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Tobacco dust: A novel molluscicide for aquaculture applications

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ABSTRACT

Parasitic trematodes require an intermediate host, such as a freshwater snail, to complete their lifecycle. It has been suggested that tobacco dust, a by-product of the tobacco industry, could be an effective molluscicide option for the aquaculture industry. Thus, the eradication of snails by this potential molluscicide could effectively reduce parasitic trematodes. Four types of tobacco dust were evaluated as a 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). Common freshwater snails (*Physa* spp.) and daphnia (*Daphnia magna*) were exposed to various concentrations of each type of tobacco dust over a three day period. Test concentrations included 0 g/L tobacco dust as a control and concentrations of 0.05, 0.25, 0.50, 1.0, and 2.5 g/L tobacco dust. Tests on goldfish (*Carassius auratus*) were also performed for a 21 day period. For flue-cured and burley tobacco dust, a dose as low as 1.0 g/L tobacco dust was effective in killing 100% of the snails within three days. For snails, the calculated LC50 (lethal concentration to kill half of the snails) values using all five concentrations of tobacco dust and four types were estimated to be 6.51, 2.51, and 2.10 mg/L nicotine for 24, 48, and 72 h exposure times, respectively. Daphnia were most sensitive to tobacco dust. Less than 70% of daphnia survived for 24 h at 0.05 g/L, the lowest tobacco dust concentrations evaluated. For daphnia, LC50 values were estimated to be 0.92, <0.20, and <0.20 mg/L nicotine for 24, 48, and 72 h exposure times, respectively. There were no mortalities or histological evidence of negative effects on goldfish at either of the 0.50 and 1.0 g/L tobacco dust concentrations over a 21 day exposure trial.

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1. Introduction

Parasites can cause significant economic losses amongst propagated aquaculture species (Lo et al., 1985; Venable et al., 2000; Terhune et al., 2003). Fish infected with parasitic trematodes can develop tissue 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, a common final host for these types of parasites is generally aquatic birds, and trematode eggs are spread to ponds when the birds defecate. The eggs hatch and the larval mericidium stage infest snails. The snails, in turn, release free-swimming cercariae which infest fish, and the life cycle is complete when the bird 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 (Mitchell et al., 2007; Wise et al., 2008; Kuhn et al., 2014).

Currently, effective disease management practices are based on eliminating populations of snails from individual ponds with copper sulfate (Mitchell, 2002; Wise et al., 2006) and hydrated lime (Mitchell et al., 2007). Copper sulfate can be very effective but has limited use during growing season because of algaecidal effects and hydrated lime is only effective against snails in the littoral zone of pond environment. Moreover, copper is persistent and can accumulate in the environment (Duke et al., 2010). In addition, non-specific preventative use of chemicals to control snail populations at the farm level is cost prohibitive and ponds are generally only treated after infestations develop. Expense associated with farm-level treatments creates a limitation in effective management because mild infestations are difficult to detect and many times go undetected until it is too late. A safe and more cost effective treatment would allow for routine treatment of snails to prevent trematode infections in commercially raised catfish and other species of aquacultured fish. One option that is receiving serious interest is tobacco dust, a waste product of the tobacco industry. The primary toxicants in tobacco are alkaloids, nitrogenous organic compounds often known to have physiological actions on organisms (Konar, 1977; Borlongan et al., 1998). It has been shown in the

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Philippines that tobacco dust has molluscicidal properties against brackish water pond snails (*Cerithidea cingulata*) (Borlongan et al., 1998), and in Nigeria, tobacco waste has been used to control periwinkles (*Tympanotonus fuscatus*) (Aleem, 1988). In the laboratory, Kuhn et al. (2014) demonstrated that tobacco dust is an effective molluscicide against ramshorn snails (*Planorbella trivolvis*).

There are four major tobacco dust by-product as defined by the tobacco industry. These included burley, flue-cured, truck burley, and truck flue-cured. Burley tobacco is air dried and flue-cured tobacco is usually 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.

In the study reported herein, we evaluated the effects of tobacco dust on three model organisms: the common goldfish (*C. auratus*), freshwater pond snail (*Physa* spp.) and daphnia (*D. magna*). Goldfish are a popular model organism for many different types of studies, including neuroendocrine signaling, cell biology, immunology, toxicology, endocrine disruption, molecular evolution, comparative genomics, neurobiology, olfaction, learning and memory, vision, and taste (Popesku et al., 2008). Pond snails have been used as a model to determine the environmental fate of pesticides (Metcalf et al., 1973), for predation studies (Tripet and Perrin, 1994) and for resource allocation studies (Tsitrone et al., 2003). In addition to serving as an intermediate host for trematodes, snails can cause problems in filtration mechanisms and in aquaculture systems (Boltz et al., 2008). Daphnia are used as a model for acute toxicity testing (United States Department of Environmental Protection Agency, USEPA) as well as aquatic ecology and water quality, population growth (Lu and Metcalf, 1975; Metcalf et al., 1971; McCauley et al., 1990), and toxicological genomics (Shaw et al., 2008).

