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Chemical control of water hyacinth by some herbicides and their effect on some aquatic invertebrate

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Abstract

Water Hyacinth (*Eichhornia crassipes*), regarded as one of worst aquatic weeds in the world, has been an invader in northern Iran, particularly in the Anzali Wetland. Herbicide application as a control method with respect to ecosystem health has been investigated. The effects of three herbicides, glyphosate (Roundup), Glufosinate-ammonium (Basta), and Bispyribac sodium (Nominee) were investigated on water hyacinth and on the survival of five aquatic invertebrates from the Anzali Wetland including Hemiptera, Amphipoda, Odonata, Ostracoda and Daphnia. The treatments consisted of 3 L/ha of glyphosate, 5 L/ha of Glufosinate-ammonium, and 0.3 L/ha of Bispyribac sodium. European Weed Research Council (EWRC) rating scale determining reduction of wet and dry weight of shoot was the basis of assessment to determine the effectiveness of the herbicides in the control of water hyacinth. All herbicides were effective on water hyacinth while Roundup caused a significant reduction of shoot biomass and scored 98% on the EWRC scale. Bayesian mediation model was used to calculate total and decomposition effect of herbicides on animal groups. Based on the Bayesian mediation model, Basta showed the best performance with lowest probability of a negative effect ($P_{\text{Eff}<0}=0.22$). The accuracy of dosages and spraying of herbicides can be considered the most effective in inhibiting water hyacinth and the least destructive to living organisms.

Key words: Glyphosate, Glufosinate-ammonium, Bispyribac sodium, Water Hyacinth, Anzali Wetland

Introduction:

Water Hyacinth (*Eichhornia crassipes*), a free-floating plant, is regarded as one of the world's worst weeds (Holm et al. 1977; ISSG 2021), being ahead of the eight worst water weeds. It is known to have originated from the Amazon basin of Brazil in South America. It is an invader to many countries and is one of the most widespread aquatic invasive species (Gopal 1987). It is known to cause significant ecological and socio-economic effects; water loss through evaporation, obstruction of navigation, recreation and fishing, blockage of irrigation,

hydroelectricity and drainage systems (Gopal 1987; Holm et al. 1977; Villamagna and Murphy 2010).

Although, water hyacinth can be used in the production of biogas (Kunatsa and Mufundirwa 2013; Nugraha and Pradita 2020; Priya et al. 2018) and in the extraction of some phenolic compounds (Lalitha et al. 2012; Rufchaei et al. 2021; Shanab et al. 2010), if other control measures are not implemented soon the problem will worsen and will become more difficult to be solved.

Herbicides application as a control method is usually less expensive than mechanical control, but it has to be accountable for human and ecosystem health.

Although herbicides are used to control pests, they present some risks to aquatic environments and affect the biological community. The effects of these toxins have been reported on the soil and its microorganisms as well as on non-target species in aquatic ecosystems (Audus 1949; Burnet and Hodgson 1991; Folmar et al. 1979; Nyström et al. 1999; Sarikaya and Yılmaz 2003; Sihtmäe et al. 2013; Tsui and Chu 2003).

Evaluating the hazards of pesticides to the aquatic environment revealed considerable differences in sensitivity to pesticides among species. Peterson *et al.*, (1994) found considerable differences in sensitivity among 11 aquatic photosynthetic organisms exposed to 23 different agricultural pesticides.

Previously, Aqua-Kleen (2,4-D) applied at the range of 1-12 kg/ha and Copper sulfate and copper chelate applied at a rate of 3.5 mg/l provided effective chemical control and inhibited the growth of *E. crassipes* but were toxic to fish, birds, some mammals, aquatic and land invertebrates and other aquatic plants (Burton 2005; Lugo et al. 1998; Roshon et al. 1999).

