

Efficiency of Certain Bio-Agents as Biological Control against Two Land Snail Species, *Eobania Vermiculata* (Müller) and *Succinea Putris* (Linnaeus) under Laboratory Conditions

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ABSTRACT

This study was carried out to evaluate the effectiveness of certain bio control agents such as entomopathogenic nematodes (EPNS) (*Heterorhabditis bacteriophora* (HP88), *Heterorhabditis bacteriophora* (B20), *Steinernema carpocapsae* and *Steinernema* sp.), Bioarc (*Bacillus megaterium*) and Biozeid (*Trichoderma album*) against the two land snails, *Eobania vermiculata* and *Succinea putris* compared with recommended molluscicide Agrinate under laboratory conditions. Results revealed that Agrinate showed superior toxicity effect more than tested biological agents recording the highest total mortality percentages in *E. vermiculata* (86.65%) and *S. putris* (96.65%). Bioarc and Biozeid ranked the next and induced total mortality percentages amounted to 56.66 and 39.98% for *E. vermiculata* and 69.98 and 56.66% for *S. putris*, respectively. Among entomogenous nematodes, *H. bacteriophora* HP88 and B20 performed the best at the level of 9000 IJs/ cup, while *S. carpocapsae* and *Steinernema* sp. showed the least molluscicidal activity against *E. vermiculata* and *S. putris* after 28 days of treatment. The land snail, *S. putris* showed more sensitivity to all tested treatments than *E. vermiculata*.

Keywords: Entomopathogenic nematodes, Bioarc, Biozeid, *Eobania vermiculata*, *Succinea putris*.

INTRODUCTION

Land gastropods have greatly increased in economic importance and they are considered a group of the most serious pests attacking agricultural crops around the world (Barker, 2002). In Egypt, land snails have been increased and distributed rapidly in most Governorates and caused considerable damage especially in most areas where they found the optimum conditions for survival and dispersion (El-Okda, 1981). They cause great damage to vegetables, field crops, orchard trees as well as ornamental and medical plants (El- Wakil *et al.*, 2000; Abed, 2011; Lokma 2013). Therefore, control of these snails is becoming very important. The terrestrial snails *Eobania vermiculata* and *Succinea putris* were recorded to be harmful snails in many districts of Egypt attacking various plants (Eshra, 2013). Chemical control of harmful snails by using synthetic organic molluscicides are expensive and toxic to non- target organisms and caused environmental pollution (Gabr *et al.*, 2006), so alternative safe and clean molluscicides must be used. Insect pathogenic nematodes occur naturally in soil and are found in most places where plants grow. Entomopathogenic nematodes (EPNs) *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* are utilized as biological control agents against various insect pests (Ebssa, 2005). They are considered as attractive alternatives to chemical insecticides and safe for use around humans, livestock and plants (Ehlers, 2001). Also, biological control of land snails or slugs using microbial agents such as bacteria and fungi is an alternative control method to pesticides that received greater attention few years ago (Genena and Mostafa, 2008).

Therefore, this work was planned to study the effect of certain entomopathogenic nematodes (EPNS) and the two commercial products, Bioarc (*Bacillus megaterium*) and Biozeid (*Trichoderma album*) as biological control agents compared with Agrinate (24% SL. recommended molluscicide) against the two land snails, *Eobania vermiculata* and *Succinea putris* under laboratory conditions.

MATERIALS AND METHODS

Tested snails:

Adults of the Brown garden snail, *Eobania vermiculata* (Müller) and the amber snail, *Succinea putris* (Linnaeus) having approximately the same age and size were collected from infested ornamental plants and infested fields crops at Mansoura district, Dakahlia Governorate. The obtained snails were kept in plastic containers filled with moist soil. Snails were daily fed on fresh leaves of lettuce (*Lactuca sativa* L.) for 14 days to be acclimatized. Dead and unhealthy snails were removed and only healthy ones were used in the experiments.