2. Materials and methods

2.1. Tobacco dust

Tobacco dust types were supplied by Altria Client Services (Richmond, VA, USA). Nicotine concentrations were determined using Gas Chromatography (GC) with Nitrogen Phosphorus Detection (NPD) using Method 2551-Nicotine (NIOSH-CDC, 1998). Nicotine concentrations for burley, flue-cured, truck burley, and truck flue-cured tobacco dust were respectively, 8200, 7200, 4400, and 3900 μg/g nicotine.

2.2. Snails

Freshwater pond snails (*Physa* spp.) were collected from a commercial tilapia farm in Virginia and shipped to the Aquatic Medicine Laboratory at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) (Blacksburg, VA, USA) were the snails were maintained in 75 L aquaria at room temperature (21 °C) until exposure trials were conducted.

Snails were exposed to each of the four types of tobacco dust at room temperature for a 72 h period. Each exposure trial was replicated a second time. For each of the acute toxicity exposure trials, 180 snails were divided amongst the 18 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 triplicate. Ten snails were stocked per well for a total of 30 snails per tobacco concentration. Probing the foot of the snail with a dissection tool was used to determine mortalities. If the snails did not respond then this indicated that the snails were moribound or dead (Ciomperlik et al., 2013). Survival rates were used to determine the toxic effects of tobacco dust concentrations.

2.3. Goldfish

Goldfish were obtained from a commercial source (5D Tropical, Plant City, FL, USA) and shipped to the Southwest Virginia Aquaculture Research Center – Virginia Tech (Saltville, VA, USA). Five hundred goldfish (mean weight of 5.2 g) were acclimated and quarantined for a minimum of two weeks in a 4200 L recirculating aquaculture system outfitted with a beadfilter, nitrification/aeration sump, and ultraviolet filtration.

Goldfish were exposed to burley tobacco dust only because it contains the highest level of nicotine compared to the other type of tobacco dust. One-hundred and eighty (180) goldfish were exposed to six concentrations of burley tobacco dust (0, 0.05, 0.25, 0.50, 1.0, and 2.5 g/L) at 27–28 °C in triplicate amongst 18 aquarium systems. This 21-day toxicity exposure trial was replicated a second time. Each 80 L aquarium system was outfitted with aeration and a 2000 L/h mechanical and biofiltration filter unit. Water quality was monitored daily for dissolved oxygen and temperature and several times a week for ammonia, nitrite, and pH using methods adapted from APHA (2012).

Survival rates and histological examination were used to determine toxic effects of tobacco dust on goldfish. Six goldfish from each of the four experimental groups (0, 0.5, and 1.0 g/L tobacco dust exposures) were arbitrarily chosen and humanely euthanized with an overdose of sodium bicarbonate buffered MS-222 (200 mg/L, Sigma Chemical Co., St. Louis, MO) in water at the end of exposure period. The coelomic cavities of the goldfish were opened by incision and the internal organs were immediately preserved *in situ* by immersion in 10% neutral buffered formalin. Selected tissues (gills, liver, and intestine) were trimmed and processed for histopathology using standard histological techniques. Tissues were sectioned at 3–4 μm, and sections stained with hematoxylin and eosin (H&E) and evaluated for histopathology.

2.4. Daphnia

D. magna were purchased from a commercial supplier (Carolina Biological Supply, Burlington, NC, USA) and shipped to the Aquatic Medicine Laboratory at the VMRCVM. Since daphnia are a model organism for the USEPA the experimental methods and animal care described herein were adapted from USEPA (2002). More specifically, the USEPA water was prepared by dissolving 1.2 g $MgSO_4$, 1.92 g $NaHCO_2$, and 0.08 g KCl overnight with aeration in 19 L of deionized H_2O (dH_2O) in a 20 L carboy. Then 1.2 g $CaSO_4$ was dissolved in 1 L dH_2O and added to the carboy, and aerated overnight before use. All new glassware was soaked overnight in 10% HCl, then rinsed in dH_2O followed by a rinse in USEPA synthetic water. Glassware that was reused was cleaned in an automatic dishwasher, rinsed with 10% HCl, rinsed twice with dH_2O, once with acetone then rinsed three times with dH_2O. Cleaned glassware was then rinsed once with USEPA synthetic water immediately prior to use.

Daphnia were exposed to each of the four types of tobacco dust for a 72 h period. For each exposure trial, 120 daphnia were divided amongst twenty-four 50 mL beakers that contained 25 mL of USEPA water and the following concentrations of tobacco dust (0, 0.05, 0.5, 1.0, and 2.5 g/L). This design resulted in five daphnia per beaker and each exposure concentration was conducted in quadruplicate. Extra USEPA water with respective tobacco concentrations were used for static renewal for each beaker at 48 h. Survival rates were used to determine the toxic effects of tobacco dust concentrations.

2.5. Data analysis

Statistical analysis was performed using JMP Pro 11 for Windows (Cary, North Carolina, USA). Differences in mean water quality parameters amongst treatments (6 levels in triplicate) were

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