THE SUBLETHAL EFFECTS OF CERTAIN PESTICIDES ON THE REPRODUCTIVE PERFORMANCE OF THE LAND SNAIL *THEBA PISANA*

HUSSEIN A. A. [1], Y. ABO BAKR[2], F. A. KASSEM[3], H. I. EL-DEEB[2] AND E. A. M. ABDALLAH[3]

1-Central Agriculture Pesticide Laboratory, ARC, Dokki – Giza. Egypt
2-Department of Agricultural Animal Pests, Plant Protection Research Institute, ARC.
3-Pesticide Chemistry Dept. Faculty of Agriculture, Alexandria University.

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Abstract

The present study aimed to investigate the sublethal effects of different pesticides on the reproductive performance of the land snail *Theba pisana* using wheat bran laboratory formulated toxic bait.

Changes in reproductive parameters included fecundity, fertility, number of clutches, depth of clutches, egg weight, as well as the number of eggs per clutch (clutch size) were recorded for *T. pisana* treated with methomyl, chlorpyrifos or metaldehyde against control. Fecundity and fertility were increased with methomyl and chlorpyrifos treatments, while the same parameters were reduced by metaldehyde treatment. Also, number of clutches was increased by methomyl and chlorpyrifos, but decreased by metaldehyde treatment. Depth of clutches was decreased by all tested pesticides. Egg weight was reduced by methomyl treatment only, while chlorpyrifos and metaldehyde did not exhibit any effect on egg weight. All pesticides reduced the number of eggs per clutch (clutch size).

Key words: Land snails; Pesticides; Reproduction; Sub lethal effects.

INTRODUCTION

Gastropods play a role as agricultural pests, since they cause a damage in agriculture, horticulture and forestry. They attack any part of the plant and they have a wide range of host plants (Abdallah *et al.*, 1998 ; El-Wakil *et al.*, 2000 and Baker, 2002).

However, gastropods in the field are exposed mainly to sublethal concentrations of different types of pesticides as non-target organisms, during control of different pests that live in the same habitat of gastropods. Also, they are exposed to sublethal concentrations of molluscicides during their chemical control, where after a period of molluscicide application that degrades and the rest concentration will be sublethal.

The present study aimed to shed light on changes in fecundity, fertility, hatching success, egg weight, number of clutches per snail pair, clutch size (number of eggs per clutch), weight of clutch as well as clutch depth of *Theba pisana* after exposure to sublethal concentrations of methomyl, chlorpyrifos and metaldehyde. The first two insecticides are the most common used ones in the field of insect control, while the third tested copmound is the most effective and recommended molluscicde has been used since 1937 (Gimingham, 1940).

MATERIALS AND METHODS

Adult snails were collected from uncontaminated nurseries at the beginning of the breeding season (early autumn). Once in the laboratory, the snails were placed in glass aquaria and allowed to acclimatize to laboratory conditions for 3 weeks. They were fed on lettuce *ad libitum*.

Treatment procedures

T. pisana was treated with chlorpyrifos, methomyl or metaldehyde. Preliminary series of experiments were carried out to determine the sublethal concentrations of each pesticide. The highest concentration that did not cause mortality was chosen as the maximum one of sublethal concentrations. Five decreasing concentrations approximately halved from level to level were used. The following concentrations were used: chlorpyrifos (500, 1000, 2000, 5000 and 10000 µg/g bait), methomyl (10, 20, 50, 100 and 200 µg/g bait) and metaldehyde (20, 50, 100, 200 and 500 µg/g bait). The snails were fed on a diet consisting of wheat bran, calcium carbonate and dried milk powder in a mixture of 1:1:1 as described by Beeby and Richmond (2001) with slight modification. Control snails were fed on pesticide-free bait or diet.

