

Effects of a Popular Aquatic Pest Control Agent among Carp Aqua-culturists on Aquatic Microfauna Dynamics

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ABSTRACT

Cypermethrin is gaining popularity among fish farmers community to control aquatic insect pests and ectoparasites. Effect of cypermethrin application at farmer select dose (@0.003 ppm) on aquaculture pests (Notonecta & small prawn) commonly found in tropical ponds along with fish fry was assessed. Effect of cypermethrin application on the composition and survivability dynamics of aquatic microfauna namely rotifer, copepod and cladocera for 8 days was studied in tropical pond conditions. Cypermethrin cause significantly higher mortality to notonecta and prawn compared to fish fry within 24 h of exposure ($P < 0.05$). Mortality of notonecta, prawn & fish fry became insignificant only after 72 h of cypermethrin application. During the 8 days span after application of cypermethrin, while population density *Rotifer* sp. remain unaffected ($P > 0.05$), population density of *Cladocera* sp. and *Copepod* sp. reduced significantly ($P < 0.05$). However, presence of dead zooplankton in the samples on the 8th day suggests that the presence of cypermethrin toxicity of beyond the period of study. Our results suggest that nursery pond stocking with fish fry after 72 h (three days) of application of cypermethrin might offer the triple advantage of predator elimination, prey availability and reduction in pesticide induced mortality of fish fry.

HIGHLIGHTS

- Cypermethrin toxicity to zooplanktons in pond water may persists beyond 8th day's.
- Cypermethrin affect density of Copepda and Cladocera but not Rotifers.
- Cypermethrin effectively kills predatory notonecta and prawn.
- Cypermethrin might offer the triple advantage of predator elimination, prey availability and reduction in pesticide induced mortality of fish fry.

Keywords: Pyrethroid, cypermethrin, plankton, toxicity, density dynamics, nursery pond

Cypermethrin[[Cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2- dimethylcyclopropane-1-carboxylate} is a pyrethroid commonly used in agriculture, dairy industry and public health sector to eliminate insects or pests (Nolan *et al.* 1979; Kranthi *et al.* 2002; Antwi and Reddy 2015). Cypermethrin kills a broad range of insects of both terrestrial as well as aquatic origin (Lazartigues *et al.* 2013). Fish farmers use it to control predatory insects in carp nursery pond and ectoparasites in

grow out pond (Shao 2001; Radheyshyam *et al.* 2011). Use of cypermethrin is increasingly gaining popularity among farmers in view of its lower cost and higher efficiency compared to recommended practices like use of soap-oil emulsion (Basavaraja

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2007). In addition, farmers also claim increased carp fry survivability in nursery ponds treated with cypermethrin. While removal of predatory insects from the ecosystem might be the major reason for this effect, we hypothesized that a change in zooplankton composition of the nursery pond could also be contributing to this observed increased fry survivability.

Cypermethrin is known to affect survival, clutch size, feeding rates and reproductive success in *Tisbe battaglai*, causing moulting failure and reduction in reproductive capabilities in *Daphnia magna* and behavioural changes and mortality in *Artemia* (Barata *et al.* 2002; Kim *et al.* 2008; Piska *et al.* 2013). Cypermethrin also reportedly affects reproduction, and embryonic & larval development in common carp (Aydin *et al.* 2005). However, studies on effect cypermethrin on pest, fish fry and zooplankton (microfauna) of aquaculture significance are scanty, mostly dealing with conditions simulating temperate lakes or marine ecosystems (Friberg-Jensen *et al.* 2003; Wendt-Rasch *et al.* 2003).

This study was aimed to find the effects of cypermethrin application on zooplankton dynamics in tropical earthen ponds at the select dose used for aquatic pest control by carp aquaculturists. The duration of toxicity in the pond water to the target pests and non-target pond macrofauna was also investigated.

