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COMPARATIVE MOLLUSCICIDAL ACTIVITY OF ABAMECTIN AND METHOMYL AGAINST *Eobania vermiculata* (Müller) AND *Theba pisana* (Müller)

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ABSTRACT

The molluscicidal activity of abamectin were assessed compared with methomyl against the most two abundant snail species brown garden snail (BGS) *Eobania vermiculata* and white garden snail (WGS) *Theba pisana* under laboratory conditions. The results indicated that LC_{50} values of methomyl were almost equal LC_{50} values of abamectin indicating more molluscicidal potency of abamectin and methomyl. Results also revealed a complete consistency of correlation between the LC_{50} values and exposed time intervals. The high molluscicidal activity of abamectin against *E. vermiculata* and *T. pisana* compared to methomyl using topical application technique could be used practically in the field of land snails control program and overcome some drawbacks of using bait forms in this respect. Comparing the LD_{50} values indicate the significant variation of the molluscicidal activity between the two applications methods used in addition to the variation between the two species of chosen snails, the brown garden snail *E. vermiculata* were resistant species than the white garden snail *T. pisana*.
Keywords: Molluscicides, Abamectin, Methomyl, *Eobania vermiculata* and *Theba pisana*

INTRODUCTION

Terrestrial gastropods are from the most significant threats of sustainable agriculture in many parts of the world (Barker, 2002). From the terrestrial gastropods, land snails are considered as serious economic pests due to its considerable damage of several types of plants. Land snails attack leaves, flowers, roots, buds, and even the trunk of trees causing great damage to the cultivated plants. In Egypt, land snails are known as dangerous pests to field crops, vegetables, orchards and ornamental plants (Kassab and Daoud, 1964; El-Okda, 1979; Abo-Bakr., 1997; Abdallah *et al*., 1998; Ibrahim 1995 and Mohamed 1995). Damage caused by snails is due mainly to feeding and to contamination with their bodies, faces or slime, leading to deterioration of the product quality besides, the financial loss (Lglesias *et al*., 2003). The importance of land snails as pest organisms has drastically increased in the past few decades (Godan, 1983; Gathwaite and Thomas, 1996).

Carbamate and oximecarbamate pesticides have been found to possess high potential use as molluscicides against terrestrial gastropods (Judge, 1969; Hunter and Johnson, 1970; Godan, 1983; Miller *et al*., 1988; El-Okda *et al*., 1989; Radwan and El-Wakil, 1991; Abdallah *et al*., 1992 and 1998 and Abo-Bakr 1997). Methomyl is one among the most potent oximecarbamates used especially in bait formulation forms against terrestrial slugs and snails.

However, growers and farmers often experience difficulty controlling land gastropods with conventional bait pellets containing molluscicides as in wet conditions the efficacy of these pellets can be very low leading to unsatisfactory control levels (Hata *et al*., 1997 and Schuder *et al*., 2003). In addition, poison baits can be toxic to other non-target life farms (Martin, 1993; Purvis, 1996).

Avermectins, a group of 16-membered macrocyclic lactones, are fermentation products from *Streptomyces avermitilis*, a naturally occurring soil actinomycete. From the fermentation, eight different avermectins were isolated, which comprise four pairs of homologues. One of these pairs, avermectin B1, that is the mixture of avermectins B1a (>80%) and B1b (<20%), is commercialized under the common name Abamectin (Pitterna *et al*., 2009). Abamectin is currently the main avermectin compound used as a miticide/ insecticide in a great variety of crops.

The aim of this research is to assess the molluscicidal activity of non conventional pesticides as Abamectin as well as standard conventional molluscicide methomyl against the most two abundant species of the terrestrial snails brown garden snail (BGS) *Eobania vermiculata* and white garden snail (WGS) *Theba pisana* using both poison bait and topical application techniques under laboratory condition.