Tested Bio-agents:

A) Entomopathogenic nematodes:

Heterorhabditis bacteriophora (HP88), *Heterorhabditis bacteriophora* (B20), *Steinernema carpocapsae* and *Steinernema* sp. were used. The entomopathogenic nematodes inocula used in this research were obtained from Plant Protection Research Inst. Agricultural Research Center (ARC) Dokki, Giza., Egypt, where greater wax moth, *Galleria mellonella* was used as host insect for in vivo culture.

B) Bioarc (2.5g/L of distilled water):

An indigenous commercial formulation of phosphorus solubilizing bacterium, *Bacillus megaterium* (25×10^6 cfu/g).

C) Biozeid (2.5g/L of distilled water):

An indigenous commercial formulation of the nematophagous fungus, *Trichoderma album* (25×10^6 cfu/g).

Both bio-products were obtained from Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt and enrolled by the Ministry of Agriculture.

Chemical pesticide:

Methomyl: (Agrinate 24% SL. 1liter/ feddan) Carbamate recommended molluscicide.

Chemical name: S-methyl N (methylcarbamoyloxy) thioacetimidate

Application methods:

1- Soil injection:

Entomopathogenic nematodes, *H. bacteriophora* (HP88), *H. bacteriophora* (B20), *S. carpocapsae* and

Steinernema sp. were tested at three levels against *E. vermiculata* and *S. putris* under laboratory conditions at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $85\% \pm 5\%$ soil moisture. Infective nematode juveniles were prepared at three levels (3000, 6000 and 9000 IJs). Sterilized moist clay soil (100 gm.) was placed at the bottom of each plastic cup (10 cm diameter). Each nematode level was pipetted on the moist surface layer of soil. For each plastic cup, ten snails of *E. vermiculata* and *S. putris* were fed on lettuce leaf disk daily and cup was covered with muslin cloth fixed with rubber bands to prevent snails from escaping for four weeks. Each nematode level was replicated three times in addition to control treatment. The tested snails were examined daily, where the dead individuals were counted and removed. Mortality percentages were calculated after one, two, three and four weeks of treatment.

2- Concomitant technique:

The two bio-agents *B. megaterium* (Bioarc) and *T. album* (Biozeid) were tested for their effectiveness against *E. vermiculata* and *S. putris* as concomitant treatment (leaf dipping + soil incorporation) (Genena and Mostafa, 2008). Similar leaf discs of fresh leaves of lettuce were immersed for 5 seconds in the tested bio-agents then left to air dry before application. Ten snails of each species were transferred to plastic cup (10 cm diameter) filled with sterilized moist clay soil which incorporated with 5 ml of liquid solution of Bioarc and Biozeid. Each cup was then covered with muslin cloth held by rubber bands. Cups receiving untreated leaves and soil were sewed as control. Each treatment was replicated three times. After 48 hrs. of treatment, the treated leaves were replaced daily with fresh untreated lettuce leaves for four weeks. The tested snails were examined daily, where the dead individuals were counted and removed. Mortality percentages were calculated after one, two, three and four weeks of treatment.

3- Leaf dipping technique:

Chemical molluscicide (Agrinate 24% as a recommended molluscicide was used. Fresh lettuce leaves were dipped for one minute in one concentration of Agrinate (9600ppm) and left for dryness (Ghamry,

1994). The treated leaves were placed inside plastic cups filled with sterilized moist soil. Ten adult snails of *E. vermiculata* and *S. putris* were placed into each cup. Each cup was covered with muslin cloth held by rubber bands to prevent snails from escaping. Three replicates for treated and untreated were used as a control treatment. Mortality percentages were recorded after one, two, three and four weeks of treatment.

Statistical analysis:

Data were calculated analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less was considered significant. All statistical analysis was done with Cohort Software (2004).