MATERIALS AND METHODS

Pond preparation and cypermethrin treatment at farmers select dose

Through a short survey conducted with fish seed growers, the dose of cypermethrin most commonly used by fish farmers to eradicate harmful insect and control ectoparasites as a popular practice was found to be 0.003 ppm ($3.0 \mu\text{g L}^{-1}$). Hence this dose was selected in the study. CLINAR Liquid (Virbac, India) containing cypermethrin at concentration: 10% wt v^{-1} was used as source of cypermethrin.

The experiments were conducted in three tropical earthen ponds (Latitude: 23.905899, Longitude: 91.306382), each of size 300 M^2 with an average depth of 1 meter in the College of Fisheries, Lembucherra, Tripura. The ponds were fertilized with raw cattle dung and Single Super Phosphate (SSP) at doses

of 2,500 kg and 250 kg ha^{-1} respectively, 20 days before conducting the experiments to allow the development of stable community of zooplanktons. After fertilisation, the density and percentage of cladocerans, copepods and rotifers were measured daily, and when no significant density variation between days was recorded for a week, development of stable community of zooplankton was assumed. Cypermethrin was applied at rate of 0.003 ppm in all the three ponds.

Evaluation of cypermethrin effect on pond microfauna dynamics

For collection of zooplankton (microfauna) 100 L of surface water from cypermethrin treated ponds were sieved through plankton net of mesh size 50 μM (diameter) and reduced to 15 mL water volume. Samples were collected from three different places within a pond and were pooled for the respective pond. Sampling was performed at 0⁽⁺⁾h (i.e. 1 h prior to application of cypermethrin), 3, 6, 20, 24, 48, 72, 120 and 192 h post application of cypermethrin. Night sampling was avoided to prevent under and over representation of species due to diurnal movement (Kelso *et al.* 2003).

Zooplankter species were identified as per manual (Shiel 1995; Witty 2004). Briefly, 100 μL of each pooled sample was mounted over a Sedgewick rafter counting chamber under 50X magnification. No fixatives were used, so as to enable counting of dead and live individuals separately. The absolute zooplankton counts were further extrapolated to get the count per litre of pond water.

Toxicity assessment of cypermethrin on aquaculture pests and fish fry

Toxic effect of cypermethrin treatment on two major aquatic pests in carp nursery namely, the predatory insect *Notonecta* spp (F: Notonectidae) and *Macrobrachium lamarrei* (F: Palaemonidae; a small weed prawn, predatory on small fish fry) was studied. In addition, the toxicity duration of the pesticide on three days old fry of *Barbonymus gonionotus* (Cyprinidae) was also assessed. Live notonecta and small prawn were collected from untreated ponds and the fish fry from the fish hatchery for this purpose.

Water samples from the three cypermethrin treated ponds were collected separately at 0⁽⁺⁾, 24, 48 and

72 h post cypermethrin application in 20 L plastic tubs. For each time point, each of the three tubs representing respective cypermethrin treated ponds, were stocked with one hundred numbers of above mentioned pests and fish fry separately. For every time point, mortality of the introduced organisms was recorded in each tub after 24 h of continuous exposure. All the experiments were performed in strict adherence with the recommendations of the ethical standards of the Institutional Animal Ethics Committee, College of Fisheries, Central Agricultural University, Imphal, India.

STATISTICAL ANALYSIS

For the experiment on macrofauna (pests and fish fry), Independent-Samples Kruskal-Wallis test at 5% level of significance ($P < 0.05$) was employed to evaluate the effect of time lapse on mortality in cypermethrin treated water collected at the different time points. Post-hoc test was computed to check the pair-wise comparison between different time intervals on the number mortality of the macrofauna.

For toxicity experiments on zooplankters, two ways non parametric ANOVA at 5% level of significance ($P < 0.05$) was employed to compare the effects of time on density for the live zooplankton count (numbers L^{-1}) for three groups of zooplankton viz. cladocerans, copepods and rotifers. For dead zooplankton, a ridge saddle response surface analysis was performed to find the effect of time and persistence of drug considering reported half-life (Friberg-Jensen *et al.* 2003) that minimize the density.

