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Snail-Killing Effects of *Streptomyces* 218 Powder

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Abstract: This study is aimed at finding out the snail-killing effects of *Streptomyces* 218 powder on *Oncomelania hupensis* snails which are the vectors or intermediate host of Schiltosoma Japonicum (intestinal schistosomiasis) in china the tests were carried out in the laboratory and on the field. The snail-killing effects of *Streptomyces* 218 powder, isolated from snail habitat at Anchang Village of Anxiang country in China was tested using the immersion and spraying methods. The tests on the *Oncomelania hupensis* snails which are intermediate host of *Schistosoma japonicum* infection were carried out in the laboratory and in the field. The mean corrected snail mortalities of the immersion technique in the laboratory were 81.70 and 98.63% in 10 ppm of 218 solutions after 24 and 48 h, respectively. The mean corrected snail mortalities of the spraying tests in the laboratory were 82.90 and 87.90% at 3 and 5 days, respectively with 10 g/m^2 218 powders. The snail-killing ability of 218 powders on the field determines by immersion and spraying methods were comparable to that of the chemical molluscicide-Niclosamide. The corrected snail mortality at 150 ppm of 218 powder (g/m^2) and at 2 ppm of Niclosamide by immersion was 100% at the second time test after 24, 48 and 72 h. In the field spraying test, the mean corrected snail mortality at 100 ppm of 218 powders were 61.96 and 70.00% after 3 and 7 days of spraying respectively. At 2 ppm niclosamide, this was found to be 65.58 and 63.81%, respectively. The effective ingredients for the snail-killing are found to be located in the spore chains. *Streptomyces* 218 powder, although at higher concentrations, seems to be a promising molluscicide biological agent. If developed further, this could compliment existing molluscicide agents.

Keywords: Biological agent, immersion, molluscide, niclosamide, snail mortality, *Streptomyces* 218 powder

INTRODUCTION

Niclosamide and sodium Pentachlorohenate (NaPCP) have commonly been used for the control of schistosome-bearing snails on the field world-wide (Komiya *et al*., 1962). Niclosamide is highly used because of its high efficiency and low toxicity of humans. However, its large scale use is hindered due to high cost and poor water solubility. The use of NaPCP has been prohibited in Japan by the Government regulation due to problem of environmental pollution (Hosaka *et al*., 1969).

The molluscicidal activity and efficacy of B-2 (Sodium 2, 5-dichloro-4 romophenol) against *Oncomelania nosophora* and *Oncomelania hupensis*, *Oncomelania quadrasi*, intermediate hosts of *Schistosoma japonicum* in Japan, China and Philippines respectively have been reported by several authors (Kajihara *et al*., 1979a, b; Hosaka *et al*., 1984; Wong *et al*., 1988). Taira *et al*. (1977) also reported that B-2 showed a cidal effect against eggs, Juveniles and adults of *Lyinnaea ollula*, the intermediate host of *Fasciola* sp., in Japan. Results of laboratory and field studies with B-2 and its low toxicity to non-target animals (fish and mammals) indicated it to be a promising molluscicide against the target snails (Kajihara *et al*., 1979a, b). The residual concentration of B-2 in the soil after application was found to have decreased more rapidly than that of sodium penta chlorophenate NaPCP (Hosaka *et al*., 1984).

Xia *et al*. (1992) reported Tribromosalan, Cartap and Chlorothalonil to have high molluscicidal activities against *Oncomelania hupensis* both in the laboratory and on the field. Due to environmental pollution problems and toxicity to non-target organisms, the use of chemical molluscides has to be discouraged.

Emphasis should now be shifted to environmental manipulations and the use of biological control agents such as fish and microorganisms (Weinzetti and Jurbarg, 1990; Loreau and Baluku, 1991; Yao *et al*., 1993a, b). This study reports the laboratory and field trials of *Streptomyces diastato-chromogenes* 218 powder as a potential molluscicide against *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum* in China.

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MATERIALS AND METHODS

This study was carried out between June-August, 1994 at the Department of Vector Control, Hunan Institute of Parasitic Disease, Yueyang and Peoples' Republic of China. The immersion and spraying methods for killing snails as described by Komiya *et al*. (1962) were used for both the laboratory and field tests. The nail specie used was *Oncomelania hupensis*, the intermediate snail host of *Schistoma japonicum* infection.