RESULTS AND DISCUSSION

Effect of different entomopathogenic nematodes on the Brown garden snail, *Eobania vermiculata*:

Results summarized in Table (1) revealed that the mortality percentages of the land snail, *E. vermiculata* were increased by increasing inoculum levels of *H. bacteriophora* (HP88), *H. bacteriophora* (B20), *S. carpocapsae* and *Steinernema* sp. and increasing exposure periods as well. After four weeks of exposure, *H. bacteriophora* (HP88) gave mortality percentages of *E. vermiculata* with values of 6.66, 13.33 and 16.66% followed by *H. bacteriophora* (B20) and *S. carpocapsae* nematodes with values 3.33, 6.66 and 13.33% at 3000, 6000 and 9000 IJs/ cup, respectively. However, *Steinernema* sp. gave mortality percentages 3.33 and 10.00 % when exposed to 6000 and 9000 IJs/ cup, respectively. Also, mortality percentages of *E. vermiculata* ranged from 0.00 to 16.66 % for *H. bacteriophora* (HP88), 0.00 to 13.33% for *H. bacteriophora* (B20) and *S. carpocapsae* and 0.0 to 10.00 % for *Steinernema* sp. among the four weeks of treatment. Also, Data showed the total mortality % at 9000 IJs/ cup was 29.98, 23.32, 23.32 and 13.33% for *H. bacteriophora* (HP88), *H. bacteriophora* (B20), *S. carpocapsae* and *Steinernema* sp., respectively.

Table 1. Mortality percentages of *Eobania vermiculata* after exposure to three levels of entomopathogenic nematodes.

Nematodes	Treatments Nematode levels	Mortality percentages after indicated weeks				Total Mortality %
		One Week	Two Weeks	Three Weeks	Four Weeks	
<i>H. bacteriophora</i> (HP88)	3000	0.00 ^a	3.33 ^a	3.33 ^a	6.66 ^{abc}	13.32
	6000	0.00 ^a	3.33 ^a	6.66 ^a	13.33 ^{ab}	23.32
	9000	3.33 ^a	3.33 ^a	6.66 ^a	16.66 ^a	29.98
<i>H. bacteriophora</i> (B20)	3000	0.00 ^a	0.00 ^a	3.33 ^a	3.33 ^{bc}	6.66
	6000	0.00 ^a	3.33 ^a	3.33 ^a	6.66 ^{abc}	13.32
	9000	3.33 ^a	3.33 ^a	3.33 ^a	13.33 ^{ab}	23.32
<i>S. carpocapsae</i>	3000	0.00	0.00 ^a	3.33 ^a	3.33 ^{bc}	6.66
	6000	0.00	3.33 ^a	0.00 ^a	6.66 ^{abc}	9.99
	9000	3.33 ^a	3.33 ^a	3.33 ^a	13.33 ^{ab}	23.32
<i>Steinernema</i> sp.	3000	0.00	0.00	0.00 ^a	0.00 ^c	0.00
	6000	0.00	0.00	3.33 ^a	3.33 ^{bc}	6.66
	9000	0.00	0.00	3.33 ^a	10.00 ^{abc}	13.33
Control	-----	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^c	0.00

Each value presented the mean of three replicates.

Values followed by the same letter (s) in a column are not significantly different according to analysis of variance (ANOVA) test at level 0.05.

Effect of different entomopathogenic nematodes on the amber snail, *Succinea putris*:

Data in Table (2) showed the mortality percentages of the land snail, *S. putris* after different periods of exposure to three levels of entomopathogenic nematodes, *H. bacteriophora* (HP88), *H. bacteriophora* (B20), *S. carpocapsae* and *Steinernema* sp. Results indicated that the mortality percentages of *S. putris* increased gradually after two and three weeks of exposure to all entomopathogenic nematodes. After four weeks of exposure *H. bacteriophora* (HP88) exhibited mortality percentages of *S. putris* with values of 16.66, 16.66 and 20.00% followed by *H. bacteriophora* (B20) with values of 10.00, 13.33 and 13.33% then *S. carpocapsa* nematodes with values of 6.66, 10.00 and 10.00 % and *Steinernema* sp. with values of 6.66, 6.66 and 10.00% when exposed to 3000, 6000 and 9000 IJs/ cup, respectively. Mortality percentages ranged from 3.33 to 20.00 % for *H. bacteriophora* (HP88), 0.00 to 13.33% for *H. bacteriophora* (B20), 0.0 to 10.00% for *S. carpocapsae* and *Steinernema* sp. The total mortality % at 9000 IJs/ cup was 46.66, 36.66, 33.32 and 26.66% for *H. bacteriophora* (HP88), *H. bacteriophora* (B20), *S. carpocapsae* and *Steinernema* sp., respectively. As a whole, entomopathogenic nematodes, *H. bacteriophora* (HP88 and B20) performed the best in all levels (3000,

