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Screening of Commercial Herbicides for Controlling Algae Growth in Aquarium

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Abstract: This study was conducted to screen the commercial herbicides for algae control in the aquarium. Three herbicides of ametryn, atrazine and metribuzine were tested at concentrations ranging from 0.625 to 10.00 ppm. It is found that ametryn was the most effective herbicide that inhibited algal growth. Ametryn concentration that caused 50% inhibition on algae growth after exposure for 21 days was 0.335 ppm. Survival rate of ornamental fish, Harlequin rasbora (*Trigonostigma heteromorpha*) and growth rate of aquatic plant (*Elodea canadensis*) exposed to 10 ppm ametryn were compared with those exposed to algaecide. The result showed that there was no significant difference in survival rate of Harlequin rasbora when exposed to ametryn, algaecide and dechlorinized tap water (control). However, growth rate of *E. canadensis* was lower after exposure of ametryn compared to those exposed to algaecide and tap water. These results suggest that ametryn has potential to be used as an algal inhibitor in aquarium.

Key words: Ametryn, *Elodea canadensis*, ornamental fish, *Trigonostigma heteromorpha*.

1. Introduction

Aquarium or ornamental fish are also called the "living jewels" due to their diversity of species, great variety of color, shape, behavior and origin. Keeping of ornamental fish is a hobby with worldwide interest. The ornamental fish sector is a widespread and global component of international trade, fisheries, aquaculture and development. Statistic reports shown by Food and Agriculture Organization (FAO) [1] indicated that the world export value in 1998 of ornamental fish was USD174 million with imports valued at USD257 million.

In Malaysia, aquarium fish trade started in the 1950s within the southern state of Johor, the activity was mainly limited to the collection of fish from the wild for subsequent distribution to Singapore, which had begun to export fish to Europe in the late 1940s. The potential for further expansion of the industry in Malaysia is enormous because the government provides various support services and fiscal incentives to promote the development of the industry. In 1993, it was estimated that 188 million pieces of ornamental fish which are more than 250 species of mostly freshwater species were produced valued at about RM33.65 million. The industry continued to grow rapidly and the production had increased more than 30% with 253 million of fish valued at RM49.13 million by the 1995 [2]. Although this industry has developed steadily nowadays, aquarists are always facing with algae control problems. As an ornamental fish keeper, the clean and clear of aquarium is the most important aspect that makes beautiful and clear view. Presence of algae makes the aquarium glass

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look dirty with “green dot” algae and the water looks green and cloudy.

According to Freedman [3], herbicides are used to protect crop plants from competition with abundant and unwanted plant species. In aquaculture industry, aquaculturists employ herbicides to control aquatic plants by severely interrupting their growth. However, herbicides with a high concentration might damage the ecosystem [4]. Hence, it is important for aquaculturists to use the registered herbicides in a safe and effective manner.

The production and demand of ornamental fish has increased remarkably in Malaysia during recent years. Again, entrepreneurs in aquarium industry will be encounting with algae problems. Although there are many commercial anti-algae growth products available in the market, not all of the algaecides are effective to control all types of algae besides being costly. Acute toxicity of various herbicide groups has been tested on cyanobacteria [5] and green algae [6]. Ma et al. [7] have demonstrated that green algae *Raphidocelis subcapitata* are very sensitive to herbicide like atrazine, ametryme, simazine, prometryne, cyanazine, isoproturon, chlorotoluron, diuron, methabenzthiazuron and paraquat. However, to date, there is still limited study on effect of herbicides on algae found in aquariums. Thus, this study was designed to access the potential of commercial herbicides as algaecides for aquarium purpose and aimed to screen for suitable commercial herbicides at the optimum dosage on inhibition of algae growth as well as to identify the algae species attached onto the aquarium.

2. Materials and Methods

2.1 Materials

This study was conducted at the freshwater hatchery of University Malaysia Terengganu (UMT). The sources of the algae were obtained from the freshwater hatchery of UMT. The commercial herbicides used in this study were ametryn, metribuzine, and atrazine. All the commercial herbicides products used in this experiment were prepared to obtain the concentrations at 0, 0.0625, 0.125, 0.25, 0.5 and 10.0 ppm. Harlequin rasbora and *Elodea canadensis* were purchased from an ornamental fish shop.