MATERIALS AND METHODS

Tested Pesticides:

Abamectin:

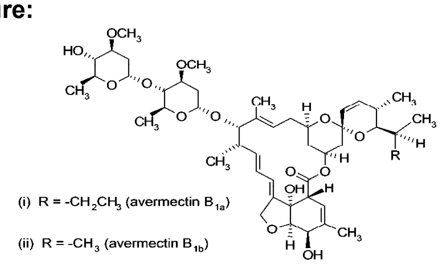
Common name : Abamectin

Molecular formula:$C_{48}H_{72}O_{14}$ (avermectin B1a); $C_{47}H_{70}O_{14}$ (avermectin B1b).

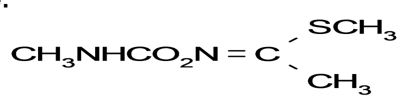
Chemical name: 5-*O*-demethylavermectin A1a (i) mixture with 5-*O*-demethyl-25-de (1-methylpropyl)-25-(1-methylethyl) avermectin A1a (ii)

Source: Technical were supplied by Syngenta and formulated (Demectin® 1.8% EC) were supplied by Agrochem.

Chemical structure:



Methomyl:
Common name : Methomyl
Molecularformula:$C_5H_{10}N_2O_2S$
Chemical name : methyl N-[[(methylamino) carbonyl]oxy]ethanimidothioate
Source: Both technical and formulated methomyl were supplied by E.I.du Pont de Nemours&Co.
Chemical structure:



Experimental snails:

Adults of the brown garden snail, *Eobania vermeculata* (Müller) and the white garden snail*, Theba pisana* (Müller) having approximately the same age and size were collected for laboratory experiments. These snails were collected during September from El-Maamoura locality, Alexandria. These snails were transferred to plastic cups covered with cloth netting and maintained under laboratory conditions of 27 $^{\circ}$ C and 65% R.H. The snails were daily fed on lettuce leaves up to the initiation of tests. The snails were allowed to be acclimatized to these conditions for two weeks. Dead snails were excluded as soon as possible, whenever needed.

Laboratory bioassay:

Toxicity of abamectin and methomyl against *E. vermiculata* and *T. pisana* snails was evaluated by two techniques:

Wheat-bran bait:

Local poison baits containing 0.05% methylene blue and 2% molasses as attractant were formulated in the laboratory, for the tested compounds in addition to the control (Bourne, *et al*., 1988). Methomyl (Kuik® 90% SP) were tested at the concentrations of (0.25, 0.5, 1.0, 1.5 and 2.0%) whereas abamectin (Demectin® 1.8%EC) were tested at concentrations of (0.25,0.5, 1and 1.5%) wheat bran (w/w). For each treatment, 30 animals were used and kept in three boxes (10 animals for each replicate). Five grams of bait was introduced to snails in each box. 30 animals in three boxes were used as control group containing wheat bran bait with 0.05% methylene blue, 2% molasses. Formulated of pesticides were dissolved in water. Mortality counts were recorded after 24, 48 and 72 hr of treatment, Mortality percentages were calculated and corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). The dead animals were detected by probing the snails with a needle to elicit typical withdrawal movement according to WHO, (1965).

Topical application:

Topical application technique was used according to the method of Abdallah *et al*., (1992). In this method, technical grades of abamectin (Technical 92%), and methomyl (Technical 97%) were dissolved in dimethyl sulfoxide (DMSO). DMSO has been shown to be the most appropriate solvent for topical application as it causes little distress to snails (Young and Wilkins. 1989). The stock solutions of these compounds were serially diluted with the same solvent to achieve the desired concentrations. The tested doses of abamectin were (0.077, 0.155, 0.232, 0.310, 0.388, 0.776 and 1.55 µg/gm b. w) whereas methomyl, were (0.776, 3.105, 6.211, 9.316, 9.316. 15.52, 23.29 and 31.05 µg/gm b.w) *E. vermiculata*. Methomyl doses were (0.851, 3.404, 6.808, 10.212, 17.021, 25.531 and 34.042 µg/gm b. w) *T. pisana*, while abamectin doses were (0.085, 0.170, 0.255, 0.340, 0.425 and 0.892 µg/gm b. w). For each treatment, 30 animals were used and kept in three plastic boxes (10 animals for each replicate) that were covered with cloth netting secured with rubber bands to prevent snails from escaping.. The tested doses were gently applied once on the surface of the snail body inside the shell using a micropipette. Positive controls were exposed to DMSO. Mortality counts were recorded after 24, 48 and 72 hr of treatment and mortality percentages were calculated, corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). The dead animals were detected by probing the snails with a needle to elicit typical withdrawal movement according to WHO, (1965).

