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Methanol extract of *Nyctanthes arbortristis* seeds enhances non-specific immune responses and protects *Oreochromis mossambicus* (Peters) against *Aeromonas hydrophila* infection

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Highlights

- *Nyctanthes arbortristis* seed methanol extract enhances non-specific immune response of *Oreochromis mossambicus*.
- Serum non-specific immune parameters – lysozyme, myeloperoxidase, antiprotease increased significantly compared to control.
- Antibody production and cellular ROS production were significantly higher in methanol extract treated groups.
- The relative percent survival of treatment groups were higher.

- *N. arbortristis* seed extract can be used as immunostimulant in aquaculture industry.

Abstract

Immunostimulation using medicinal plant extracts is a promising approach for prevention and control of diseases with reference to sustainable fish farming. *Oreochromis mossambicus*, dubbed as aquatic chicken is a cultured fish worldwide and a laboratory model organism. *Aeromonas hydrophila* is one of the major bacterial pathogens in fish farming that causes huge loss to aquaculture industries. In this study, we investigated the efficacy of methanol extract of *Nyctanthes arbortristis* seeds on disease resistance of *O. mossambicus* against live virulent *A. hydrophila*. We also investigated its effect on the non-specific immune parameters such as serum lysozyme, myeloperoxidase, antiprotease and specific immune parameters in terms of specific serum antibody titres assayed by bacterial agglutination test. Our studies indicate that intra-peritoneal administration of 20mg/kg methanol extract increases the Relative Percent Survival (RPS) of *O. mossambicus* challenged with LD₈₀ of *A. hydrophila*. Further, both non-specific and specific immune parameters were enhanced by the methanol extract. Further experiments at molecular levels in the laboratory and also efficacy testing at field level are essential before applying this plant product in aquaculture industry.

Introduction

Aquaculture accounts for more than 50% of fish available in the market (FAO, 2014). One of the main limiting factors of aquaculture industry is diseases (Düğenci et al., 2003). Intensification of aquaculture and poor culture practices results in large scale microbial infection of the fish leading to large scale mortality of crops (Guo et al., 2015, Liao et al., 2004). The supremacy of immunostimulants over chemotherapeutics like antibiotics and vaccines has already been established (Reverter et al., 2014, Sakai, 1999). Commercial immunostimulants are often used as adjuvants during vaccination in order to enhance the efficacy of vaccines (Bricknell and Dalmo, 2005, Licciardi and Underwood, 2011). Tilapia is one of the widely cultured fish and is a fairly hardy species suitable for various laboratory manipulations (Thomas et al., 2014). *Aeromonas hydrophila* is one of the prevalent pathogens of the warm water fishes including tilapia (Wu et al., 2010).

Plants possess a vast repertoire of bioactive compounds including immunostimulants which are being explored only recently (Shukla et al., 2014). Also, a sudden boom of knowledge about the traditional medicinal plant-derived immunomodulators is increasingly evident (Gurib-Fakim, 2006, Kumar et al., 2012). We have earlier reported the effects of extracts from various plant sources including *Tinospora cordifolia* leaf (Alexander et al., 2010), *Nyctanthes arbortristis* seed (Devasree et al., 2014, Kirubakaran et al., 2010), *Solanum trilobatum* leaves (Divyagnaneswari et al., 2007) in *Oreochromis mossambicus*, and that of a marine macroalga (Balasubramanian and Michael, 2014) in *Channa striata*. The present study explores the immunomodulation and disease resistance properties of the methanol extract of *N. arbortristis* seeds on *O. mossambicus*.

N. arbortristis Linn. called popularly as night jasmine or coral jasmine, finds an important place in traditional medicine of India. Iridoids and urosolic acid from *N. arbortristis* was shown to possess anti-leishmania (Singh et al., 2014) and anti-filarial (Saini et al., 2014) properties respectively. A number of biochemical entities have been reported from this plant including alkaloids, flavonoids, and cardiac glycosides (Sasmal et al., 2007, Yadav et al., 2013). *N. arbortristis* is a potent clastogenic agent and a mito-depressant (Tamokou and Kuete, 2014). Ethanol extracts of various parts of *N. arbortristis* has been shown to modulate different branches of immune system in a mouse model (Puri et al., 1994). In this study, we examined the potential of methanol extract from the seeds of *N. arbortristis* in enhancing specific and non-specific immune responses thereby increasing the disease resistance of *O. mossambicus* against *A. hydrophila*.

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Fish

Experimental fishes (*O. mossambicus*) weighing 30 ± 5 g were procured from local fish farmers (n=800) and maintained in rectangular fibre reinforced plastic (FRP) tanks. Only male fishes were used for all the experiments. Fishes were then randomly separated into three sets corresponding to non-specific response (Set I), specific response (Set II) and

disease resistance (Set III) and acclimated for 20 days in 250l rectangular FRP tanks. Each set contained four groups corresponding to one control

Non-specific immune parameters

As shown in Fig. 1, although the serum lysozyme activity increased significantly in all the experimental groups, the group administered with 2 or 20 mg/kg dose of the methanol extract exhibited highly enhanced serum lysozyme activity compared to that of the untreated control ($P < 0.001$). The highest activity of 1490 units/ml was observed on day 6 in fishes administered with the lowest tested dose of 2 mg/kg of methanol extract.

While there was a significant increase ($P < 0.001$) in serum

Discussion

Plant preparation-mediated immunostimulation is becoming a very prominent research topic in the area of health management in aquaculture. Enhancement of the non-specific immune responses of fish is very important in the context that this branch of immune system in fish plays a vital role in controlling pathogen infections in fishes (Maqsood et al., 2011, Whyte, 2007). In the present study, we explored the properties of the methanol extract of *N. arbortristis* seeds in enhancing the immune

Conclusion

In conclusion, methanol extract of *N. arbortristis* seeds enhances both the specific and non-specific immune responses in *O. mossambicus*. Further, intra-peritoneal administration of methanol extract to fishes increased the survival of fish on experimental infection. All these findings clearly indicate that the extract possesses fairly potent immunostimulatory and disease protective properties and can be used in aquaculture industries perhaps after testing its efficacy in field trials involving

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...Oral administration of *Eclipta alba* leaf aqueous extract to *O. mossambicus* enhanced disease resistance of *O. mossambicus* against *A. hydrophila* [15]. It was evident from the disease resistance test that *Nyctanthes arbor-tristis* seed methanolic extract significantly reduced the mortality of *O. mossambicus* [27]. There are convincing evidences about herbal plant extracts modulating the immune system and increasing the disease resistance of fish...

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