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TOXICITY AND BIOCHEMICAL RESPONSE OF *Eobania vermiculata* LAND SNAIL TO NICLOSAMIDE MOLLUSCICIDE UNDER LABORATORY AND FIELD CONDITIONS.

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ABSTRACT

The toxicity and biochemical response of *E. vermiculata* land snail to Niclosamide molluscicide were laboratory tested using spraying technique on most soil. Also, the efficiency of the molluscicide was tested under field conditions.

Data revealed that the tested molluscicide at concentrations of 0.14, 0.17, 0.2 and 0.29 ppm exhibited 20, 43.3, 66.7 and 93.3 % mortality of snails with LC_{50} value of 0.631 ppm. Results of sub lethal concentration (¼ LC_{50}) effect showed that % activity of Peroxidase and Catalase enzymes after 0.0, 24h, 48h, 72h, and 96h were (100, 183.3, 49.63, 85.57 and 64.1) and (100, 137.5, 124.4, 150 and 103.1 %) respectively.

The effect of ¼ LC_{50} on GOT, GPT, AchE, LDH, total protein and total lipids of the snail after 24, 48, 72 and 96 hours post-treatment were (-3.8, -61.9, -41.9 and –33.3 %), (20, 60, 80 and 20%), (-48.9, -43.5, -65 and –41.7 %), (47.1, 14.5, 55.8 and 63.1 %), (17.9, -10.3, 21.3 and 26.7 %) and (237.7, 302.6, 228.6 and 263.6 %) percent increase or decrease than control (26.25, 5, 2.23, 334, 2.41 and 0.77) respectively.

The tested molluscicide at rates of 0.05, 0.1, 0.2 and 0.3 gm / m^2 induced 58, 72, 79 and 84 % reduction in snails population density respectively under field conditions.

INTRODUCTION

The terrestrial snails, E.vermiculata has been recently considered as one of the serious pests in Egypt. It increases year after another and attacks many agriculture, horticulture and ornamental plants. Awad(2000), Abd El-Aal (2001), Mortada (2002), Metwally *et al.* (2002) and Daoud (2004).

Respiratory, Transaminases enzymes and Acetylcholin Esterase as well as total protein and total lipids are important in the biological processes. The effect of pesticidal treatments on some biochemical aspects in land snails were studied by Radwan *et al.* (1992), Mourad and Zedan (1996) and El-Deeb *et al.* (1999).

Niclosamide a relatively non-cumulative chlorinated aromatic amide pesticide, not only principally used against aquatic snails but also as an antiparasitic drug in human and veterinary medicine, McCullough and Mott,(1983) and WHO (1988).

Therefore the present study deals with the interaction of Niclosamide with some biochemical target *e.g* total soluble proteins and total lipids as well as the activities of some enzyme systems to throw a light on the toxicity and the mode of action of the tested molluscicide on the terrestrial snail *Eobania vermiculata* (O.F. Muller, 1774).

MATERIALS AND METHODS

Tested pesticide:

Niclosamide (Bayluscide) 70 % WP, 2,5 dichloro-4-nitrosalicylanilide.

Tested snails:

Individuals of the land snail *Eobania vermiculata* were collected from infested nurseries, ornamental plants in Mansoura city, Dakahlia Governorate in spring 2004 and kept in laboratory under 20 ± 2 °C and 80 ± 3 % RH.

Assessment of molluscicidal activity:

Plastic containers of 384 cm surface area, field with moist soil were prepared. Ten adult snails were placed in each one. Snails were sprayed with various rates of Niclosamide. Mortality was calculated after 48 h. Zedan, (1999). Data statistically analyzed according to finney (1952).

Field experiment:

Field experiment was carried out in a nursery cultivated with ornamental plants at Mansoura city Dakahlia Governorate. during spring 2004 season to evaluate the effectiveness of Niclosamide 70 % WP on the population density of *E.vermiculata* snails on various ornamental plants. The area was divided into four replicates 10m² each one, in addition to control. Area of 10 m² (5 meters longs and 2 m apart) were acted as buffer area between plots.

The ornamental plants were sprayed with Niclosamide at rates of 0.5, 0.1, 0.2 and 0.3 gm / m² on the damp soil after 3 days of irrigation at evening. The random of snail population (snails / m²) before and after 1, 3, 5 and 7 days of treatments were estimated according to Henderson and Tilton (1955) also, L.S.D values were calculated.

Biochemical studies:

The concentration of ¼ LC_{50} of the tested compound was selected to study the biochemical interaction effects against ***E.vermiculata*** snails. The live treated snails were subjected after 24, 48, 72 and 96 hours post-treatment for biochemical measurement estemation.

