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Control of the brown garden snail *Eobania vermiculata* (Gastropoda : Helicidae) adults using baits of usable and expired Kz mineral oil and its effect on aminotransferase enzymes activity

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Abstract:

Mineral oils are considering a promising control agent against wide varieties of pests all over the world. In this paper, focus on the baits of usable and expired Kz mineral oil against the adults of the brown garden snail *Eobania vermiculata* (Müller) (Gastropoda : Helicidae) under both of laboratory and field conditions. The baits of expired Kz oil against *E. vermiculata* adults, considering as novel technique. Under laboratory conditions, the highest mortalities (%) of snails were (73.33 %) recorded with a concentration 20 % of usable Kz oil followed by (66.67 %) for expired Kz oil at the same concentration after three weeks of treatment. Field results were recorded reduction in population of snails by (31.93 and 27.83 %) from used baits of usable and expired Kz oil, respectively at concentration (10 %) after three weeks from treatment. At concentration (20 %) and the same field conditions, mortalities (%) were (68.19 and 62.08 %) with usable and expired Kz oil, respectively. Biochemical studies were recorded increasing percentage reached its maximum level for tested snails after one week of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes recorded (-11.92 and -3.52) and (-33.33 and -5.39) for snails treatment using baits of usable Kz oil, at concentrations 10 and 20 % respectively, (- 9.26 and - 4.58) and (- 29.93 and - 12.53) for snails treatment using baits of expired Kz oil, at the same concentrations, respectively.

Introduction

Snails are gastropod animals which live in marine and fresh water, but others have successfully colonized land and known as pests in many places of the world. Some of land snails are very important pests of agricultural crops, in addition to the role as intermediate hosts for many parasitic diseases which infest human, animals and birds (Godan, 1983; Barker, 2002 and Mahrous *et al*., 2002). Therefore, land gastropods cause costly damage to field crops, vegetables and fruit trees as well as ornamental plants (Nakhla *et al.*, 1993 and Godan, 1983). While, control of these snails is becoming

very important. The terrestrial snail (brown garden snail) *Eobania vermiculata* (Müller) (Gastropoda : Helicidae) was recorded to be harmful snails in many districts of Egypt attacking various plants (Eshra, 2013). Unfortunately, about 25 million agricultural workers in developing countries are poisoned every year by pesticides (Jeyaratnam, 1990). Attention is increasingly being paid to the use of synthetic chemical compounds became unsafe method in controlling pests as they are one of the major reasons in the environmental pollution and cause a chronic disease for humans and harmful for most of the living organisms. That's why the usage of these chemicals decreased all over the world and humans continued to find practical alternatives. Mineral oils are considering one of the safest methods in controlling pests especially the scale insects infesting different plants. Also, they play a fundamental role in the IPM programs on many pests (Helmy *et al.*, 2012). A finding by this work aimed to study the toxic (as field trials) and physiological (as laboratory trials) effects of baits of usable and expired Kz oil against adults of *E. vermiculata* snail.

Materials and methods

1.Tested animals:

Land snail, the brown garden snail *E. vermiculata* adults were collected from orchard cultivated with sago palm at Zagazig district, Sharkia Governorate, Egypt. The collected snails were immediately transferred in white cloth bags to the laboratory. Healthy and similar individuals were chosen and kept in glass terrarium filled with moist clay soil adjusted at 75 % of water field capacity. Snails were fed daily with bran for two weeks before treatment for acclimatization.

2.Tested oils:

Usable and expired oil (95%) purchased from company of Kafr El-Zayat for pesticides and chemicals, Kafr El-Zayat district at Gharbia Governorate, Egypt.

3.Toxicity studies:

3.1. Laboratory test using poisonous baits technique:

Three concentrations 5, 10, and 20 % for tested oils were prepared by incorporating the appropriate amount of each compound with bran bait. Three plastic boxes (3/4 kg capacity) were used for each concentration. Five grams of baits were spread into each box. Control treatment was prepared using bran bait. Five adult individuals of *E. vermiculata* snail were put into each plastic box, then covered with muslin cloth and secured with rubber band. Dead individuals were counted using stainless steel needle according to El-Okda (1980). Mortality percentages were calculated after 1, 3, 7, 14 and 21 days and corrected by Abbott's formula (1925).

3.2. Field application:

The field trial was conducted in orchard cultivated with sago palm at, Zagazig district, Sharkia Governorate, Egypt. The field area was divided into five plots, each including control, each plot was divided into three replicates for each treatment. Area of about 50 m^2 was left as buffer between each plot. Baits were offered on blue plastic pieces each provided with 100 gm. Reduction percentages were calculated according to the formula of Henderson and Tilton (1955) as follows:

$$\% \text{ Reduction} = [1 - (t_2 \times r_1) / (t_1 \times r_2)] \times 100$$

Whereas:

r_1 = Number of alive snails before treatment in untreated plots.

r_2 = Number of alive snails after treatment in untreated plots.

t_1 = Number of alive snails before treatment in treated plots.

t_2 = Number of alive snails after treatment in treated plots.