Adult *T. pisana* snails were collected at the beginning of the breeding season (early autumn) from nurseries. After 7 days of exposure to pesticide-dosed diet (treated snails) and pesticide-free diet (controls), three pairs for each concentration were transferred to three cups (8 cm in deep and 10 cm in diameter), a pair of snails in one cup. These cups were filled with 150 g of sandy soil, clay soil and peatmoss in 1:1:1 mixture, and moistened with 40 ml water 3 times a week. Cups were checked for eggs twice a week for 30 days and pesticide-free diet was provided *ad libitum* as food. The number of egg clutches was recorded and each clutch was weighed and transferred on a moist filter paper in a Petri-dish (5 cm in diameter), then eggs were counted. Eggs were moistened with 0.5 ml water every 2 days and stored in the dark at room temperature(20$\pm$2^{o}C) until hatching occurred. Emergent offspring were counted after they moved in the Petri-dish. The following reproductive performance parameters were calculated: fecundity (number of eggs per snail pair), fertility (number of offspring per snail pair), hatching success (%), weight of egg (mg), number of clutches per snail pair, clutch size (number of eggs per clutch), weight of clutch (mg) and depth of clutch (cm).

Statistical analysis

The differences between means were tested using one-way analysis of variance (ANOVA) and least significant differences were calculated at p = 0.05.

RESULTS AND DISCUSSION

Changes in fecundity, fertility, hatching success, egg weight, number of clutches per snail pair, clutch size (number of eggs per clutch), weight of clutch as well as clutch depth of methomyl-, chlorpyrifos- or metaldehyde-treated *T. pisana* versus control are shown in Tables 1, 2, and 3 respectively.

All methomyl treatments, except the highest one, induced the fecundity of *T. pisana* as shown in Table 1. Number of eggs per snail pair was significantly increased with the most concentrations and the highest increase was obtained with 100 µg/g concentration which induced the fecundity by 44% as compared to the control. Severe reduction of the fecundity was observed with the highest methomyl treatment (200 µg/g), that was 43% of control. Fertility also was induced, whereas the number of offspring was increased with most concentrations, except the third and the last ones. The highest increase was detected with the 10 µg/g treatment, that the number of offspring exceeded the control by 38%. The reduction caused by the highest concentration was severe (63%). Hatching success of eggs laid by treated *T. pisana* slightly decreased than the control. The lowest percentage of hatching success was achieved by 50 µg/g treatment as shown in Table 1. Weight of egg was reduced by all methomyl treatments, except the highest one. The maximum reduction occurred with 100 μg/g treatment. This reduction was due to the increase of the number of eggs laid, so the highest concentration did not reduce egg weight because the number of eggs was lower than the control. Number of egg clutches was significantly increased with most methomyl treatments as shown in Table 1. The only reduction was observed with highest methomyl concentration, unless it was insignificant. Clutch size (number of eggs per clutch) was decreased by methomyl treatments. Clutch size of the highest concentration was 69% as compared with the control. Weight of clutch was significantly reduced by all concentrations of methomyl as compared with the control, but there was no difference between them. This reduction could be attributed to the reduction of the weight of eggs. Table 1 shows also the reduction of clutch depth due to methomyl treatments. All treatments reduced significantly the depth of clutch and this reduction was approximately concentration-dependent.

Fecundity of chlorpyrifos-treated *T. pisana* was enhanced as a result of the treatment. Number of eggs was significantly increased from the second concentration treatment. Concentration of 10000 μg/g caused 39% increase in fecundity as compared with the control as shown in Table 2. Fertility also was induced, whereas the highest 3 concentrations caused an increase in the number of offspring per snail pair. Maximum increase (32%) more than the control was obtained by 5000 μg/g treatment. Hatching success was not approximately affected, where the minimum hatching success was 89% with two treatments of 1000 and 10000 μg/g. The number of clutches per snail pair was increased with the most chlorpyrifos treatments, but the significant increases were restricted to the highest two concentrations, where the increases were 1.7 and 1.6 folds with 5000 and 10000 μg/g treatments, respectively as compared with the control (Table 2). The maximum reduction of clutch size was observed (Table 2) with the highest concentration, where clutch size was 77% of control. Weight of clutch was not significantly affected with most concentrations of chlorpyrifos (Table 2). Depth of clutch was significantly decreased with all treatments. Maximum reduction (50%), as compared with the control, was observed with the last concentration, as shown in Table 2.

