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SHORT COMMUNICATION

Field evaluation of a novel molluscicide (niclosamidate) against *Oncomelania hupensis*, intermediate host of *Schistosoma japonicum*

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Abstract The molluscicidal activity of a novel molluscicide (niclosamidate) was evaluated in field trials against *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum*. The environmental safety of niclosamidate for local fishes was also studied under field conditions. The results showed that, at the dosages of 8.0 g/m^2 and 4.0 g/m^3, niclosamidate exhibits highly potent molluscicidal activity in the spraying and immersion trials, resulting in mortality rates of up to 81.8 and 72.7%, respectively. Its performance seems to be target-specific, with good molluscicidal ability observed for *Oncomelania hupensis* snails, but very low toxicity for local fishes and other aquatic organisms. The results suggest that niclosamidate can be used as an alternative molluscicide for snail control, which would be particularly applicable in semi-commercial or commercial aquaculture ponds.

Keywords Molluscicide · *Oncomelania hupensis* · *Schistosoma japonicum* · Snail control · Fish toxicity · Field trials

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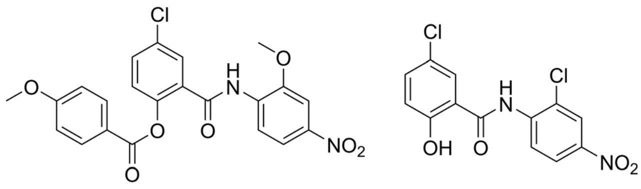
Introduction

Schistosomiasis japonica remains a serious public health problem and great socioeconomic burden in China, Indonesia, and the Philippines (Garjito et al. 2008; Leenstra et al. 2006; Zhou et al. 2012). Over 58 million people worldwide are infected with *Schistosoma japonicum* (Steinmann et al. 2006). *Oncomelania hupensis* is the sole intermediate host of *S. japonicum*. In the schistosomiasis control program, snail control is a very important method of interrupting the transmission of *S. japonicum*. It is also regarded as a cost-effective community intervention strategy at transmission foci (Yang et al. 2012). To date, the application of chemical molluscicides is the most effective and widely used measure for snail control (Yang et al. 2010). In the past four decades, thousands of chemicals have been screened for their molluscicidal potential. Among them, niclosamide is the only commercially available molluscicide recommended by WHO. However, it is highly toxic to fishes (Mitchell et al. 2007), which limits its application in the field, especially in aquaculture areas. Novel and improved molluscicides with potent molluscicidal activity and low toxicity on other organisms are urgently needed.

In our previous studies, a novel molluscicide (named niclosamidate; Fig. 1) was identified, which showed potent molluscicidal activity against *O. hupensis* in laboratory tests (Wang et al. 2015). Notably, it exhibited much lower fish toxicity on *Danio rerio* in comparison with niclosamide. The objective of the present study was to further evaluate the molluscicidal activity of niclosamidate in the marshland infested with *O. hupensis* snails. The environmental safety of niclosamidate for local fishes was also studied under field conditions.



Niclosamidate Niclosamide

Fig. 1 Chemical structures of niclosamidate and niclosamide

Material and methods

Study drugs

Fifty percent niclosamide wettable powder (WP) was purchased from Senliang Phamaceuticals (Suzhou, Jiangsu Province, China). Niclosamidate (active pharmaceutical ingredient (API)) was synthesized as previously described (Wang et al. 2015), and its 20% WP was manufactured by Xi'an Xinuo Agrochemical Co. Ltd. (Xi'an, Shanxi Province, China). A mixture containing kaolin (23 μm, 1300 g), white carbon black (200 g), Dispwet wp-410 (100 g), and niclosamidate (API, 400 g) was fine-grounded in a ball mill. The mixture was then sieved with a 45-μm mesh sieve and resulted in 20% WP of niclosamidate.

Study sites and molluscicide treatment

Field trials were performed in the marshland of Jiangling County, Jinzhou City, Hubei Province, China. To begin, a baseline survey of snail density was conducted (Wang et al. 2000). In the spraying method (Fig. 2), four plots (snail density > 5) with identical ecological environment in the marshland were selected as study plots, including three test plots and one positive control plot (niclosamide). Each plot covered an area of 100 m^2 (10 m × 10 m, 5 m interval) and was surrounded by low soil dykes to prevent the migration of snails. The non-experimental regions between and surrounding the study plots were also treated with molluscicides. A negative control plot was selected in a separated open area with similar ecological conditions. Tall weeds (> 5 cm) were pulled up and removed from the plots before the molluscicide treatment. In the immersion method (Fig. 2), three ditches (snail density > 5) with similar ecological environment were selected as study sites of niclosamidate, positive and negative controls. One ditch was divided into several units by building dykes and impounding water (50 cm interval), to which different concentrations of niclosamidate were applied. Positive and negative control units were identified in the remaining two ditches. The volume of the water in each unit was about 3 m^3.