The continuous exposure of 2,4-D, dissolved in water, on tench (*Tinca tinca* L) revealed marked alteration of hematopoietic tissue (Gomez et al. 1998). A widespread hemorrhage in excretory and digestive systems and enlargement in the liver were revealed in common carp (*Cyprinus carpio*), subjected to different concentrations of 2,4-D (Sarikaya and Yılmaz 2003). The genotoxicity of this herbicide was also observed in freshwater air-breathing fish *Channa punctatus* (Farah et al. 2003).

Among herbicides, glyphosate is used extensively as a non-selective herbicide to control weeds in agriculture and its use is likely to increase further against crops which have been genetically modified (Bradberry et al. 2004), which applied at 2 kg/ha, will kill water hyacinth in 8 weeks (Burton 2005; Gopal 1987). Glufosinate-ammonium (Basta) also is a non-selective contact herbicide with some systemic action. Translocation occurs only within leaves, predominantly from the leaf base to the leaf tip. It rapidly degrades in surface levels of soil, and in water, because of polarity, and its metabolites do not bioaccumulate (Turner 2018). Conversely, Bispyribac sodium (Nominee) is a selective, systemic post-emergence herbicide, absorbed by foliage and roots (Turner 2018).

A pilot demonstration of the herbicidal control of water hyacinth using glyphosate was undertaken at Ere, Nigeria (Adekoya 2015). Five eco-friendly chemical compounds were investigated to control water hyacinth among which Glyphosate and Acetic acid performed well (Agidie et al. 2018). Robichaud and Rooney (2021) investigated the direct over-water application of glyphosate to control the invasive aquatic plant, *Phragmites australis*.

Over 100 peer-reviewed papers have been published by scientists on the detrimental effects of glyphosate in increasing diseases in plants, animals and humans (Sirinathsinghji 2012). The Assessment Group on Glyphosate (AGG) of EU released a statement which concluded that glyphosate meets the approval criteria for human health and its amendments for the approval as an active substance to be used in plant protection products. However AGG offers that the current classification “Toxic to aquatic life with long lasting effects” should be taken (Anonymous 2021a), a lower environmental impact measures than most synthetic herbicide

alternatives have been reported for aquatic and terrestrial organisms (Blake and Pallett 2018; Duke 2020; Solomon 2020).

Water hyacinth is an important substratum for invertebrate colonization, as Rocha-Ramirez et al. (2007) found a highly diverse invertebrate assemblage within root masses of *Eichhornia crassipes* in a coastal lagoon of Mexico. Also a positive correlation was reported between the water hyacinth surface area and epiphytic macro invertebrate densities (Villamagna and Murphy 2010).

Water hyacinth was detected in several natural areas of the northern part of Iran where it is distributed with high abundance (Amini Rad 2017; Mirzajani et al. 2019; Mozaffarian and Yaghoubi 2015) making its control program absolutely necessary.

The Anzali Wetland, as one of the most important freshwater ecosystems in the southern Caspian Sea, is confronted with many problems and negative factors, and has been listed as priority site for conservation in the Montreux record (Mirzajani et al. 2021; Naderi et al. 2017). Recently, the high density of water hyacinth in the Anzali Wetland has become a management challenge because many areas are inaccessible to mechanical removal (Mirzajani et al. 2019). Therefore, stopping its growth or removal of the plant through chemical treatment was taken into consideration. However, although information regarding the toxicological risks of many herbicides such as glyphosate is universally known, the effects of some other new toxins commonly used to control weeds around the Anzali Wetland particularly in the rice fields are unknown.

Thus, the present study investigates the short-term effects of herbicides on the survival of macro-invertebrates from the Anzali Wetland. Because of the variability of the responses of organisms to pollutants, the toxicological risks of three herbicides on five species were assessed.

Material and Methods:

The effects of three herbicides glyphosate (Roundup), Glufosinate-ammonium (Basta), Bispyribac sodium (Nominee) were investigated on water hyacinth and on the survival of five species of aquatic invertebrates (Table 1).

