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Efficiency of Experimental Rice (*Oryza sativa* L.) Fields in Mitigating Diazinon Runoff Toxicity to *Hyalella azteca*

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Abstract This study assessed the viability of using planted, mature rice fields in mitigating diazinon (an organophosphate insecticide) runoff toxicity using aqueous 48 h *Hyalella azteca* whole effluent toxicity bioassays. Rice fields decreased diazinon concentrations 80.1%–99.9% compared with 10.8% in the unvegetated field control. *H. azteca* survival responses coincided with observed diazinon concentrations. Estimated LC50 effects dilutions (%) ranged from 1.15 to 1.47 for inflow samples and 1.66 (unvegetated), 6.44 (rice field A), and >100 (rice field B) outflow samples. Decreases in inflow versus outflow aqueous toxicity were 77.1%–100% in rice fields compared with 18.7% in the unvegetated field.

Keywords Wetlands · Organophosphate toxicity · *Hyalella azteca*

Wetland vegetation has been shown to be an important component in trapping organophosphate pesticides such as diazinon and improving water quality (Moore et al. 2007).

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Agricultural rice (*Oryza sativa* L.) fields, having many of the same components as natural temporary wetlands (Suhling et al. 2000), could potentially be used for mitigating agricultural runoff. Mitigation efforts could improve water quality and reduce ecological impacts on receiving waters. Approximately 1.3 million ha of rice is grown annually in six states: Arkansas (49%), California (14%), Louisiana (14%), Mississippi (8%), Missouri (6%), and Texas (6%) (Gealy et al. 2003). In regions of intensive agricultural production, runoff from adjacent fields planted in other crops could be directed into rice fields for irrigation and to maintain water levels. As a result, rice fields have the potential to be widely used as an agricultural best management practice (BMP) to more efficiently control runoff and discharge and mitigate impacts on non-target aquatic biota.

Diazinon, *O*,*O*-diethyl 0-[6-methyl-2-(1-methylethyl-4-pyrimidinyl)] ester, is an organophosphate (OP) insecticide used on agricultural crops such as fruits and vegetables predominantly in California, USA (Brown et al. 2008). In 2006, approximately 134,000 kg diazinon was used in California (USDA NASS 2006) with the potential to contaminate rivers and streams during runoff events. This study's purpose was to assess the efficiency of using planted, mature rice fields in mitigating diazinon runoff toxicity. About 48-h toxicity bioassays used *Hyalella azteca*, an epibenthic, detritivorous crustacean found in water bodies throughout much of North America (de March 1981) as the sentinel species.

Materials and Methods

The study site was located at the University of Mississippi Field Station (UMFS) in Lafayette County, Mississippi, USA. Three experimental wetland fields with a hydraulic retention time of 2–3 h were used. Two fields were planted with rice (similar densities) and allowed to mature for 3 months prior to pesticide amendment in August. One additional field remained vegetation free to serve as a control. Rice field A was 2,016 m^2 (32 mW × 63 mL), rice field B was 1,456 m^2 (52 mW × 28 mL) and the unvegetated field control was 2,016 m^2 (32 mW × 63 mL). All fields were amended once with 0.478 kg (nominal) a.i. diazinon as field formulation Diazinon 4E™. This amendment was based on the recommended field application rate for an 32.3 ha field, assuming 0.05% runoff. Four liters of water was collected from the inflow (influent) and outflow (effluent) of each field 2.5 h after amendment initiation. Samples were preserved on ice and transported to the US Department of Agriculture, Agricultural Research Service National Sedimentation Laboratory (NSL) in Oxford, Mississippi for chemical analysis and bioassay assessment.

A 500 mL aqueous aliquot was extracted and analyzed for diazinon from each site within each field according to methods described by Smith et al. (2007). Diazinon concentrations were determined using two Agilent HP model 6890 gas chromatographs (GC) equipped with dual Agilent HP 7683 ALS autoinjectors, dual split-splitless inlets, dual capillary columns, and an Agilent HP Kayak XA Chemstation. Diazinon was extracted by sonication using reagent-grade KCl and 50 mL pesticide-grade ethyl acetate dried over anhydrous Na_2SO_4 and concentrated to near dryness by rotary evaporation. The extract was then subjected to silica gel column chromatography cleanup, and concentrated to 1 mL volume under high purity dry nitrogen for GC analysis. Diazinon recoveries and extraction efficiencies, based on fortified samples, were ≥90% with a detection limit of 0.1 µg/L.