For the spraying method, niclosamidate WP was sprayed on the test plots to reach corresponding concentrations with mist spraying machines following the instructions of the schistosomiasis prevention handbook (Wang et al. 2000). Snail mortality was evaluated on day 1 (D1), day 3 (D3), and day 7 (D7) post-treatment. A total of ten square frames (0.1 m^2) were investigated in each plot by random systematic sampling. All snails within the frames were collected, washed with dechlorinated water, and allowed to recover for 24 h. Snails were considered dead by the presence of discoloration, the absence of muscle contraction, hemorrhage, and the deterioration of the body

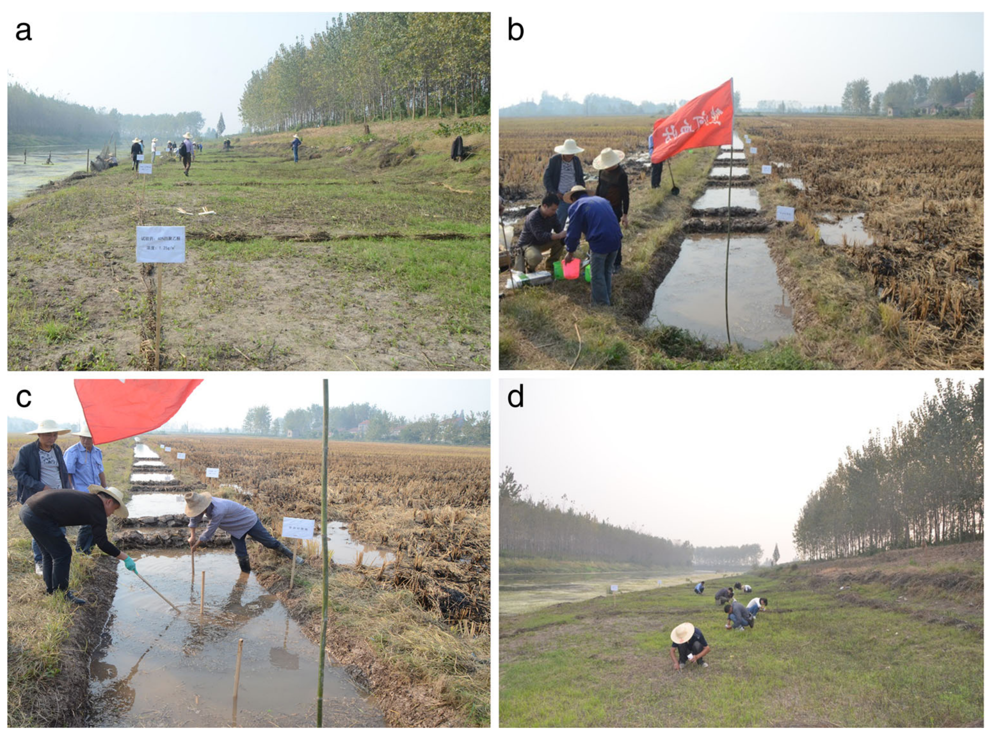


Fig. 2 Study plots (**a**) and ditches (**b**) of field trials. Molluscicide application in the immersion trials (**c**) and snail collection in the spraying trials (**d**)

tissues. Mortality and adjusted mortality of the snails were calculated as follows:

$$Mortality_{adjusted} = \frac{Mortality_{Experimental} - Mortality_{Control}}{1 - Mortality_{Control}} \times 100\%$$

For the immersion method, niclosamidate WP was applied to the test units and mixed evenly with the ditch water to reach corresponding concentrations. The snail-containing soil on both sides of the ditch was shoveled into the ditch water. Snail mortality was evaluated on D1, D2, and D3 post-treatment. Snails in each unit were collected by a triangular net (0.15 m^2, 20 mesh). Death of the snails was determined as described above.

Fish toxicity test

One ditch with identical ecological environment was identified as the test site for the fish toxicity test. The ditch was divided into several units with a volume of about 1.2 m^3 each by building dykes and impounding water, to which corresponding concentrations of niclosamidate WP and niclosamide WP were applied. Local fishes (~ 50–100 g per fish), including crucian, cyprinoid, and chub, were used as test fish. The test fish (20 fish/m^3) of each unit were placed in an iron cage (0.6 m × 0.5 m × 0.44 m) and immersed in the ditch water. Fish mortality was evaluated on D1, D2, and D3 post-treatment.