The experiment was performed in 48 aerated plastic aquaria (open area 0.05 m², volume 8 liters) using four liters of water from the Anzali Wetland water was used in each aquarium. Each aquarium was stocked with 3 plants of water hyacinth with a total number of 5 to 7 leaves, covering about half of the aquarium surface. Three dominant macro-invertebrates species of the Anzali Wetland were collected as experimental specimens. For any herbicide treatment, the macro-invertebrates were separately investigated and were stocked in the aquaria with initial counts of 10 Hemiptera (*Pelocoris* sp.), 12 Amphipoda (*Obesogammarus* sp.) and 15 Odonata (*Coenagrion* sp.). Two zooplankton species Ostracoda and *Daphnia magna* were reared in a laboratory and included in the experiment design as macro invertebrates with initial counts of 50 individuals. There were three replicates for each herbicide treatment and also for the control groups without adding herbicide.

To start the toxicity tests, herbicide solutions were previously diluted many times based on the application dosage of 3, 5, and 0.3 L/ha for Roundup, Basta and Nominee, respectively. Depending on the surface of the leaves in the aquaria, 50% of each herbicide solution was sprayed on the leaves and the remaining was directly added to the water. In fact, the probability of the effects of herbicide was considered assuming its entry into the aquatic environment. Herbicide treatments (except the Nominee) were applied twice with an interval of 5 days. The concentration of herbicides in each aquarium was calculated after the volume of each aquarium was accurately determined (Table 1).

The photoperiod was 16/8-h (light/darkness), the temperature was measured at 25.0 ± 0.3 °C. After 10 day the aquaria were emptied and the final number of macro-invertebrates was counted. The metamorphic stages of many *Coenagrion sp.* was completed during the experiment and were removed from each aquarium. The final number of *Obesogammarus sp.* juveniles was also counted.

European Weed Research Council (EWRC) scoring system was used to quantify damage of water hyacinth (Sandral et al. 1997).

Percent inhibition of herbicides on water hyacinth was calculated through the following equation for the quantitative measurement (Ghosh et al. 2016; ISA 2009) where; A and B is the final wet or dry weight of water hyacinth in treatments and in the control group, respectively.

$$WCE = \left(\frac{A - B}{B} \right) \times 100$$

This equation was also used for percent frequency changes of organisms in treatments compared to the control.

Markov Chain Monte Carlo (MCMC) simulation was applied to determine which one of the herbicides has the best efficiency, comprehensively. We drew 100000 random samples based on the original data for each animal frequency as an input to establish a Bayesian mediation model using R programming language (R Core Team 2021). Finally, we calculated the total effects from differences in the mean effect on each animal group to the control level resulting in density plot of total probabilities.

Table 1) Names, chemical and physical properties, agricultural application dosage of herbicides (Data from NIH (2021)). Final dose of herbicides measured based on water volume in each aquarium.

Common name	Glyphosate	Glufosinate-ammonium	Bispyribac sodium
Alternative Commercial name	Roundup	Basta	Nominee
Formulation	Soluble liquid (41% active ingredients)	Soluble liquid (20% active ingredients)	Oil fluable (10% active ingredients)
Chemical name	N-(phosphonomethyl) glycine	2-amino-4-methylphosphinobutyric acid	Sodium 2,6-bis((4,6-dimethoxypyrimidin-2-yl)oxy) benzoate
Application dosage	3 L.ha ⁻¹	5 L.ha ⁻¹	0.3 L.ha ⁻¹
Half- life	35-63 days in water	300 days in water 3 days in soil	88 ± 15 days in water
Aqueous solubility	10.5 g. L ⁻¹	1370 g. L ⁻¹	73.3 g. L ⁻¹
Vapor pressure	9.8 X 10 ⁻⁸ mm Hg	232.5 mm Hg	1 X 10 ⁻⁷ mm Hg
Final dosage of active ingredient in aquarium	0.0031 mg.l ⁻¹	0.005 mg.l ⁻¹	0.000075 mg.l ⁻¹
Macro-invertebrate Final dosage of active ingredient in aquarium	0.008 mg.l ⁻¹	0.013 mg.l ⁻¹	0.0002 mg.l ⁻¹
Zooplankton			

Results

In this study, all herbicides were effective on water hyacinth while Roundup caused significant reduction of shoot wet and dry weight with the highest score (98%) of EWRC (Fig 1).