Interestingly, all metaldehyde treatments significantly in concentration-dependent manner reduced the fecundity of *T. pisana*. The fecundity percentage of the highest treatment was 9% as compared to the control (Table 3). Fertility also was reduced in concentration-dependent manner. All concentrations exhibited a significant reduction in fertility. The highest concentration caused 92% reduction of *T. pisana* fertility. Hatching success was not affected with most concentrations, except the highest one with hatching success of 79%. Most metaldehyde concentrations significantly reduced the number of clutches. Maximum reduction was detected with the highest concentration (65%) as shown in Table 3. Clutch size also was reduced with maximum reduction of 73% than the control with 500 μg/g treatment (Table 3). All metaldehyde concentrations significantly decreased weight of clutch. The reduction was due to the reduction of the number of eggs per clutch, whereas the egg weight was not reduced with treatments. Also, depth of clutch was decreased with all metaldehyde concentrations as shown in Table 3. Maximum reduction was observed with 200 μg/g concentration (2.4 cm) as compared with the control (4.92 cm).

No information are available dealing with the effect of sublethal concentrations of methomyl or chlorpyrifos or any other insecticides on reproductive performance of *T. pisana* or any other terrestrial gastropods, however same related studies have been mentioned for fresh water snail *Lymnaea acuminata* by Tripathi and Singh (2004).

Kemp and Newell (1989) studied the effect of sublethal poisoning of molluscicides (metaldehyde or methiocarb) on the fecundity and egg viability of the terrestrial slug *Deroceras reticulatum*. They reported that neither molluscicides caused a reduction in fecundity and some slugs were able to oviposit within 2 weeks of the toxic meal. The life-cycle investigations (Köhler *et al.*, 1998) revealed an adverse impact of both heavy metals Cd and Zn exposure on reproduction of *D. reticulatum*. The average number of egg clusters decreased, which consequently resulted in a continuously diminished fecundity of the slugs in response to elevated metal concentrations.

In the current study, both of methomyl and chlorpyrifos surprisingly induced the fecundity and fertility of *T. pisana*. This result may, partly, explain our field observations of increasing the number of terrestrial snails especially *T. pisana* from year to year in infested fields, since most of farmers use both insecticides for control of several insect species and the snails exposed to them as non-target organisms. On the other hand, methomyl is used in bait formulation as molluscicide in Egypt (Ministry of Agriculture and Land Reclamation, 2001). After a time of application, the concentration of the methomyl bait decreased due to degradation, at this time, snails may expose to a sublethal concentration which may induce the fecundity and fertility of these snails and the next year we will receive more snails. The induction of fecundity or fertility could be linked to a presumed species protective response as reported by da Motta and Melo (1997), who reported the induction of *Biomphalaria straminea* snails as a result of low-doses of gamma irradiation.

Metaldehyde, in the current study, reduced the fecundity and fertility of *T. pisana*. This result contradicts with the result of Kemp and Newell (1989) on the terrestrial slug *D. reticulatum*. This contradictory may be attributed to the difference of experimental gastropod or metaldehyde concentrations or due to the differences between the two tested organisms.

The three tested pesticides reduced the size and depth of egg clutch. This changing in egg-laying behavior may be useful in monitoring of environmental pollution as a general bioindicator according to the definition of van Gestel and van Brummelen (1996) to bioindicator as: an organism giving information on the environmental conditions of its habitual by its presence or absence and its behaviour. This response is considered an early warning for ecotoxicologists and biodiversity conservationists. Results of egg and clutch weights indicate that the reduction of clutch weight of methomyl-treated *T. pisana* is attributed to the reduction of the egg weight and clutch size, while in the case of metaldehyde the cause of clutch weight reduction is restricted only to the decrease of the clutch size.

Finally, various man-made agents like pesticides, industrial chemicals and some natural substances have the potential to alter hormonal pathways that regulate reproductive processes in certain species of wildlife (Czech *et al.*, 2001). Until now, only a few investigations have been undertaken to determine the effects of these substances on reproductive capacities (fecundity and fertility) in exposed invertebrate species (Köhler *et al.*, 1998).

