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IMPACT OF EIGHT BACTERIAL ISOLATES OF *Bacillus thuringiensis* AGAINST THE TWO LAND SNAILS, *Monacha cantiana* AND *Eobania vermiculata* (GASTROPODA: Helicidae).

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ABSTRACT

Under laboratory conditions, eight native bacterial isolates of *Bacillus thuringiensis*, were tested for their molluscicidal activity against the two land snails, *Monacha cantiana* (Montagu) and *Eobania vermiculata* (Muller) at a constant concentration ($7x10^6$ cfu/ ml) using three methods of application. Results revealed that all tested isolates applied via food or soil had no adverse effect on the tested land snails and resulted no mortality in *M. cantiana* and *E. vermiculata* as well**.** However, results clearly indicate the molluscicidal properties of the tested isolates and identify Bt-k64 ,Bt- B33 as well as Bt- W123 as potentially effective against *M. cantiana* and *E. vermiculata* when bacterial isolates were introduced to snails via food and soil simultaneously (concomitant treatment). For eight bacterial isolates, the percentage mortality ranged from 33% to 86.6% for the clover land snail, *M. cantiana* , while in the brown land snail, *E. vermiculata* the percentage mortality ranged from 6.6% to 53%. after four weeks of treatment

B. thuringiensis designated as Bt- K64 was the most effective one against the two land snails, *M. cantiana* and *E. vermiculata* with percentage mortality 86.6% and 53.0% , respectively whereas, *B. thurinigensis* designated as Bt- B123 was the least toxic isolate with mortality 33% and 6.6% respectively. *M. cantiana* was more sensitive to the tested isolates than *Eobania vermiculatum.*

Moreover, the molluscicidal activity of the three isolates of the bacterium, *B. thurinigensis* designated as Bt-W123 , Bt-B33 and Bt-K64 at three concentrations ($1x10^6$, $1x10^7$ and $1x10^8$ cfu/ ml) against *M. cantina* under laboratory condition at 15ºC±3 and 60 ±5% RH. was undertaken. Isolate Bt –B33 and isolate Bt –W123 showed higher toxicity to the target snail than isolate Bt –K64 with mortality increasing with an increase in the concentrations of isolates as well as the period of exposure. At the highest concentration ($1x10^8$) the bacterial isolate Bt –B33 was the most toxic which killed 53% of the land snail *M. cantiana* after two weeks from treatment and 73.3% and 86.4% after three and four weeks of experiment

Keywords: land snails, *Monacha cantiana, Eobania vermiculata,* isolates,*Bacillus thuringiensis*, percentage mortality.

INTRODUCTION

Land snails (Mollusca: Gastropoda) are serious and widespread pests in nurseries, greenhouses and field crops in many parts of the world. In Egypt, the two land snails, *Monacha cantiana* (Montague) and *Eobania vermiculata* (Muller) became the most important agricultural pests causing substantial damage to different crops (El- Deeb *et al*., 1999, Mahrous,2002 and Khidr *et al*.,2005).

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Control of snails or slugs on different crops is heavily dependent on the use of pesticides that limit the effect of these pests below damaging level. The only therapeutic measure in field and horticulture crops is the use of molluscicide baits (Hammond *et al*., 1996). These baits offer acceptable control when applied appropriately. However, they rendered ineffective under wet and humid conditions that also favor slugs or snails activity (South, 1992). The excessive use of pesticides which leads to environmental health hazards in men, animals and disturbance of biological balance of microorganisms have enhanced scientists to reach for another alternative pest management.