RESULTS AND DISCUSSION

The extent of use of cypermethrin and its popularity among farmers in carp nursery operations for control of insect pests and ectoparasite is poorly acknowledged. While not scientifically recommended, the practice is adopted widely by farmers who claim enhanced fry survivability in carp nurseries treated with cypermethrin. This study was undertaken to examine the effect of this practice on the zooplankton dynamics in tropical earthen ponds. On the basis of a short survey, the dose of cypermethrin most commonly used by fish farmers was found to be 0.003 ppm ($3.0 \mu g L^{-1}$) and this dose was used for the experiments.

To study dynamics of microfauna with reference to

time lapse after cypermethrin treatment in pond, live and dead density of different zooplankton community were estimated. Cladocerans, copepods and rotifers were the predominating zooplankton groups, observed in the ponds. *Daphnia*, *Mesocyclops*, *Brachionus* were the most dominant in cladocera, copepoda and rotifer, respectively (accounting for 36-71%, 32- 93% and 71-96% of the respective group) both before and after application of cypermethrin.

The average density {numbers per liter; mean (\pm SE)} of live cladocerans, copepods and rotifers were 21.86 (± 8.28), 60.93 (± 10.97) and 16.58 (± 1.13) respectively at 0^(h) before application of cypermethrin. Eight days (192 h) after application of cypermethrin, average density of live cladocerans, copepods and rotifers were 1 (± 0.5), 42.7 (± 25.5) and 39.7 (± 28.5) respectively. In two ways non-parametric ANOVA, after application of cypermethrin the average density of live cladocera & copepoda reduced significantly ($P < 0.05$) over time (8 days), but not for live rotifer ($P > 0.05$). Our results suggest that sensitivity to cypermethrin toxicity was maximum in cladocera, followed by copepoda, and least in rotifera. This observation was in agreement with Friberg-Jensen *et al.* (2003). Though cypermethrin degrades very fast in the environment, it can exert its toxic effects on aquatic fauna at low concentration ranges of 0.1 to 10 ng L^{-1} (Anonymous 1989).

For dead zooplankton, saddle-Surface response using ridge analysis was employed to find the effect of time and persistence of the drug (considering reported half-life) that minimize the density. Under model fitting, the contribution of linear effect was found to be significant for cladocera, copepod and rotifers (0.0278, 0.0769 and 0.0512, respectively) while quadratic and cross products were non-significant in all the three cases. The lack of fit test was also non-significant for the three groups (0.6367, 0.9304 and 0.9675, respectively) implying that the model was adequate and both components estimated the nominal level of error. The stationary point was a saddle point. The ridge analysis (Table 1, 2 & 3) and the ridge plot in each case indicate that minimum density of dead individuals resulted from relatively low reaction times and low persistence of the drug (Fig. 1A, 2A & 3A). A contour plot of the predicted response surface (Fig. 1B, 2B & 3B), confirms this conclusion. The presence of dead individuals in a sample gives an indication of

**Table 1:** Estimated Ridge of Minimum Response for Variable density of dead cladocera

Coded Radius	Estimated Response	Standard Error	Uncoded Factor Values	
			Time	Persistence
0.0	95.660180	175.195624	97.500000	1429.215000
0.1	79.119872	145.075255	90.745481	1330.313837
0.2	63.477703	116.531392	83.732855	1235.438862
0.3	48.713213	89.526263	76.353847	1146.640381
0.4	34.791126	63.995437	68.442688	1067.420345
0.5	21.647301	39.823310	59.757096	1003.831660
0.6	9.161504	16.797524	50.013265	965.577898
0.7	-2.881918	5.494704	39.114118	963.887737
0.8	-14.804028	27.547641	27.499975	1001.909034
0.9	-26.951766	50.034839	15.923736	1069.187819
1.0	-39.595309	73.433609	4.833046	1152.032099

Table 2: Estimated Ridge of Minimum Response for Variable density of dead copepods