RESULTS AND DISCUSSION

The molluscicidal activity of abamectin were assessed under laboratory conditions against the most two abundant snails species brown garden snail (BGS) *Eobania vermiculata* and white garden snail (WGS) *Theba pisana* compared with methomyl the most patent pesticide known against terrestrial gastropods.

Effect of tested pesticides on BGS and WGS using wheat bran bait:

The molluscicidal activity of abamectin tested insecticides against both BGS and WGS using wheat bran bait technique are recorded in Tables (1,2) and Figures (1,2). The results indicated that LC_{50} values of methomyl (the standard conventional molluscicide) used especially in bait formulation form were lower than LC_{50} values of abamectin. The results of abamectin indicating more molluscicidal potency of methomyl. Results also revealed a complete consistency of correlation between the LC_{50} values and exposed time intervals. The lower LC_{50} values were obtained always with increasing exposure time; however LC_{50} values of methomyl were 2.062, 0.952 and 0.564% after 24, 48 and 72 hrs, respectively. The other LC_{50} values of abamectin were higher than those corresponding each time interval of methomyl. Toxicity indices values indicated lower values than methomyl using wheat bran bait technique; however the toxicity indices decreased remarkably by increasing time for abamectin compared with methomyl.

Table (1): Efficacy of Abamectin (Demectin® 1.8% EC) against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using wheat bran bait technique, shown as mortality percentage % and LC_{50} values, at 24, 48 and 72 hr.

Hours	% Mortality at indicated concentration (%)				LC_{50}	Conf. Limits 95%		Slope
	0.25	0.5	1.0	1.5		Upper	Lower	
Eobania vermiculata								
24	5	14	25	42	2.091	3.346	1.589	1.796
48	18	33	48	70	0.885	1.085	0.746	1.753
72	27	47	65	84	0.541	0.633	0.454	1.936
Theba pisana								
24	22	38	55	73	0.742	0.895	0.623	1.711
48	40	53	68	78	0.412	0.521	0.292	1.296
72	55	73	85	93	0.204	0.279	0.105	1.518

Fig :(١) .Probit regression lines representing the effect of abamectin wheat bran bait against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

Table (2): Efficacy of Methomyl (Kuik® 90% SP) against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using wheat bran bait technique, shown as mortality percentage % and LC_{50} values, at 24, 48 and 72 hr.

Hours	% Mortality at indicated concentration (%)					LC_{50}	Conf. Limits 95%		Slope
	0.25	0.5	1	1.5	2		Upper	Lower	
Eobania vermiculata									
24	6	17	31	43	48	2.062	3.17	1.63	1.54
48	19	32	53	63	66	0.952	1.148	0.799	1.51
72	22	48	69	81	87	0.564	0.648	0.481	2.068
Theba pisana									
24	13	23	33	40	46	2.421	4.304	1.749	1.12
48	35	48	60	67	72	0.571	0.731	0.41	1.06
72	50	70	83	86	90	0.233	0.313	0.146	1.422

Fig. (2): Probit regression lines representing the effect of methomyl wheat bran bait against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

The molluscicidal effect against *T. pisana* of the two tested pesticides was much higher than their effect against the other snail species *E. vermiculata*. The LC_{50} values against WGS of the tow insecticides were lower than the corresponding LC_{50} values of BGS at the tow time intervals 48 and 72 hrs except 24hr in case methomyl treatment. The toxicity indices were less than 100% proving the superurity of methomyl using wheat bran bait technique than other tested pesticides (Table 2).

