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Toxicity of tobacco dust to freshwater snails (*Planorbella trivolvis*) and channel catfish (*Ictalurus punctatus*)

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ABSTRACT

Tobacco dust is a waste product of the tobacco industry and has been suggested as a molluscicide for aquaculture production. Snails serve as a required intermediate host for a number of trematode parasites. If snails can be eliminated using a molluscicide then aquaculture producers could effectively minimize parasitic infections of trematodes in their fish stocks by breaking the trematode life cycle. Four types of tobacco dust were evaluated as a potential molluscicide including burley (8200 μg/g nicotine), flue-cured (7200 μg/g nicotine), truck burley (4400 μg/g nicotine), and truck flue-cured (3900 μg/g nicotine). Ramshorn snails (*Planorbella trivolvis*), a common snail found in freshwater aquaculture ponds, were exposed to various concentrations of each type of tobacco dust over a three day period. Test concentrations included of 0 g/L tobacco dust and concentrations of 0.05, 0.25, 0.50, 1.0, and 2.5 g/L tobacco dust. Flue-cured and burley tobacco dust were more toxic compared to truck-flue-cured and truck burley tobacco dust. Tests on channel catfish (*Ictalurus punctatus*) were also performed at the same concentrations that were evaluated for snails. A dose between 0.5 to 1.0 g/L tobacco dust was effective in killing 100% of the snails within three days. In other experimental trials, there were no mortalities or histological evidence of effects on catfish at either of the 0.50 and 1.0 g/L tobacco dust concentrations over a 21 day trial. For the ramshorn snails, LC50 (lethal concentration to kill half of the snails) values were estimated to be 8.31, 2.58, and 1.73 mg/L nicotine for 24, 48, and 72 h exposure times, respectively. LC99 (lethal concentration to kill 99% of the snails) values were estimated to be 16.5, 8.35, and 5.41 mg/L nicotine for 24, 48, and 72 h exposure times, respectively.

1. Introduction

Snails serve as required intermediate hosts for a number of digenetic trematode parasites. The ramshorn snail (*Planorbella trivolvis* ~ *Heliosoma*) is the first intermediate host for the strigeid trematode parasite (*Bolbophorus confusus*), which infects several species of fish including the fathead minnow (*Pimephales promelas*), rainbow trout (*Onchorhynchus mykiss*), brown trout (*Salmo trutta*), arctic grayling (*Thymallus arcticus*), longnose sucker (*Catostomus catostomus*), white sucker (*Catostomus commersoni*), channel catfish (*Ictalurus punctatus*) and numerous other species experimentally (Olsen, 1966; Terhune et al., 2003). This parasite can cause significant economic losses among propagated species in both the production and in the hatchery (Lo et al., 1985; Venable et al., 2000; Terhune et al., 2003). For the U.S. catfish industry, economic analysis demonstrated that light infestations reduced sales receipts by 61% and ponds categorized as moderate and severe did not cover the costs of production. These farm level studies document the serious effect this disease can have on catfish operations and demonstrates the need for effective control measures. Research has shown that sub-lethal infections result in decreased production (Wise et al., 2006) and secondary bacterial infections (Labrie et al., 2004).

Fish infected with the parasite can develop cysts with metacercarial stages of the parasite, have impaired growth and are susceptible to other diseases that can weaken and kill the fish (Meepagala et al., 2004). In North America, the final host for this parasite is the American white pelican (*Pelecanus erythrorhychos*), and trematode eggs are spread to catfish ponds when the birds defecate. The eggs hatch and the larval mericidium stage infest the

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ramshorn snails. The snails, in turn, release free-swimming cercariae which infest catfish, and the life cycle is complete when the pelican eats the second intermediate host fish (Terhune et al., 2003). In order to control the parasite, the life cycle may be broken by controlling the snail population.

There are many methods of controlling snails and other molluscs in aquaculture ponds. A commonly used method in the United States is the application of chemicals such as copper sulfate (Boyd, 1990), often in conjunction with citric acid (Mitchell, 2002). Other chemical applications include hydrated lime and Bayluscide® (Terhune et al., 2003). It has also been suggested that altering the salinity of the pond and introducing biological control agents such as black carp (*Mylopharyngodon piceus*) (Venable et al., 2000) are effective methods of controlling snails. Many of the chemical agents used may also be toxic to fish, or are persistent in the environment and can cause damage to other non-target organisms (Duke et al., 2010).

Naturally occurring pesticides have an advantage over synthetic products because they tend to have a shorter half-life and are less likely to accumulate (Meepagala et al., 2004). Tobacco dust is a waste product of the tobacco industry. Nicotine is the primary toxicant in tobacco, comprising 95% of the total alkaloids (Cai et al., 2003). It has been shown in the Philippines that tobacco dust has molluscicidal properties against brackish water pond snails (*Cerithidea cingulata* Gmelin) (Borlongan et al., 1998), and in Nigeria, tobacco waste has been used to control periwinkles (*Tympanotonus fuscatus*) (Aleem, 1988). In the United States, the catfish industry is concerned with ramshorn snails. Accordingly, tobacco dust toxicity exposure studies were conducted on ramshorn snails and catfish. It was hypothesized that tobacco dust would be an effective agent for killing ramshorn snails without compromising juvenile channel catfish health.

