

**TOXICITY OF PROPINEB 70%WP AND PROTECTO
9.4% WP ON TERRESTRIAL SNAILS *EOBANIA
VERMICULATA* AND *HELICELLA VESTALIS* UNDER
LABORATORY CONDITIONS**

[3]

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ABSTRACT

The present work aims at investigating and evaluating the effect of fungicide (propineb70% W.P) and bacterialcide (protecto 9.4% W.P) at different concentrations against eggs, immature stages (one, four and eight weeks) and adult stage of *Eobania vermiculata* and *Helicella vestalis* by using leaf dipping technique under laboratory conditions ($20^{\circ}\pm 2^{\circ}$ & $65\%\pm 5$ R.H).

Results revealed that, eggs was more sensitive to propineb 70%, since *H. vestalis* recorded higher mortality percentage compared to *E. vermiculata*. The % mortalities were (27, 47, 83 and 93%), (20, 40, 80 and 90%) respectively at the concentrations (0.1, 0.2, 0.3 and 0.4 %), with LC50 values (0.17 and 0.19%), respectively. Regarding immature stages (one week) also, *H. vestalis* recorded higher mortality percentage than *E. vermiculata* (23, 40, 73 and 97%) and (20, 33, 70 and 93%) respectively at concentrations (0.2, 0.4 0.6 and 0.8%). But for four weeks age was more sensitive than eight weeks and adult. As for in case of protecto 9.4% W.P also, eggs were more susceptible than immature stages and adult. A high mortality percentage was recorded to *H. vestalis* compared to *E. vermiculata* (20, 33.30, 70 and 76.70%) and (10, 30, 60 and 70%) respectively at concentrations of (0.2, 0.4, 0.6 and 0.8%) while for immature stages (one week) also, it was more sensitive than four, eight week and adult for *H. vestalis* compared to *E. vermiculata*. Generally eggs and immature stages (one week) of *H. vestalis*

were more sensitive to propineb 70% and protecto 9.4% compared to *E. vermiculata* and propineb 70% was more effective than protecto 9.4% for all stages.

Key words: *Eobania vermiculata*, *Helicella vestalis*, Egg, immature Stage, Propineb70% W.P, Protecto 9.4% W.P.

INTRODUCTION

Terrestrial gastropoda are from the most significant threats of sustainable agriculture in many parts of the world (Barker, 2002). From the Terrestrial gastropoda, 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 *et al.*, 1964 and El-Okda, 1979). Damage caused by snails is due mainly to feeding and to contamination with their bodies, feces or slime, leading to deterioration of the product quality besides, and financial losses (Glesias *et al.*, 2003). The importance of land snails as pest organisms has drastically increased in the past few decades (Godan, 1983 and Gathwaite *et al.*, 1996). Land snails are widespread pests that infested greenhouses and field crops all over the world. (Genena *et al.*, 2010). as well as (Said *et al.*, 2018) studied the molluscicidal effect of *Bacillus thuringiensis* on the land snail *Monacha cantiana*. They reported that, using the bacterial suspension is an effective method.

The aim of this work is to determine the molluscicidal activity of two pesticides (propineb70% W.P) and (protecto 9.4% W.P) against the eggs, immature stages and adult of two land snails species; *Eobania vermiculata* and *Helicella Vestalis* under laboratory conditions.

MATERIALS AND METHODS

Tested Pesticides:-

1. Fungicides (Propineb 70% W.P)

Company: Coromandel international limited

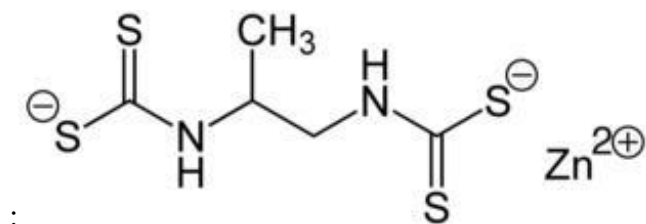
Local company: Chemitra

Trade name: Chemoneb 70 % W.P

Common name: propineb (dithiocarbamate)