The herbicide effects on organisms have been illustrated in Fig 2. The final number of *Daphnia magna* increased in the Basta treatment while in the two other treatments it decreased compared to the control. Doubling the young *Daphnia* specimens has also shown to have good reproductive activity. The final number of Ostracoda adults was also nearly similar to that of *Daphnia magna* while its nauplius larvae decreased in the Basta treatment. Herbicide Nominee did not significantly decrease in the number of adults while it showed an increase in the nauplius larvae (Fig 2).

While the final number of Amphipoda was lower in the Basta treatment than in the control samples, it had nearly similar numbers with more than 93% survival in the other treatments (Fig 2). Survival of Odonata was lower in Nominee (about 80%) while the final number and dimorphism rate was similar to the control samples in the other treatments.

The Bayesian mediation model (Fig. 2) shows that the total negative effect of the pesticide Roundup is larger with the estimated probability of 94% of total negative effect ($PEff < 0 = 0.94$) compared to the Nominee with the 0.48 probability and Basta with 0.22 probability which is relatively the best performance. The decomposition graph shows that although the overall effect of Basta is small, there are still two groups of animals that are negatively affected (Amphipoda and nauplius larvae of Ostracoda). In contrast, the Nominee has negative effects on all groups except the Amphipoda and nauplius larvae of Ostracoda (Fig. 2).

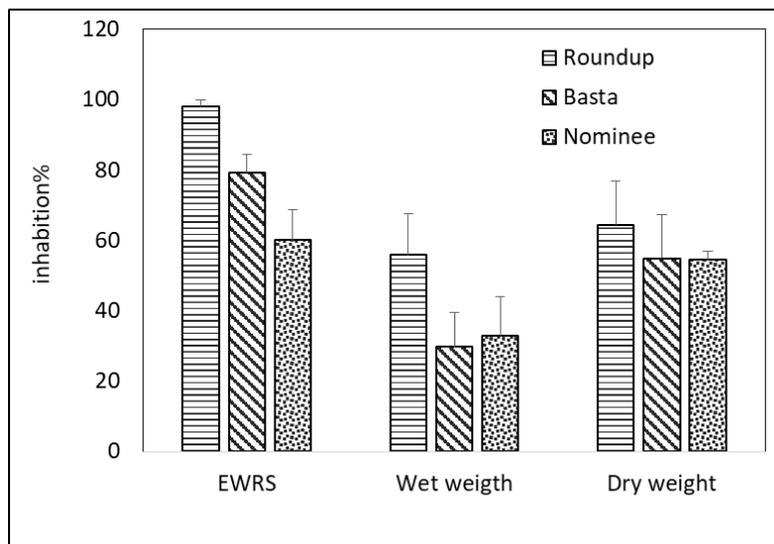


Fig. 1) Average (\pm SD) inhibition percentage herbicides for water hyacinth compared to control sample according to EWRC and weight.