Enzyme assay methods:

Determination of Peroxidase and Catalase activity:

The following procedure was used according to Bergmeyer(1963). Ten individuals of snails were homogenized in blender for 3 minutes with 5 ml of 0.006 M phosphate buffer, PH 7 at 1-4 °C then centrifuged at 3500 r.p.m for 10 minute. The sediment was stirred with cold phosphate buffer 0.006 M and allowed to stand in the cold water with occasional shaking. The extraction process was repeated for two times. The whole extract was centrifuged several times for cleaning and this process not take more than 24 hours under cooling conditions. The enzyme activity was determined by following the disappearence of hydrogen peroxidase using the method reported by Saunders (1964).

Determination of Transaminases and Acetylecholin-estarase activities:

The shells of snails were removed then the soft tissue was homogenized in 10 volumes (w /v) of 0.1 M phosphate buffer, PH 8 using homogenizer for 30 seconds. The homogenates were centrifuged at 10000 xg for 20 minutes using cooling centrifuge at 4 °C. The supernatants were used as the enzymes source. GOT and GPT activities were assayed by the method of Reitman and Frankel (1957), using " Boehringer Mannheim GmbH Diagnostica Kit" and AchE activity was assayed by the method of Ellman *et al.* (1961), using Acetylthiochline iodide as a substrate.

Total soluble proteins were measured by the biuret method described by Kabat and Mayer (1971), and total lipids were measured by the method of knight *et al.* (1972), with slight modification, 2.5 ml of the prepared homogenate was boiled in 5 ml of H_2SO_4 95 % conc. A.R. using water bath for 10 minutes, then

cooled at room temperature. An aliquot of 0.05 ml from the above mixture was added to 3 ml of phospho-vanilline reagent. The colour was allowed to develop in the test tubes in the dark place for 45 minutes.The developed faint pink colour was measured spectrophotometrically at 525 nm. The amount of total lipids was calculated from the corresponding standard curve. All the previous constituents were expressed in milligrams / 100 mg tissue.

RESULTS AND DISCUSSION

1- Molluscicidal activity:

Molluscicidal activity of Niclosamide is summarized in Table (1) using spray technique on moist soil under laboratory conditions, results indicate that the tested compound at concentrations of 0.14, 0.17, 0.2 and 0.29 ppm exhibited 20, 43.3, 66.7 and 93.3 % mortality of ***E. vermiculata*** snails after 48 h. post-treatment. The corresponding LC_{50} value was 0.631 ppm.

Table (1): % Mortality of *Eobania vermiculata* snails treated with different concentration of Niclosamide 70% WP solution and the corresponding LC_{50}, LC_{90} values.

Concentrations (ppm)				LC_{50} and (C.L)	LC_{90} * and (C.L)**
0.14	0.17	0.2	0.29		
20	43.3	66.7	93.3	0.631 (0.438-0.909)	3.725 (2.587-5.364)

* Finney (1952) ** Confidence limits.

Regarding field experiment Table (2) showed that Niclosamide 70 % WP when tested against *E.vermiculata* snails on ornamental plants at rates of 0.5, 0.1, 0.2 and 0.3 gm / m² exhibited 58, 72, 79 and 84 % reduction in snail population after 7 days post-treatment ($P > 0.05$).

Table (2) : Effect of Niclosamide 70 % WP against *E.vermiculata* land snails on ornamental plants.

Conc. Gm / m²	% of infestation before treatment	% of infestation after treatment (days)				Total of infestation	Mean	% Reduction
		1	3	5	7			
Control	35	36	38	38	35	147	36.75	-
0.05	32	18	16	12	10	56	14	58
0.1	33.4	13.5	11	9	6	39.9	9.88	72
0.2	32.5	10	8	6	4	28	7	79
0.3	34	7	6	5	3	21	5.25	84

L.S.D at 5 % 18.2 ($P > 0.05$) .

The obtained results are in harmony with those obtained by Zedan (1999), who reported that Niclosamide 70 % WP exhibited potent effect against *M.obestracta* land snails, LC_{50} value was 15.74 ppm after 24h exposure period in laboratory, while in field experiment rates of 0.1, 0.2 and 0.3 gm /m² of Niclosamide reduced the snail population on pea with 54, 80.8 and 86.3 % after 7 days of treatment. Daoud (2004) reported that LC_{50} values of Vertimec, Neomyle, Marshal, Dursban and Curacron were 0.64, 0.65, 2.94, 4.73 and 10.17 % when tested as poisons bait against *E.vermiculata* snails under laboratory conditions. In contrast under field conditions, Neomyle and Vertimec when tested as poisons bait at concentration of 1, 2, 4 and 8 % against *E.vermiculata* snails infesting ornamental plants, exhibited initial and residual activity of [(31 and 54.66 %), (38 and 74.33), (52 and 82.33%) and (57 and 88%)] and [(44 and 47 %), (49 and 58.66 %), (51 and 70.66%) and (58 and 81 %)] reduction after 3 and 21 days post-treatment, respectively.

