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Effects of Using the *Valeriana officinalis* Extract During Transportation of Swordtail, *Xiphophorus helleri*

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Abstract: This experiment was conducted to test the *Valeriana officinalis* root extract during transportation of swordtail, *Xiphophorus helleri*. For the transport experiment, fish were distributed in 24 plastic bags of which 12 contained the *V. officinalis* root extract (1 g L^{-1}) (group B) and the remaining had no herbal extract and acted as control (group A). Transport lasted 24 h and every 6 h, 3 bags of each treatment were sampled. Survival, water quality parameters and whole-body cortisol were then studied. Results indicated that no significant differences ($p>0.05$) in the dissolved oxygen, pH, temperature and ammonia levels were observed between treatments at all the assay periods. Whole-body cortisol levels in group B was significantly ($p<0.05$) lower than control group. The survival rate (%) was significantly ($p<0.05$) higher in group B compared to the control group. The present research revealed that the application of *V. officinalis* root extract will be effective in suppressing stress responses in *X. helleri* and it can be recommended for ornamental fish transport.

Key words: Herbal extract, ornamental fish, survival, *Valeriana officinalis*, whole-body cortisol, Iran

INTRODUCTION

There is a growing commercial interest in the ornamental fish trade in Asia and all over the world. The annual international exports of ornamental fish in 2002 were about US$ 200 million in value (Vannuccini, 2004). Transportation is an important part of the ornamental fish export. The transport logistics involved inevitably stress the animals and causing mortality (Lim *et al*., 2003; Pramod *et al*., 2010). A variety of techniques have been developed to reduce the stress and mortality during the transport of fish. They include lowering the temperature (Phillips and Brockway, 1954; Lim and Chua, 1993; Lim *et al*., 2003) using of anaesthetics (Takashima *et al*., 1983; Guo *et al*., 1995), addition of drugs (Ling *et al*., 2000), in exchange resin (Bower and Turner, 1982; Lim and Chua, 1993) and using the probiotic (Gomes *et al*., 2009) in transport water. Many substances from different sources such as chemical agents, bacterial components, animal or plant extracts have been studied as prospective anti-stress for fish. Of these, plants are rich sources of safer and cheaper chemical compounds while simultaneously are non-toxic, biodegradable, biocompatible and cause no problems (Farag *et al*., 1989; Citarasu *et al*., 2002; Sagdic and Ozcan, 2003). In fact, some plants are rich sources of compounds like saponins, volatile oils, tannins, phenolic compounds, alkaloids, polypeptides and polysaccharides. These natural plant products have various activities like anti-stress, appetizer, antimicrobials and immunostimulants (Citarasu *et al*., 2003). Hence, it is advisable to select alternative anti-stress products from herbal plants. The principal objective of this study was to evaluate the effect of *V. officinalis* root extract on survival, water quality and whole-body cortisol levels of *X. helleri* during the transportation.

MATERIALS AND METHODS

Fish: The swordtail (*Xiphophorus helleri*) (2.49±0.62 g) were purchased from a commercial ornamental fish farm at Gorgan, Golestan, Iran. They were acclimated and kept in 200 L plastic containers with recirculated and aerated water for 10 days and fed with a commercial feed (34% crude protein and 4100 kcal CE kg^{-1} of crude energy) at 3% body weight twice daily. Moreover, fish were starved 48 h before the transport.

Preparation of the plant extract: Fresh *V. officinalis* roots were cleaned, washed and dried at 37°C for 3 days and ground well. Dried herbal powder was then soaked in sterilized fresh water (1:1 ratio) for 48 h (Eloff, 1998; Citarasu *et al*., 2006; Punitha *et al*., 2008). The slurry was

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then filtered with Wathman No. 1 filter paper and centrifuged for 5 min at 5000 rpm. In order to obtain dried extract, the extraction solvent was removed by using rotary evaporator (IKA® HB10 basic, China) at 40°C. Then, solvent-free extract was dried by using a freeze drier system (Operon: FDB-5503, Korea). Finally, the herbal extracts were stored at 4°C until use (Arabshahi-Delouee and Urooj, 2007).

Experimental design and transportation: After acclimatization, fish were distributed randomly into 24 plastic bags containing 22±0.2 g of fish L^{-1} of water of which 12 contained the plant extract (1 g L^{-1}) (group B) and the remaining had no plant extract and acted as control (group A). For the transportation, plastic bags were utilized with a total capacity of 20 L but filled with 5 L of water and the remaining filled with pure oxygen. The bags were then made airtight and placed in Styrofoam boxes for thermal isolation to prevent sudden changes in the temperature of the transport water. All the bags were kept at a controlled temperature of 24±1.5°C for 24 h.

Sampling: The survival rate, whole-body cortisol and water quality parameters were sampled from 3 bags of each treatment (A and B) at 6, 12, 18 and 24 h of transport. Each bag was sampled only once and then discarded from the experiment.