Results

Molluscicidal effect of niclosamidate against *O. hupensis* in field trials

Both spraying and immersion methods were employed in this investigation to adapt to the terrain of the snail habitat. For the spraying method (Table 1), snail adjusted mortality rates of 49.6, 67.3, and 81.8% were achieved on D7 after treatment with niclosamidate at concentrations of 2.0, 4.0, and 8.0 g/m^2, respectively. It is worth noting that at the concentration of 8.0 g/m^2, niclosamidate was fast-acting and as effective as niclosamide with the adjusted mortality rates of 79.7, 82.8, and 81.8% on D1, D3, and D7, respectively. For the immersion method (Table 2), niclosamidate was effective at all three concentrations. At the concentration of 4.0 g/m^3, niclosamidate had a rapid onset of action and exhibited comparable molluscicidal results with niclosamide. The snail population was significantly reduced on D3 with an adjusted mortality of 72.7%.

While carrying out the field trials, a number of local tadpoles, frogs, shellfish, and shrimps were also put in each test unit (or plot), and a 72 h observation was performed to qualitatively observe the toxic effect of niclosamidate on aquatic organisms. No lethal toxicity was observed. No obvious toxic symptoms were observed either, including balance disorder, bradykinesia, convulsion, and discoloration. Tadpoles, shrimps, and frogs showed normal behavior of motor activity and food intake. In contrast, a mass of fish and shellfish died within 2 h after the application of niclosamide.

Fish toxicity of niclosamidate on local fishes

After 72 h exposure to niclosamidate (Table 3), only half of the test fish died at the highest concentration of 12.8 g/m^3, which is threefold higher than the effective molluscicidal dose (4.0 g/m^3) identified in field trials. Niclosamide, in contrast, caused a fish mortality rate of 100% within 4 h at the concentration of 0.5 g/m^3 (Table 3), which is just half of the WHO recommended molluscicidal effective concentration (1 g/m^3). Our results demonstrate the significantly low toxicity of niclosamidate on local fish.

Discussion

Before the trials reported here, we performed preliminary experiments to evaluate the molluscicidal activity of

Table 1 Molluscicidal effect of niclosamidate in field trials by the spraying method

Molluscicides	Concn (g/m^2)[a]	D1		D3		D7	
		Mortality (%, *n*/*N*)[b]	Adjusted mortality (%)	Mortality (%, *n*/N)[b]	Adjusted mortality (%)	Mortality (%, *n*/*N*)[b]	Adjusted mortality (%)
20% Niclosamidate WP	2.0	29.8 (113/161)	27.1	42.5 (69/120)	40.5	51.6 (60/124)	49.6
	4.0	50.8 (59/120)	48.9	61.9 (45/118)	60.6	68.6 (38/121)	67.3
	8.0	80.5 (25/128)	79.7	83.3 (20/120)	82.8	82.5 (21/120)	81.8
50% Niclosamide WP	1.0	91.0 (12/134)	90.7	83.3 (20/120)	82.8	85.7 (18/126)	85.1
Control	–	3.7 (79/82)	–	3.3 (58/60)	–	4.0 (96/100)	–

[a] Concentrations of API

[b] *N*, total number of snails in 10 frames; *n*, number of living snails

Table 2 Molluscicidal effect of niclosamidate in field trials by the immersion method

Molluscicides	Concn (g/m^3)[a]	D1		D2		D3	
		Mortality (%, *n*/N)[b]	Adjusted mortality (%)	Mortality (%, *n*/N)[b]	Adjusted mortality (%)	Mortality (%, *n*/N)[b]	Adjusted mortality (%)
20% Niclosamidate WP	1.0	39.1 (28/46)	35.9	47.9 (25/48)	42.3	44.9 (27/49)	41.1
	2.0	55.3 (21/47)	53.0	63.5 (19/52)	59.5	64.6 (17/48)	62.1
	4.0	60.0 (20/50)	57.9	73.3 (12/45)	70.5	74.5 (13/51)	72.7
50% Niclosamide WP	1.0	70.5 (13/44)	68.9	82.2 (8/45)	80.3	84.0 (8/50)	82.9
Control	–	5.0 (38/40)	–	9.8 (37/41)	–	6.5 (43/46)	–