Coded Radius	Estimated Response	Standard Error	Uncoded Factor Values	
			Time	Persistence
0.0	35.027576	405.779560	97.500000	1429.215000
0.1	28.752302	336.016809	90.676269	1331.383575
0.2	22.893578	269.903313	83.656769	1236.656576
0.3	17.446815	207.356272	76.351163	1146.685369
0.4	12.403797	148.232157	68.609976	1064.432587
0.5	7.748606	92.267007	60.182004	995.517766
0.6	3.447879	38.959762	50.678126	950.674674
0.7	-0.569949	12.681513	39.737372	946.783187
0.8	-4.436533	63.798846	27.710398	994.269543
0.9	-8.315109	115.870920	15.730490	1079.142520
1.0	-12.332442	169.963264	4.479334	1179.972109

Table 3: Estimated Ridge of Minimum Response for Variable density of dead rotifers

Coded Radius	Estimated Response	Standard Error	Uncoded Factor Values	
			Time	Persistence
0.0	190.033985	662.391624	97.500000	1429.215000
0.1	159.721743	548.515558	90.645675	1331.863706
0.2	130.348342	440.610044	83.477483	1239.583893
0.3	101.857620	338.531859	75.901643	1154.406269
0.4	74.164082	242.027428	67.799238	1079.316879
0.5	47.136063	150.636586	59.036956	1018.530902
0.6	20.573371	63.537814	49.510003	977.331019
0.7	-5.811543	20.771292	39.228967	960.674946
0.8	-32.386285	103.967010	28.394537	970.458989
0.9	-59.548293	188.414532	17.341184	1003.823523
1.0	-87.656898	275.919654	6.371431	1054.850460

the biologically relevant toxicity of cypermethrin exerted on the studied group at a given time and its rate of degradation. This important information can be confounded when counting plankton from fixed samples. In studies by earlier workers, plankton counts were made from fixed samples (Friberg-Jensen *et al.* 2003), thus effectively losing this

information. To address this lacuna, in our study, dead and live individuals in a sample were counted separately without using fixatives. The estimated half-lives for the total (particle bound plus dissolved fraction) and dissolved fractions of cypermethrin are about 48 and 25 h in water respectively (Friberg-Jensen *et al.* 2003). Estimating by this reported

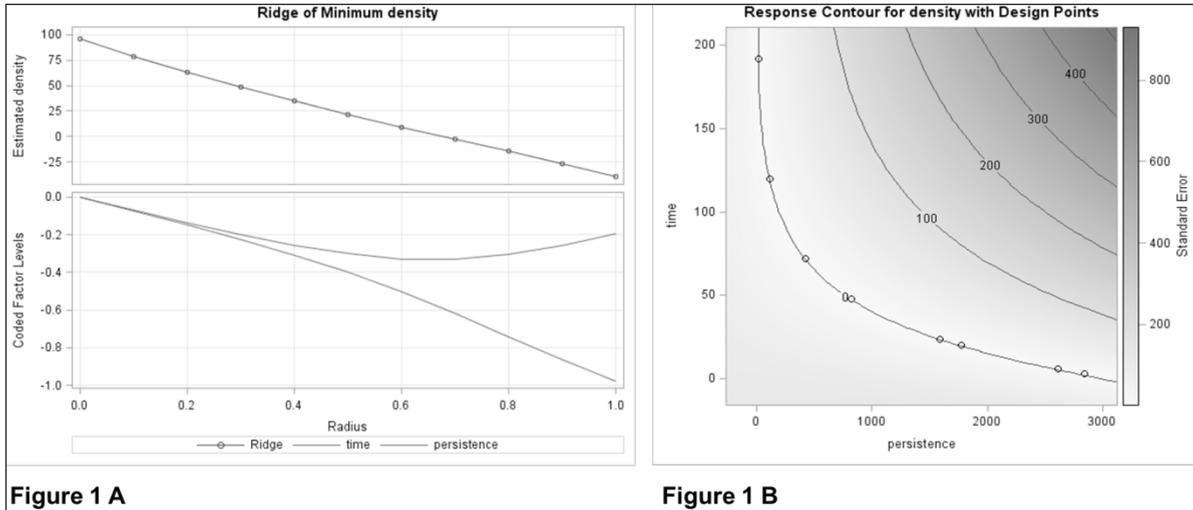


Fig. 1: Density response of dead cladocera with time and cypermethrin persistence; **(A)** Ridge Minimum density for cladocera, **(B)** Response contour for density with design points for Cladocera