Biological control of land snails or slugs using microbial agents, i.e. bacteria is an alternative method that received greater attention few years ago, providing effected control against land snails. *Bacillus thuringiensis* (Bt) is a gram- negative soil bacterium, which produces chemicals toxic to pests. Recently, it became one of the biological control agents against several insect pests, (Dean,1984). The toxicity of this bacterium against some land snails in Egypt has been studied by several researches (Zedan *et al.,*1999& Azzam & Belal, 2003 and Kramarz, *et al*., 2007). Zedan *et al*.(1999)studied the efficacy of *B. thuringiensis* var *israelensis* against the land snail, *M. obstructa*, and found that bacterial formulation was most effective compared with methomyl. However, Kramarz, *et al*. (2007) studied the effect of Bt toxin, Cry1Ab against the land snail, *Helix aspersa* under laboratory conditions and reported that Bt toxin Cry 1Ab had no negative effect on the land snail, *H. aspersa* during observed life stage**.**

Therefore, the present study was conducted to 1: evaluate the molluscicidal activity of eight native isolates of the bacterium, *B*. *thuringiensis* against the two land snails, *M. cantiana* and *E. vermiculata*. 2: the impact of three Bt isolates with different concentrations against the land snail, *M. cantiana*.

MATERIALS AND METHODS

Experiment 1:

In order to determine the molluscicidal activity of eight isolates of *B. thurinigensis* against the two land snails, *M. cantiana* and *E. vermiculata*, three methods of application were under taken in-vivo with constant concentration ($7x10^6$ cfu / ml.), at 25±1 ºC and 60 ± 5% RH.

1-Tested snails:

Adult snails of *M. cantiana* and *E. vermiculata* were collected from infested nurseries and field crops in Mansoura University, Mansoura city, Dakahlia Governorate. The obtained snails were transferred in plastic bags to the laboratory, then transferred to plastic containers containing moist sterilized sandy loam soil 1:1 (v:v) and fed on fresh leaves lettuce(*Lactuca sativa* L.) for 14 days to be laboratory acclimatized.

2- Tested bacterial isolates:

Eight native isolate of *B. thuringiensis* obtained from Agricultural Research Center- Giza were tested for their potentials to control the two land snails *M. cantiana* and *E. vermiculata.* The code and origin of each isolate are shown in table(1)

Table (1): List of native *B. Thuringiensis* isolates used in the present experiments:

Bt isolates (Code)	Origin
Bt –W123	**El- Wady EL- Gadeed, Assiut, Egypt**
Bt –W24	**El- Wady EL- Gadeed, Assiut, Egypt**
Bt –B33	**Badr City, Cairo, Egypt**
Bt –B123	**Badr City, Cairo, Egypt**
Bt –T83	**El- Tahrer, Behera, Egypt**
Bt –K64	**EL- Khatatbah, Behera,Egypt**
Bt –M73	**Menia, Egypt**
Bt –M103	**Menia, Egypt**

3- Bacterial inoculum preparation:

Each isolate was cultured on the nutrient agar medium for two days. An agar plug (5 mm diameter) of each isolate was then taken from the margin of the bacterial colonies and transferred to autoclaved flask (250 ml) filled with 100 ml of nutrient broth medium. The inoculated flasks were incubated at 25 ºC for 24 hrs. Bacterial suspensions (100ml) was prepared for each isolate and adjusted to $7x10^6$ cfu / ml.

4- Application methods:

a- Leaf-dip treatment:

Similar leaf discs **(**3 cm- diameter**)** of fresh leaves of lettuce were immersed for 5 seconds in the tested bacterial inoculum at a concentration $7x10^6$ cfu / ml for each isolate, then left to air dry before application. Ten adult snail individuals with approximately similar size were transferred from stock culture to plastic cup 12 cm diameter(250 gm capacity) filled with 80 gm of moist sterilized sandy loam soil 1:1 (v:v). one treated lettuce leaf disc was then introduced to the ten tested individuals/ cup.

Each cup was then covered with muslin cloth held by rubber bands. Cups receiving untreated leaves were sewed as control. After 48 hrs of exposure period, the treated leaved were replaced daily with fresh untreated lettuce leaves for 28 days. The tested snails were examined daily, where the dead individuals were counted and removed.

b- Soil incorporation treatment :

Eighty grams of sterilized sandy loam soil 1:1 (v:v) were incorporated with 5 ml of liquid bacterial broth at a concentration of $7x10^6$ cfu / ml. for each isolate, or with distilled water and placed in the bottom of plastic cup,12 cm diameter (250g capacity). Ten adult snail individuals were transferred to each cup & fed with a lettuce leaf disc (3 cm diameter). Each cup was then covered with muslin cloth held by rubber bands. Each isolate was replicated three times. Lettuce leaves were replaced daily with fresh ones for 28 days. The tested snails were examined daily, where the dead individuals were counted and removed.