Chemical name: polymeric 1, 2 – propylenebis

Chemical formula



2. Bacterial pesticides (Protecto 9.4 % W.P).

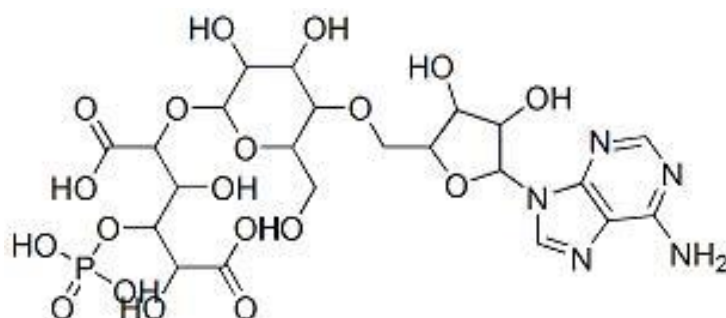
Company: Biocides Production Unit / Plant Protection Research Institute

Local name: protecto

Trade name: Bacillus thuringiensis kurstaki

Common name: Bacillus Thuringensis

Chemical name: 2-[5-[[5-(6-Aminopurin-9-yl)-3, 4-dihydroxyoxolan-2-yl] methoxy]-3, 4-dihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy-3, 5-dihydroxy-4-phosphonoxyhexanedioic acid.

Chemical formula:

Rearing and maintenance of snails: The tested snails, eggs, immature and adult of *Eobania vermiculata* and *Helicella vestallis* were collected from infested horticulture, vegetables and field crops at Ashmoon district, Menufiya governorate. The snails transferred in closed bags to the laboratory and were kept separately (each stage of each species) under laboratory conditions ($20^{\circ}\pm 2^{\circ}\text{C}$ and $65\%\pm 5\%$ R.H) in glassy terrariums ($40\times 25\times 20\text{cm}$) container the humidity reach ($75\pm 5\%$) with moist clay and sand soil (1:1) of about 8: 12 cm from its height soil was moistened with tap water to allow with fresh green lettuce leaves for two weeks for acclimatization before testing (Godan, 1983). The terrariums were closed with black muslin cloth secured with rubber band to avoid snail escaping (Baker and Hawake, 1991). Ten healthy individuals were selected for each replicate and starved for 24 hours before starting the tests.

Bioassay of the tested pesticides on egg and different stages of terrestrial snails *Eobania vermiculata* and *Helicella vestallis*:

1- Treatment of eggs of *Eobania vermiculata* and *Helicella vestallis*: The applied technique was leaf dipping method. Propineb 70% w.p was tested at different concentrations, 0.1, 0.2, 0.3 and 0.4 %., and of Protecto 9.4 % wp

was 0.2, 0.4, 0.6, and 0.8 % were prepared using distilled water of two genus of snails eggs as mentioned previously ml/100ml, similar pieces of fresh green lettuce leaves were dipped in glass jar containing 100ml of tested pesticides for 15 seconds, then left to dry before being offered to the target animals. Ten individuals for each tested stage with the shell diameter were exposed to the treated leaves in plastic box (13 cm diameter × 15 cm depth). Boxes were covered with muslin cloth held by rubber band to prevent animals from escaping, (Baker and Hawake; 1991). On the other hand 25 eggs were placed on the treated fresh green lettuce leaves in petry dish which were covered with muslin cloth held by rubber band. Each concentration was included 4 replicates were Kept with untreated fresh green lettuce leaves as a control. All replicates were examined after 3, 6, 9 and 11 days to recorded the mortality for adult and immature stage mean while eggs have been observed daily for 28 days to calculate the hatchability percentage.

2-Treatment for different stages of *Eobania vermiculata* and *Helicella vestallis*: The applied concentrations of propineb 70% W.P against the different ages of immature stage and adult of *E. vermiculata* and *H. vestallis* were; 0.2%, 0.4%, 0.6% and 0.8% for one week age, meanwhile for four week old, eight week age and adult; 0.5%, 1%, 1.5% and 2%.Whereas the used concentrations of Protecto against the different ages of immature stage and adult were; 0.5%, 1 %, 1.5% and 2. %. These concentrations were prepared using distilled water then the lettuce leaves were dipped in each concentration for 15 seconds before introduced to the experimental snails for feeding.

Mortality percentages were calculated and corrected according to Abbot's formula (1952) as follows:

$$\text{Corrected mortality \%} = \frac{\text{Observed M\%} - \text{Control M\%}}{100 - \text{Control M\%}} \times 100$$

M%: Mortality: The Toxicity lines were statistically analyzed according to Litchfield and Wilcoxon (1949).