6000 and 9000 IJs/ cup). However, *S. carpocapsae* and *Steinernema* sp. exhibited the least mortality percentages against the land snail, *E. vermiculata* and *S. putris*. Also, *S. putris* was more sensitive to entomopathogenic nematodes than the *E. vermiculata*. The present data agreed with the findings of Genena and Mostafa (2013) who reported the pathogenicity of *Phasmarhabditis hermaphrodita*, *Diplogaster* spp., *H. bacteriophora* and *S. carpocapsae* against *M. cantiana* with mortality percentages amounted to 73.0, 30.0, 20.0 and 13.3% after three weeks of treatment, respectively. However, Wilson *et al.* (1994) reported that the entomopathogenic nematodes, *S. feliae* and *Heterorhabditis* sp., had no effect on slug *D. reticulatum* using Petri- dish and soil bioassays at 14 and 23°C, Also they concluded that *S. feliae* and *Heterorhabditis* sp. have no potential as bio control agents against slugs. Georgis and Gaugler (1991) showed that *Heterorhabditis* spp. usually performs better than *Steinernema* spp. which is a consequence of their predatory lifestyles. Jaworska, (1993) reported that the two slug species *Deroceras reticulatum* and *D. agresta* were infected and killed by polish isolates of *S. carpocapsae*, *S. feliae* and *H. bacteriophora*, and these nematodes were able to reproduce within slug cadavers.

Table 2. Mortality percentages of *Succinea putris* after exposure to three levels of entomopathogenic nematodes

Treatments		Mortality percentages after indicated weeks				Total
Nematodes	Nematode levels	One Week	Two Weeks	Three Weeks	Four Weeks	Mortality %
<i>H. bacteriophora</i> (HP88)	3000	3.33 ^{bc}	6.66 ^a	10.00 ^{ab}	16.66 ^a	36.65
	6000	6.66 ^{ab}	6.66 ^a	13.33 ^a	16.66 ^a	43.31
	9000	6.66 ^{ab}	10.00 ^a	10.00 ^{ab}	20.00 ^a	46.66
<i>H. bacteriophora</i> (B20)	3000	0.00 ^c	3.33 ^a	6.66 ^{ab}	10.00 ^{ab}	19.99
	6000	3.33 ^{bc}	6.66 ^a	6.66 ^{ab}	13.33 ^{ab}	29.98
	9000	3.33 ^{bc}	10.00 ^a	10.00 ^{ab}	13.33 ^{ab}	36.66
<i>S. carpocapsae</i>	3000	0.00 ^c	6.66 ^a	6.66 ^{ab}	6.66 ^{ab}	19.98
	6000	0.00 ^c	3.33 ^a	6.66 ^{ab}	10.00 ^{ab}	19.99
	9000	10.00 ^a	6.66 ^a	6.66 ^{ab}	10.00 ^{ab}	33.32
<i>Steinernema</i> sp.	3000	0.00 ^c	0.00 ^a	3.33 ^{ab}	6.66 ^{ab}	9.99
	6000	0.00 ^c	3.33 ^a	6.66 ^{ab}	6.66 ^{ab}	16.65
	9000	0.00 ^c	10.00 ^a	6.66 ^{ab}	10.00 ^{ab}	26.66
Control	-----	0.00 ^c	0.00 ^a	0.00 ^b	0.00 ^b	0.00

Each value presented the mean of three replicates.

Values followed by the same letter (s) in a column are not significantly different according to analysis of variance (ANOVA) test at level 0.05.