Topical application technique:

The molluscicidal efficacy of the abamectin and methomyl against both BGS and WGS using topical application technique are recorded in Tables (3,4) and illustrated in Figs. (3,4). The results showed remarkable increase of molluscicidal activity of abamectin compared to methomyl. The following points could be deduced clearly as recorded in Tables (3,4).

1- All toxicity indices for abamectin at 24, 48 and 72 hrs were highly more than 100% indicating much potent molluscicidal activity compared to the standard molluscicide methomyl.

2- Increasing toxicity was in range of three and four orders of magnitudes for BGS and WGS, respectively, in case of abamectin, The results confirmed the superurity of abamectin compared with methomyl.Using topical application technique emphasized the chance of using pesticide abamectin as spray against terrestrial gastropods and substitute the most common control method used in the field against land snails using poison baits and overcome all known drawbacks of using baits against these types of pests with major disadvantage of bait application that could be used only during the activity period of gastropods which is mainly short in both spring at autumn seasons. (Abdallah *et al*, 1992 and Abo-Bakr 2004).

3- Comparing the LD_{50} values indicate the significant variation of the molluscicidal activity between the two application methods used in addition to the variation between the two species of chosen snails, the brown garden snail *E. vermiculata* were resistant species than the white garden snail *T. pisana*. All LC_{50} and LD_{50} values of the tow tested insecticides within the time intervals measured throughout the experiment were lower in the case of WGS compared with the corresponding values of BGS as late recorded in Table (3,4).

4- There was a consistency in Tables (1, 2, 3 and 4) using both methods of applications either poisoned wheat bran bait or topical application technique that the slope values obtained of LD-P line (dosage-response curve) of BGS were lower than the corresponding slope values of WGS indicating more homogeneity population of WGS toward the two tested pesticides under both methods of applications than these of BGS. The high slope value means homogeneity in response of the tested snails species towards the toxic compound and at the same time the pesticides is activity as a selection factor producing on snails species, while the low slope value indicate heterogeneous snails populations.

Table (3): Efficacy of abamectin against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using topical application technique, shown as mortality percentage % and LD_{50} values, at 24, 48 and 72 hr.

Hours	% Mortality at indicated dose (µg/ gm b. w)							LD_{50} (µg/ gm b. w)	Conf. Limits 95%		Slope
	0.077	0.155	0.232	0.310	0.388	0.776	1.552		Upper	Lower	
Eobania vermiculata											
24	0	20	33	45	55	73	86	0.376	0.428	0.33	1.946
48	14	27	40	48	60	80	94	0.305	0.349	0.269	1.99
72	19	33	46	57	68	86	100	0.238	0..269	0..209	2.014

Hours	% Mortality at indicated dose (µg/ gm b. w)						LD_{50} (µg/ gm b. w)	Conf. Limits 95%		Slope
	0.085	0.170	0.255	0.340	0.425	0.892		Upper	Lower	
Theba pisana										
24	25	40	58	67	79	100	0.199	0.226	0. 173	2.065
48	30	50	70	80	93	100	0.153	0.174	0.13	2.274
72	35	60	80	86	93	100	0.125	1.42	0.105	2.524

Fig. (3): Probit regression lines representing the effect of Abamectin topical application agains terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

Table (4): Efficacy of methomyl against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snailsusing topical application technique, As mortality percentage % and LD_{50} values, at 24, 48 and 72 hr.

Hours	% Mortality at indicated dose (µg/ gm b. w)							LD_{50} (µg/ gm b. w)	Conf. Limits 95%		Slope
	0.776	3.105	6.211	9.316	15.528	23.29	31.05		Upper	Lower	
Eobania vermiculata											
24	26	40	46	55	66	66	73	5.898	7.805	4.3	0.787
48	30	53	60	66	73	83	86	2.883	3.769	2.044	0.971
72	35	60	68	76	86	90	93	1.88	2.48	1.301	1.107
Hours	% Mortality at indicated dose (µg/ gm b. w)							LD_{50} (µg/ gm b. w)	Conf. Limits 95%		Slope
	0.851	3.404	6.808	10.21	17.021	25.53	34.04		Upper	Lower	
Theba pisana											
24	23	40	53	60	73	80	86	4.944	6.095	3.878	1.136
48	28	53	63	68	80	86	93	3.059	3.913	2.246	1.093
72	30	53	66	73	86	86	93	2.644	3.379	1.938	1.159