4.Biochemical studies:

4.1. Preparation of snails for biochemical assay:

The adult mollusca shells of *E. vermiculata* snails were removed and the soft tissues were weighed, pooled, and homogenized as 1:10 (w/v) in distilled water.

The homogenates were centrifuged at 5000 r.p.m for 20 minutes at 5 °C according to Abd El-Haleim *et al*. (2006). The supernatants were used as enzyme source for aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Activities of enzymes were measured according to the method described by Reitman and Frankel (1957).

5. Statistical analysis:

The statistical analysis was determined by using one-way test, (ANOVA), **Cohort software (2005).**

Results and discussion

1. Effect of usable and expired Kz oil against adults of *Eobania vermiculata* snail under laboratory conditions:

Using baits technique, data in Table (1) revealed a highly significance between the three concentrations (5, 10 and 20%) of Kz oil (usable and expired) than control. Where mortality percentages were 26.67, 40.00 and 73.33 %, and 20.00, 33.33 |and 66.67 % for usable and expire Kz oil, respectively after three weeks of treatment. Finally, results obtained revealed that mortalities increased with increasing the concentrations. For instances, Helmy *et al.* (1982) stated that mineral oils of various qualities have traditionally been used as curatives to down insects and mites. Shahawy (2005) showed that the pesticide comate 500 (85% cotton seed oil) was the lowest effective compound with 2.5 % mortality percentage against *Monacha cantiana* (Montagu) (Gastropoda : Hygromiidae) snail after 12 days. Farag *et al.* (2010) indicated that long term used frying oils displayed more deformations in rat organs than that used for shorter period. Kaleem *et al.* (2015) found that rancidity of oils can produce potentially toxic compounds associated with long-term health effects such as neurological disorders, heart and cancer, may support the reuse of usable and expired Kz oil in baits controlling snails.

Table (1): Effect of usable and expired Kz mineral oil on adults of *Eobania vermiculata* snail using baits technique under laboratory conditions.

Kz oil	Concentrations	Mortality percentages				
		One day	Three days	One week	Two weeks	Three weeks
Usable	5 %	0.00^{c}	0.00^{d}	13.33^{cd}	20.00^{c}	26.67^{cd}
	10 %	6.67^{bc}	13.33^{bc}	20.00^{bc}	33.33^{b}	40.00^{b}
	20 %	20.00^{a}	26.67^{a}	33.33^{a}	53.33^{a}	73.33^{a}
Expired	5 %	0.00^{c}	0.00^{d}	6.67^{de}	13.33^{c}	20.00^{d}
	10 %	6.67^{bc}	6.67^{cd}	13.33^{cd}	20.00^{c}	33.33^{bc}
	20 %	13.33^{ab}	20.00^{ab}	26.67^{ab}	53.33^{a}	66.67^{a}
Control		0.00^{c}	0.00^{d}	0.00^{e}	0.00^{d}	0.00^{e}
$LSD_{0.05}$		7.64***	8.82***	10.81***	10.80***	*** 10.79

2. Effect of against adults of *Eobania vermiculata* snail under field conditions by baits technique:

The results in Table (2) showed that the initial effect after one day were (2.87 and 7.49 %) and (2.29 and 6.32 %) for usable and expired Kz oil at concentrations (10 and 20%), respectively. The residual effect on reduction percentages were (31.93and 68.19 %) and (27.83 and 62.08 %) after three weeks for Kz oil (usable and expired) at the same concentrations, respectively. Data revealed a significance between the two concentrations (10 and 20%) of Kz oil (usable and expired) by time elapsing except after three days of treatment than control. The reduction percentages were increased.

Table (2): Effect of usable and expired Kz mineral oil on adults of *Eobania vermiculata* snail using baits technique under field conditions.

Kz mineral oil	Conc. (%)	Number of snails before treatment	Initial effect		Residual effect								
			One day		Three days		One week		Two weeks		Three weeks		Mean for red.
			No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.	No.	% Red.	
Usable	10 %	43.74	43.19	2.87^{bc}	43.05	5.89^{b}	41.87	10.97^{b}	40.37	19.37^{b}	35.65	31.93^{c}	14.21^{b}
	20 %	57.97	54.52	7.49^{a}	53.89	11.11^{a}	49.35	20.82^{a}	37.79	43.05^{a}	22.08	68.19^{a}	30.13^{a}
Expired	10 %	48.19	47.87	2.29^{c}	47.63	5.49^{b}	46.23	10.78^{b}	44.96	18.50^{b}	41.64	27.83^{c}	12.98^{b}
	20 %	52.09	49.61	6.32^{ab}	48.86	10.31^{a}	45.17	19.35^{a}	33.98	43.01^{a}	23.65	62.08^{b}	28.21^{a}
Control		63.29	64.34		66.19		68.05		72.45		75.78		
$LSD_{0.05}$				3.65*		5.95^{ns}		7.47*		5.62 ***		4.98 ***	4.51 ***