c- Concomitant treatment (leaf dip +soil incorporation):

The same protocol as outlined for soil incorporation treatment was used except adult snail individuals were fed with lettuce leaf disc(3cm diameter) previously immersed for 5 seconds in the tested bacterial inoculum at a concentration of $7x10^6$ cfu / ml for each isolate and air dried before application. After 48 hrs of exposure period , the treated leaves were

replaced daily with fresh untreated ones for 28 days. The tested snails were examined daily, where the dead individuals were counted and removed.

Experiment 2:

Herein, three isolates of the bacterium , *B. thuringiensis* designated as Bt –K64, Bt –B33 and Bt –W123 at three different concentration $1x10^6$, $1x10^7$ and $1x10^8$ cfu/ ml were tested and compared for their potentials to control the land *M. cantiana* under laboratory conditions at 15±3 ºC and 60 ± 5% RH.

Bacterial isolates were introduced to snail via snail and food simultaneously(concomitant treatment) **.** Each treatment was replicated three times. Each replicate included ten individuals of the land snail, *M. cantiana*. Cups free of bacterial isolates were served as control. As in previous experiment after 48 hrs of exposure period , the treated leaves were replaced daily with fresh untreated ones for 28 days. The tested snails were examined daily, where the dead individuals were counted and removed. Snails were considered dead after prodding with a needle. For the two experiments mortality percentages were calculated after one, two, three and four weeks of treatment.

Data analysis:

For the two experiments data were statistically subjected to analysis of variance (ANOVA) (Gomez and Gomev,1984) followed by Duncan's multiple-range test to compare means (Duncan, 1955).

RESULTS AND DISCUSSION

Experiment 1:-

1- Molluscicidal activity of eight bacterial isolates of *B. thuringiensis*, applied via food or soil, against the two land snails, *M . cantiana* and *E. vermiculata*, under laboratory conditions:

Results revealed that after one, two , three and four weeks of treatment, all tested isolates applied via food or soil had no adverse effect on the tested land snails and resulted no mortality in *M. cantiana* and *E. vermiculata* as well.

2- Molluscicidal activity of eight bacterial isolates of *B. thuringiensis*, applied via food and soil simultaneously, against the clover snail, *M. cantiana* under laboratory conditions:

The percentage mortality of the land snail, *M . cantiana* after one , two, three and four weeks from exposure to eight isolates of *B. thuringiensis* applied via food in concomitantly with soil are represented in table (2). Results indicated that all tested bacterial isolates of *B. thuringiensis* were found to be toxic to *M . cantiana* but in different proportions. A positive correlation has noticed between percentage mortality of tested snails and exposure time to tested isolates. Meanwhile, the longer exposure to tested isolates was existed, the higher percentages mortality in *M . cantiana* was achieved. From table (2) it was evident that isolate Bt- K64 showed 50% mortality in the land snail, *M . cantiana* after two weeks of exposure followed

by isolates Bt- M103 and Bt- W123 with values 43% and 40%, respectively. Thereafter, the percentages mortality of *M . cantiana* significantly increased after three and four weeks of exposure to isolate Bt- K64 to reach 86.6% followed by isolate Bt-B33 then Bt- W123 with values 80% and 73%, respectively after four weeks of treatment.

However, bacterial isolate Bt- B123 exhibited the lowest percentage mortality with values 33.0% after three and four weeks of treatment (Table2).

Table (2): The percentage mortality of the clover snail, *M . cantiana* as influenced by the addition of eight bacterial isolates of *B. thuringiensis* under laboratory conditions.