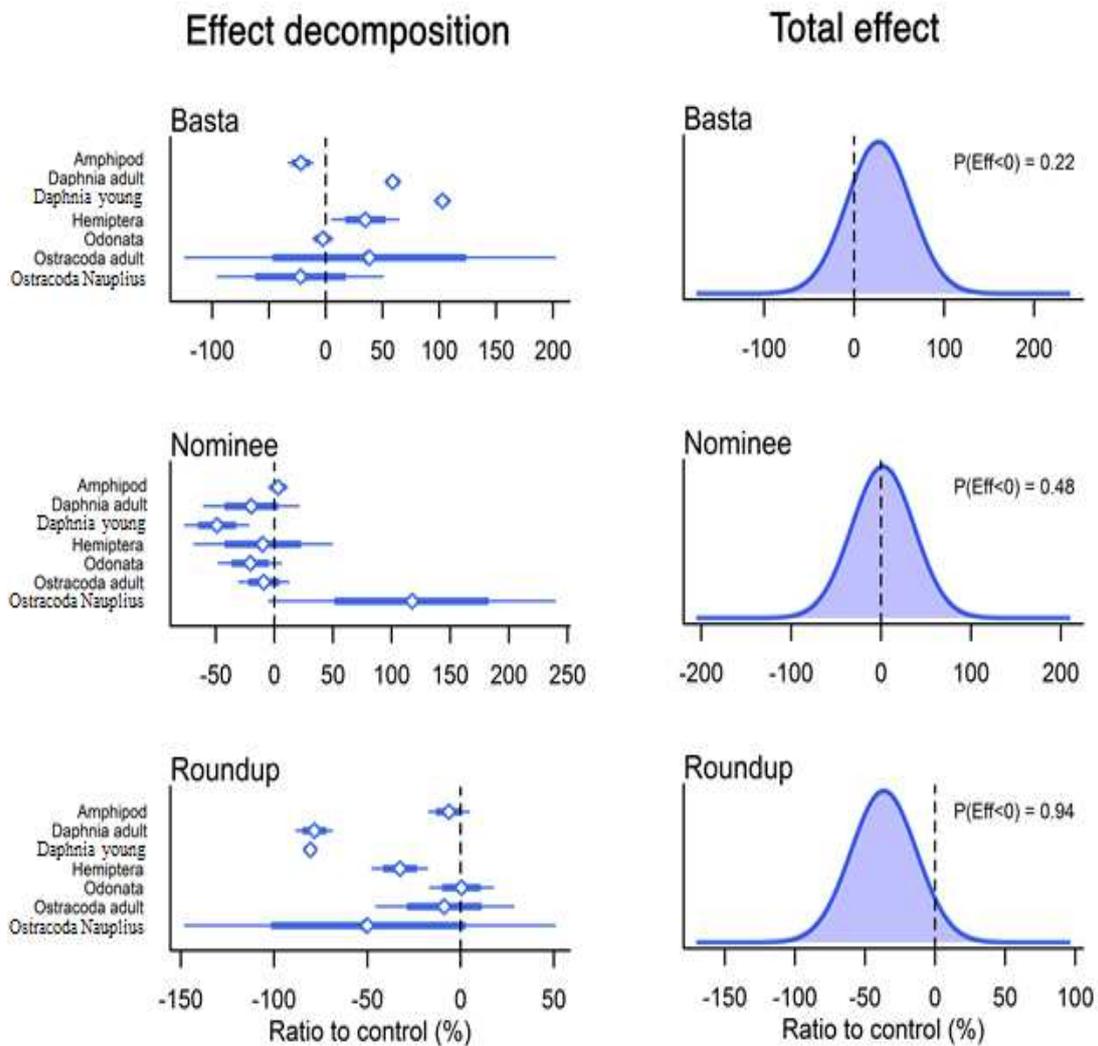


Figure 2. Bayesian mediation analysis of three herbicides (Basta, Nominee, and Roundup). Left graphs show the effect decomposition ratio in percentage to control treatment (dashed line). Right graphs show the Bayesian posterior distribution of total effect ratio. $P(\text{Eff} < 0)$ is the probability of negative effect (proportion of the distribution below zero).

Discussion

In this study, glyphosate had an immediate effect on water hyacinth and also had a greater inhibitory effect. In other investigations the effects of glyphosate herbicide on water hyacinth were also observed. For example based on the results of Adekoya (2015), after 4 weeks water hyacinth was completely controlled.