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Table 1 . Changes in reproductive performance parameters of methomyl-treated *Theba pisana.*

Reproductive Parameters	Sub lethal Concentrations (µg/g)						L.S.D $_{0.05}$
	0	10	20	50	100	200	
Fecundity (no. of eggs/snail pair)	261.8± 6.1^{c}	372.3 ±8.3^{a}	352.8 ±8.2^{a}	306.7 ±7.4^{b}	378.0± 21.8^{a}	113.5 ±10.1^{b}	33.46
Fertility (no. of offspring/ snail pair	251.3± 6.8^{b}	346.0 ±3.8^{a}	318.7 ±8.7^{a}	243.3 ±5.0^{b}	337.2 ±18.9^{a}	92.7 ±7.9^{c}	28.41
Hatching success (%)	96	93	90	79	89	82	
Weight of egg (mg)	7.2 ±0.3^{a}	6.8 ±0.1^{b}	6.2 ±0.4^{c}	6.0 ±0.2^{c}	6.0 ±0.2^{c}	7.5 ±0.4^{a}	0.37
No. of clutches/ snail pair	3.3 ±0.4^{b}	6.3 ±0.4^{a}	6.7 ±0.9^{a}	6.3 ±0.7^{a}	7.0 ±0.7^{a}	2.2 ±0.5^{b}	1.44
Clutch size (no. of eggs / clutch)	80.3 ±0.5^{a}	59.3 ±3.7^{b}	66.2 ±6.1^{ab}	54.2 ±5.7^{b}	56.2 ±4.5^{b}	55.0 ±5.6^{b}	14.29
Weight of clutch (mg)	580.0± 51.0^{a}	402.0 ±30.0 b	381.0± 23.0^{b}	323.0 ±31.0^{b}	345.0 ±34.0^{b}	374.0 ±32.0^{b}	97
Depth of clutch (cm)	4.9 ±0.2^{a}	4.3 ±0.2^{b}	3.5 ±0.1^{c}	3.3 ±0.2^{c}	3.4 ±0.2^{c}	2.4 ±0.2^{d}	0.55

Values are means ± SE.
Values that share the same superscript in the same row are non significantly different ($p<0.05$).

Table 2. Changes in reproductive performance parameters of chlorpyrifos-treated *Theba pisana.*

Reproductive Parameters	Sublethal Concentrations (µg/g)						L.S.D$_{0.05}$
	0	500	1000	2000	5000	10000	
Fecundity (no. of eggs/snail pair)	261.8 ±6.1^{d}	242.0 ±5.7^{d}	296.3 ±7.1^{c}	337.3 ±10.2 b	357.2 ±9.1^{b}	364.8 ±4.1^{a}	20.35
Fertility (no. of offspring/ snail pair	251.3 ±6.8^{c}	231.8 ±4.6^{d}	263.7 ±5.4^{c}	310.3 ±6.0^{b}	332.3 ±7.1^{a}	326.0 ±6.9^{ab}	17.86
Hatching success (%)	96	96	89	92	93	89	
Weight of egg (mg)*	7.2 ±0.3	7.2 ±0.3	7.2 ±0.2	7.6 ±0.4	7.8 ±0.2	7.5 ±0.2	
No. of clutches/ snail pair	3.3 ±0.4^{bc}	3.0 ±0.7^{c}	4.2 ±0.5^{abc}	4.8 ±0.5^{a} b	5.5 ±0.7^{a}	5.7 ±0.7^{a}	1.69
Clutch size (no. of eggs /clutch)	80.3 ±0.5^{a}	80.2 ±3.2^{a}	69.7 ±5.2^{ab}	69.3 ±3.8^{b}	64.7 ±2.6^{b}	61.7 ±3.6^{b}	10.8
Weight of clutch (mg)	580.0 ±51.0 ab	612.0 ±27. 0^{a}	494.0 ±32.0^{b} c	525.0 ±30.0 abc	503.0 ±32.0 bc	462.0 ±42.0 c	105.0
Depth of clutch (cm)	4.9 ±0.2^{a}	2.6 ±0.1^{b}	2.7 ±0.1^{b}	2.8 ±0.1^{b}	2.7 ±0.2^{b}	2.5 ±0.1^{b}	0.46

Values are means ± SE.
Values that share the same superscript in the same row are non significantly different ($p<0.05$).
* F is insignificant.