Effect of two bio-agents (Bioarc and Biozeid) and the chemical pesticide, Agrinate on the Brown garden snail, *Eobania vermiculata*:

The ability of two bio agents *B. megaterium* (Bioarc) & *T. album* (Biozeid) and Agrinate (24% chemical pesticide) were tested for controlling *E. vermiculata* is shown in Table (3). Data revealed the toxic effect of all tested compounds with mortality percentage increasing with an increase in the period of exposure. However, Agrinate (46.66%) showed the greatest percentage in mortality one week post treatment. After four weeks of treatment, Bioarc (30.00%) exhibited the highest percentage of toxicity in the land snail *E. vermiculata* followed by Biozeid and Agrinate with values of 26.66%. The total mortality % was 56.66, 39.98 and 86.65 % for Bioarc & Biozeid and

Agrinate, respectively. Also, Bioarc exhibited higher mortality effect than Biozeid.

Table 3. Mortality percentages of *Eobania vermiculata* after exposure to two bio agents and the chemical pesticide Agrinate

Treatments	Mortality percentages after indicated weeks				Total
	One Week	Two Weeks	Three Weeks	Four Weeks	Mortality %
Bioarc	0.00 ^b	6.66 ^a	20.00 ^a	30.00 ^a	56.66
Biozeid	0.00 ^b	6.66 ^a	6.66 ^a	26.66 ^a	39.98
Agrinate	46.66 ^a	0.00 ^a	13.33 ^a	26.66 ^a	86.65
Control	0.00 ^b	0.00 ^a	0.00 ^a	0.00 ^b	0.00

Each value presented the mean of three replicates.

Values followed by the same letter (s) in a column are not significantly different according to analysis of variance (ANOVA) test at level 0.05.

Effect of two bio agents (Bioarc and Biozeid) and the chemical pesticide, Agrinate on the amber snail, *Succinea putris*:

The ability of *B. megaterium* (Bioarc), *T. album* (Biozeid) and Agrinate for the control of *S. putris* after certain periods of exposure is shown in Table (4). Results indicated that Agrinate (60.00%) was the effective one in *S. putris* mortality after one week of exposure followed by Bioarc (6.66%). After that, the mortality percentages of *S. putris* gradually increased to reach 30.00 and 36.66% for Bioarc and Biozeid respectively, after four weeks of treatment. Also, Data showed the total mortality % was 69.98, 56.66 and 96.65% for Bioarc & Biozeid and Agrinate, respectively. Bioarc exhibited higher mortality effect than Biozeid. These results agreed with Shetaia et al., (2013) who assured that Agrinate was more effective than any insecticides or biocides tested against *M. cartusiana* snail under field conditions. Affokpon et al., (2011) and Oliveira et al., (2009) found that the soil fungi *Trichoderma* spp. is potential nematode bio-control agent on many food, vegetables and crops. Besides, *Trichoderma* spp. and *B. megaterium* is common soil beneficial bio-fertilizer belonging to plant growth promoting rhizobacteria and have also been used for controlling root-knot nematode. Arafa (2006) found that biocides contain *B. thuringiensis* failed to exhibit any molluscicidal activity while Diple 2x, Bioclean and Diple- NT gave mortality 97, 97, and 32%, respectively. Heiba et al. (2002) reported that the mortality rates increased by the time and greatest mortality occurred over five days posted insecticide administration. The maximum mortality rate was observed with *E. vermiculata* (95%) and *M. cantiana* (75%) snails after 108 hours. Ghamry (1997) assured that *Helicella* sp. was the most susceptible to *B. thuringiensis* followed by *Monacha* sp. then *Eobania* sp. Genena and Mostafa (2008) reported the molluscicidal activities of eight bacterial isolates of *B. thuringiensis* against *M. cantiana* and *E. vermiculata*.