by increasing concentration. Similar results were recorded by Aly *et al.* (1984) found that mineral oil showed a considerable reduction (99.2% reduction after 60 days from treatment) when used on the soft scale insect *Pulvinaria psidii* Maskell (Hemiptera: Coccidae) infesting guava trees. Ismail and Abdel Kader (2011) where reduction percentages for *Monacha cartusiana* (Müller) (Gastropoda : Hygromiidae) adult snails were 39.6,57.2 and 62.4 % for (1, 2 and 4 %), respectively concentrations of essential oil of *Syzygium aromaticum* using baiting technique under field conditions after 21 days. Farag (2012) tested castor oil at concentration (40%) against *M. cartusiana* and found that the initial effect gave (6.54%) reduction while the residual effect recorded (53.12%) reduction at three weeks. Helmy *et al.* (2012) showed that the modes of action of mineral oils on insects are death of newly hatched individuals and it's also may act as poisons, interacting with the fatty acids of the insect and interfering with normal metabolism.

3. Biochemical studies:

The obtained results in Table (3) found remarked increase in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in adults of *E. vermiculata* treated with baits of usable and expired Kz oil compared to control. The increasing percentages reached its maximum level for tested snails by time elapsing were recorded (-29.76, -18.16 and -11.92**)** and (- 16.05, - 10.86 and -3.52) of AST enzyme and (- 61.68, -55.39 and -33.33) and (- 43.27, - 41.76 and -5.39) of ALT enzyme for snails treatment of usable Kz oil, at concentrations 10 and 20 % respectively, (-25.53, -14.79 and -9.26**)** and (-11.51, - 6.51 and - 4.58) of AST enzyme and (- 53.27, -50.20 and -29.93) and (- 31.35, - 16.77 and -12.53) of ALT enzyme for snails treatment using baits of expired Kz oil, at the same concentrations, respectively. The activity of AST and ALT

Table (3): Changes in (AST and ALT) enzymes activity in adults of *Eobania vermiculata* snail treated with used usable and expired Kz mineral oil using baits technique.

Kz mineral oil	Concentrations (%)		AST			ALT		
			One day	Three days	One week	One day	Three days	One week
Usable	10 %	SA	20.39^{d}	23.52^{c}	25.78^{b}	6.43^{c}	7.82^{b}	11.76^{c}
		RA%	-29.76	-18.16	-11.92	-61.68	-55.39	-33.33
	20 %	SA	24.37^{bc}	25.62^{abc}	28.24^{ab}	9.52^{bc}	10.21^{b}	16.69^{a}
		RA%	-16.05	-10.86	-3.52	- 43.27	-41.76	-5.39
Expired	10 %	SA	21.62^{cd}	24.49^{bc}	26.56^{b}	7.84^{bc}	8.73^{b}	12.36^{bc}
		RA%	-25.53	-14.79	-9.26	-53.27	-50.20	-29.93
	20 %	SA	25.69^{ab}	26.87^{ab}	27.93^{ab}	11.52^{b}	14.59^{a}	15.43^{ab}
		RA%	-11.51	-6.51	-4.58	-31.35	-16.77	-12.53
Control		SA	29.03^{a}	28.74^{a}	29.27^{a}	16.78^{a}	17.53^{a}	17.64^{a}
L.S. $D_{0.05}$			3.90**	3.15*	2.57^{ns}	4.95**	3.15***	3.45*

SA = Specific activity as (µg pyruvate/ml)

RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100

were decreased as a result of all treatments compared to control. Data found that a significance between the two concentrations (10 and 20%) of Kz oil (usable and expired) by time elapsing except one week of treatment of AST than control. Results agree with those reported by Lebsack *et al.* (1980) who mentioned that the possible mechanism involved in the elevation of AST and ALT levels may be due to tissue damage. Tilkian *et al.* (1983) studied the pathogenesis effects which response enzymes activation and stated that the amount of AST was directly proportional to the number of cells damaged. Amer *et al.* (1994) found that the increase of AST and ALT activities may be referred to the diffusion of these enzymes from its intracellular sites due to damage caused by the insecticide on the subcellular level. Soliman *et al.* (2007) studied the effect of mineral oils on rats (as an example of mammals), they found that oils caused increase in white blood cells counts, AST and ALT activities of treated rats after 15 and 30 days from treatment comparison with control. Farag (2012) reported that using baits of castor oil on *M. cartusiana* snail caused decrease of AST activity and increase of ALT activity. Generally, alteration in the activity of AST and ALT are known to be helpful in the diagnosis of hepatic infarcts or damage.

Paper inspected likelihood of using baits of usable and expired Kz oil as a save and inexpensive manner to control adults of the brown garden snail *E. vermiculata*. So, it is concluded that the used usable and expired Kz oil are toxic on adults of *E. vermiculata* snail where it induces the increase in reduction percentage in field. Where, the connection between baits of Kz mineral oil (usable and expired) and activity of aspartate aminotransferase and alanine aminotransferase enzymes of *E. vermiculata* snail was investigated.

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