Glyphosate is used in more than 160 countries, with more than 1.4 billion pounds applied per year. As the most common herbicide in chemical control (Weidlich et al. 2020), its use on genetically engineered crops took off in the early to mid-nineties, and it is now the second most widely used U.S. lawn and garden weed killer (Grossman 2015).

Regarding the sensitivity of species to herbicides and pesticides, Peterson *et al.* (1994) showed that the label rate 4.27 (kg/ha) glyphosate was highly toxic to diatom species, cyanobacterium, *Aphanizomenon flosaquae* while it was relatively non-toxic to the remaining species. The results also showed that the glyphosate kill of the plants in less than the expected environmental concentrations and while it is unavailable to some organisms, it has general intrinsic phytotoxicity (Peterson et al. 1994).

The sensitivity of organisms to herbicides was also very diverse in this study and various reactions have been observed in the exposure of organisms to herbicides. No significant difference was observed for many treatments in the final number of organisms in comparison with the control group. While Basta and Nominee did not affect the Amphipoda and Odonata species, Roundup had a negative effect on most of the organisms.

Using glyphosate has been reported to be comparatively less dangerous than many other chemicals such as 2,4-D and copper sulfate (Burton 2005) and no mortality in fish and aquatic organisms was observed (Adekoya 2015). Although it is non-toxic to fish and slightly toxic to aquatic invertebrates (Burton 2005), glyphosate can show high uptake and absorption by aquatic organisms. Its toxicological risks to aquatic biota have been reported (Braz-Mota et al. 2015; Giesy et al. 2000; Mel'nichuk and Lokhanskaya 2007; Moraes and Rossi 2010; Roshon et al. 1999). Braz-Mota *et al.* (2015) reported increased DNA damage in red blood cells and cholinesterase inhibition in the brain of the Amazon teleost fish, *Colossoma macropomum*, exposed to two sub-lethal concentrations of glyphosate (10 mg L⁻¹ and 15 mg L⁻¹). They finally suggest that glyphosate is potentially toxic to tambaqui and possibly to other tropical fish species (Braz-Mota et al. 2015).

According to a literature review on glyphosate hazards in all agroecosystems, human diseases and fauna on the earth, it has been warned that the effects of glyphosate are difficult to predict and thus it is to be used with caution (Weidlich et al. 2020).

In this study glyphosate had an obvious effect especially on *Daphnia magna*. Because of high filtration of this species, as a commonly used species in toxicity bioassays, they have a greater potential to be affected by environmental contamination compared with that of other aquatic organisms (Lovern and Klaper 2006; McMahon and Rigler 1965; Sanchez-Bayo 2006). The toxicity of glyphosate herbicides (48% isopropilamine salt as the active ingredient) was investigated for *D. magna* and LC₅₀ (120 hours) was within the range of 1.75–6.75 mg/dm³ (Mel'nichuk and Lokhanskaya 2007). Similarly (Fig. 2), glyphosate induces a reduction in reproduction of *Daphnia magna* (Maycock et al. 2012). Glyphosate applied at 22 kg/ha did not significantly impact diatom densities or community structure in experimental forest streams (Sullivan et al. 1981), while its application dosage was much lower than 3 L/ha (1.23 kg/ha) in this study.

Although there is no significant difference between the numbers of Ostracoda nauplius larvae in the glyphosate treatment compared to the control group, it had the lowest number among the treatments (Fig 2 or table 1).

In another study by Houssou et al. (2021), the individuals of ostracod species, *Eucypris sp.* were exposed to 4.51 ppm and 9.03 ppm of glyphosate, 10%, and 20% of the median lethal concentration of herbicide for this species, respectively. They showed that Ostracod was able to reproduce 60% and 50% in these concentrations of glyphosate respectively, compared to 90% seen in the control treatment (Houssou et al. 2021). In the present study the final dose of glyphosate was much lower (Table 1) than that used in these investigations, while the final number of larvae was 50% that of the control group (Fig 2).