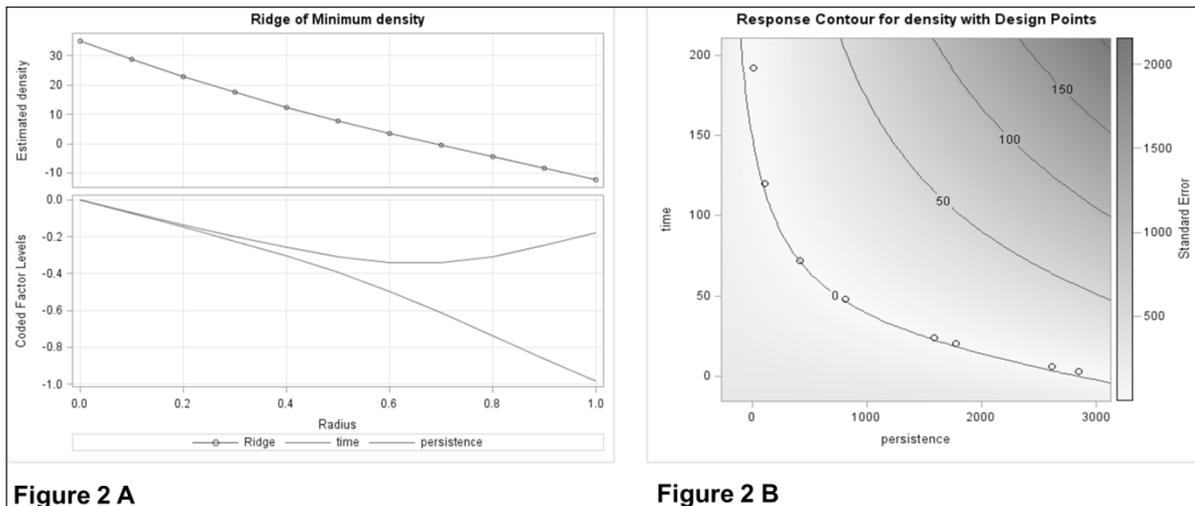


Fig. 2: Density response of dead copepods with time and cypermethrin persistence; **(A)** Ridge Minimum density for copepods, **(B)** Response contour for density with design points for copepods