Bacterial isolates	% Mortality after			
	One week	Two weeks	Three weeks	Four weeks
Bt –W123	13.0 ghIj	40.0 cde	73.0 ab	73.0 ab
Bt –W24	13.0 ghij	26 efg	33.0 def	43.0 cd
Bt –B33	10.0 hij	33 def	60.0 ab	80.o ab
Bt –B123	6.0 hij	20.0 fgh	33.0 def	33.0 def
Bt –T83	0.0 j	10.0 hij	46 .0 cd	46.0 cd
Bt -K64	13 ghij	50.0 c	86.6 a	86.6 a
Bt –M73	33 ij	16.6 ghi	33.0 def	40.0 cde
Bt –M103	13 ghij	43.0 cd	70.0 b	70.0 b
CK	0 .0 j	0.0 j	0 .0 j	0 .0 j

Each number presented the mean of three replicates
Means followed by the same letter(s) are not significantly different at 0.05 level, according to (Duncan,1955).

3- Molluscicidal activity of eight bacterial isolates of *B. thuringiensis*, applied via food and soil simultaneously against the brown snail, *E. vermiculata*, under laboratory conditions:

The impact of eight bacterial isolates of *B. thuringiensis* for the control of the land snail, *E. vermiculata* is shown in table (3).

Table (3): The percentage mortality of the brown snail, *E. vermiculata* as influenced by the addition of eight bacterial isolates of *B. thuringiensis* under laboratory conditions.

Bacterial isolates	% Mortality after			
	One week	Two weeks	Three weeks	Four weeks
Bt –W123	13.0 ef	26.0 cd	26.0 cd	26.0 cd
Bt –W24	20.0 de	26.0 cd	33.0 bc	50.0 b
Bt –B33	20.0 de	26.0 cd	33.0 bc	33.0 bc
Bt –B123	6.6 fg	6.6 fg	6.6 fg	6.6 fg
Bt –T83	13.0 ef	30.0 de	20.0 cd	20.0 cd
Bt -K64	6.6 fg	33.0 bc	40.0 b	53.0 a
Bt –M73	13. ef	13.0 ef	20.0 de	20.0 de
Bt –M103	6.6 fg	6.6 fg	13.0 ef	16.0 def
CK	0.0 j	0.0 j	0.0 j	0.0 j

Each number presented the mean of three replicates
Means followed by the same letter(s) are not significantly different at 0.05 level, according to (Duncan,1955).

Data revealed the toxic effect of all tested isolates with mortality percentage increasing with an increase in the period of exposure. It was evident that isolate Bt -K64 exhibited the highest percentage of toxicity in the

land snail *E. vermiculata* with value 53% followed by isolate Bt –W24 than isolate Bt –B33 with values 50 and 33%, respectively after four weeks from the beginning of experiment. However, bacterial isolate designated as Bt –B123 exhibited the lowest percentage mortality with value 6.6% after one, two, three and four weeks from experiment.

Apparently, the land snail, *M cantiana* showed more sensitivity to eight bacterial isolates than the land snail *E. vermiculata* (Fig.1). The bacterial isolate Bt -K64 exhibited the highest percentage mortality against the two land snails , while isolate Bt –B123 gave the lowest one after four weeks of treatment.

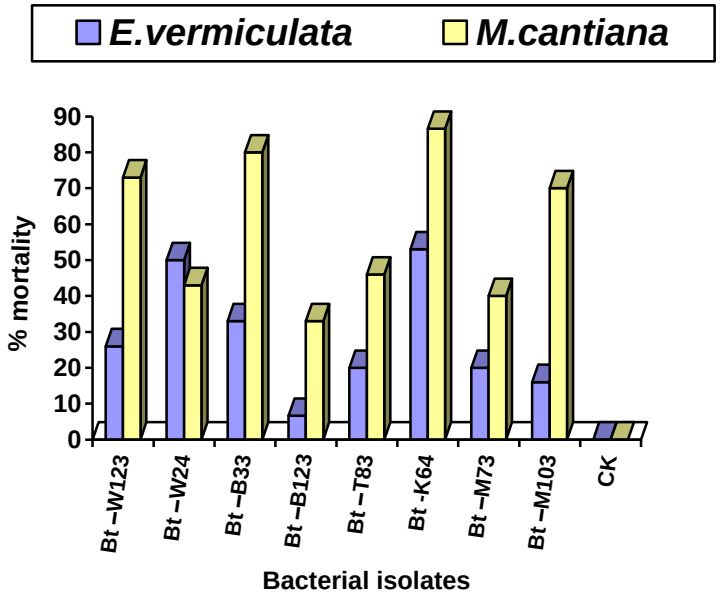

Fig. (1): The percentage mortality of the two land snails, *M. cantiana* and *E. vermiculata* as influenced by the addition of eight bacterial isolates of *B. thuringiensis* after four weeks of treatment.