2. Materials and methods

Four types of tobacco dust were evaluated in toxicity exposure trials for ramshorn snails, including burley (8200 μg/g nicotine), flue-cured (7200 μg/g nicotine), truck burley (4400 μg/g nicotine), and truck flue-cured (3900 μg/g nicotine) (Altria Client Services, Richmond, VA, USA). Tobacco dust exposure studies are reported as tobacco dust concentrations and calculated nicotine concentrations. Burley tobacco is air dried and flue-cured tobacco is generally dried using heat. Truck-based tobacco is differentiated from the other tobacco dust types because these products did not pass the <5% sand content standard. Channel catfish were exposed to burley tobacco dust only. Burley tobacco dust was selected because it contains the highest level of nicotine compared to the other three types of tobacco dust.

2.1. Ramshorn snails

Ramshorn snails were collected from a commercial catfish pond (L. Khoo, Mississippi State University, Stoneville, MS) and shipped to the Aquatic Medicine Laboratory at the Virginia-Maryland Regional College of Veterinary Medicine (Blacksburg, VA, USA) where the snails were maintained in 75-L aquariums at room temperature until exposure trials were conducted.

Ramshorn snails were exposed to each of the four types of tobacco dust for a 72 h period. Each 72 h exposure trial was replicated a second time. For each of the acute toxicity exposure trials, one hundred and eight (108) ramshorn snails were divided up amongst 36 wells to test snail response to six tobacco dust concentrations (0, 0.05, 0.25, 0.50, 1.0, and 2.5 g/L), each concentration was tested in sextuplicate (six replicates). Three snails were stocked per well for a total of 18 snails per tobacco concentration. Survival rates

Table 1
Mean survival rates over time for ramshorn snails exposed to flue-cured and burley tobacco dust at various concentrations. Alphas denote significant differences ($P < 0.05$) at a given time ($df = 35$).

Tobacco concentration as tobacco dust [g/L]	Tobacco dust type									
	Flue-cured					Burley				
	Tobacco concentration as nicotine [mg/L]	Survival at 0 days [%]	Survival at 1 days [%]	Survival at 2 days [%]	Survival at 3 days [%]	Tobacco concentration as nicotine [mg/L]	Survival at 0 days [%]	Survival at 1 days [%]	Survival at 2 days [%]	Survival at 3 days [%]
	Trial one					Trial one				
0	0	100	100^{a}	94.4^{a}	94.4^{a}	0	100	100^{a}	100^{a}	72.2^{a}
0.05	0.36	100	100^{a}	100^{a}	94.4^{a}	0.41	100	100^{a}	$83.3^{a,b}$	66.7^{a}
0.25	1.8	100	94.4^{a}	94.4^{a}	83.3^{a}	2.1	100	94.4^{a}	$61.1^{a,b}$	$33.3^{a,b}$
0.50	3.6	100	88.9^{a}	50.0^{b}	11.1^{b}	4.1	100	83.3^{a}	50.0b	22.0^{b}
1.0	7.2	100	22.2^{b}	16.7^{c}	0^{b}	8.2	100	55.6^{b}	5.6^{c}	0^{b}
2.5	18	100	11.1^{b}	0^{c}	0^{b}	20.5	100	11.1^{b}	0^{c}	0^{b}
Pooled error		*n/a*	*15.91*	*13.11*	*15.51*		*n/a*	*18.25*	*19.87*	*22.07*
P > F		*n/a*	*<0.0001*	*<0.0001*	*<0.0001*		*n/a*	*<0.0001*	*<0.0001*	*<0.0001*
	Trial two					Trial two				
0	0	100	100^{a}	100^{a}	88.9^{a}	0	100	100^{a}	83.3^{a}	72.2^{a}
0.05	0.36	100	100^{a}	100^{a}	94.4^{a}	0.41	100	100^{a}	83.3^{a}	61.1^{s}
0.25	1.8	100	100^{a}	100^{a}	83.3^{a}	2.1	100	88.9^{a}	$61.1^{a,b}$	$55.6^{a,b}$
0.50	3.6	100	88.9^{a}	50.0^{b}	16.7^{b}	4.1	100	88.9^{a}	$22.2^{b,c}$	0^{b}
1.0	7.2	100	38.9^{a}	11.1^{c}	0^{b}	8.2	100	38.9^{b}	5.6^{c}	0^{b}
2.5	18	100	33.3^{b}	5.6^{c}	0^{b}	20.5	100	27.8^{b}	0^{c}	0^{b}
Pooled error		*n/a*	*15.10*	*13.11*	*22.07*		*n/a*	*21.35*	*22.07*	*23.56*
P > F		*n/a*	*<0.0001*	*<0.0001*	*<0.0001*		*n/a*	*<0.0001*	*<0.0001*	*<0.0001*