Streptomyces 218 powder used in the experiments was produced with the help of Human Bio-Pharmaceutical factory, Changsha, Peoples' Republic of China.

Laboratory tests for molluscicidal effect:

Komiya's immersion method: Four concentrations (5, 10, 15 and 20 ppm) of *Streptomyces* 218 powder were prepared in 100ml beakers. Dechlorinated water was used as solvent and as control. Two nylon-net bags each containing 50 living adult *Oncomelania hupensis* snails were placed in each of the five beakers, one bag each was taken out and washed several times after immersion for 24 and 48 h, respectively. The snails were relaxed in petri dishes at room temperature for 4 days and the numbers of dead snails were determined using the water detection and knocking methods.

The snails were judged as dead when they showed no movement after their transfer into water. Those that showed no movement were crushed to confirm their death.

Spraying method for killing snails: Three hundred grams each of lake soils were put into 16×22 cm stainless trays. Dechlorinated water was then added to make 40% humidity. One hundred living adult *Oncomelania hupensis* snails were then introduced into each tray. Two hundred eighteen powders of varying concentrations of between 5 to 30 g/m^2 were sprayed into the trays. Fifty snails were removed from each tray after 3 and 5 days of spraying and numbers of dead snails were counted following the same procedure as in the immersion method.

Field tests for molluscicidal effect:

Immersion method: Pools varying between 0.69 to 1.3 m^3/per pool were dug for the study at lake shore outside embankment at Junshan farm. Concentrations of solutions of 218 powder of between 50 to 200 ppm were prepared. This was compared with 2 ppm of niclosamide (chemical molluscicide). Lake shore water served as control. Six nylon-net bags each were put into each pool. Two bags (100 snails) were taken out from each pool respectively after 24, 48 and 72 h of immersion. The bags were washed for several times and allowed to relax in petri dishes for 4 days at room temperature. The number of dead and living snails was then determined using the same procedure adopted fro Komiya's Immersion method in the laboratory.

Spraying method: Snail habitat with weed-shore at Junshan Park was chosen for the study. The dominant plant species was *Cyperaceae* and a snail density of 81-540 m^2/5 different concentrations of 218 powder (100 to 300 g/m^2) and 2 g/m^2 of niclosamide were prepared. The study habitat was 20 m^2/group. Pilot sampling method was used for snail-detection to determine if they have died after 3 and 7 days of spraying.

RESULTS

The mean corrected snail mortalities for Komiya's immersion method used in the laboratory tests for determining the molluscicidal effect of *Streptomyces* 218 powder is as shown in Table 1. After 3 repeated immersion tests in the laboratory, the mean corrected snail mortalities were found to be 81.70 and 98.63%, respectively after 254 and 48 h immersion in 10 ppm of 218 solutions. This was comparatively low at 5 ppm with the values being 21.88 and 42.14% respectively after 24 and 48 h after immersion. The mean corrected snail mortality at 15 and 20 ppm after 48 h of immersion were found to be 100%. This indicates that concentrations of between 15 to 20 ppm could be effective in eliminating all the snail species.

The mean corrected snail mortality in the laboratory using the spraying method at 5 g/m^2 was extremely low after 3 and 5 days of spraying. The values were respectively 2.03 and 11.17% (Table 2), respectively. The mean corrected snail mortality was found to be higher 5 days after spraying than after 3 days of spraying. Dosages of 10 to 30 g/m^2 were found to be effective in causing snail death of between 82.90 and 98.00%, respectively.

The snail-killing ability of 218 powders by immersion in the field at 150 ppm was comparable with that of Niclosamide at 2 ppm after 24 h of immersion (Table 3). The mean corrected snail mortality (%) at 50ppm of 218 powders was found to be low. These were 11.22, 3.09 and 4.08% after 24, 48 and 72 h of immersion, respectively.

In the spraying method in the field, the snail-killing of 218 powders was found to be higher after 7 days of spraying between 200 and 300 g/m^2. The mean values were 86.670, 92.44 and 98.74%, respectively (Table 4).

DISCUSSION

Streptomyces 218 powder was found to be a new snail-killing strain of Actinomycetes. The molluscicidal activity was found to be next to that of *Streptomyces griseolus* 230 (antibiotic 230) reported by Yao *et al*.