Experiment 2:-

Molluscicidal activity of three bacterial isolates of *B. thuringiensis* against the clover snail, *M . cantiana* under laboratory conditions:

The mollusicidal activity of bacterial isolates designated as Bt –W123, Bt –B33 and Bt -K64 at three concentrations against *M. cantiana* is shown in table (4). Isolate Bt –B33 and isolate Bt –W123 showed higher toxicity to the target snail than isolate Bt –K64 with mortality increasing with an increase in the concentrations of isolates as well as the period of exposure.

At the highest concentration ($1x10^8$) the bacterial isolate Bt –B33 was the most toxic which killed 53% of the land snail *M. cantiana* after two weeks from exposure and then mortality significantly increased to reach

73.3% and 86.4% after three and four weeks of experiment. However, isolate Bt –W123 and Bt -K64 were of moderate percentage mortality which killed 76.3 and 73%, respectively at the same concentration ($1x10^8$) after four weeks of treatment.

Table (4): Molluscicidal activity of three bacterial isolates of *B. thuringiensis* against the clover snail, *M . cantiana* under laboratory conditions:

Weeks after treatment	Isolate Bt –K64			Isolate Bt –B33			Isolate Bt –W123			CK
	$1x10^6$	$1x\ 10^7$	$1x10^8$	$1x10^6$	$1x\ 10^7$	$1x10^8$	$1x10^6$	$1x\ 10^7$	$1x10^8$	
	%M	%M	%M	%M	%M	%M	%M	%M	%M	%M
One week	13.jkl	13 Jkl	33ghi	6.6kl	6.6kl	13.3jkl	6.6kl	6.6kl	26.6hij	0.0 l
Two weeks	26hij	26.6hij	46fg	6.6kl	26.6hij	53def	6.6kl	13.3jkl	33.2ghi	0.0 l
Three weeks	40fgh	60cde	66bcd	13jkl	53.2def	73.3abc	20jk	13.3jkl	46.6efg	0.0 l
Four weeks	66bcd	73abc	73abc	33ghi	37.3abc	86.4a	33.3ghi	46.6efg	76.3ab	0.0 l

Each number presented the mean of three replicates.
Means followed by the same letter(s) are not significantly different at 0.05 level, according to (Duncan,1955).

Regarding to methods of application, our study revealed that all tested isolates applied via food or soil had no adverse effect on the tested land snails and resulted no mortality in *M. cantiana* and *E. vermiculata* as well. This result agreed with Ester and Nijensterin (1995) and Kramarz *et al* (2007) in respect to *Derocceras reticulutum* for the former and *H. aspersa* for the latter. On the other hand, results clearly indicate the molluscicidal properties of the tested native isolates and identify Bt –W123, Bt –B33 as well as Bt – K64 as potentially effective against the land snails, when bacterial isolates were introduced to snails via food and soil simultaneously (concomitant treatment).These results, regardless to method of application and isolate type, support the findings reported by Zedan *et al.* (1999) who found that *B. thuringiensis* var. *israeliensis* showed mortality toxicity against the land snail, and the fresh water snails in Egypt . Further, the present results are in accordance with those reported by Gao *et al*. (2008), who demonstrated that of 570 Bt isolates , six isolate exhibited activity mortality (1.1%) against the snail *Oncomelania hupensis* which is a vector of schistosomiasis with very harmful to humans in China. Results also indicate that the land snail was more sensitive than to eight tested bacterial isolates. The toxic effect of Bt isolates designated as Bt –W123 and Bt –B33 was more than Bt – K64 with mortality increasing with an increase in the concentration of isolates as well as the period of exposure.