2- Biochemical studies:

The interaction of pesticides with biochemical targets, that can affect the normal physiological functions in the terrestrial snails will be valuable for assessment of the possible success of control programs El-Wakil and Radwan (1991).

The results presented in Table (3) show variable differences in peroxidase and catalase activities in the snails treated with ¼ LC_{50} 0f Niclosamide among time intervals. It was observed that the tested material increased the activity of the two enzymes after 24h with 183.3 % and 137.5 % respectively. Thereafter the activity of Peroxidase enzyme was decreased gradually to reach 64.1 % after 96 h interval while the activity of Catalase enzyme was increased to reached its maximum (150 %) after 72 h, then decreased to (103.1%) after 96 h, to be approximately near the control range (100 %).

Table (3): Effect of sub lethal concentrations of Niclosamide on respiratory enzymes of *E.vermiculata* land snails.

Tested Enzymes	Optical density after					% Activity ** after				
	0.0*	24h	48h	72h	96h	0.0*	24h	48h	72h	96h
Peroxidase	0.679	1.248	0.337	0.581	0.435	100	183.3	49.63	85.57	64.1
Catalase	0.16	0.22	0.199	0.24	0.165	100	137.5	124.4	150	103.1

* Control

** Activity = $\frac{\text{optical density of treated}}{\text{optical density of control}} \times 100$

Concerning the effects of the test compound on the activities of GOT, GPT, AchE and LDH enzymes of terrestrial ***E.vermiculata*** snail in adition to the two biological parameters total protein (T.P) and Total lipids (T.L), data presented in Table(4) revealed that Niclosamide at ¼ LC_{50} dramaticaly decreased the activity of GOT than untreated check to reached – 33.3 % after 96 hour post-treatment while GPT was increased activity than control (5%) to reached 80% after 72 h, thereafter the activity decreased to 20 % after the end of the experiment.

Concerning AchE activity, it is obvious from the same table that the tested compound decreased the activity of the enzyme with variable degrees at all the experiment intervals, it is clear that Niclosamide has inhibitory effect on AchE enzyme.

Regarding the effect on Lactic Acid Dehydrogenasis enzymes (LDH) the values were significantly increased from the first day of exposure till the end of the experimental period. % of differences than control were 47.1, 14.5, 55.8 and 63.1 % after 24, 48,72 and 96 hour, respectively.

Concerning total protein parameter it is clear that the tested compound significantly decreased the total soluble proteins at all the experimental intervals. The enzyme reached it's minimum (- 10.3 %) differences after (48 h). An irreversible effect was noticed in case of total lipids content, where, Niclosamide increased the lipids content of the treated snails. The corresponding mean values were 2.6, 2.33, 2.53 and 2.8 mg / gm after 24, 48, 72 and 96 hour post-treatment respectively than that of control (0.77 mg / gm).

The obtained results are agreement and / or disagreement with the findings of many authors. El-Sebae *et al.* (1978) found no evidence of an inhibitory action of the 70 % WP formulation of Niclosamide on the Peroxidase or on the Catalase activities of *Biomphalaria glabrata* homogenates. Nabih and El-Wasimi (1968) claim that the oxidation of p-phenylene diamine dihydrochloride by extracts of Bulinus and Biomphalaria snails could be inhibited by Niclosamide through a depression of the rate of the catalytic decomposition of hydrogen

peroxide. Webbe (1987) reported that the transamination of glutamate appeared to be sensitive to inhibition by Niclosamide in *Biomphalaria alexandrina* snails.

In conclusion, it appears that the molluscicidal activity of Niclosamide on the terrestrial snail *E.vermiculata* may be -at least in part- attributed to the inhibition or an alteration caused in some biochemical targets which could lead to serious metabolic and cellular damage.

During the course of the present study. It is interesting to notice that terrestrial snails were active only, after irrigation, in most weather, in damp evening and early morning hours. It is thus under these circumstances that snails came into contact with molluscicids. Moreover, Bayluscide the ethanol amine salt of Niclosamide is considered the compound of choice in many parts of the world as fresh water snails molluscicide Mcullough and Mott (1983), can successfully be used at the previous test concentrations in combination with other methods for controlling terrestrial snails.

