abbott's formula (1925) for control. The changes in the reproductive potentiality for emerged adults of grasshoppers was evaluated using the formula recommended by El-brashy and Abou-Zeid (1972). The haemolymph main metabolites were quantitatively estimated colourimetrically using the techniques outlined by Gornall *et al.* (1949) for protein determination, Richmond (1973) for determination of serum cholesterol (0.1ml haemolymph sample). Hole body lipid content was determined as gravimetric method of Loveridge (1973), after the sampling of the haemolymph, pupal stage at 7-days old and adult stage at first day old chitin content was determined gravimetrically after prolonged digestion (Karl and Daizo, 1986).

RESULTS AND DISCUSSION

We have focused to open new lines in research of physiology of grasshopper and cotton leafworm by bioassay of IGRs.

Development: It has been found that the rate of development of the 5th nymphal instar of grasshopper after treatment with anti-moulting [BAY-SIR] and juvenoids, [pyriproxfen] was 16.26 % for the former and 6.17 % for the latter against 14.08% in check treatment. Concerted with the findings of (Vennard *et al.*, 1998), insects retained characteristics of the 5th instar or super numerary formed Fig. 1. Data in Table 3A revealed that prolonged the stadia of survivors development of *Spodoptera littoralis* dosed on day 4 of 5th instar.

Mortality rates: The moult inhibiting BAY-SIR demonstrates 80% for grasshopper according to Retnakaran *et al.* (1995), the untanned new cuticle signifies the absence of dopa decarboxylase function. In case of pyriproxfen mortality was 6.7% due to its structure, as JHM for insects, but in contrast with *S. littoralis* the mortality concentrated at latent effect on population.

Gross reproduction: Results as response of (J.H.A.) on grasshopper, all phenomena in Table 2 caused the resulted number of offspring which reduced dramatically. In general, the present results are in agreement with those obtained by Vennard *et al.* (1998) when IGRs had more severe impact on life-table parameters of *Micromus tasmaniae*.

Synthetic metabolic rate: Worthwhile to explore IGRs as a forceful tool for testing on the role of juvenile hormone (JH) and ecdysone based on metabolic rate, Tables (2, 3 B) show that IGRs treatment increased haemolymph total protein. In conclusion, accumulation of protein concentrations in haemolymph acted on blocked protein; to uptake in oocytes and reduced incorporation of vitellogenesis (VG) in the oocytes, but in contrast with *S. littoralis* acted on source of synthesis sites.

On Cholesterol: The haemolymph cholesterol was sharply reduced in the treated adults (males and females) of grasshopper than control ones. Similar results for *S. littoralis* were achieved.



Figure 1. Abortive metamorphosis in male and females nymphs treated with pyriproxifen, few nymphs were however characterized by coloration of head and thorax shield of the 5th instar while the rest of the body was similar to that of normal 6th instar causing a permanent ecdysial stasis, precocious in complete molt that was lethal.

Table 1. Serial experiment of the insect growth regulators on the main metabolites of the adults male and female Berseem grasshopper, *Euprepocnemis plorans plorans* treated in 5th instar nymph.

Age analysis (days)	Insects (10)	Material	Total protein (mg/100 ml)		Total cholesterol (mg/100 ml)		Whole body lipid content (mg)		Chitin cuticle (mg)		Statements
			Act.	Change	Act.	Change	Act.	Change	Act.	Change	
6	M	Cont.	12	5.33	4.44	43.2	38.5	Day 6:			
		Mimic	10.3	+14.2	23.5	+55.9	5.67	-27.7	Vitelin content		
		Antimoulting	9.3	+22.5	25.8	+51.6	5.28	-18.9	Day 10:		
7	F	Cont.	5.7	98.9	9.75	42.5	42.5	Major hem.ecdytstroids			
		Mimic	20.6	-261.4	21.3	+78.5	5.65	+42.1	And vitellogenesis		
		Antimoulting	6.7	-17.5	19.9	+79.9	6.97	+28.8	+4.5		
10	M	Cont.	3.1	62.9	11.63	42.1	42.1				
		Mimic	15.2	-390.3	31.6	+49.8	4.63	+60.2	+13.6		
		Antimoulting	8.3	-167.7	28.1	+55.3	4.49	+61.4	37.8 +10.5		
10	F	Cont.	13.2	154.1	7.97	50.4	50.4				
		Mimic	20.6	-56.1	42.4	+72.5	3.15	60.5	+13.5		
		Antimoulting	11.4	+13.6	34.5	+77.6	6.58	17.4	45.2 +10.3		