Quality parameters of the transport water: Temperature, pH and dissolved oxygen levels were measured with a digital meter (Horiba U10, Japan). Total ammonia was measured according to the method of Verdouw *et al.* (1978).

Whole-body cortisol: Whole-body cortisol was extracted according to the procedure described by Jesus *et al.* (1991) with slight modification described by Gomes *et al.* (2009).

Survival rate: The percent survival rate was calculated as follow: Survival (%) = (total live fish (no.) after t/total fish at 0 h (no.) × 100 where t is the time of experiment.

Statistical analysis: All data obtained from experiments were analyzed by a one-way analysis of variance (ANOVA) using the SAS package. Differences between means were determined and compared by Tukey's test. Significance was also set at 5% level.

RESULTS AND DISCUSSION

Water quality parameters: The data of the temperature and dissolved oxygen levels are shown in Fig. 1 and 2, respectively. There were no significantly differences (p>0.05) in temperature and dissolved oxygen levels between group A and B, although oxygen decreased to levels about 2.6 mg L^{-1} after 24 h of transport.

A gradual increase in pH levels was observed during the transport process (Fig. 3) which reached a maximum (about 8) at 24 h of transport. No significant differences (p<0.05) were observed in pH levels between treatments. Total ammonia levels showed an increasing trend but there were no significant differences (p<0.05) between treatments at all the assay periods (Fig. 4). Moreover, the highest total ammonia (about 0.8 mg L^{-1}) was observed at 24 h of transport.

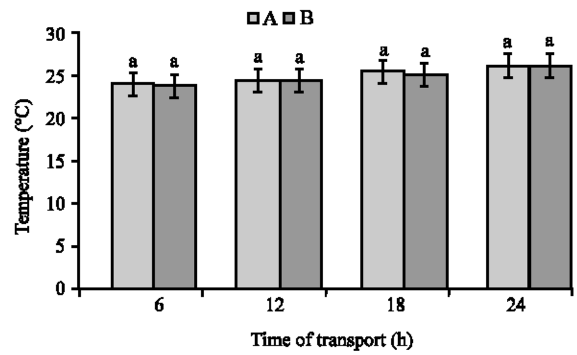

Fig. 1: Water temperature levels during transportation of swordtail (*Xiphophorus helleri*) with *V. officinalis* root extract (group B) and without addition of the herbal extract to the water (group A). Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

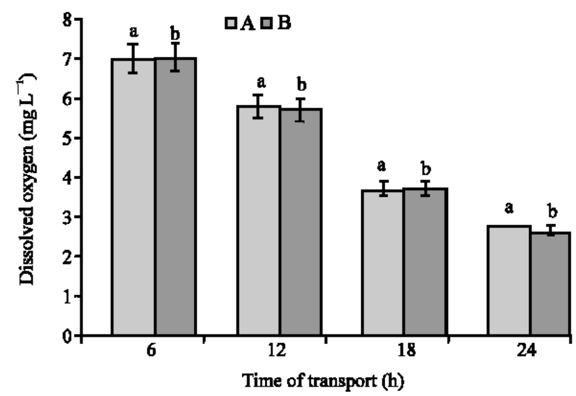

Fig. 2: Water dissolved oxygen levels during transportation of swordtail (*Xiphophorus helleri*) with *V. officinalis* root extract (group B) and without addition of the herbal extract to the water (group A). Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

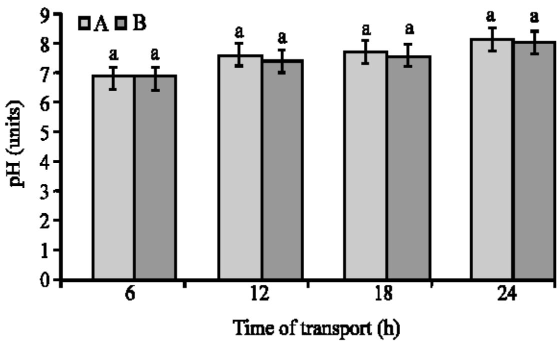

Fig. 3: Water pH levels during transportation of swordtail (*Xiphophorus helleri*) with *V. officinalis* root extract (group B) and without addition of the herbal extract to the water (group A). Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

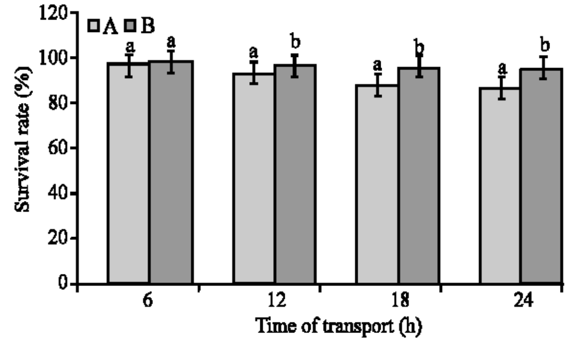

Fig. 5: Percentage survival of swordtail (*Xiphophorus helleri*) during transportation with *V. officinalis* root extract (group B) and without addition of the herbal extract to the water (group A). Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