2.2 Dose-response Tests

A total of 15 homogenous aquariums were set up with 35 L of water at 28 °C. Gentle aeration and a photoperiod of 12L:12D were provided. Prior to exposure to the different concentrations of herbicide, 100 mL of cultured algae cells from the stock cultured algae were introduced into each aquarium. Then, the prepared herbicide and 0.1 mL L^{-1} additional nutrients (Chelated Ferrite Liquid)® were added as a fertilizer for aquatic plants into each aquarium. As a static experiment, the experimental mediums (aquariums water) were not renewed or changed. This experiment was conducted in three week times and observation was carried out daily. Area of algae on each side of the aquariums was measured by using quadratic method. Algae attached onto glass surface were stripped off by using a glass stripper and the algae were observed under the compound microscope (Motic microscope) at 100 × magnification for identification. The experiment was arranged as completely randomized design with three replicates for each treatment.

2.3 Effects of Ametryn on Ornamental Fish and Aquatic Plant

Ametryn was selected to study the effects of ametryn on ornamental fish and aquatic plant in a man-made aquatic eco-system experiment based on results from the dose-response tests. A total of 25 Harlequin rasbora (*Trigonostigma heteromorpha*) and three aquatic plants (*Elodea canadensis*) with stem length 30 cm were introduced into each aquarium tank filled with ametryn solution at 10 ppm or tap water. A total of three treatments, namely, ametryn, algaecide and tap water (control) with three replicates were examined in this experiment. Survival rate of Harlequin rasbora were recorded daily. Initial and final lengths of *E. canandensis* plants were measured to determine the growth rate of the plants.

2.4 Statistical Analysis

Concentration of each herbicide that causes 50% mortality (ED50) was obtained based on probit analysis. Survival rate and growth rate of respective *Trigonostigma heteromorpha* and *Elodea canadensis* were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-test comparison ($P < 0.05$).

2.5 Comparison of Herbicide and Algaecide Cost

Application cost of ametryn and algaecide was compared to reveal either ametryn or algaecide recorded less value of price for each usage. Volume of ametryn and algaecide for each usage were recorded to determine its price value per usage.

3. Results and Discussion

Percentage of algae inhibition increased with herbicide concentrations where 10.00 ppm of ametryn, atrazine and metribuzine recorded 99%, 98% and 99% inhibition, respectively, compared to the control (Fig. 1). All the tested concentrations of each herbicides exhibited more than 50% of algae inhibition. At the lowest concentration of 0.625 ppm, ametryn, atrazine and metribuzine recorded 71%, 72% and 73% algae inhibition, respectively. Based on probit analysis, dosage which caused 50% algal inhibition (ED50) of ametryn, atrazine and metribuzine were observed at 0.224, 0.230 and 0.335 ppm, respectively. The survival rate of Harlequin rasbora was not significantly different when aquariums were subjected to ametryn (87%), algaecide (85%) and control (93%) (Table 1).

Elodea canadensis treated with ametryn exhibited lower growth rate as compared to the control. The aquatic plants recorded growth rate at 0.1, 0.4 and 0.8 cm per day, after being exposed to ametryn, algaecide and control, respectively (Table 2). However, leaf discoloration observed in *E. canadensis* plant was evident when exposed to ametryn as compared with that exposed to algaecide (Fig. 2). According to McEwen and Stephenson [8], triazine herbicides like ametryn accumulated in leaf cells where water loss by transpiration was the greatest at the leaf chloroplast. Application of herbicide-ametryn as the inhibitor of algae in the aquarium is cheaper than algaecide. A single aquarium subjected to ametryn treatment just costs only RM0.05 per usage and it can sustain for three weeks. Comparatively, algaecide treatment recorded higher value of price per usage that is RM0.13 (Table 3). Furthermore, application of algaecide in the aquarium must be carried out weekly to maintain effective control of algae growth. This result indicates that ametryn is more cost-effective than algaecide for control of algae growth in the aquariums.

Algae growth is found to be greatly inhibited at the highest dose of 10.00 ppm for each herbicide. Among three herbicides used in dose-response experiment, ametryn had the most effectiveness while metribuzine had the least. Ametryn recorded the lowest value of ED50. However, ED50 values for these herbicides are lower than the lowest herbicide concentration tested (0.625 ppm). The same observations have been reported by other studies on the effects of some herbicides on other species of algae. Shane [9] reported that 10 ppm of ametryn gave mortality to blue-green algae (*Plectonema boryanum*) in a static experiment for 14 days.

The results of present study show that survival rates of Harlequin rasbora after exposure of ametryn and algaecide had no significant difference compared to the control. A previous study by Tooby et al. [10] has demonstrated that herbicide-carbetamide caused mortality to Harlequin rasbora at concentrations below 165 ppm. According to study [11], mortality of goldfish (*Carassius auratus*) was evident at 14 ppm ametryn after exposure for 96 h.

