

wirkungen auf insektenpathogene Pilze zu berücksichtigen. Dies gilt selbstverständlich auch für Arten, die andere Schädlinge befallen.

Die vorliegenden Resultate wurden in Laborversuchen erarbeitet und lassen sich deshalb nur mit Vorbehalten auf die Feldsituation übertragen. Trotzdem besteht der dringende Verdacht, daß insbesondere die Produkte auf der Basis von Dinitro-Verbindungen auch unter Praxisbedingungen insektenpathogene Pilze schädigen und dadurch indirekt deren Wirte fördern. Zu den Wirten gehören nicht nur Blattläuse, sondern zahlreiche tatsächliche und potentielle Schädlinge (KELLER, 1991). Herbizide, aber auch andere Pflanzenschutzmittel mit derart ausgeprägter Nebenwirkung auf Nutzorganismen sind unvereinbar mit neuzeitlichen Pflanzenschutzbestrebungen. Der gegenwärtig laufende Prozeß, die Gelbspritzmittel in der Schweiz durch modernere Pflanzenschutzmittel zu ersetzen bzw. vom Markt zurückzuziehen, ist deshalb sehr zu begrüßen.

### Zusammenfassung

In Laborversuchen wurde die Wirkung von 7 Herbiziden in der empfohlenen Konzentration und Verdünnungen davon auf

das Sporulierungsvermögen des blattlauspathogenen Pilzes *Erynia neopaphidis* untersucht. Gesaprim (Wirkstoff Atrazin) hatte keinen Einfluß auf das Konidienbildungs- und -abschleuderungsvermögen des Pilzes auf infizierten Blattläusen. Drei Produkte auf der Basis von Dinitro-Derivaten töteten den Pilz vollständig ab, Dinoseb selbst noch bei einer 33fachen Verdünnung der empfohlenen Anwendungskonzentration. Die übrigen 3 Produkte (Betanal, Kerb, Tribunil) mit verschiedenen Wirkstoffen beeinträchtigten das Sporulierungsvermögen unterschiedlich stark, töteten jedoch den Pilz nicht.

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## Bio-efficacy of bacterial insecticide, *Bacillus thuringiensis* Berl. as biological control agent against snails vectors of Schistosomiasis in Egypt

By G. Y. OSMAN and A. M. MOHAMED

With 3 tables

### Abstract

The molluscicidal activity of Thuricide used as bacterial insecticide, *Bacillus thuringiensis*, against *Biomphalaria alexandrina* and *Bulinus truncatus* was tested.

The obtained results indicate that Thuricide possess a molluscicidal activity against both snails species. *B. truncatus* was more sensitive to the action of Thuricide. However the slope function of the LC<sub>50</sub> for *B. alexandrina* was significantly higher than that for *B. truncatus*. The mortality rate of exposed *B. alexandrina* increased by increasing the exposure time.

Exposure of *B. alexandrina* to low concentration of Thuricide caused a significant decrease in both ovipository activity and size of egg masses and reduced the percentage of egg hatchability.

### 1 Introduction

Schistosomiasis is one of the most endemic disease in Egypt. It was estimated by the WHO (1980) that the prevalence of the disease is about 22% in 1974. Various methods have been practiced to control bilharziasis through the breakage of the life cycle of the parasite by means of mass chemotherapy, health education, environ-

mental sanitation and snail control. At the present time the first three modes of attack show a slow success. According to WHO reports (1980, 1981), it is indicated that complete eradication of snail intermediate hosts would effectively put an end to the transmission of bilharziasis in any given area.

As a result of continuous application of pesticides, the synthetic toxicants began to face incapability for controlling snails. Relatively few pathogens have been exploited as pest control agents. The bacterial pathogen insecticide, *Bacillus thuringiensis* is one of the most common microbial insecticide in use today (IGNOFFO et al., 1981; LARGET, and DE BARJAC, 1981; LACEY, and SINGER, 1982; KNOWDEN et al., 1983; TREMEYER, 1984; DAVIDSON, and YAMAMOTO, 1984; ARMSTRONG et al., 1985 and NISHWRA, 1988). In field experiment, DIAS and DAWOOD (1955) found that *Bacillus pinottii* CRUZ, and DIAS destroyed all the snails in the ditches to which it was introduced.