According to some recent assessments the acute toxicity of glyphosate for aquatic and terrestrial organisms is minimal to nil and it has a lower environmental impact than most synthetic herbicide substitutes (Blake and Pallett 2018; Duke 2020). Glyphosate is essentially reported to be nontoxic to terrestrial and aquatic animals by no expression of enzyme EPSPS (Solomon 2020). Robichaud and Rooney (2021) showed that glyphosate residuals did not reach concentrations thought to pose toxicological concerns to aquatic biota in water and sediment (Robichaud and Rooney 2021). In general, despite the possible negative effects, an effective strategy to prevent soil erosion and loss of nutrients will become a large problem without glyphosate and the fundamental changes in farming practices in the EU is impossible without much cost, at least in the short term (Kudsk and Mathiassen 2020).

Basta had no negative effects on *Daphnia* and the final numbers of both, the adults and nauplius larvae were much higher than in the control group (Fig. 2). Ostracods are also sensitive to the water quality and can be excellent indicators of their environment (Külköylüoğlu 2004; Sanchez-Bayo 2006; Schornikov 2000) as far as the widespread use of pesticides may decrease their species richness (Rossi et al. 2003). The freshwater ostracod *Eucypris* sp. showed a tolerance to glyphosate with the median lethal concentrations of 50.525 ppm in 24 h and 45.149 ppm in 48 h. The sub-lethal concentration of 9.03 ppm of glyphosate may have an effect on reproduction and on the survival of neonates and, consequently, the population growth over time (Houssou et al. 2021).

Both herbicides, Basta and Nominee had no significantly negative effects on Ostracoda and even the final number of Ostracoda nauplius larvae was much higher than that in the control sample in the treatment Nominee (Fig. 2). Acute oral LC₅₀ of Basta and Nominee for *Daphnia* are >668mg/l and >95mg/l (48 h), respectively (Turner, 2018). The organism sensitivity to glyphosate suggests that wetland contamination with this herbicide could alter natural assemblages and affect upper trophic levels. *Daphnia* and Ostracods organisms are a vital connection in the food chain between algae and fishes and are extremely important in the diet of some invertebrates and fish for example in the Anzali Wetland (Abbasi 2021) where we ultimately intend to use these herbicides.

In this study although Basta was observed to have the least effect on organisms in comparison with the other two herbicides, due to some its chemical characteristic such as its higher half-life, aqueous solubility and vapor pressure than the other herbicides (table 1) the environmental considerations need to be deliberated upon. According to application label of Basta, it has been recommended to not contaminate wetlands or water resources with this product. Its LC₅₀ for most fishes was higher than 1000 mg/l (96 h) and for *Oncorhynchus mykiss* it was 13.4 mg/l. The EC₅₀ (48 h) of Basta for aquatic invertebrates particularly *Daphnia magna* was determined as 17.8 mg/l. (Anonymous 2019). Therefore, the accurate dose of Basta can be the most effective on inhibiting of water hyacinth and causing the least destruction of living organisms. On the other hand, the chemical characteristics for Nominee and Roundup were lower (table 1), and generally Nominee had no negative effect on organisms (Fig. 2). The half lethal concentration and effective concentration (LC₅₀ and EC₅₀) of Nominee was reported in the range of 99-130 ppm (96h) for different organisms including fishes, crustaceans and mollusks (Anonymous 2021b).

The present research studied the direct effects of herbicides on the survival and reproduction of non-target organisms. It may be necessary to examine their full and realistic effects on a more detailed scale, such as molecular and DNA structure, especially for Basta and Nominee that had no significantly negative effects on organisms. Therefore, although the use of these herbicides is very effective in controlling water hyacinth, their long-term use will have biological and genetic consequences which should be considered. Moreover, the proper concentration of toxins and their accurate spraying only on plants can be a part of environmental considerations to conserve native biota.

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