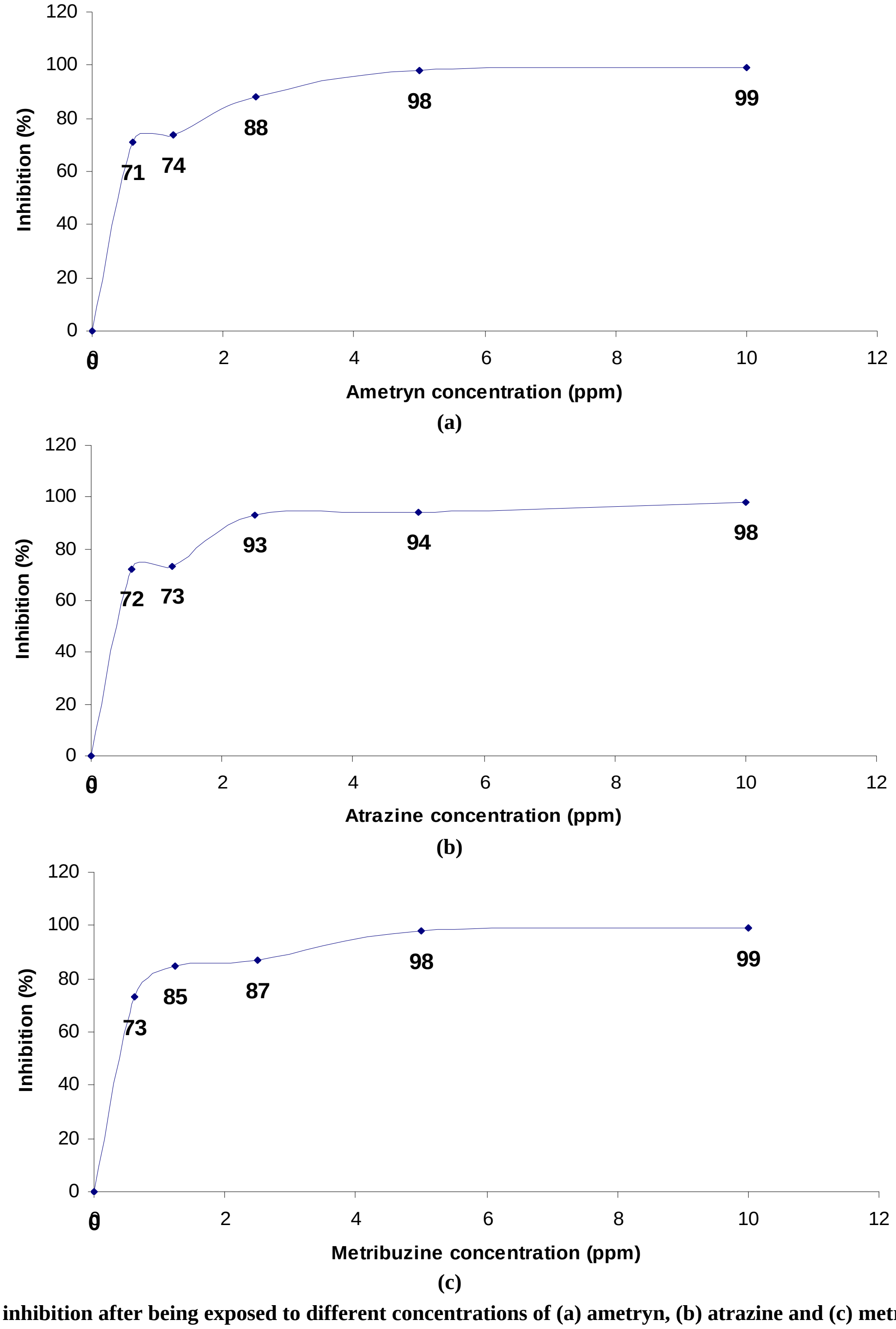

Fig. 1 Algae inhibition after being exposed to different concentrations of (a) ametryn, (b) atrazine and (c) metribuzine.

Some triazines are moderately irritating to the eyes, skin and respiratory tract. However, systemic toxicity is unlikely unless large amounts have been ingested [12]. According to McEwen and Stephenson [8], a large number of studies on mice and rats have failed to demonstrate ametryn effects except at very high doses. Thus, the 10 ppm ametryn is not toxic to human and safe to be used. For several good reasons, ametryn should be handled and applied only with full attention to safety measures that minimize personal contact.