Three replicate 48 h whole effluent toxicity bioassays using *H. azteca* were conducted per sample according to modified USEPA protocols (2000, 2002). All *H. azteca* were cultured at the NSL culturing facility according to the procedures of de March (1981). Toxicity bioassays were conducted in a Powers Scientific Inc. incubator with a photoperiod of 16:8 light:dark at 20 ± 1°C at the NSL. *H. azteca* approximately 1–2 weeks old (passing a 600 µm stainless steel mesh sieve but retained by a 425 µm stainless steel mesh sieve) were collected for all toxicity bioassays. Samples were hardness adjusted (~100 mg/L as $CaCO_3$) UMFS water free from priority pollutants and serially diluted as follows: 7 at 0.5× serial dilutions for rice field A and rice field B samples; 11 at 0.5× serial dilutions for non-vegetated field samples. Exposure chambers (four replicates per dilution) were 120 mL polyethylene plastic containers having a sample volume of 100 mL with 2 × 2 cm sterile cotton gauze as substrate and containing five animals each. Standard toxicity bioassay water quality parameters of water temperature, pH, conductivity, dissolved oxygen, alkalinity, and hardness were measured according to APHA (1998). About 48 h survival data were analyzed with Sigma Stat® statistical software (SPSS 1997) to determine no observed effects dilutions (NOEC) and lowest observed effects dilutions (LOEC) using analysis of variance (ANOVA) or Kruskal–Wallis (ANOVA on ranks) with Dunnett's multiple range test when appropriate. NOEC values were based on lack of significant differences ($p < 0.05$) relative to controls and LOEC values were lowest dilutions that provided significant differences ($p < 0.05$) relative to controls. In addition, dilution LC1 (calculated when the NOEC was below the lowest tested dilution) and LC50 estimates and 95% confidence intervals were determined using the probit method (APHA 1998).

Results and Discussion

Inflow (influent) aqueous diazinon concentrations 2.5 h post-amendment were similar in unvegetated and rice field A, but more than twofold greater in rice field B (Fig. 1). Outflow (effluent) aqueous diazinon concentrations 2.5 h post-amendment decreased only 10.8% after passing through the 2,016 m^2 unvegetated field. In comparison, diazinon laden effluent passing through the 2,016 m^2 rice field A and the 1,456 m^2 rice field B decreased by 80.1% and 99.9%, respectively (Fig. 1). These results show that the presence of rice vegetation is an important factor in diazinon mitigation. Because fields were planted at the same time and shared similar sediment characteristics, differences in observed decreased diazinon concentrations were likely due to length/width ratios. Several previous studies have demonstrated the effectiveness of using vegetated aquatic systems to mitigate agricultural pesticide runoff and improve water quality. Rose et al. (2006) reported endosulfan, aldicarb, diuron, and fluometuron residue reductions of 22%–53% (year one) and 32%–90% (year two) in a wetland receiving cotton farm tailwater. Hunt et al. (2008) reported vegetated treatment systems capable of reducing the organophosphate pesticide chlorpyrifos by 52% between inflow and outflow concentrations. Moore et al. (2007) reported a vegetated drainage ditch distance of 55 m was needed to reduce amended diazinon concentrations by half their original amount, compared to 158 m needed for unvegetated ditches.

Measured water quality data were within parameters for acute aqueous toxicity bioassays according to USEPA protocols (2000, 2002). Ranges of mean water quality parameters for aqueous bioassays were: temperature, 20.0–20.2°C; pH 7.9–8; dissolved oxygen, 7.56–7.70 mg/L; conductivity, 227–300 µmhos/cm; alkalinity, 34.2–74.1 mg/L as $CaCO_3$; hardness, 62.7–79.8 mg/L as $CaCO_3$; turbidity, 1.8–4.2 NTU. Diazinon toxicity to *H. azteca* after

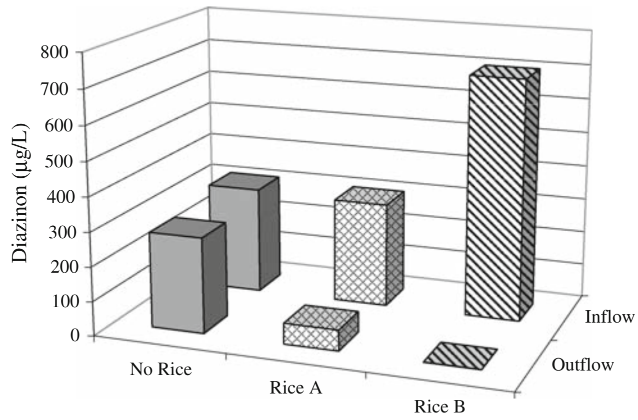

Fig. 1 Inflow and outflow diazinon concentrations (µg/L) from an unvegetated field control (No Rice), rice field A, and rice field B

