Evaluation of free radical-generating compounds for toxicity towards the cyanobacterium *Planktothrix perornata*, which causes musty off-flavour in pond-raised channel catfish (*Ictalurus punctatus*)

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Photosynthetic organisms have evolved complex enzymatic systems to neutralize the potentially toxic and disruptive effects of physiologically generated reactive oxygen intermediates (ROI) (e.g. H$_2$O$_2$, OH$^\cdot$, O$_2$$^\cdot$). However, H$_2$O$_2$ will inhibit the growth of certain species of cyanobacteria (Samuilov, Bezryadnov, Gusev, Kitashov & Fedorenko 1999). Previous research to discover other algaecidal compounds for use to manage *P. perornata* determined that ROI-generating molecules, such as paraquat (Schrader, de Regt, Tidwell, Tucker & Duke 1998a), artemisinin (Xiao, Chen, Qu & Liu 2010) and certain quinones (e.g. anthraquinone) (Schrader, de Regt, Tidwell, Tucker & Duke 1998b), were highly toxic towards *P. perornata*. The physiological mechanisms (e.g. deficiency of antioxidant enzyme activities including catalase, superoxide dismutase and ascorbate peroxidase) contributing to the sensitivity of *P. perornata* to ROI-generating compounds were recently elucidated by Schrader and Dayan (2009). The discovery of alternative algaecides that are selectively toxic towards *P. perornata* and environmentally safe would benefit the catfish production industry.

In this study, a catalase inhibitor, 3-amino-1,2,4-triazole (Correa, Manrique, Font, Escrig & Aragon 2008), and several known intracellular free radical-producing compounds, tert-butyl hydroperoxide (Cheng, Nguyen, Song & Bonanno 2007), phenazine...

methosulphate (Shcherbachenko, Lisovsky & Ti-
khonov 2007), 6-hydroxydopamine (Fujita, Shiosaka,
Ogino, Okinura, Utsumi, Sato, Akagi, Inoue, Utsumi
& Sasaki 2008), ninhydrin (Elser, Gurgul-Convey &
Lenzen 2008) and the two quinoline compounds (+)-
(5)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-
methyl-5-[3,4-dichlorophenoxo]quinoline succinate or abbreviated as NPC 1161A and (−)-(5)-8-[(4-amino-
1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4
-dichlorophenoxo]quinoline succinate] or abbreviated
as NPC 1161B (Nanayakkara, Ager Jr, Bartlett, Yardley,
Croft, Khan, McChesney & Walker 2008) were evaluated
for their toxicity towards P. perornata. Test compounds tert-butyl hydroperoxide, phenazine methosulphate, 6-hydroxydopamine, ninhydrin and 3-amino-1,2,4-triazole were obtained from Aldrich (St Louis, MO, USA). Synthesis of NPC 1161A and NPC 1161B was reported earlier (Nanayakkara et al. 2008). Each test compound was dissolved in 100% technical grade ethanol and corrections for purity were made.

An isolate of P. perornata was obtained from a water sample collected from a Mississippi catfish pond (van der Ploeg, Dennis & de Regt 1995). A culture of the green alga Selenastrum capricornutum [Printz] was obtained from Dr J. C. Greene, USEPA, Corvallis, OR, USA, and was used as a representative of green algae (division Chlorophyta) in the bioassay to determine selective toxicity of the test compounds. Each culture was maintained separately in continuous, steady-state growth using the conditions outlined previously (Schrader, de Regt, Tucker & Duke 1997) to provide a source of cells growing at a constant rate. Continuous cultures samples were measured spectrophotometrically (model UV-3101PC, Shimadzu, Kyoto, Japan) at 750 nm to monitor cell density, which was maintained at 0.18–0.27 absorbance for P. perornata and 0.19–0.25 absorbance for S. capricornutum.

The bioassay procedures used by Schrader et al. (1997) were followed, except for several modifications as described below. Final test concentrations of the test compounds were 0.01, 0.1, 1.0, 10.0 and 100.0 µM. Three replicate wells were used for each test compound concentration and controls. Microplates (96-well, type Costar polystyrene; Corning, Corning, NY, USA) were held in an environmental light chamber (Percival Scientific, Boone, IA, USA) maintained at 29 °C and under continuous illumination by overhead fluorescent lamps (20 W, cool-white) at a photon flux density (photosynthetically active radiation) of 16–30 µmol m−2 s−1 as measured using a model LI-250 light meter and Quantum sensor (LI-COR, Lincoln,
NE, USA). Absorbance measurements (650 nm) were obtained at the beginning of each assay and then every 24 h for 4 days using a SpectraCount microplate photometer (Packard Instrument Company, Meriden, CT, USA). Mean absorbance data (average of three re-
plicate wells) were graphed to determine the lowest observed-effect concentration (LOEC; the lowest concentration of test compound to partially inhibit growth) and lowest-complete-inhibition concentration (LCIC; the lowest concentration of test compound to completely inhibit growth). The LOEC was determined to be the lowest concentration in which the graphed line and standard deviation bars of the mean absorbance data points at 3–4 days did not overlap the graphed line and standard deviation bars of the control mean absorbance data points at 3–4 days. The bioassay was repeated.