Table 1 Survival rate of Harlequin rasbora after being treated with ametryn, algaecide and control.

Treatments	Survival rate (%)	
	Day 1	Day 21
Ametryn	100	*[a] 87 ± 6
Algaecide	100	[a] 85 ± 6
Control	100	[a] 93 ± 2

*Means with similar letter within the same column are not significantly different as determined by Tukey-test at the 5% of significant level.

Table 2 Growth rate of *Elodea canadensis* after being treated with ametryn, algaecide and control.

Treatments	Initial length (cm)	Final length (cm)	Growth rate (cm/day)
Ametryn	30.0 ± 0	31.8 ± 0.8	*[a]0.10 ± 0.04
Algaecide	30.0 ± 0	37.8 ± 2.3	[b]0.40 ± 0.11
Control	30.0 ± 0	47.4 ± 2.0	[c]0.80 ± 0.10

*Means with similar letter within the same column are not significantly different as determined by Tukey-test at the 5% of significant level.

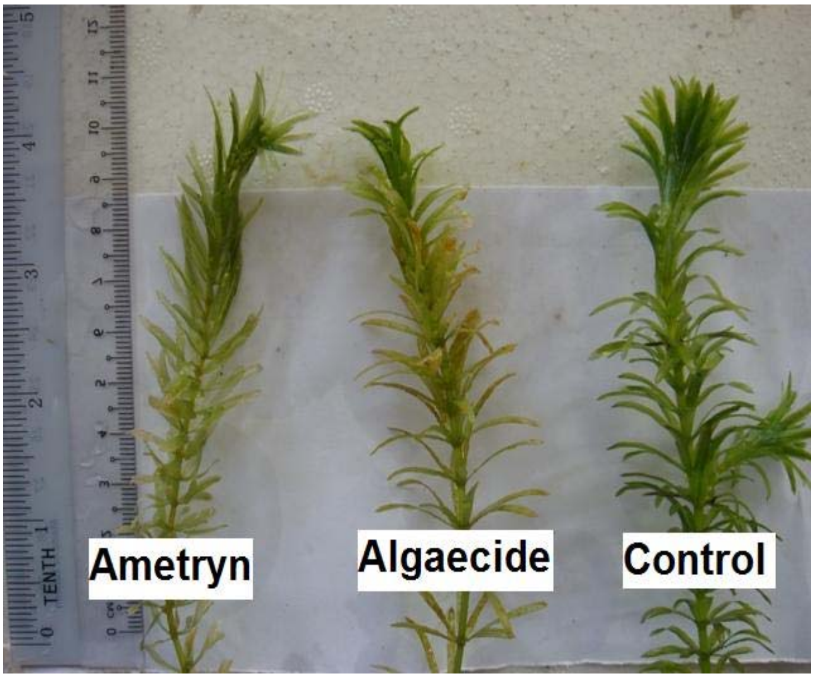

Fig. 2 Green color discoloration of *Elodea canadensis* leaves after exposed to ametryn, algaecide and control, respectively for 21 days in aquariums.

Table 3 Cost comparison between ametryn and algaecide.

	Ametryn	Algaecide
Commercial price	RM 11.80/500 mL	RM 16.00/60 mL
Usage	2.25 mL	0.5 mL
Sustainability	3 weeks	1 week
Price/usage	RM 0.05	RM 0.13

Based on the result, there are two groups of algae found in this study, namely those free living and non-free living algae which attached onto the glasses of the aquarium. The free living algae have smaller size than non-free living algae. The algae samples found in this study are shown in Fig. 3. In order to differentiate the algae species (Table 4), observation was made under compound microscope to identify them based on freshwater algae taxonomy.

4. Conclusions

Ametryn is the most effective triazine herbicide that could inhibit algal growth in the aquarium as compared with atrazine and metribuzine. The results of dose-response experiments show that the ED50 value of ametryn on growth inhibition of algae is 0.224 ppm. The survival rate of Harlequin rasbora after exposure of 10 ppm ametryn, recommended dose of algaecide and tap water (control) are not significantly different, suggesting that 10.00 ppm ametryn is not toxic to *Harlequin rasbora*. In addition, ametryn could inhibit growth of *E. canadensis* plants with lower growth rate recorded than those exposed to algaecide. In term of cost-effectiveness, ametryn has