Fig. (4): Probit regression lines representing the effect of Abamectin topical application against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

The high molluscicidal activity of abamectin against *E. vermiculata* and *T. pisana* compared to methomyl was expected. The introduction of ivermectin in the early 1980s brought a revolution in the control of animal parasites and was accepted as a potent anthelmintic (Campbell and Benz, 1984). In addition, avermectins have found wide application as pesticides and antiparasitic drugs for humans and animals (Burg *et al*., 1979; Campbell, 1989; Ostlind and Long, 1979). The most extensively used compounds of this class are avermectin B1 (abamectin). Abamectin were effectively used for control of the ectoparasite sea lice, the cause of serious problem for the salmon (primarily *Salmo salar*) aquaculture industry (Westcott et al., 2004). Campbell, (1989) stated that, the mechanism by which avermectins produce the pesticide and antiparasitic effects in invertebrates and a neurotoxic effect in vertebrates is the release of γ-aminobutyric acid (GABA) and the enhancement of its inhibitory action. Also, (Yamazaki *et al*, 1989) reported that ivermectin is an agonist for the GABA neurotransmitter. Our results is in agreement with Abdelgalil (2011). From the previous results, it can be concluded that molluscicidal activity of methomyl on both types of snails BGS and WGS may be at least in part attributed to the inhibition of AChE that comes in agreement with (Abdallah *et al*, 1998 and Kassem *et al*, 1992).

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مقارنـة الكفـاءة الابادية للقواقع للابـاميكتين والميثوميل ضد قوقـع الحدائق البنـي وقوقع الحدائق الابيض

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(٣)المعهد القومي لعلوم البحار والمصايد

تـم مقارنـة النشـاط الابـادي لمركـب الابـامكتين تحـت الظـروف المعمليـة بطريقتـي الطعـوم السـامة والمعاملة السطحية ضد أكثر أنواع القواقع الأرضية انتشارا وهما قوقع الحدائق البني (ايوبانيا فيرميكيولاتا) وقوقع الحدائق الأبيض (تيبا بيسانا) مع المقارنة بمركب الميثوميل.

طريقة الطعوم السامة : أشارت النتائج أن قيمة LC_{50} للميثوميل كانت متقاربة مع قيم الابامكتين مما تشير الـى انـه يوجـد نشـاط ابـادي للابـامكتين والميثوميـل. وأوضـحت النتـائج وجـود علاقـة بـين قـيم LC_{50} وزمن التعرض حيث تقل قيمة LC_{50} مع الزيادة في زمن التعريض.

التـأثير الابـادي لكـلا المبيدين المختبـرين كانـا أكثـر تـأثيرا ضـد التيبـا بيسـانا مـن تاثيرهمـا ضـد القوقـع ايوبانيا فيرميكيولاتا على مدار الثلاث فترات الزمنية ٢٤ ، ٤٨ ، ٧٢ ساعة بعد المعاملة.

طريقـة المعاملـة السـطحية: أوضـحت النتـائج زيـادة فائقـة للنشـاط الابـادي لمركـب الابـامكتين عـن الميثوميـل للقواقع ويستدل على ذلك بالنقاط التالية:-

١- سمية الابامكتين للقواقع بعد ٢٤ ، ٤٨ ، ٧٢ ساعة كانت اعلى من الميثوميل الذي يعد كمبيد قواقع.

٢- مقارنـة قيم LC_{50} LD_{50} تشـير الـى اختلافـات معنويـة للنشـاط الابـادي بـين طريقتـي المعاملـة المسـتخدمة بالاضافة الى الاختلافات بين النوعين المختارين من القواقع حيث كان القوقع البني ايوانيا فيرميكيولاتـا اكثـر مقاومة من القوقع الابيض تيبا بيسانا للمبيدات المختبرة.