[a] Concentrations of API

[b] *N*, total number of collected snails; *n*, number of living snails

niclosamidate API in field conditions and identified a minimal effective dose. By using the spraying method, niclosamidate API caused snail mortality rates of 49.3, 77.6, and 53.0% at the concentrations of 1.25, 2.5, and 5.0 g/m^2, respectively, on D7. For the immersion trials, the corresponding results were 43.3, 55.6, and 87.8% at the concentrations of 1.25, 2.5, and 5.0 g/m^3, respectively, on D3. The poor water solubility of niclosamidate API likely resulted in the unusual results observed in the spraying trials. Thus, the more soluble formulation, 20% wettable powder, was manufactured to replace the API for field trials.

Niclosamidate WP exhibited potent molluscicidal ability in field trials and was as effective as the first-line molluscacide niclosamide. Satisfactory results were observed in both the spraying and immersion trials, which indicate that it is applicable to different terrains, including marshland, farmland, ditches, ponds, and shorelines. The negligible toxicity of niclosamidate WP to local fishes is another major achievement of the present study. Villagers living in schistosomiasis-epidemic areas are generally wary of molluscicides, due to its lethal toxicity to the fish stock. The results reported here demonstrate that, at the effective molluscicidal concentrations, niclosamidate WP is lethal to snails but not to fish, shrimps, frogs, tadpoles, and aquatic plants, which is beneficial to the snail control program. Furthermore, the target specificity of niclosamidate extends its scope of application to semi-commercial and commercial aquaculture ponds, particularly fish ponds, where the use of niclosamide has been banned.

The high-yield and low-cost synthetic routes of niclosamidate make it applicable for commercial production. Before putting it on the market, there are still some factors that should be considered. The effective molluscicidal concentration of niclosamidate WP in field trials (8 g/m^2, counted by API) was about eightfold higher than the lethal concentration (LC_{90} = 0.77 g/m^2) observed in laboratory studies. In lab conditions, the snails were immersed in the niclosamidate within the limited volume of the containers. In field conditions, the snails can escape from the toxic chemical by migration or by burying themselves in mud. The cost of 20% niclosamidate WP and 50% niclosamide WP was 60,000 and 50,000 CNY/ton. The high dosage of niclosamidate WP resulted in high cost in field practice. Compared with niclosamide WP (0.1 CNY/m^2, 0.1 CNY/m^3), the unit cost of niclosamidate WP (2.4 CNY/m^2, 1.2 CNY/m^3) is limiting. It is necessary to optimize the formulation, dosage regimen, and drug delivery system of niclosamidate to achieve high cost-effectiveness and maximize the efficacy of snail control. In addition, more field trials are needed, particularly in other snail habitat environments and against other *O. hupensis* subspecies, to further substantiate the effectiveness and applicability. The influence of environmental factors (e.g., terrain, water depth, and snail density) and climatic conditions (e.g., sunlight, soil humidity, temperature, and precipitation) on the molluscicidal efficacy of niclosamidate also needs to be investigated in future studies. As a molluscicide applied in the field, degradation is another concern we should care about. Besides

Table 3 Fish toxicity of niclosamidate on local fishes

Molluscicides	Concn (g/m^3)	No. of dead fish (*n* = 24)[a]			Mortality (%)
		24 h	48 h	72 h	
20% Niclosamidate WP	12.8	0	9	12	50
	25.6	8	17	24	100
50% Niclosamide WP	0.1	12	14	15	62.5
	0.5	24	24	24	100

[a] *n*, total number of test fish at each concentration

a fraction of the applied molluscicide reaches its snail target, the majority is released into the ecosystem, and there it has to be degraded biologically, abiotically, or photochemically to prevent accumulation or contamination. The results of our study on the photodegradation of niclosamidate showed that (see Fig. S1 in Supplementary Material), under the irradiation of a high-pressure mercury lamp, niclosamidate started to degrade after 10 min and formed a slightly more hydrophobic degradation product. Other routes of the degradation of niclosamidate, including biodegradation and hydrolysis, will be given more attention in our future work to understand its persistence in the snail habitat and evaluate the risk of offsite transport.

In conclusion, the results shown here demonstrate that niclosamidate is a promising molluscicide for *O. hupensis*. The 8.0 g/m^2 and 4.0 g/m^3 treatments could be effective dosages for the spraying and immersion methods. In contrast with niclosamide, niclosamidate exhibited negligible fish toxicity and can be used as an alternative molluscicide that particularly applicable to semi-commercial or commercial aquaculture ponds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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