48 h exposures decreased from inflow to outflow in rice fields A and B, but not in the unvegetated control field, and corresponded with changes in measured diazinon concentrations. *H. azteca* 48 h dilution-response curves showed nearly identical slopes during exposure to influent from all three fields (Fig. 2). Effluent response slope from the unvegetated field was nearly identical to the influent response slope. In contrast, dilution-response curves during exposure to effluent differed for rice fields A and B in conjunction with measured diazinon concentrations (Fig. 2). Diazinon dilution-response curves observed in this study were comparable to diazinon exposure-response curves of Burkepile et al. (2000). Inflow NOECs and surrogate LC1 dilutions, LOECs, and LC50 dilutions were similar across all fields (Table 1). Inflow LC1 dilutions (%) ranged from 0.30 to 0.58 and LC50 dilutions ranged from 1.15 to 1.47. By comparison, outflow LC50 dilutions varied from 1.66 (unvegetated field), 6.44 (rice field A), and >100 (rice field B) with NOECs, LOECs, and LC1 dilutions exhibiting the same pattern (Table 1). Decreases in inflow versus outflow aqueous toxicity were 77.1%–100% in rice fields A and B compared with only 18.7% in the unvegetated field. Previous studies showed vegetated aquatic treatment systems have varying degrees of effectiveness in mitigating diazinon contamination and toxicity (Bouldin et al. 2007; Moore et al. 2007; Hunt et al. 2008). Based upon percent dilution effects values, estimated measured diazinon effects concentrations for *H. azteca* 48 h NOECs, LOECs, and LC50s ranged from 1.9 to 2.4 µg/L, 3.8 to 11 µg/L, and 3.9 to 8.1 µg/L. Reported diazinon effects concentrations from this study are similar to 96 h LC50s reported by Collyard et al. (1994), however, they are two to threefold less than those reported by Burkepile et al. (2000). Our results show rice fields to be highly effective in decreasing diazinon concentrations and concomitant toxicity even in relatively small fields (<2,000 m^2). Results from this study support the use of rice fields in mitigating diazinon runoff toxicity to non-target aquatic biota.

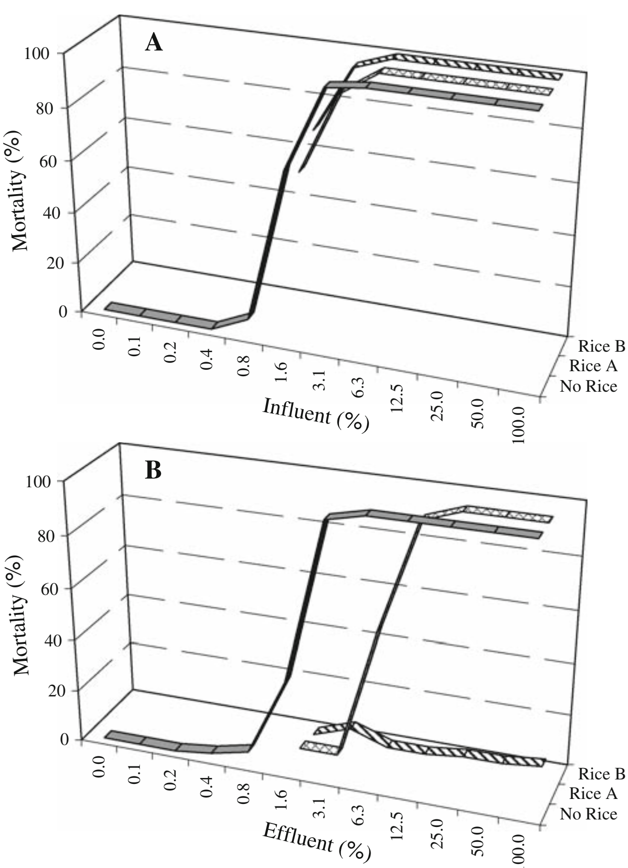

Fig. 2 Influent (**a**) and effluent (**b**) 48 h dilution-response curves for *Hyalella azteca* exposed to diazinon from an unvegetated field control, rice field A and rice field B

Table 1 Mean (SD) 48 h *Hyalella azteca* survival effects dilutions (%) (n = 3)

Location	Endpoint	Treatment		
		Rice field A	Rice field B	No vegetation
Inflow	NOEC	<1.56	<1.56	0.78 (0)
	LOEC	1.56 (0)	1.56 (0)	1.56 (0)
	LC1	0.35 (0.05)	0.30 (0.13)	0.58 (0.06)
	LC50	1.47 (0.44)	1.15 (0.20)	1.35 (0.11)
Outflow	NOEC	3.13 (0)	100 (0)	0.78 (0)
	LOEC	6.25 (0)	>100	1.56 (0)
	LC1	3.00 (0.71)	>100	0.61 (0.15)
	LC50	6.44 (0.91)	>100	1.66 (0.19)

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