LC₅₀, confidential limits and slope for the tested pesticides were calculated according to Finney (1952).

RESULTS AND DISCUSSION

The recorded results in tables (1 & 2) and figures (1 & 2) showed that the efficacy of fungicide (Propineb 70% w.p) after application as leaf dipping method of *E. vermiculata* and *H. vestalis* eggs, immature stages (one week, four week and eight week) and adult. Results showed that, a significant increase mortality percentage increasing concentrations for two genuses of snails. For eggs of *H. vestalis* were recorded highly mortality percentage compared to *E. vermiculata* 27, 47, 83 and 93 %, 20, 40, 80, and 90 % respectively at the concentrations 0.1, 0.2, 0.3 and 0.4 % comparing with control. LC₅₀ values for eggs of *H. vestalis* and *E. vermiculata* were 0.17 and 0.19 %. On the other hand for immature stages (one week) for *H. vestalis* was recorded highly mortality percentage compared to *E. vermiculata* 23, 40, 73 and 97 %, 20, 33, 70 and 93 %, respectively at the concentrations 0.2 ,0.4, 0.6 and 0.8 % compared with control. LC₅₀ values for one week for *H. vestalis* and *E. vermiculata* were 0.37 and 0.41 %. while four week, eight week and adult also, recorded highly mortality percentage of *H. vestalis* compared to *E. vermiculata* 37, 73, 86 and 97 % and 33, 70, 77 and 97 %, respectively

compared with control LC50 values were recorded 0.64 and 0.70%. For four weeks at the concentrations of 0.5, 1, 1.5 and 2 %. At the same concentrations for eight weeks was recorded highly mortality percentage 33% and 70%, 30 and 63 % at the concentrations 0.5 and 1 % but at the concentrations 1.5 and 2 %. Mortality percentage was recorded 83 and 97 % for the two genus of snails *H. vestalis* and *E. vermiculata* comparing with control. LC50 values were recorded 0.68 and 74 %. While for adult at the same concentrations 0.5, 1, 1.5 and 2 % were recorded highly mortality percentage of *H. vestalis* compared to *E. vermiculata* 30, 43, 53 and 87 % and 23, 40, 47 and 83 %, respectively compared with control LC50 values was recorded 1.11 and 1.16 %, respectively.

Data in Table (3 & 4) and figures (3 & 4) illustrates that bacterialcide (Protecto 9.4% wp) that applied, as leaf dipping technique of *E. vermiculata* and *H. vestalis* eggs, immature stages (one week, four week and eight week) and adult. The results showed that, Protecto 9.4% wp gave a significant effect on egg, all stages and adult. For eggs of *H. vestalis* caused highly mortality percentage compared to *E. vermiculata* 20, 33.3, 70 and %, 10, 30, 60 and 70 %, respectively at the concentration of 0.2, 0.4, 0.6 and 0.8 % comparing with control LC50 values were 0.44 and 0.53% of *H. vestalis* was recorded highly mortality percentage compared to *E. vermiculata* at concentrations 0.5, 1, 1.5, 2 %, except 0.5 % concentration was recorded the same mortality percentage of *H. vestalis* and *E. vermiculata* was 26, 70% but the other concentrations were recorded 66.70, 80 and 86.70 %, 56.70, 63.30 and 76.70%, respectively for *H. vestalis* and *E. vermiculata* comparing with control LC50 values for this ages of *H. vestalis* and *E. vermiculata* were 0.77 and 0.93%, respectively.

