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Botanical pesticides as potential rotifer-control agents in microalgal mass culture

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ABSTRACT

With the aim of identifying an effective and safe technique for reducing rotifer contamination in microalgal mass cultivation, the toxic effects of four botanical pesticides on the rotifer *Brachionus plicatilis* were studied. Acute toxicity tests showed that celangulin, matrine and toosendanin are highly toxic to *B. plicatilis*, with 24 h LC_{50} values of 0.175 mg L^{-1}, 0.061 mg L^{-1} and 2.132×10^{-3} mg L^{-1}, respectively. Azadirachtin was the least toxic, with a 24 h LC_{50} value of 18.386 mg L^{-1}. Chronic toxicity tests showed that life history parameters (R_o, T, r_m and λ) and population density of rotifers decreased significantly when exposed to 0.110 mg L^{-1} celangulin, ≥0.050 mg L^{-1} matrine or 0.380×10^{-3} mg L^{-1} toosendanin. In addition, the rotifer-control effects of toosendanin and its safety in *Chlorella* and *Nannochloropsis* sp. cultivation were evaluated. Results demonstrated that $1.755 - 2.132 \times 10^{-3}$ mg L^{-1} toosendanin had no obvious toxic influence on final cell density and photosynthesis of *Chlorella* and *Nannochloropsis* sp., but effectively reduced the rotifer density and its fecundity. Based on the high toxicity to rotifers and the relative safety to microalgae, together with their low commercial price and ecological acceptability, celangulin, matrine and toosendanin are considered to be good potential botanical pesticides for controlling rotifers in microalgal mass culture.

1. Introduction

Due to the finite stocks of fossil fuels, as well as environmental protection issues, it is highly desirable to develop renewable and sustainable sources of bioenergy, with liquid biofuel as one of the most important of these. With advantages such as high photosynthetic efficiencies, highly biodegradable biofuel production, absence of competition for arable lands and fresh water, microalgae, especially marine species, are considered to be a third-generation feedstock for biofuel production [1–6]. In addition, microalgal cultivation can be integrated with management of waste streams and CO_2-generating processes [7] to reduce CO_2 emissions and the overall cost of large-scale production of biofuel from microalgae. Bulk production of microalgal biomass, through large-scale cultivation, is a prerequisite for realizing these potentials of microalgae. However, sustained, large-scale, biomass production has been successfully achieved only for a limited number of microalgal species, e.g., *Dunaliella* and *Spirulina* species, able to grow under extreme conditions such as high pH or high salinity [8], which discourage contamination and predation of the crop species. With expansion of microalgal cultivation to additional species, biomass production is increasingly limited by biological contamination [9], in which rotifers are among the most harmful of algal grazers. Rotifers can survive in extreme environments and reproduce rapidly owing to their sexual and asexual reproduction capabilities. Rotifer grazing and consumption of microalgae can sharply reduce an algal biomass to very low levels in just a few days. Consequently, large-scale cultivation of microalgae usually fails due to rotifer contamination.

At present, physical prevention is the main method for dealing with rotifer contamination in microalgal industrial cultivation, including the disinfection of equipment and the micropore filtration of microalgal seed inocula. However, these physical methods are only effective for preventing rotifer contamination in laboratory and pilot-scale cultures. They are ineffective for dealing with rotifer contamination in mass culture. Chemical control is considered a feasible method for exterminating rotifers. Some reagents such as toluene, hexane and xylene were shown to be toxic to *Brachionus calyciflorus* and *Brachionus plicatilis* [10], but they were broad-spectrum and could also injure microalgae and other aquatic organisms. Synthetic chemical pesticides such as organophosphates and pyrethroids were also toxic to rotifers. Methyl parathion and diazinon reduced the population growth rate of *Brachionus* species [11–13]. Other synthetic chemical pesticides such as thiophanate-methyl, methamidophos and fenitrothion had inhibitory effects on population growth and resting egg production in *B. calyciflorus* [14–16]. The studies above mainly focused on the influences of synthetic chemical pesticides on rotifers, however, their possible effects on the growth of microalgae and the quality of microalgal products were not considered.

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In addition, the excessive use of synthetic pesticides has led to serious problems, including toxicity to non-target organisms, and environmental contamination, and potentially threaten human health [17,18]. Therefore, such synthetic chemical pesticides cannot be used for rotifer extermination in microalgal mass culture. Recently several plant derivatives have been used as antifeedants or toxins against insects [19]. Pesticides derived from plants have received worldwide interest owing to their effectiveness, relative selectivity and safety [20]. Azadirachtin has antifeedant effects on Lepidoptera and Coleoptera, while non-target mammals are very insensitive to it [21]. Certain essential oil monoterpenes show lethal toxicity to cockroach and housefly, with complete degradation in liquid and soil systems in a short period [22–24]. However, the use of botanical pesticides for rotifer extermination during microalgal cultivation has not been well studied.

Pesticides may produce harmful effects on microalgae by inhibiting algal growth [25,26] or disrupting photosynthesis [27,28]. The organophosphorus insecticide trichlorfon decreased cell growth and pigment content of *Scenedesmus bijuga* and *Dunaliella salina* [29,30]. Cypermethrin (>50 μg L^{-1}) decreased cell density and soluble protein content of marine microalgae by inactivating their superoxide dismutase [31]. Compared to traditional phytotoxicity tests [32], chlorophyll *a* fluorescence of microalgae is an excellent indicator of pesticide toxicity, owing to its non-invasive application, sensitive response, and rapid measurement. The parameters F_v/F_m and φPSII derived from chlorophyll *a* fluorescence are commonly used to evaluate the phytotoxicity of pesticides on microalgae [33–35]. In the present study, the effects of four botanical pesticides including celangulin, azadirachtin, matrine and toosendanin on mortality rate, population growth and life history parameters of the rotifer *B. plicatilis* were compared by acute and chronic toxicity tests. In addition, the possible influence of toosendanin on photosynthetic performance in *Chlorella* and *Nannochloropsis* sp. was evaluated by chlorophyll *a* fluorescence.