The findings of the previous authors outlined above stimulated us to evaluate the molluscicidal activity of

*Bacillus thuringiensis* Berl., Thuricide strain, as safer compound than other chemical molluscicides used, against fresh water-molluscs of schistosomiasis.

## 2 Material and Methods

The experimental snails used in the present studies were *Biomphalaria alexandrina* (EHRENBERG, 1831) and *Bulinus truncatus* (AUDOUIN, 1827). They were about 3 months old and their individual weight ranged between 50 to 70 mg for *B. alexandrina* and from 80 to 100 mg for *B. truncatus*.

The original snails stocks were collected from several irrigation ditches not previously treated with any molluscicides located at Menoufia Governorate, Egypt. About hundred snails were kept in glass aquaria (50 × 30 × 20 cm), containing about 15 l of dechlorinated tap water of temperature ranged from 23–26 °C. The snails were fed daily on fresh lettuce leaves. After 3 weeks of laboratory acclimatization the experimental snails were collected for the screening tests.

### 2.1 Tested material

The tested commercial bacterial pathogen insecticide *B. thuringiensis* was used as Thuricide (16,000 I.U./mg).

A test solutions were made up with dechlorinated tap water, pH 7.5 to 7.7. Single stock solution of the experimental material was made on basis of weight/volume. The appropriate dilution series, which would provide the computation of LC<sub>50</sub> values were prepared.

### 2.2 Screening tests

The screening tests were carried out at temperature of 24–26 °C, according to the method described and recommended by the Molluscicidal Screening Method of WHO (1965). The snails were immersed in 11 jars (10 snails / 1,000 ml) and 50 snails for each concentration level. The exposure period was 24 h. For each test, control snails maintained under the same experimental conditions were used. At the end of exposure period, the snails were removed from the experimental test solution, then washed thoroughly with dechlorinated tap water, placed in clean dechlorinate tap water and were observed for a period of 24 h. Observations were extended over a period of 12 days to determine the effect of prolonged exposure to low concentrations of the tested compound.

The number of egg-masses laid by snails exposed 15 days to sublethal concentration of Thuricide was recorded. The number of embryos, the length and width of egg-mass and percent of snail hatchability were also recorded. The results of the snail-screening tests were analysed statistically according to the method described by RITCHFIELD, and WILCOXON (1944).

## 3 Results

### 3.1 Molluscicidal activity of Thuricide

The LC<sub>50</sub>, slope function and LC<sub>98</sub> value are presented in table 1. Table 1 shows that the LC<sub>50</sub> values were 500 and 400 mg/l, whereas the calculated slopes of the lines were 2.13 and 1.4 for *B. alexandrina* and *B. truncatus*, respectively.

It is obvious that though *B. truncatus* snails were more sensitive to the compound, its slope function was low.

### 3.2 Effects of prolonged exposure concentrations

#### 3.2.1 Mortality rate

The experimental snails were exposed for a period of 12 days in different low concentrations of Thuricide, observations and daily mortality were recorded.

It is evident that the mortality rate of exposed snails

Table 1. Molluscicidal activity of Thuricide to the snails *B. alexandrina* and *B. truncatus*. (Exposure period of 24 hours):

Snails	LC <sub>50</sub> mg/l	Slope function LC <sub>98</sub> (S)	
<i>B. alexandrina</i>	500	2.13	0.0
<i>B. truncatus</i>	400	1.4	0.0

Table 2. The effect of prolonged exposure to Thuricide on mature *Biomphalaria alexandrina* (percent accumulative daily mortality)\*:

Conc. mg/liter	Percent accumulative mortality					
	2 days	4 days	6 days	8 days	10 days	12 days
1000	70	90	100	—	—	—
500	50	60	80	90	100	—
250	30	50	70	75	90	100
125	15	30	50	57	57	60
65	10	20	30	30	30	30
30	5	10	20	20	20	20
15	5	10	10	10	15	15

\* Each value represents average of 3 experiments, each of 20 snails.

increased by increasing the exposure time (table 2). A hundred percent mortality was reached after 6, 10 and 12 days of exposure to 1,000, 500 and 250 mg/l respectively. There were no significant mortalities among the control snails.