Whereas, four weeks and eight weeks also, recorded highly mortality percentage of *H. vestalis* than *E. vermiculata* at the same concentrations 0.5, 1, 1.5 and 2 %, except 1.5 % recorded the same mortality percentage 66.70 % comparing with control. However the other concentrations recorded 26.70, 60 and 73.30 %, 20, 36.70 and 70%, respectively for four week of *H. vestalis* and *E. vermiculata*. LC50 values were 0.91 and 1.17%, respectively. At the same concentrations as mentioned previously, also, recorded highly mortality percentage of *H. vestalis* compared to *E. vermiculata* 16.70, 33.30, 56.70 and 80%, 13.30, 26.70, 63.30 and 73.30%, respectively comparing with control. LC50 recorded 1.21 and 1.28 %, respectively. While for adult at the concentrations 0.5 and 1% were recorded the same mortality percentage for *H. vestalis* and *E. vermiculata* 13.30 and 30 %, but at the other concentrations 1.5 and 2% recorded highly mortality percentage of *H. vestalis* compared to *E. vermiculata* ,43.3 and 66.70%, 36.70 and 63.30%, respectively comparing with control LC50 were 1.52 and 1.69%, respectively. Our finding are in harmony with those which obtained by Godan (1983) and Andrew (2009) recorded that, carbendazim cause fetotoxic and teratogenic effects in laboratory animals following bolus oral dosing. Also, Mahmoud, (2002) found that, diflubenzuron compound caused teratogenic effect of different ages of land slug, *Limax flavus*. Asran *et al.*, (2011) they found that, Metaldehyde was the most effective compound against *Theba pisana* followed by Secnor and Oxamyl which was the lowest effective one for both laboratory and field conditions. As well Mobarak, *et al.*, (2014).they indicated that, hatching age was the most susceptible to carbendazim (fungicide) followed by juvenile while the adult age was the lowest susceptible one of

different ages of land slug, *Arion linnaeus*. Rady *et al.*, (2018) they reported that, Neomyl exhibited the highest toxic effect followed by Round up, while, Topsin gave the least effect in this respect. Finally, we can conclude that (dithiocarbamate) compound (Propineb 70% w.p) and *Bacillus thuringiensis* (Protecto 9.4% wp), they can be used in the biological control of all stages of snails.

Table (1): Toxicity of Propineb 70% W.P on different stages of *E.vermiculata* under laboratory conditions

Stage	% mortality of snails		LC50 (%)	95% Confidence limits		99% Confidence limits		slope	
	conc.	Mortality		Lower	Upper	Lower	Upper		
Eggs	0.1%	20%	0.19	0.15	0.22	0.14	0.23	0.23	
	0.2%	40%							
	0.3%	80%							
	0.4%	90%							
Immature stages	One week	0.2%	0.41	0.34	0.48	0.34	0.51	0.55	
		0.4%							33%
		0.6%							70%
		0.8%							93%
	Four weeks	0.5%	0.7	0.52	0.84	0.45	0.89	0.11	
		1%							70%
		1.5%							77%
		2%							97%
	Eight weeks	0.5%	0.74	0.57	0.88	0.5	0.93	0.61	
		1%							63%
		1.5%							83%
		2%							97%
Adult	0.5%	1.16	0.9056	1.5163	0.8073	1.7196	0.88		
	1%							40%	
	1.5%							47%	
	2%							83%	

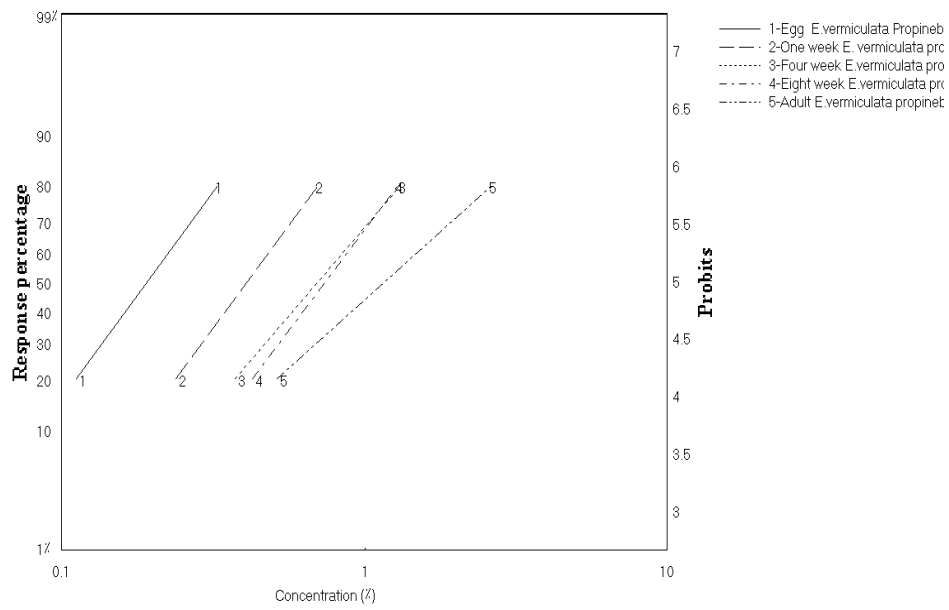


Fig. (1): Toxicity of Propinb 70% W.P on different stages of *E. vermiculata* under laboratory conditions

