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THE COMBINATION EFFECT OF BACTERIAL EXOTOXIN AND SNAIL PARASITIC NEMATODE *Rhabditis sp*. ON THE PRODUCTION OF THE NEMATODE FROM *Biomphalaria alexandrina*

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ABSTRACT

The production of the snail parasitic nematode *Rhabditis* sp. from *Biomphalaria alexandrina* and *Lymnaea cailliaudi* snails which were exposed to the nematode mixed with different concentrations of the bacterial exotoxin Victoback$_{12}$ AS was studied

The maximum number of recovered nematodes was recorded for both *Biomphalaria alexandrina* and *Lymnaea cailliaudi* at the concentration of 10 I.S of nematode/snail in 2% of Victoback$_{12}$ AS solution . The longest period of releasing nematodes was also recorded at the same concentration for both snail species. Thus this concentration is considered the most suitable for field applications .

Keywords: Snails, *Biomphalaria alexandrina, Lymnaea cailliaudi ,* Nematodes, *Rhabditis,* bacterial exotoxin,.

INTRODUCTION

The aquatic snail, *Biomphalaria alexandrina* (Ehrenberg) and *Lymnaea cailliaudi* Bourguigant, have great importance in the agriculture, veterinary and medical fields. (Azzam 1987and1995) Lutfallah1974& Hassan and Kalliny 1967).

The aquatic nematode *Rhabditis onchomelaniae* Jokko and Okabe was successfully used against the intermediate hosts of the trematode *Schistosoma japonicum* Katsurada in Japan. The snail *Onchomelania nosophora* (Robson) was highly infected (80- 100%) in the laboratory and similar results were reported in the field (Okabe and Shiraishi, 1971).

The slug parasitic nematode *Phasmarhabditis hermaphrodita* (Schneider) appears to be a successful biological control agent against slugs (wilson *et al.*, 1994, Glen and wilson 1997) .

Azzam and Belal (1999,a and b) investigated the effect of combinations of the snail parasitic nematode *Rhabditis* sp. and the bacterial exotoxin Victoback$_{12}$ AS on both *Biomphalaria alexandrina* and *Lymnaea cailliaudi* in the laboratory and found that 50 I.S./snail of the nematode in 5% of Victobak$_{12}$ AS caused 100% mortality of *B. alexandrina* within 15hr. while 10 I.S. of the same nematodes in 2 % Victoback$_{12}$ AS caused 100% mortality of *B. alexandrina* within 48 hr., but in only 24 hr. in *L.cailliaudi* snails. Azzam (1999) studied the production of the *Rhabditis* sp from different pests including *B. alexandrina* snail.

Further studies on these nematode and combinations are needed . Therefor, the present investigation deals with the production of the nematode *Rhabditis* sp when it was combined with a bacterial exotoxin to determine the most suitable concentration of the exotoxin in combinations with the snail parasitic nematode for field applications.

MATERIALS AND METHODS

The parasitic nematodes were progeny of the original colony which was isolated for the first time in Egypt from *Eobania vermiculata* snails by Azzam in September 1996 , using the technique described by Azzam (1998 , 99) .

Rearing of the aquatic snail *Biomphalaria alexandrina* Ehrenberg was carried out by the technique previously described by Azzam and Tawfik (1997) .

Rearing of *Lymnaea cailliaudi* Bourguigant was carried out by the method previously mentioned by Awadallah *et al.,* 1991

The molluscicidal activity of the combination was tested by the same techniqne used by Azzam and Belal (1999 , a and b) . Counting the nematodes emerging from infected specimen was carried out by the technique previously described by Azzam (1999).

RESULTS AND DISCUSSION

Tables (1,2) showed that the interval period from infection to total mortality decreased with the increase of the concentration of either of Victoback$_{12}$ AS or *Rhabditis* sp . nematode .

Lymnaea cailliaudi died faster than *Biomphalaria alexandrina* at equal concentrions , probably due to the larger aperature of *Lymnaea* snails than that of *Biomphalaria* which exposed a larger part of the snail to the nematode and exotoxin suspension consequently the effect of the suspension was more rapid.

The shortest period from death to releasing nematode was reported at the lowest concentration for both *B.alexandrina* and *L.cailliaudi* snails. (10 I.S./snail, in 0.25% Victoback and 10 I.S./snail in 0.1% Victoback, respectively). The longest period of releasing nematodes was recorded at the concentration of 10 I.S. of nematodes/snail in 2% Victoback solution in both *B.alexandrina* and *L. cailliaudi* , reaching more than five months (163.25 , 163.5 days), respectively . Such delay is considered an advantage for using this combination against these harmful snails which act as intermediate host of the parasitic trematodes subsequently , *Schistosoma mansoni* Sambon and *Fasciola hepatica* Linnaeus in Egypt, in addition to infestation and damage caused by these snails to the rice plants.