Table 2. Viability of eggs laid by Berseem grasshopper, and number of offspring resulting from treated with biologically active compound pyriproxyfen as day old last instar nymphs and paired with normal one on reproductive potential (values are mean of 10 females)

Percent viable eggs per female in indicated egg batch	Mean of egg pods/female				Mean of total eggs laid/female		Mean of total eggs hatched/clutch		Fertile eggs (%)		Relative number of offspring		Change in reproductive potential (% decrease)				
	1st	2nd	3rd	4th	5th	6th	Act.	Change	Act.	Change	Act.	Change					
Treated female	100	0	0	0	-	-	3.52	50.60	104.00	65.30	36.00	87.70	34.60	64.70	4.10	95.90	95.75
Treated male	100	.96.2	-	-	-	-	2.61	63.40	81.00	73.00	52.00	82.30	64.20	34.50	5.90	94.10	95.22
Treated female x male	15.4	0	-	-	-	-	1.13	84.20	72.00	76.00	4.00	98.60	5.60	94.30	0.50	99.50	99.67
Untreated	100	100	96	96	92	92	7.13	-	300.00	-	294.00	-	98.00	-	100.00	-	-

Table 3. Effectiveness of insect growth regulators against 5th instar larvae of *S. littoralis* and bioactivity at different developmental stages.

A:

Material	Dose (ppm)	% mortality		Developmental rate				% Abnormality	% Emergence
		Acute	Latent	Accumulate larvae (5 th +6 th instars)		Pupa			
				Change	Act.	Change	Act.		
Juvenile hormone	1500	1.89	100	-	-	-	-	-	-
hormone mimic	500	21.35	100	-	-	-	-	-	-
	40	20.6	100	-	-	-	-	-	-
	20	9.73	96.15	37.04	-124.48	-	-	0.96	0
Anti-moult-	40	2.5	95.65	41.15	-149.4	-	-	-	-
moult-	20	0	85.71	33.33	-101.8	11.11	+14.8	8.33	0
ing	10	3.66	72.99	26.66	-61.2	12.5	+4.1	16.07	50
Cont.	-	-	-	16.5	-	13.04	-	-	100

B:

Material	Dose (ppm)	Total protein			Total cholesterol			% chitin			% whole body lipid content				
		6 th larvae		Prepupa	6 th larvae		Prepupa	6 th larvae		Prepupa	6 th larvae		Prepupa		
		Act.	Change	Act.	Act.	Change	Act.	Act.	Change	Act.	Change	Act.	Change		
JHA	1500	0.09	+61.5	-	32.17	+76.6	-	54.49	+33.2	-	23.16	-20.43	-	-	
hormone mimic	500	2.08	+61.7	-	15.37	+88.8	-	41.4	-1.2	-	6.61	+13.1	-	-	
	40	2.52	+53.6	-	63.63	+53.8	-	40.5	+1	-	7.62	-0.1	-	-	
	20	2.27	+58.2	3.97	+22.9	5.19	+96.2	18.65	+79.1	42.17	-3.1	7.75	+82.5	64.55	-18.9
Anti-moult-	40	2.81	+48.3	3.31	+35.7	125.12	+9.2	90.7	-1.5	34.94	+14.6	24.65	+44.5	-	-
moult-	20	3.29	+39.4	3.61	+29.9	142.29	-3.3	150.97	-69	42.68	-4.3	43.97	+1	57.24	-2.8
ing	10	3.02	+44.4	3.6	+30.1	75.6	+45.1	146.09	-63.5	45.4	-10.9	61.82	-39.2	18.4	67
Cont.	-	5.43	-	5.15	-	137.77	-	89.33	-	40.92	-	44.4	-	55.7	-

Percent of change = $\frac{\text{Check-treatment}}{\text{Check}} \times 100$

(-) higher than check, (+) lower than control

Whole body lipid content: It appeared that treated adults exhibited decreasing rate against control of grasshopper. Previous studies were carried out by Scheinder *et al.* (1995) on *Locusta*, JH stimulated vitellogenin production by the fat body, uptake by the developing oocytes and the adipokinetic reaction. On the other hand, *S. littoralis* data revealed the presence of significant increase in lipid during the investigated intervals. Several authors have demonstrated stimulation of protein synthesis by 20-hydroxy-ecdysone. However, other claims that ecdysteroids have an inhibitory effect of protein synthesis (Daniel *et al.*, 1981).