#### 3.2.2 Fecundity

Exposure of snails to 125, 65 and 30 mg/l for 15 days caused a significant decrease in both ovipository activity, length and width of egg masses and the hatchability % of the eggs (table 3). Exposure to 15 mg/l gave no significant differences. It is obvious that exposure to 125 mg/l for 15 days caused a complete sterilization.

## 4 Discussion

The results showed that Thuricide had a molluscicidal activity against both snail species. *B. truncatus* was more sensitive to the action of Thuricide. However the results showed that slope function of the LC<sub>50</sub> for *B. alexandrina* was significantly higher than that for *B. truncatus*. This indicates that development of resistance to *B. alexandrina* may require a long time that extends to many generation. Similar results were obtained when different formulation were use (RITCHIE et al., 1963; MOHAMED, 1974; ABU-ELGHAR, and MOHAMED, 1976).

The results on the effect of prolonged exposure to sublethal concentrations proved that there was an increase of mortality with the time of exposure. These results may give an idea about the time of exposure and the molluscicidal activity of Thuricide and would help when the compound used on field scale.

Our results are in harmony with those obtained by TERYTZE, and HOFMAN (1986) who found that different preparations of *Bacillus thuringiensis* gave remarkable mortality effects on slugs (Limacidae and Milacidae). They added that most successful control results were

Table 3. Effect of 15 days exposure to Thuricide on the fecundity of *Biomphalaria alexandrina* snails

Conc. mg/l	No. of snail exposed	No. of egg masses	Mean length of egg mass and range (mm)	Mean width of egg mass and range (mm)	Mean number of eggs/egg mass	Hatchability (%)
Control	20	38	5.2 (3-6)	3.4 (3-4)	16.8 (10-21)	100
125	20	2	2.4 (2.2-3.4)	1.1 (1.1)	2.0 (2)	0
65	20	2	2.8 (2.5-4)	2.6 (2.8-3.1)	4.32 (3-6)	30
30	20	6	3 (3.4-4)	1.9 (1.5-2)	5.11 (3-7)	70
15	20	25	6 (3-6)	3.8 (3.4-4.2)	13.73	90
L.S.D. at 5 %		3.85	1.09	0.82	3.46	

Water temperature during the experiments 25 + 2 °C.

obtained with Bitoxibacillin, Dendrobacillin and Thuricide HP.

The present results proved that Thuricide was an effective compound on egg laying capacity, size and hatchability of egg masses. Similar results were obtained by OLIVIER and HASKINS (1960), HARRISON et al. (1966) and MOHAMED et al. (1981) when they used other materials. They reported that snails maintained in low concentrations of sodium pentachlorophanate and Bayluscide produced few egg masses. The results also showed that the size of egg mass and the number of embryos per egg mass laid by exposed snails to low concentrations of Thuricide are significantly lower than the control ones. In hermaphroditic gastropod the development of genital tract and accessory sex organs are the main factor affecting their fecundity (GERAERTS, 1978). Therefore it is suggested that Thuricide had significant effect on the spermatogenes and oogenes of exposed snails.

The toxicity of *B. thuringiensis* supernatant to insects was attributed, in addition to B-exotoxin, to  $\alpha$ -exotoxin (KRIEG, 1971) Labile-exotoxin (SMIRNOFF, and BERLINQUET, 1966), water soluble toxin (FAST, 1971) and endotoxin (ABOU BAKR et al., 1985). THOMES and ELLAR (1983, 1986) have isolated delta-endotoxin (protien) from *B. thuringiensis israelensis* which affected membrane lipids in the insect gut epithelium and had both cytolytic and mosquitocidal activities. In the present work, it is speculated that the potency of Thuricide as molluscicide may be due to one or more of the toxins produced by the bacteria. Further studies may be conducted to isolate the effective fractions of *B. thuringiensis* Berl. on molluscs.