Statistically, very highly significant differences (P>0.001) relative to the periods form infection to host death appeared between the concentrations of 2,4 and 5% Victoback$_{12}$ AS and each of 1.5,1,0.5 and 0.25%. Significant differences (P>0.05) were found between concentrations of (i)1 and 1.5% (ii)

5 and 2%. Insignificant differences between the concentration of (i) 4% and each of 2 and 5% (ii) 0.5 and 0.25 % in the case of *Biomphalaria* snails . While very highly significant differences (P>0.001) were found between all data in *Lymnaea* snails.

Concerning the period from death to emerging nematodes, very highly significant differences (P>0.001) appeared between the concentrations of (i) 1.5% and each of 5,4, 0.5 and 0.25% (ii) 0.25% and each of 1,2 and 4% (iii) both 5 and 0.5% and each of 2 and 1 % concentration. Non significant differences between other data in the case of *Biomphalaria alexandrina* . In the case of *Lymnaea cailliaudi,* very highly significant differences (P>0.001) existed between the concentration of (i) 2% and each of 0.5,0.25 and 0.1% (ii) 1.5% and each of 1,0.5,0.25 and 0.1% , (iii) 1 and 0.1% , and insignificant differences between other data.

Table (1): Impact of the combination of the snail parasitic nematode *Rhabditis* sp . and bacterial exotoxin Victoback$_{12}$ AS on *Biomphalaria alexandrina* and *Lymnaea cailliaudi* at 28 ±2°C

Snails	Size in mm.	Concentr-ation	Mortality %	Maximum perio for mortality in hr.	Production Index(PI.)	
					Value	Rank
Biomphalaria alexandrina	13.56±1.44 d(11.4-16)	50 I.S./snail in 5% vict.	100	16	7.58	7
	13.56±1.44 d(11.4-16)	I.S./ snail in 4% vict.	100	24	9.75	6
	13.56±1.44 d(11.4-16)	I.S/s nail in 2% vict.	100	36	263.65	1
	13.56±1.44 d(11.4-16)	I.S/s nail in 1.5 % vict.	100	60	42.96	4
	13.56±1.44 d(11.4-16)	I.S/s nail in 1 % vict.	100	72	35.28	5
	13.56±1.44 d(11.4-16)	I.S/s nail in 0.5 % vict.	100	96	90.87	3
	13.56±1.44 d(11.4-16)	I.S/s nail in 0.25 % vict.	100	96	91.16	2
Lymnaea cailliaudi	14.94+1.24 h(12.9-17.9)	I.S/s nail in 2% vict.	100	24	152.46	1
	14.94+1.24 h(12.9-17.9)	10 I.S/snail in 1.5% vict.	100	48	88.35	5
	14.94+1.24 h(12.9-17.9)	I.S/ snail in 1% vict.	100	48	66.96	6
	14.94+1.24 h(12.9-17.9)	I.S/ snail in 0.5% vict.	100	72	111.85	2
	14.94+1.24 h(12.9-17.9)	I.S/ snail in 0.25% vict.	100	84	102.6	3

	14.94+1.24 h(12.9-17.9)	I.S/s nail in 0.1% vict.	100	96	100.36	4

d = diameter h = heightPI= N/CXT N = number of recovered nematode C = concentration of nematodes T = time of development in days necessary for the nematode in infected individuals post infection date

Table (2): Mean± SD duration in hours from infection to death of host, and in days ,from host death to emergence of nematode and period of recovery , total numbers of recovered nematodes from *B. alaxardrina* and *L.cailliaudi* at 28±2°C