Table (4): Effect of sub lethal concentrations of (¼ LC_{50}) Niclosamide of different enzyme systems of *E.vermiculata* land snails.

Parameter	Control x ± S.E	Post-treatment (hours) 24		48		72		96	
		x ± S.E	% diff.	x ± S.E	% diff.	x ± S.E	% diff.	x ± S.E	% diff.
Glutamic Oxaloacetic Transaminase (GOT)	26.25 ± 6.5	25.25 ± 3.7	- 3.8	10.00 ± 1.2	- 61.9*	15.52 ± 1.9	- 41.9	17.5 ± 4.7	- 33.3
Glutamic Pyruvic Transaminase (GPT)	5 ± 1.00	6 ± 1.15	20	8.00 ± 1.63	60	9.00 ± 1.91	80	6.00 ± 1.15	20
Acetyl colenestrase (AchE)	2.23 ± 0.59	1.14 ± 0.33	- 48.9	1.26 ± 0.3	- 43.5	0.78 ± 0.35	- 65*	1.3 ± 0.51	- 41.7
Lactic acid Dehydrogenasis LDH)	334 ± 55.1	355.9 ± 779	47.1	382.5 ± 73.2	14.5	520.5 ± 16.8	55.8	544.7 ± 148	63.1
Total Protein (T.P)	2.41 ± 0.11	0.71 ± 0.09	17.9	0.54 ± 0.05	- 10.3*	0.73 ± 0.06	21.3	0.76 ± 0.02	26.7
Total Lipids (T.L)	0.77 ± 0.17	2.6 ± 0.89	237.7*	2.33 ± 0.56	302.6*	2.53 ± 0.25	228.6**	2.8 ± 0.3	263.6**

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السمية والاستجابة الحيوية للقوقع الأرضى أوبانيا فيرميكيولاتا لمبيد النيكلوزاميد تحت الظروف المعملية والحقلية.

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١- تم دراسة السمية والاستجابة الحيوية لقوقع الأوبانيا فرميكيولاتا لمبيد القواقع النيكلوزاميد معمليا باستخدام طريقة الرش على تربة رطبة ، كما تم اختبار تأثير هذا المركب على نفس القوقع حقليا.

٢- اثبتت النتائج أن استخدام المركب بتركيزات ٠,١٤ ، ٠,١٧ ، ٠,٢٠ ، ٠,٢٩ جزء فى المليون أعطت ٢٠ ، ٤٣,٣ ، ٦٦,٧ ، ٩٣,٣ % موت للقواقع ، وكانت قيم الجرعة النصفية ٠,٦٣١ جزء فى المليون.

٣- تم دراسة التركيزات الغير مميتة(¼ الجرعة النصفية) على انزيم البيروكسيديز والكتاليز فى القواقع بعد (صفر ، ٢٤ ، ٤٨ ، ٧٢ ، ٩٦ ساعة) وكانت النسبة المئوية لنشاط الانـــزيم (١٠٠ ، ١٨٣,٣ ، ٤٩,٦٣ ، ٨٥,٥٧ ، ٦٤,١ %) & (١٠٠ ، ١٣٧,٥ ، ١٢٤,٤ ، ١٥٠ ، ١٠٣,١ %) على التوالى.

٤- تم دراسة تأثير قيم ¼ الجرعة النصفية على انزيمـــات GOT ، GPT ، AchE ، LDH ، والبروتينـــات والدهون الكلية بالقوقع بعد ٢٤ ، ٤٨ ، ٧٢ ، ٩٦ ساعة من المعاملة وكانت النتائج (- ٣,٨ ، ٦١,٩ ، -٤١,٩ ، - ٣٣,٤ %)&(٢٠، ٦٠، ٨٠، ٢٠ %)&(-٤٨,٩، ٤٣,٥، ٦٥، -٤١,٧%)&(٤٧,١، ٤١,٥، ٥٥,٨، ٦٣,١%)&(١٧,٩، ١٠,٣، ٢١,٣، ٢٦,٧%)&(٢٣٧,٦، ٣٠٢,٦، ٢٢٨,٦، ٢٦٣,٦%) زيـــادة أونقـــص عـــن الكنترول (٢٦,٥، ٥، ٢,٢٣، ٣,٣٤، ٢,٤١، ٠,٧٧) على التوالى.

٥- خفض المبيد الكثافة العددية للقوقع حقليا بمقدار ٥٨، ٧٢، ٧٩، ٨٤%) عند استخدامه بتركيـــزات ٠,٠٥ ، ٠,١ ، ٠,٢ ، ٠,٣ جم / م٢.