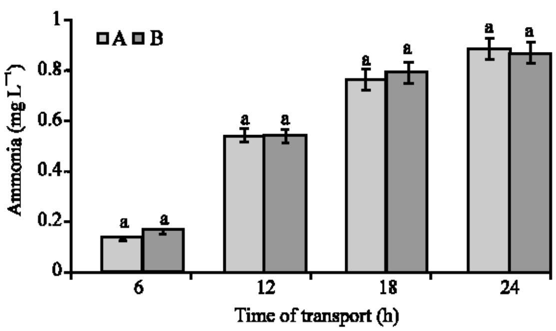

Fig. 4: Water ammonia concentrations during transportation of swordtail (*Xiphophorus helleri*) with *V. officinalis* root extract (group B) and without addition of the herbal extract to the water (group A). Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

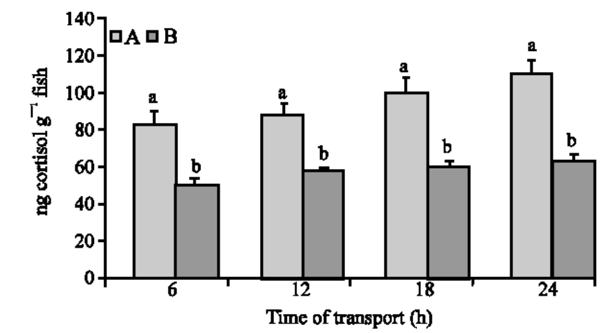

Fig. 6: Whole-body cortisol levels of swordtail (*Xiphophorus helleri*) during transportation with *V. officinalis* root extract (group B) and without addition of the herbal extract to the water (group A). Data are expressed as mean±S.E. Statistical differences (p<0.05) between groups are indicated by different letters (a and b)

Survival rate: The survival percentage of fish in group B was significantly (p<0.05) higher than control group at all the assay periods (except on 6 h of transport) (Fig. 5).

Whole-body cortisol: Whole-body cortisol levels were significantly (p<0.05) lower in group B over all the assay periods as compared to the control group (Fig. 6).

The dissolved oxygen levels measured during transportation are in accordance with the one reported by Geisler and Annibal (1984) and Gomes *et al.* (2009) transportation of tropical fish and probably did not cause mortalities. During the transportation, oxygen levels decreased to about 2.6 mg L^{-1} and these levels can not cause mortality in *X. helleri*. To support this, swordtails can tolerate a moderately wide range of dissolved oxygen (>2.0 ppm) (Englund, 2002). Moreover, the low oxygen levels were similar in both treatments. In the present study, the temperature levels were similar to those measured by Gomes *et al.* (2009) and the same results were also reported by Froese (1998) during transportation of tropical fish.

These levels can not cause mortality in *X. helleri*. In fact, they can tolerate a wide range of temperature (10-30°C) (Englund, 2002). Moreover, the results of the temperature levels showed that there were no significant differences (p>0.05) between treatments over all the assay periods. Results of the present study also indicated that pH and ammonia levels were increased in both treatments. In addition, there were no significant differences (p>0.05) between treatments at all the assay periods. These pH and ammonia levels can cause mortalities in some fish species, but not for *X. helleri* as these levels are similar to those observed in the wild. Englund (2002) reported that *X. helleri* can tolerate total ammonia to 1.0 ppm and a pH range of 7.0-8.1.

Current ornamental fish transporting practice focus on reducing stress imposed on the fish through the management of metabolic rate and control of metabolic wastes of transport water (Lim *et al.*, 2003). As the alternative to enhance the stress resistance of the fish, application of natural products like plant extracts is a new developing venture which would enable the fish to overcome the stressful conditions during transportation better. Many herbs have been used as traditional medicine for human beings for thousands of years in world and one of them, *Valeriana officinalis* is known for its anti-stress properties.

It is generally accepted that whole-body cortisol levels of unstressed fish are lower than stressed fish (Gomes *et al.*, 2009). In the present study compared with control group, whole-body cortisol levels were significantly ($p<0.05$) lower in group B at all the assay periods. This revealed that the *V. officinalis* root extract we used in this study helped to decrease the whole-body cortisol during the transportation and fish could tolerate the stressful conditions during transportation better. This is probably due to the capability of plant extract to reduce the effects of stressors. Similarly, lower cortisol response in fish was reported by Gomes *et al.* (2008) during the transport process. The results of the present study demonstrated that there is a good correlation between whole-body cortisol and survival rate. As the value of whole-body cortisol increased, the value of survival decreased. Hence, high cortisol concentrations were the main reason for mortalities in control group during the transport process.

CONCLUSION

Based on above results it can be concluded that the *V. officinalis* root extract we used in this study could increase the survival of *X. helleri* during transport. The significantly lower cortisol levels of fish in group B compared with group A demonstrate that *V. officinalis* root extract was efficient in suppressing stress responses in fish. Moreover, further studies are needed to determine the molecular mechanisms beside the isolation and characterization of the active compounds from the *V. officinalis* root.

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