Table 3 . Changes in reproductive performance parameters of metaldehyde-treated *Theba pisana.*

Reproductive Parameters	Sublethal Concentrations (µg/g)						L.S.D$_{0.05}$
	0	20	50	100	200	500	
Fecundity (no. of eggs/snail pair)	261.8 ±6.1^{a}	79.2 ±2.8^{b}	67.3 ±3.7^{c}	62.7 ±4.4^{c}	49.8 ±2.7^{d}	24.3 ±2.5^{e}	11.28
Fertility (no. of offspring/ snail pair	251.3 ±6.8^{a}	69.3 ±2.5^{b}	61.2 ±3.3^{b}	60.0 ±3.7^{b}	46.2 ±2.6^{c}	19.3 ±2.2^{d}	11.05
Hatching success (%)	96	88	91	96	93	79	
Weight of egg (mg)*	7.2 ±0.3	8.0 ±0.4	8.2 ±0.4	8.2 ±0.4	7.9 ±0.4	7.4 ±0.2	
No. of clutches/ snail pair	3.3 ±0.4^{a}	2.3 ±0.3^{ab}	2.0 ±0.3^{b}	2.0 ±0.6^{b}	1.3 ±0.3^{b}	1.2 ±0.5^{b}	1.19
Clutch size (no. of eggs /clutch)	80.3 ±3.5^{a}	32.7 ±2.4^{b}	33.5 ±1.5^{b}	32.0 ±1.4^{b}	27.5 ±1.1^{bc}	22.0 ±2.6^{c}	8.83
Weight of clutch (mg)	580.0 ±51.0^{a}	271.0 ±24.0^{b}	283.0 ±18.0^{b}	261.0 ±30.0^{b}	263.0 ±21.0^{b}	212.0 ±25.0^{b}	110.0
Depth of clutch (cm)	4.9 ±0.2^{a}	2.8 ±0.2^{bc}	3.1 ±0.2^{b}	2.9 ±0.2^{bc}	2.4±0.2^{c}	2.6 ±0.2^{bc}	0.60

Values are means ± SE.
Values that share the same superscript in the same row are non significantly different ($p<0.05$).
F is insignificant.

التأثيرات غير المميتة لبعض المبيدات على الكفاءة التناسلية لقوقع التيبا بيسانا

علاء عبد الفتاح حسين ، ياسر ابو بكر ، فهمى احمد قاسم ، حسن ابراهيم الديب ، السيد احمد محمد عبدالله

معهد بحوث وقاية النباتات – الدقي – الجيزة – مصر

يهدف هذا البحث الى دراسة التأثيرات غير المميتة لبعض المبيدات على الكفاءة التناسلية لقوقع التيبا بيسانا حيث تم رصد التغير فى عدد البيض وعدد الفقس الناتج لكل زوج من القواقع وكذلك عدد كتل البيض وحجمها وعمقها فى التربة وايضا وزن البيض وذلك بعد المعاملة بمبيدات الميثوميل ، الكلوربيروفوس ، الميتالدهيد.

اظهرت النتائج ان مبيدى الميثوميل والكلوربيروفوس احدثا زيادة معنوية فى عدد البيض وعدد الفقس الناتج مقارنة بالقواقع غير المعاملة. بينما سبب الميتالدهيد خفض معنوى فى كل منهما. كذلك عدد كتل البيض زادت معنويا نتيجة المعاملة بكلا المبيدين الاولين فى حين انخفض هذا القدر نتيجة المعاملة بالميتالدهيد.

جميع المبيدات المختبرة ادت الى انخفاض عمق كتل البيض فى التربة. وزن البيض انخفض نتيجة المعاملة بالميثوميل فقط بينما لم يؤثر كل من الكلوربيروفوس والميتالدهيد على وزن البيض واخيرا حدث انخفاض معنوى فى حجم كتل البيض (عدد البيض / كتلة بيض) مع جميع المبيدات المختبرة.