Table (2): Toxicity of Propinb 70% W.P on different stages of *H. vestalis* under laboratory conditions

Stage	% mortality of snails		LC50 (%)	95% Confidence limits		99% Confidence limits		slope	
	conc.	Mortality		Lower	Upper	Lower	Upper		
Eggs	0.1%	27%	0.17	0.13	0.21	0.12	0.21	0.21	
	0.2%	47%							
	0.3%	83%							
	0.4%	93%							
Immature stages	One week	0.2%	0.37	0.31	0.44	0.28	0.46	0.55	
		0.4%							40%
		0.6%							73%
		0.8%							97%
	Four weeks	0.5%	0.64	0.48	0.77	0.42	0.81	0.38	
		1%							73%
		1.5%							86%
		2%							97%
	Eight weeks	0.5%	0.68	0.51	0.51	0.44	0.87	0.67	
		1%							70%
		1.5%							83%
		2%							97%
Adult	0.5%	1.11	0.73	1.29	0.62	1.44	0.88		
	1%							43%	
	1.5%							53%	
	2%							87%	

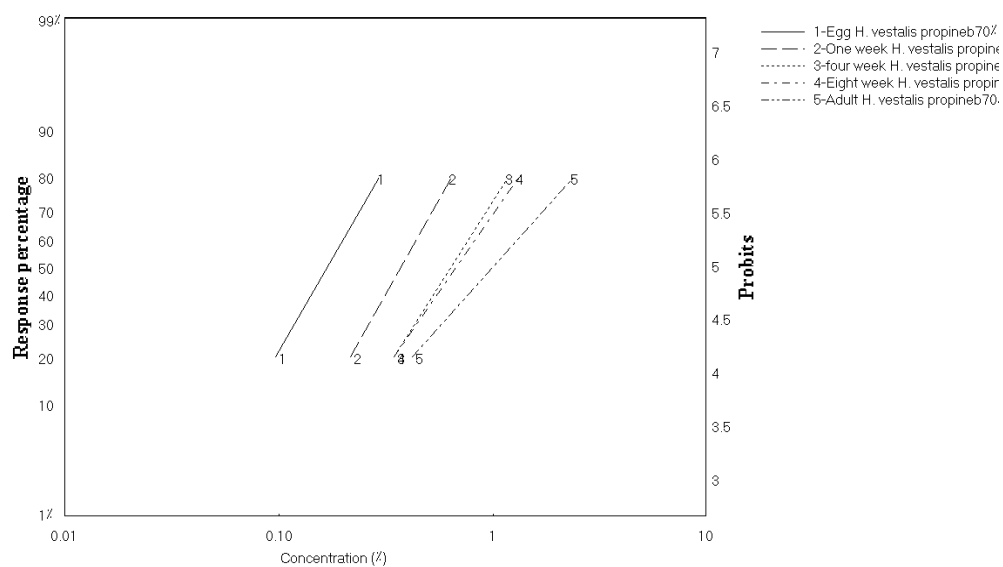


Fig. (2): Toxicity of Propinb 70% W.P on different stages of *H. vestalis* under laboratory conditions

Table (3): Toxicity of Protecto 9.4 % W.P on different stages of *E. vermiculata* under laboratory conditions

Stage	% mortality of snails		LC50 (%)	95% Confidence limits		99% Confidence limits		slope	
	conc.	Mortality		Lower	Upper	Lower	Upper		
Eggs	0.2%	10%	0.53	0.44	0.66	0.41	0.73	0.77	
	0.4%	30%							
	0.6%	60%							
	0.8%	70%							
Immature stages	One week	0.5%	0.93	0.65	1.21	0.51	1.33	0.97	
		1%							56.70%
		1.5%							63.30%
		2%							76.70%
	Four weeks	0.5%	1.17	0.91	1.51	0.82	1.69	0.56	
		1%							36.70%
		1.5%							66.70%
		2%							70%
	Eight weeks	0.5%	1.28	1.06	1.58	0.99	1.74	0.37	
		1%							26.70%
		1.5%							63.30%
		2%							73.30%
Adult	0.5%	1.69	1.32	2.78	1.22	4.17	0.45		
	1%							30%	
	1.5%							36.70%	
	2%							63.30%	