In this study, the most toxic compound towards P. perornata was tert-butyl hydroperoxide, with an LOEC and an LCIC of 0.01 µM (Table 1). This compound was also selectively toxic towards P. perornata based upon the LOEC of 100.0 µM and LCIC of >100.0 µM for S. capricornutum. Among the other compounds evaluated, NPC 1161A, NPC 1161B and phenazine methosulphate were moderately toxic to-
wards P. perornata, with LCIC of 1.0 and 10.0 µM for NPC 1161A and NPC 1161B, respectively; these compounds were also selectively toxic towards P. perornata. The greater toxicity of NPC 1161A compared with NPC 1161B towards P. perornata is likely due to the fact that NPC 1161A is a greater generator of ROI as discovered previously in animal toxicity studies (Nanayakkara et al. 2008). Ninhydrin, 3-amino-1,2,4-triazole and 6-hydroxydopamine were all marginally toxic towards P. perornata based upon LCIC values of 100.0 µM. Both 6-hydroxydopamine and ninhydrin were selectively toxic towards P. perornata when compared with LOEC and LCIC results for S. capricornutum while 3-amino-1,2,4-triazole was not selectively toxic. A previous study by Samulov et al. (1999) found the catalase inhibitor salicylic acid to suppress the growth of the cyanobacteria Anacystis ni-
dulans and Anabaena variabilis at 50000.0 µM. In our study, the catalase inhibitor 3-amino-1,2,4-triazole was toxic towards P. perornata, with LOEC and LCIC values of 1000 µM. Although the catalase activity of P. perornata was determined to be significantly lower compared with the activity in S. capricornutum as re-
ported by Schrader and Dayan (2009), the lack of selective toxicity of 3-amino-1,2,4-triazole would suggest another potential toxic mode of action towards the test organisms used in this study.
Table 1 Results of the bioassay evaluation of free radical-producing compounds for toxicity towards Planktothrix perornata

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Test organism</th>
<th>LOEC (µM)</th>
<th>LCIC (µM)</th>
<th>LOEC (µM)</th>
<th>LCIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Amino-1,2,4-triazole</td>
<td>Planktothrix perornata</td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
</tr>
<tr>
<td>tert-Butyl hydroperoxide</td>
<td></td>
<td>0.01 (0)</td>
<td>0.01 (0)</td>
<td>100.0 (0)</td>
<td>&gt; 100.0</td>
</tr>
<tr>
<td>6-Hydroxydopamine</td>
<td></td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
<td>&gt; 100.0</td>
<td>&gt; 100.0</td>
</tr>
<tr>
<td>Ninhydrin</td>
<td></td>
<td>1.0 (0)</td>
<td>1.0 (0)</td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
</tr>
<tr>
<td>NPC 1161A</td>
<td></td>
<td>1.0 (0)</td>
<td>10.0 (0)</td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
</tr>
<tr>
<td>NPC 1161B</td>
<td></td>
<td>10.0 (0)</td>
<td>10.0 (0)</td>
<td>100.0 (0)</td>
<td>100.0 (0)</td>
</tr>
</tbody>
</table>

*Lowest-observed-effect concentration = lowest test concentration that partially inhibits growth; number in parentheses is the standard error of the mean. A standard error of ‘0’ is not unusual with this bioassay due to test compound concentrations made in 10-fold dilutions.
†Lowest-complete-inhibition concentration = lowest test concentration that completely inhibits growth; number in parentheses is the standard error of the mean. A standard error of ‘0’ is not unusual with this bioassay due to test compound concentrations made in 10-fold dilutions.
‡(+)-(S)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline succinate.
§(−)-(R)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline succinate.

During previous studies to evaluate antimarialarial activities, the 8-aminoquinolines NPC 1161A and NPC 1161B were correlated with methemoglobinemia, likely due to the formation of hydrogen peroxide and ROI in erythrocytes (Nanayakkara et al. 2008). In our study, both of these compounds were more toxic towards P. perornata than primaquine, another antimarialarial compound, which had an LCIC of 100.0 µM (Schrader & Harries 2001). Although these two 8-aminoquinolines have relatively low mammalian toxicity, they are expensive to synthesize, and, therefore they are not good candidates to pursue for efficacy testing in catfish production ponds.

Although tert-butyl hydroperoxide was the most toxic towards P. perornata, the undesirable toxicity towards nontarget organisms and high reactivity of this compound make it an unlikely candidate for efficacy testing in catfish production ponds. However, the results of this study clearly demonstrate that the discovery of ROI-producing novel compounds, especially environment-safe natural compounds, should have high priority for evaluation as selective algaecides for managing musty off-flavour problems in pond-raised catfish related to the presence of P. perornata.

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References


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