Snail	Concentration	Duration			No. of recovered nematodes/ snail
		Infection to host death in hrs.	Death to nematode emergence or recovery in days	Period of recovery in days	
Biomphalaria alexandria	50 I.S/snail in 5% Victobac	15±0.72 (14-16)	3.25±0.84 (2-4)	150.5 ± 5.29 (145-159)	1468±33.44 (1422-1514)
	30. I.S/snail in 4% Victobac	20.25±3.95 (15-24)	3.5±0.51 (3-4)	148.5±5.19 (144-154)	1271±112.52 (1112-1425)
	10. I.S/snail in 2% Victobac	27±5.26 (24-36)	3.75±0.44 (3-4)	163.25±2.52 (161-167)	128 53±74.18 (12764-12942)
	10 I.S/snail in 1.5% Victobac	57±5.26 (48-60)	4±0.72 (3-5)	158±2.77 (155-162)	2738.5±318.37 (2294-3183)
	10 I.S/ snail in 1% Victobac	69±5.26 (60-72)	3.75±0.44 (3-4)	161.75±2.72 (159-166)	2337±190.51 (2030-2500)
	10 I.S/snail in 0.5% Victobac	84±8.59 (72-96)	3.25±0.84 (2-4)	157.5±2.32 (155-161)	6133.75 ±332.38 (5900-6700)
	10 I.S/ snail in 0.25% Victobac	93±5.26 (84-96)	3±0.72 (2-4)	156.5±2.09 (154-159)	6267.5±54.71 (6200-6330)
Lymnaea cailliuodi	10 I.S/snail in 2% Victobac	21± 3.46 (18-24)	3.5±0.58 (3-4)	163.5±2.65 (161-167)	6670 ± 54.07 (6600-6750)
	10 I.S/snail in 1.5 %Victobac	30±10.53 (24-48)	3.75±0.44 (3-4)	157-75±3.31 (153-162)	4417.5±711.85 (3612-5223)
	10 I.S/snail in 1 % Victobac	39±10.08 (24-48)	3.25±0.84 (2-4)	161.75±2.9 (158-166)	3264.25±764.5 (2470-4037)
	10 I.S/snail in 0.5% Victobac	66± 6.08 (60-72)	3±0.72 (2-4)	157.25±1.95 (155-160)	6431.25±257.81 (6000-6650)
	10 I.S/snail in 0.25%Victobac	78±6.08 (72-84)	3±0.72 (2-4)	157±1.60 (155-159)	6412.5±74.59 (6300-6500)
	10 I.S/snail in 0.1% Victobac	93±5.26 (84-96)	2.75±0.84 (2-4)	156±1.6 (154-158)	6649±857.19 (5452-7876)

Concerning the period of recovery, insignificant differences between (i) 0.25% and each of 0.5 and 1.5% , (ii)0.5 and 1.5% , (iii) 1 and 2%. Highly significant differences (P>0.01) were existed between the concentration of 5 and 4% and very highly significant differences (P>0.001) between other data in the case of the snail *B.alexandrina*. While in the case of *L.cailliaudi,* significant differences (P>0.05 –0.01) between the concentration of 0.1 and each of 0.5 , 0.25. Non significant differences were found between 0.5 and

each of 0.25 and 1.5 and very highly significant differences between other data .

The highest number of recovered nematodes was reported for the concentration of 10 I.S. in 2% Victoback$_{12}$ AS in both *B. alexndrina* and *L.cailliaudi* (12853 ± 74.18 and 6670± 54.07) respectively. This means that is the optimal concentration for the combination between the bacterial exotoxin and parasitic nematode , which produced the highest number of nematodes in the longest period of nematode releasing.

Statistically , there is a , highly significant difference (P>0.01) between the concentration of 4 and 5 % . Insignificant differences were found between the concentration of 0.5 and 0.25% and very highly significant differences (P>0.001) between other data in the case of *B. alexandrina* snails. In the case of *L.cailliaudi*, insignificant differences were existed between the concentration of (i) 2% and each of 0.5,0.25 and 0.1% (ii) 0.5 and each of 0.25 and 0.1% . Significant differences (P>0.05) between 0.1 and 0.25% .Very highly significant differences (P>0.001) were found between other data.

Azzam (1999) investigated the production of the snail parasitic nematode *Rhabditis* sp. alone from different pests including *B. alexandrina* snails and found that individuals of this snail produced 1846 nematodes over one month when infected with 20 I.S. of the nematode. Comparing these results with the results of the present investigation the combination between nematodes and bacterial exotoxin increases the number of recovered nematodes and also the releasing period. This may be attributed to suppression- by the exotoxin - of other organisms which may compete with the development of nematodes in the snail .Thus the nematodes found more adequate sustenance inside the cadaver of the snail for along time consequently their development continued , which inturn lengthened the period of nematode releasing .

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تأثير خلط الاكسوتوكسين البكتيرى مع النيماتودا المتطفلة على القواقع رهابديتس على انتاج النيماتودا من قوقع بيومفلاريا الكسندرينا

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تتناول هذه الدراسة إنتاج النيماتودا المتطفلة على القواقع رهابديتيس من قوقعي بيومفلاريا الكسندرينا العائل لبلهارسيا الإمعاء وليمنياكايودى العائل للدودة الكبدية عند تعريضهم لتركيزات مختلفة من الاكسوتوكسين البكتيري فيكتوباك مع النيماتودا.

أتضح من الدراسة أن أكبر عدد من النيماتودا نتج عند استخدام تركيز 10 أفراد معدية من النيماتودا / قوقع في محلول الاكسوتوكسين البكتيري فيكتوباك 2 % . كذلك كانت أطول فترة لانطلاق النيماتودا من القوقع بعد موته عند نفس التركيز .

من ذلك يتضح أن هذا التركيز هو الأفضل عند استخدام النيماتودا بالاشتراك مع الاكسوتوكسين البكتيري في التطبيق الحقلي لمكافحة هذين النوعين من القواقع الضارة .