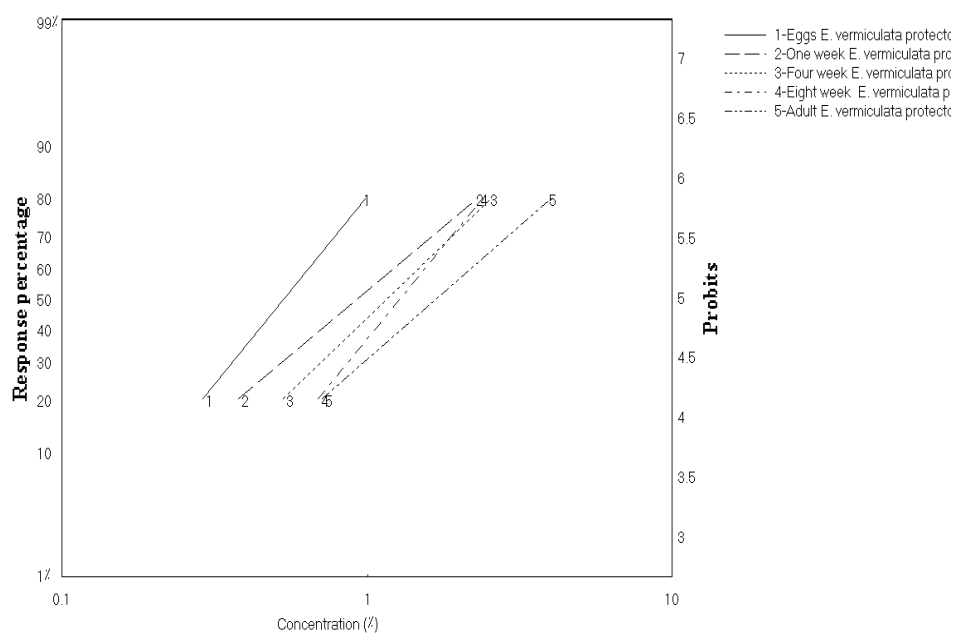


Fig. (3): Toxicity of Protecto 9.4 % W.P on different stages of *E. vermiculata* under laboratory conditions

Table (4): Toxicity of Protecto 9.4 % W.P on different stages of *H. vestalis* under laboratory conditions

Stage	% mortality of snails		LC50 (%)	95% Confidence limits		99% Confidence limits		slope	
	conc.	Mortality		Lower	Upper	Lower	Upper		
Eggs	0.2%	20%	0.44	0.35	0.55	0.32	0.61	0.31	
	0.4%	33.30%							
	0.6%	70%							
	0.8%	76.70%							
Immature stages	One week	0.5%	26.70%	0.77	0.57	0.94	0.49	1.11	0.82
		1%	66.70%						
		1.5%	80%						
		2%	86.70%						
	Four weeks	0.5%	26.70%	0.91	0.61	1.18	0.46	1.31	0.67
		1%	60%						
		1.5%	66.70%						
		2%	73.30%						
	Eight weeks	0.5%	16.70%	1.21	0.99	1.51	0.91	1.64	0.41
		1%	33.30%						
		1.5%	56.70%						
		2%	80%						
Adult	0.5%	13.30%	1.52	1.13	2.78	1.02	5.48	0.31	
	1%	30%							
	1.5%	43.3							
	2%	66.70%							