Table 4. Mortality percentages of *Succinea putris* after exposure to two bio agents and the chemical pesticide Agrinate

Treatments	Mortality percentages after indicated weeks				Total Mortality %
	One Week	Two Weeks	Three Weeks	Four Weeks	
Bioarc	6.66 ^b	6.66 ^a	26.66 ^a	30.00 ^a	69.98
Biozeid	0.00 ^b	10.00 ^a	10.00 ^{ab}	36.66 ^a	56.66
Agrinate	60.00 ^a	13.33 ^a	16.66 ^{ab}	6.66 ^b	96.65
Control	0.00 ^b	0.00 ^a	0.00 ^b	0.00 ^b	0.00

Each value presented the mean of three replicates.

Values followed by the same letter (s) in a column are not significantly different according to analysis of variance (ANOVA) test at level 0.05.

According to the obtained results, concluded that Agrinate showed superior toxicity effect than biological agents (entomopathogenic nematodes, Bioarc and Biozeid). Land snails *E. vermiculata* and *S. putris* treated with Bioarc, Biozeid exhibited the highest percentage mortality, while entomopathogenic nematodes gave the lowest one. However, *S. putris*

showed more sensitivity to all tested treatments than *E. vermiculata*.

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فعالية بعض الوسائل الحيوية فى مكافحة نوعين من القواقع الارضية *Eobania vermiculata* (MÜLLER) و *Succinea putris* (LINNAEUS) تحت الظروف المعملية
منى عبد الحميد على ، هبه عبد الجليل الغنام و دعاء عبد المقصود ابو العطا
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استهدفت الدراسة معرفة مدى قدرة بعض الوسائل الحيوية مثل الـنيماتودا الممرضة للحشرات *Heterorhabditis bacteriophora* (HP88) و *bacteriophora* (B20) و *Steinernema carpocapsae* و *Steinernema* sp. وكذلك المركبين الحيويين Bioarc و Biozeid فى مكافحة نوعين من القواقع الارضية الضارة وهما: قوقع الحدائق البنى *E. vermiculata* و القوقع الذهبى *S. putris*. مقارنة بمبيد الاجرنيت 24% SL الموصى به فى مصر كمبيد ضد القواقع الارضية لمدة 28 يوم تحت الظروف المعملية. اظهرت النتائج ان مبيد الاجرنيت كان اكثر المعاملات فعالية وسمية ضد نوعي القواقع المستخدمة حيث بلغت قيمة نسبة الموت الكلية (86,65%) و (96,65%) فى قوقع *E. vermiculata* و *S. putris* على التوالي. جاء كل من المركبين الحيويين Bioarc و Biozeid فى المرتبة الثانية وحققا نسبة موت 56,66 و 39,98% للقوقع *E. vermiculata* و نسبة موت 69,98% و 56,66% للقوقع *S. putris* على التوالي. تفاوتت نسب الموت الكلية فى قوقع *E. vermiculata* و قوقع *S. putris* عقب المعاملة بالانواع المختلفة من نيماتودا الحشرات حيث اعطت نيماتودا الحشرات *H. bacteriophora* (HP88 , B20) افضل النتائج بعد 28 يوم من المعاملة عند تركيز (9000 طور معدى / علبة) وحققت نسبة موت 29,98% و 23,32% فى حالة القوقع *E. vermiculata* و نسبة موت 46,66% و 36,66% فى حالة القوقع *S. putris* بينما اعطت نيماتودا *S. carpocapsae* و *Steinernema* sp. اقل نسبة موت لكلا النوعين من القواقع. ايضا لوحظ ان القوقع الذهبى *S. putris* كان اكثر حساسية واثرا لكل المواد المختبرة بالمقارنة بقوقع الحدائق البنى *E. vermiculata*. ومن هنا يتضح ان معاملة القواقع بالمركب الحيوى البكتيري Bioarc و المركب الحيوى الفطرى Biozeid احدث تأثيرا ساما وفعالا على كلا القوقعين اما المعاملة بالنيماتودا الممرضة للحشرات *Heterorhabditis bacteriophora* و *Steinernema carpocapsae* فكان التأثير بسيط وغير فعال للاستخدام كوسيلة للمكافحة فى حالة تزايد اعداد القواقع وانتشارها بصورة كثيفة.