Finally it can be concluded that application method had an appreciable effect on the efficacy of bacterial isolates in reducing the snail survival. The concomitant application via food and soil, was the unique method that affect snail survival. An effective method that combined between the exposure of tested land snails via food to bacterial isolates that form parasporal crystals composed of proteins known as the insecticidal crystal (cry) proteins and hemolytic toxins (cyt) proteins during sporulation (Gao *et al.*, 2008) and the presence of such snails in a soil harboring the bacterium *Bt* for 28 days which could accidentally penetrate through snails openings and

cause osmotic lysis of epithelial intestine cells (Baines, 1997). More studies on the influence of the present native Bt isolates and others found in soil on economic snails and slugs in- vivo as well as PCR multiplication are needed to define type of crystal genes in the native effective isolates that could be used for developing Bt molluscicide with high efficiency against the target snails or slugs.

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تقييم فاعلية ثمانى عزلات محلية من البكتيريا *Bacillus thuringiensis* فى مكافحة نوعين من القواقع الارضية، *Monacha cantiana* و *Eobania vermiculata*.

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أجريت تجربتين معمليتين لتقييم فاعلية بعض العزلات البكتيرية على أكثر القواقع انتشارا فى محافظة الدقهلية.

التجربة الأولى:

استهدفت الدراسة تأثير ٨ عزلات من البكتيريا *Bacillus thurinigensis* ضد القوقعين *Monacha cantiana* و *Eobania vermiculata* وذلك تحت الظروف المعملية الثابتة من حرارة ورطوبة (RH 5% ± 60 , ºC 1±25)؛ وذلك بثلاث طرق وهى طريقة غمر أوراق الخس بالعزلة البكتيرية والطريقة الثانية هى خلط التربة بالعزلة البكتيرية المختبرة والطريقة الثالثة باستخدام الطريقتين معا فى نفس الوقت وذلك بتركيز ثابت ($7x10^6$ cfu/ ml) من كل عزلة بكتيرية

وأظهرت النتائج ما يلي:-

١- لم يكن للعزلات البكتيرية المضافة عن طريق غذاء القواقع أو الخلط بالتربة أثرا ايجابيا على القواقع المختبرة ولم ينجم عنها اى نسب موت .

٢- كانت العزلات البكتيرية المضافة عن طريق الغذاء والخلط بالتربة معا أثرا ايجابيا على القواقع المختبرة و أعطت نسب موت وصلت إلى ٨٦,٦% فى القوقع *M .cantiana* و ٥٢% للقوقع *E vermiculata*. وذلك بعد أربعة أسابيع من المعاملة باستخدام العزلة البكتيرية Bt-K64.

٣- العزلة البكتيرية المحلية Bt –B123 كانت اقل العزلات سمية ،حيث أعطت اقل نسبة ٣٣% فى القوقع *M .cantiana* و٦و٦% فى القوقع *E .vermiculat* وذلك بعد أربعة أسابيع من المعاملة.

٤- يعتبر القوقع *M. cantiana* أكثر حساسية للعزلات البكتيرية المختبرة بالمقارنة بالقوقع *E. vermiculata*

التجربة الثانية:

أجريت لتقيم فاعلية أفضل ثلاث عزلات بكتيرية تم التوصل إليها من التجربة الأولى وهذه العزلات هي Bt -K64 و Bt-B33 و Bt-W 123 باستخدام ثلاث تركيزات من كل عزلة ، و هى (, $1x10^6$ $1x10^7$ and $1x10^8$ cfu/ ml) على القوقع *M.cantiana* وذلك باستخدام (طريقة غمر الأوراق وخلط التربة معا) وذلك تحت الظروف المعملية ولمدة أربعة أسابيع من المعاملة.

وأظهرت النتائج انه بزيادة التركيز تزيد نسبة الموت , كما أن العزلة البكتيرية Bt-B33 أظهرت أعلى نسبة موت بالمقارنة بالعزلات الأخرى وذلك عند استخدام التركيز المرتفع$1x10^8$ ، حيث أعطت ٨٦,٤% يليها العزلة البكتيرية , Bt-W 123 والتى أعطت نسبة موت وصلت إلى ٧٦,٣% ثم العزلة Bt K64- والتى أعطت نسبة موت وصلت إلى ٧٣% وذلك بعد أربعة أسابيع من المعاملة.