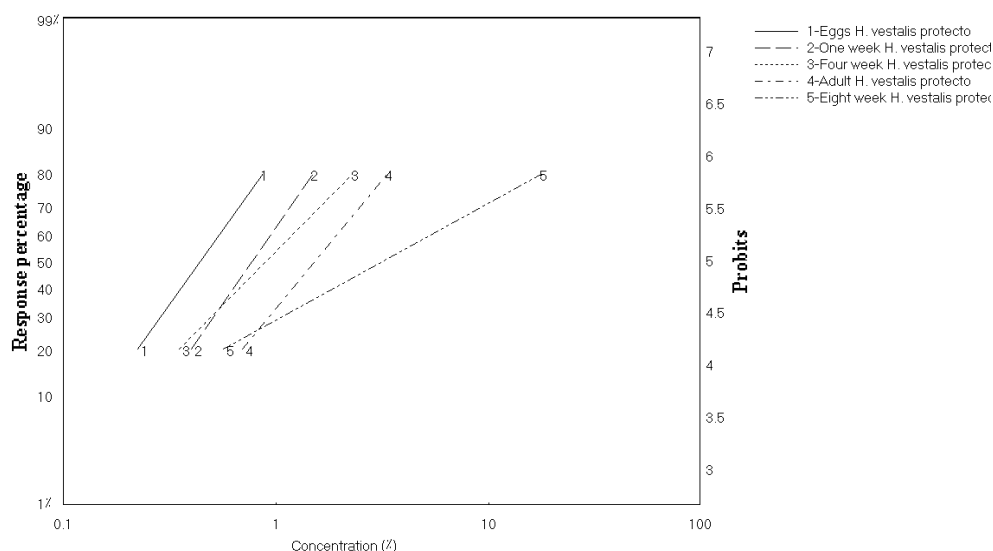


Fig. (4): Toxicity of Protecto 9.4 % W.P on different stages of *H. vestalis* under laboratory conditions

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سمية مبيد البرونيب W.P ٧٠% ومبيد البروتكتو W.P ٩,٤% على قواقع الحقائق البني ذو الشفة *E. vermiculata* وقواقع النخيل *H. vestalis* تحت الظروف المعملية

[٣]

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المستخلص

يهدف هذا البحث الي تقييم تأثير نوعين من المبيدات وهما مبيد البرونيب W.P ٧٠% ومبيد البروتكتو W.P ٩,٤% بتركيزات مختلفه علي كل من البيض والأطوار غير البالغه (عمر أسبوع وأربعة أسابيع وثمانية أسابيع) والطور البالغ لنوعين من القواقع الأرضيه هما قواقع الحقائق البني ذو الشفة *E. vermiculata* وقواقع النخيل. وذلك باستخدام طريقه غمر الأوراق النباتيه باستعمال أوراق الخس تحت الظروف المعملية (درجه حراره ٢٠± م و رطوبه نسبيه ٦٥±%).

أوضحت النتائج المتحصل عليها أن البيض أكثر حساسية لمبيد البرونيب % ٧٠ W.P في حالة قوقع النخيل *H. vestalis* حيث سجل أعلى نسبة موت مقارنة بقوقع الحقائق البني *E. vermiculata* % 27, 47, 83, 93 و % 20, 24, 80, 90 علي التوالي عند تركيزات 0.1, 0.2, 0.4%. وبلغت قيمة التركيز النصفى المميت (LC50) 0.19، 0.17، علي التوالي لكلا القوقعين وذلك بالمقارنة بالكنترول. بينما في حالة الأطوار غير البالغه (عمر اسبوع)، فقد سجل قوقع النخيل *H. vestalis* أيضا أعلى نسبة موت مقارنة بقوقع الحقائق البني *E. vermiculata* حيث كانت % 23, 40, 73, 97 و % 20, 33, 70, 93 علي التوالي عند تركيزات % 0.2, 0.4, 0.6, 0.8. لكن بالنسبة لعمر أربعة أسابيع فقد كان أكثر حساسية عن عمر ثمانية أسابيع والطور البالغ وذلك مقارنة بالكنترول. وبالنسبة لمبيد البروتكتو % ٩,٤ W.P كان البيض أكثر حساسية أو استجابيه عن الاطوار غير البالغه والطور البالغ حيث سجل أعلى نسبة موت في حالة قوقع النخيل *H. vestalis* مقارنة بقوقع الحقائق البني ذو الشفه % 20, 33.30 70, 76 *E. vermiculata* و % 10, 30, 60, 70 علي التوالي في حين كانت الاطوار غير البالغه (عمر اسبوع) كان أكثر حساسية عن عمر أربعة أسابيع وثمانية أسابيع والطور البالغ بالنسبة لقوقع النخيل *H. vestalis* مقارنة بقوقع الحقائق البني *E. vermiculata* وذلك مقارنة بالكنترول.

الكلمات الداله: قوقع الحقائق البني، قوقع النخيل، البيض، الأطوار غير البالغه، البرونيب WP 70% ، البروتكتو % 9.4 WP.