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We thank Prof. Dr. M. R. ABU EL-GHAR, plant protection Department, Faculty of Agriculture, Menoufia University, for supplying with strain Thuricide of *Bacillus thuringiensis*.

#### Zusammenfassung

Zur Wirkung von *Bacillus thuringiensis* Berl. bei der biologischen Bekämpfung von Schnecken als Vektoren der Schistosomyiasis in Ägypten

Es wurde die molluscizide Wirkung von Thuricide (*Bac. thuringiensis*) gegen die Schnecken *Biomphalaria alexandrina* und *Bulimus truncatus* getestet. Die Ergebnisse zeigen, daß Thuri-

cide für beide Schnecken molluscizid war. *B. truncatus* erwies sich als empfindlicher, doch war die Neigungs-Funktion des LC<sub>50</sub>-Wertes für *B. alexandrina* signifikant höher als für *B. truncatus*. Die Mortalitätsrate von *B. alexandrina* erhöhte sich mit zunehmender Expositionszeit. Die Exposition von *B. alexandrina* gegenüber geringen Konzentrationen von Thuricide verursachte eine signifikante Verringerung sowohl der Eiablage-Aktivität als auch der Größe der Eigelege sowie des Schlüpfprozents der Eier.

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## Rundschau

### Prof. Dr. Dr. G. Wellenstein zum 85. Geburtstag

Am 27. Juli 1991 vollendete der ehemalige Direktor des Forstzoologischen Instituts der Universität Freiburg/Br., Prof. Dr. Dr. GUSTAV WELLENSTEIN, sein 85. Lebensjahr. Der gebürtige Trierer war neun Jahre Leiter der neugegründeten „Forstschutzstelle Ost“ und Forstmeister in Breitenheide, seinem forstlichen Paradies in der ostpreußischen Wahlheimat, an der er auch heute noch mit großer Liebe hängt. Von 1946–1960 war er Direktor der „Forstschutzstelle Südwest“; 1960–1972 des Forstzoologischen Instituts. Zu seinem 70. Geburtstag wurde sein bisheriges Wirken auch in dieser Zeitschrift (49/1976, 125) gewürdigt und zu seinem 80. erschien eine Festschrift der „Freiburger Waldschutz-Abhandlungen“ (6/1986, 209 S.).

Obwohl bereits vor fast 20 Jahren emeritiert, bearbeitet und erweitert WELLENSTEIN noch immer mit viel Begeisterung ein sich nunmehr über vier Jahrzehnte erstreckendes Lebenswerk, das während seiner Amtszeit Niederschlag in über 200 Publikationen fand. Seitdem entstanden fast 100 weitere Veröffentlichungen, die seinen Namen als Verfasser oder Coautor tragen. Fast täglich ist er in seinem Emerituszimmer, von wo er weiterhin regelmäßig die Entwicklung im Institut verfolgt und mitgestaltet, ist mit der Aufarbeitung wissenschaftlicher Aufzeichnungen beschäftigt, hält Vorlesungen und betreut Forststudenten und Diplomanden. Zu seiner großen Freude konnte er erleben, daß die Gebäude seiner ehemaligen „Forstschutzstelle Südwest“ (Anwesen Fohrenbühl 25 und 27, Stegen-Wittental) jetzt als Außenstelle dem Forstzoologischen Institut angegliedert wurden.

WELLENSTEIN als Biologe und Forstmann befaßt sich mit



allen Aspekten von Waldschutz und der Ökologie der Wildtiere. Seine umfangreiche und vielgestaltige Lehrtätigkeit begann er als Dozent für Forstliche Zoologie und Waldhygiene an der Universität Königsberg, wo er 1943 Dr. rer. nat. habil. wurde. Im gleichen Jahr verlieh ihm die Forstliche Hochschule in