Chitin content: Closely parallel to the pattern of whole body lipid content and cholesterol in treated individuals of grasshopper or *S. littoralis*. Apparently, cuticle deposition and chitin synthesis are stimulated by ecdysteroids and juvenile hormones as in *Stomoxys calcitrans* pupae (Richard *et al.*, 1979) as they appear to do in other insects.

The issue was effectiveness of IGRs as an environmentally benign control agent for the grasshopper and the cotton leafworm. Therefore, these compounds could be recommended as selective insecticides.

REFERENCES

1. Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
2. Abdel Hafez, M.M., M.N. Shaaban, M.A. El-Malla, A.M. Abdel-Kawy and N.M. Ahmed. 1988. Effects of some IGRs on phosphatases activity of susceptible and profenofos resistant strains of *Spodoptera littoralis* (Boisd.). *Minia. J. Agric. Res. & Dev.*, 10 (3): 1373-1386.
3. Berrill, M.S., S. Bertran, L. McGillivray, M. Kolohon and B. Pauli. 1994. Effects of low concentrations of forest-use pesticides on frog embryo and tadpoles. *Environmental Toxicology and Chemistry*, 13: 657-664.
4. Dempster, J.P. 1957. *Anti-Locust Bull.*, 27.
5. Daniel, R.P. and G.L. Barry. 1981. Cuticle protein in adult *Locusta migratoria*. *J. Insect Physiol.*, 27 (7): 475-483.
6. El-Ibrashy, M.T. and E.N. Abou-Zeid. 1972. Preliminary field evaluation of the sterilant activity of the herbicide EPTC against the Egyptian cotton leafworm, *Spodoptera littoralis* Boisd. *Appl. Ent. Zool.*, 7: 168-170.
7. Gornall, A., C.P. Pardwell and M. David. 1949. Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.*, 177: 751-766.
8. Hunter-Jones, P. 1966. Rearing and breeding locusts and grasshoppers in the laboratory. *Bull. Anti-Locust Res. Centre, London*: p 12.
9. Loveridge, J.P. 1973. Age and changes in water and fat content of adult laboratory-reared *Locusta migratoria migratrioides* R. and F. *Rhod. J. Agric. Res.*, 11: 131-143.
10. Karl, J.K. and D. Koga. 1986. Physical stage, insect chitin synthesis, degradation and metabolic regulation. *Insect Biochem.* 16 (6): 851-877.
11. Richard, T. Mayers, Shirieem, Meola, David L. Copping and R. Deloach. 1979. The pupal instar of *Stomoxys calcitrans*: cuticle deposition and chitin synthesis. *J. Insect Physiol.*, 25: 677-683.

12. Richmond, W. 1973. Preparation and properties of cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1351-1356.
13. Retnakaran, A., R. Hiruma, S. Palli and L.M. Riddoford. 1995. Molecular analysis of the mode of action of RH-5992, a lepidopteran-specific, non-steroidal ecdysteroid agonist. Insect Biochem. Molec. Biol., 25: 109-117.
14. Schneider, M., Wiessel and A. Dorn. 1995. Effect of JHIII and JH analogues on phase-related growth, egg maturation and lipid metabolism in *Schistocerca gregaria* females. J. Insect Physiol, 41 (1): 23-31.
15. Vennard C, B. Nguama, R.J. Dillon, H. Oouchi, and A.K. Charnley. 1998. Effects of the juvenil hormone mimic pyriproxyfen on egg development and metamorphosis in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) J.Econ. Entomol. 91(1): 41-49.

إحداث تشوهات ونموات غير طبيعية باستخدام منظمات النمو الحشرية ضد آفة النطاط ودودة ورق القطن

إبراهيم على أحمد عبد الكريم ، ضياء الدين عبدالفتاح شبل

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقى - الجيزة

بهدف المحاكاة مع الطبيعة فى استخدام المركبات الكيماوية تم تطبيق الرش بجهاز بوترتور لدراسة تأثير منظمات النمو الحشرية على الغدة الصدرية فى كل من الحشرات الكاملة التطور مثل دودة ورق القطن والناقصة التطور مثل النطاطات وأثر ذلك على تعداد الآفة .
أوضحت النتائج ان التأثير المتأخر كان أكثر فاعلية حيث أحدث تثبيط شديد فى عمليات التمثيل الغذائى خاصة على الكوليسترول مما أدى الى حدوث عقم بلغ ٨٠٠٪ تقريبا.