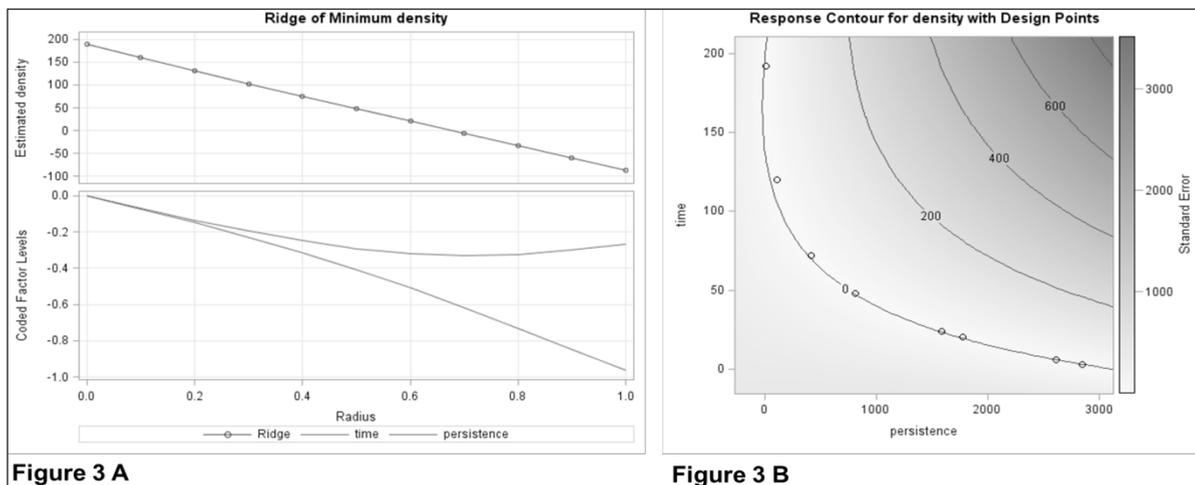


Fig. 3: Density response of dead Rotifers with time and cypermethrin persistence; **(A)** Ridge Minimum density for Rotifers, **(B)** Response contour for density with design points for Rotifers

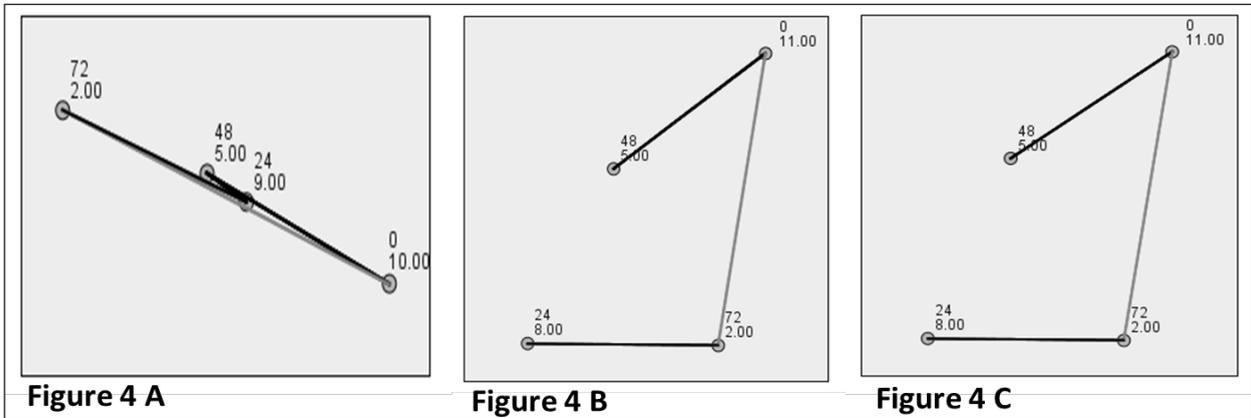


Fig. 4: Toxicity effect of cypermethrin on macrofauna in time gap: (A) Notonecta; (B) Prawn, (C) Fish fry; Each node shows sample average rank of time; Nodes joined by black line has no significant difference in pair wise comparison ($P>0.05$); Nodes joined by grey line has significant difference in pair wise comparison ($P<0.05$)

half-life, the concentration of cypermethrin even on the 8th day in our study would be nearly 24.7 ng L⁻¹ (0.8% of initial concentration). Cypermethrin adversely affects reproduction in *Daphnia* exposed at concentrations above 50 ng L⁻¹ (Sheng *et al.* 2004). On the other hand, the “lowest-observed-adverse-effect concentration” (LOAECs) of cypermethrin for cladocera and copepod is 100 ng L⁻¹ (Giddings *et al.* 2001). In present study, the dead fraction of zooplankton was negligible from untreated ponds while average of 12% of the zooplankton from the treated ponds comprised of dead individuals even on at 192 h (8th day). Our observations indicate that cypermethrin remain toxic to zooplankton well below these reported levels. Saddle-Surface response using ridge analysis also support this observation. In fact, cypermethrin has been reported to exert toxic effects at levels as low as 0.1-10.0 ng L⁻¹ on embryonic development and reproductive traits in Atlantic salmon (Moore & Waring 2002; Lower & Moore 2003) as well as survivability of several zooplankton (Maund *et al.* 2002; Wendt-Rasch *et al.* 2003). Occurrence of dead zooplankton at 192 h (8th day) in present studies, indicate the persistence of cypermethrin in water, placing a possibility of bioaccumulation in the stocked fish. In addition, it has a strong tendency to sorb in aquatic biota by virtue of its high hydrophobicity and lipo-affinity (Maund *et al.* 2002; Moore & Waring 2002; Wendt-Rasch *et al.* 2003; Lower & Moore 2003). In both invertebrates and vertebrates, cypermethrin is known entry to affect nervous system in multiple ways. Cypermethrin reportedly delays the closing of gate for sodium, resulting in multiple signals