Table 1: Mean corrected snail mortalities by Komiya's immersion method in the laboratory

Streptomyces 218 powder (PPM)	Concentration of corrected snail mortality (%)							
	1st time		2nd time		3rd time		Mean	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
5	17.98	56.000	12.240	22.490	35.42	47.920	21.88	42.140
10	73.90	100.00	85.710	97.960	85.42	97.920	81.70	98.630
15	-	-	100.00	100.00	97.92	100.00	98.96	100.00
20	95.92	100.00	100.00	100.00	97.92	100.00	97.95	100.00

Table 2: Mean corrected snail mortalities by spraying method

Trials	Days	Corrected snail mortality (%)					
		30 g/m^2	25 g/m^2	20 g/m^2	15 g/m^2	10 g/m^2	5 g/m^2
1st	3	100.00	100.00	100.00	89.80	98.80	2.050
	5	100.00	100.00	100.00	93.88	91.84	16.33
2nd	3	90.000	90.000	86.000	80.00	76.00	2.000
	5	96.000	92.000	92.000	84.00	84.00	6.000
Mean	3	95.000	95.000	93.000	84.90	82.90	2.030
	5	98.000	96.000	96.000	88.94	87.90	11.17

Table 3: Snail-killing ability of *streptomyces* 218 powder by immersion in the field

Molluscicides (ppm)	Concentration corrected snail mortality (%)						
	1st time				2nd time		
	24 h		48 h		24 h	48 h	72 h
Streptomyces 218 powder	200	100.00	100.00	100.00			
	150	97.980	100.00	100.00	100.00	100.00	100.00
	100	83.840	100.00	100.00	897.86	100.00	100.00
	75	-	-	-	86.730	96.910	94.900
	50	-	-	-	11.220	3.0900	4.0800
Nicosamide	2	98.990	100.00	100.00	100.00	100.00	100.00

Table 4: Snail killing ability of *strephtomyces* 218 powder by spraying in the field

Trials	Days	Corrected snail mortality (%)					
		218 powder (g/m^2)			Niclosamide (g/m^2)		
		300	250	200	150	100	2
1st	3	87.95	79.52	83.13	-	-	51.81
	7	98.48	87.88	83.33	-	-	62.61
2nd	3	98.91	94.57	97.83	83.70	61.96	79.35
	7	99.00	97.00	90.00	81.00	70.00	65.00
Mean	3	93.43	87.05	90.48	83.70	61.96	65.58
	7	98.74	92.44	86.67	81.00	70.00	63.81

(1993a, b). In the laboratory tests the mean corrected snail mortality were 81.70 and 98.63% respectively after 24 and 48 h immersion in 10 ppm 218 powder. This was found to be 82.90 and 87.90%, respectively at 3 and 5 days after spraying with 10 g/m^2 218 powders. In the field test, the mean corrected snail mortalities were 86.73 and 96.91% respectively after 24 and 48 h immersion in 75 ppm 218 powders. These were 90.48 and 86.67%, respectively at 3 and 7 days after spraying with 200 g/m^2 218 powders.

This result demonstrates that *Streptomyces* 218 has a very high lethal activity to *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum*, both in the laboratory and in the field.

The molluscicidal effects of *Streptomyces* 218 powders were found to be influenced by the humidity of the test soil, altitude of the test place and ground vegetation. This is in contrast to that of antibiotic 230-2 whose molluscicidal effect was found to be related to temperature (Yao *et al*., 1993b).

Laboratory studies have indicated the control of vector snails with microorganisms (Yao *et al*., 1993b). For example *Bacillus pinotti* were reported to kill the snails that transmits *Schistosomes* in Egypt and Venezuela (Yao *et al*., 1993b). The metabolic products of *Aspergillus flavus* and *Aspergillus parasitious* were reported by Brazilian Scientists to be toxic to *Planorbis caenous* (Yao *et al*., 1993b). The first intermediate host of *Opisthorchis* sp and the Cercariae of the parasite were also reported to be killed by Nystatin in the Soviet Union (Yao *et al*., 1993b). Zhu *et al*. as reported by Yao *et al*. (1993b) also found that the products of *Cereus lieske* destroyed *Oncomelania* snails.

The effective ingredients for the snail-killing ability of *Streptomyces* 218 powder were found to be located in the spore chains. *Streptomyces* 218 powder seems to be promising molluscicide biological agents. If developed further, this could compliment existing molluscide agents.

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