2. Material and methods

2.1. Rotifers

B. plicatilis rotifers were obtained by hatching dormant eggs purchased from the Salt Research Institute, China National Salt Industry Corporation. The rotifers were continuously cultured in the laboratory at 26 °C in filtered seawater (salinity 32‰, pH 8.0). Rotifers were fed daily on *Nannochloropsis oculata*, which were cultured in L_1 medium [36] at an average light intensity of 45 μmol photons m^{-2} s^{-1} under a 14 h:10 h light:dark photoperiod provided by fluorescent lamps.

2.2. Botanical pesticides

Sources of botanical pesticides and their stock solutions are listed in Table 1. The stock solutions were prepared in distilled water.

Table 1
Sources of botanical pesticides and stock solutions used in acute and chronic toxicity tests against *Brachionus plicatilis*.

Botanical pesticides	Stock solution (mg/L)	Manufacturer or supplier
Celangulin	100.0	Institute of Bio-pesticide, Shijiazhuang City, Hebei Prov.
Azadirachtin	300.0	Neem Industrial Development Co. Ltd, Luxi City, Yunnan Prov.
Matrine	5.0	Institute of Bio-pesticide, Shijiazhuang City, Hebei Prov.
Toosendanin	0.1	Guiyou Biotechnology Co. Ltd, Shijiazhuang City, Hebei Prov.

2.3. Rotifer toxicity experiments

2.3.1. Test protocol

Acute toxicity tests and life history tests were conducted by using 24-well plates. One milliliter of toxicant medium was added to each well. Population growth tests were conducted in flasks containing 30 ml of toxicant medium. Neonates less than 7 h old were used for each experiment. The rotifers were counted and transferred with a glass micropipette. Rotifers in each test were fed with *N. oculata* at a density of 3×10^6 cells ml^{-1} and incubated at 26 ± 1 °C. The behaviors of rotifers exposed to different toxicants were microscopically observed.

2.3.2. Acute toxicity test

To obtain 24 h LC_{50} values for each botanical pesticide, sixty neonates (ten per replicate) were randomly divided into six treatment groups. Gradient concentrations of each pesticide were chosen to test its acute toxicity. The tested concentrations varied from 0 to 0.302 mg L^{-1} for celangulin, 0–95.50 mg L^{-1} for azadirachtin, 0–0.126 mg L^{-1} for matrine and 0–5.01×10^{-3} mg L^{-1} for toosendanin. After 24 h, the number of immobilized rotifers in each treatment was recorded. The 24 h LC_{50} value was calculated using the probit-logarithm method [37]. Based on 24 h LC_{50} values of celangulin, azadirachtin, matrine and toosendanin, four sublethal concentrations were chosen to perform the chronic toxicity test.

2.3.3. Chronic toxicity test

In the life history test of the four botanical pesticides, six replicates (each containing one neonate rotifer) were used for each sublethal concentration (Table 3). The egg attached, the offspring and mortality in each replicate were checked daily, then the newly produced neonates were removed. Toxicant medium was refreshed every 24 h until the rotifer died. Survivorship (l_x) and fecundity (m_x) tables were constructed for each replicate using conventional life-history techniques [38], in which x is the time in days. The life history parameters were calculated as followed: (1) the net reproduction, $R_o = \sum l_x m_x$; (2) the generation time, $T = \sum x l_x m_x / \sum l_x m_x$; (3) the intrinsic rate of increase, $r_m = \ln R_o / T$; (4) the finite rate of increase, $\lambda = e^{rm}$ [39,40].

In the population growth test, 90 neonates were randomly put into three groups. Thirty individuals were added to a flask containing 30 ml of toxicant medium. The rotifers were counted under a dissection microscope. The number of rotifers was determined daily, then the living rotifers were transferred to another flask with the same concentration as the fresh toxicant. The population density was measured during the six-day experimental period.

2.4. Safety evaluation of toosendanin for microalgal cultivation

2.4.1. Test protocol

Nannochloropsis and *Chlorella* sp. at the exponential growth stage were used to study the safety of toosendanin for microalgal cultivation. The microalgae were cultured in L_1 medium at 26 ± 1 °C. Light at 100 μmol photons m^{-2} s^{-1} intensity was provided by fluorescent lights with a 14 h light:10 h dark photoperiod. Rotifers were added into the algal culture to reach an initial density of 5 individuals/ml. After 5 h, a gradient of toosendanin ranging from 0 to 2.132×10^{-3} mg L^{-1} was added into a series of flasks containing the algae–rotifer culture. A rotifer-free algal culture was used as the control. Each group of the test was triplicated.

2.4.2. Chlorophyll fluorescence under dark or light steady-state photosynthesis

Chlorophyll fluorescence measurements were carried out at room temperature using a plant efficiency analyzer (PEA, Hansatech, UK). The stable and maximal chlorophyll fluorescences (F_s and F'_m) of a light-acclimated algal cell were measured after samples were light-acclimated for 10 min under 100 μmol photons m^{-2} s^{-1}. The minimal

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