instead of single (Vijverberg & Vanden 1990). It is also known to induce spontaneous release of acetylcholine in rat brain synaptosomes (Eells *et al.* 1992). Exposure to cypermethrin causes excitability and convulsions, likely due to its inhibitory effects on g-aminobutyric acid receptors (Ramadan *et al.* 1988). Hence, research is warranted to address the consumer safety of using cypermethrin in pond.

Water sample from each of the treated earthen ponds were collected for studies on macrofauna. While 0⁽⁺⁾ h water sample resulted in near 100% mortality of notonecta and prawn within one hour of introduction, no mortality of fish fry was recorded within the same time. Moreover in 0⁽⁺⁾ h water sample, mortality for both notonecta and prawn were 100%, and for fish fry was 52.3% after 24 h continuous exposure. Though mortality for all the three macrofauna in these studies was reduced in water collected at 24 & 48 h, but reduction in mortality became significant only in water collected at 72 h ($P<0.05$; Fig. 4, A, B, C). While average percentage mortality for notonecta was 1.6 % after 24 h continuous exposure in water collected at 72 h, for prawn and fish fry mortality was nil. Cypermethrin, is toxic at very low concentration and killed 100 % of representative aquatic invertebrate (notonecta and prawn), common predator of fish fry. Twenty-four-hour LC₅₀ for cypermethrin for most of the aquatic insects (*Cloeonidipterum*, *Gyrinus natator*, *Culex/Aedes* spp.) is below 1 µg L⁻¹ to 5 µg L⁻¹ for notonecta (Anonymous, 1989). On the other hand, 96 h LC₅₀ of cypermethrin for freshwater prawn, namely *Paratya australiensis* and *Palaemonetes argentine*s is 0.019 µg L⁻¹ and 0.0020



$\mu\text{g L}^{-1}$ respectively (Collins *et al.* 2006; Kumar *et al.* 2010). Cypermethrin dose used in present study and by farmers are near to lethal concentration, hence resulted in 100% mortality of notonecta and prawn (common fish fry predator in nursery pond) within 1 h of application. Elimination of notonecta and prawn, seems to add to higher survivability of fish fry as perceived by farmers. The mortality of all three macrofaunal species (fish fry, notonecta and prawn) became negligible after 72 h (3rd day).

Our results show that stocking of carp fry in nurseries three days post application of cypermethrin could be offering the triple advantage of predator elimination, prey availability and less pesticide induced mortality for the fry, explaining the farmers' observations. It is also evident that cypermethrin contamination is dangerous to aquatic ecosystems, and this fact should be taken into consideration when this insecticide is used in agriculture or in the control of mosquito populations. In addition, potential risk from cypermethrin metabolites should be investigated to get a more complete picture in terms of toxicity.

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Abbreviation used: 0⁽⁻⁾ h: one hour before application of cypermethrin; 0⁽⁺⁾ h: immediately after application of cypermethrin; L: liter; μ : micro; μg : micro-gram; L^{-1} : per liter; ng: nano-gram; μM : micro-meter; mL: milli-liter; μL : micro-liter; kg: kilogram; ha: hectare; ha^{-1} : per hectare; wt: weight; V^{-1} : by volume; SSP: Single super phosphate; h: hour; SE: standard error

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