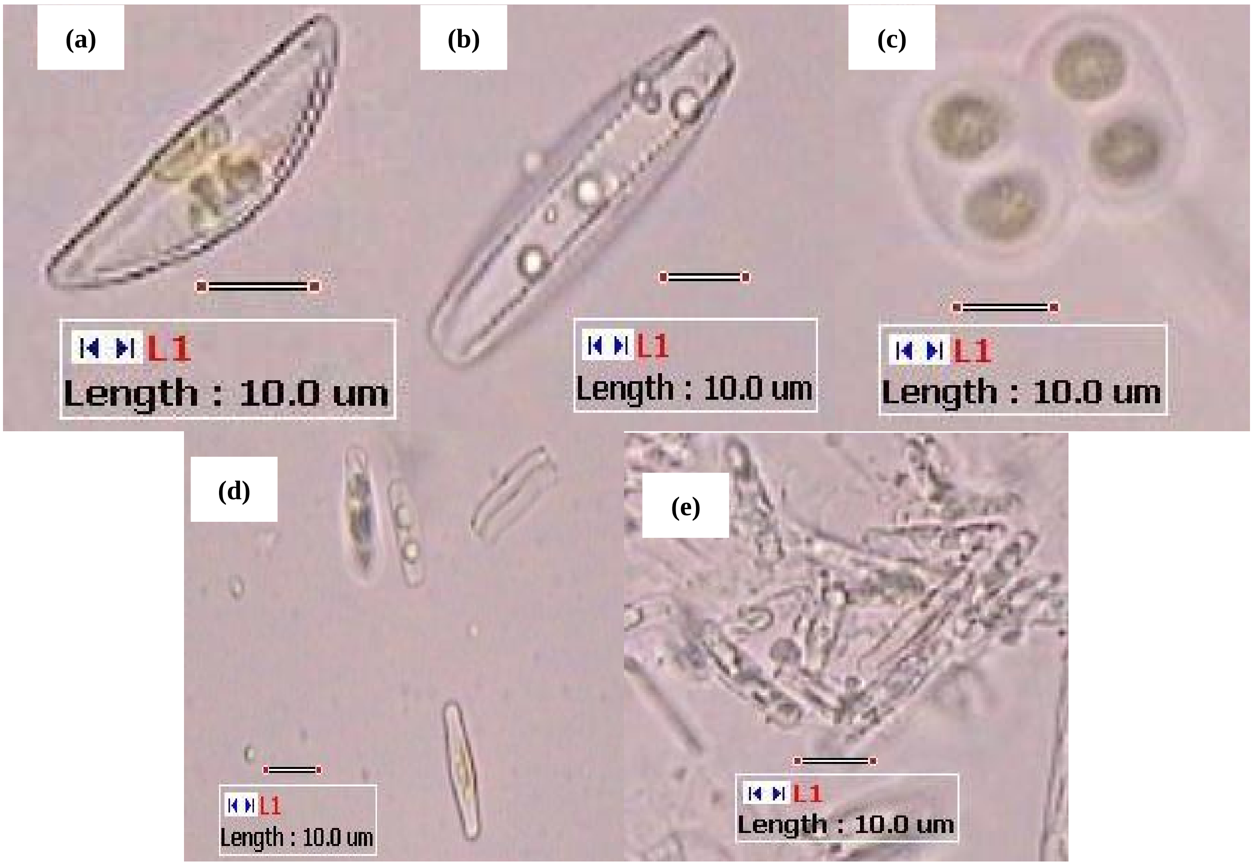

Fig. 3 Algae that attached on the glass of the aquariums: (a) *Navicula* sp., (b) *Gloeocapsa* sp., (c) *Closterium* sp., (d) *Tetradesmus* sp. and (e) *Ectocarpus* sp..

Table 4 Taxonomy of the identified algae.

Taxonomy
Bacillariophyta (Diatom) Phylum: *Bacillariophyta* Family: *Bacillariophyceae* Genus: *Navicula*
Cyanophyta (Blue-Green, Prokaryotic Algae) Phylum: *Cyanophyta* Order: *Chroococcales* Family: *Chroococcaceae* Genus: *Gloeocapsa*
Bacillariophyta (Diatom) Phylum: *Bacillariophyta* Family: *Bacillariophyceae* Genus: *Closterium*
Bacillariophyta (Diatom) Phylum: *Bacillariophyta* Family: *Bacillariophyceae* Genus: *Tetradesmus*
Phaeophycophyta (Brown algae) Phylum: *Phaeophyta* Family: *Phaeophyceae* Genus: *Ectocarpus*

advantage over algaecide for algal growth control. Based upon the results of the study, ametryn has potential to replace algaecide as an inhibitor of algal growth in the aquarium.

Acknowledgments

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