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RESEARCH ARTICLE

EFFICACY OF PLANT EXTRACT, *EMBLICA OFFICINALIS* IN REMEDIATION OF MALACHITE GREEN INDUCED Demeanor ALTERATIONS IN *CYPRINUS CARPIO*.

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Abstract

Traditionally *Emblca officinalis* is an effective medicine in ayurvedic system to treat various diseases and hepatic ailments. Malachite green (MG) is a dye, extensively used in textile, paper, leather and confectionary industry. Effectiveness as aquaculture therapeutant, contribute to its addition in list of aquatic toxicant. Semi static bioassay was maintained to assess the efficacy of *Emblca officinalis* extract as ameliorant in malachite green induced toxicity in *Cyprinus carpio*. Toxicological investigation comprised of study of alterations in fish demeanor such as opercular movements, air gulping and fin movements (dorsal and caudal) after chronic exposure to malachite green and its restorations on oral supplementation (feed) of plant extract of *Emblca officinalis*. Observations revealed increased opercular movements & air gulping and reduced fin movements on exposure to dye. However on supplementation with *E. officinalis* extract behavioural activities were restored near to control fish. From the present investigation it can be concluded that *E. officinalis* possess potential to restore the altered behavioural activities in *C. carpio* exposed to sublethal concentration of MG, suggesting efficacy of *E. officinalis* in combating fish stress against toxicants.

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Introduction:-

Plants and its extract form the basis of ayurvedic system of medicine. Plant have been known since ancient times for its antioxidative, antibacterial, antiseptic and anti helminthic efficacy due to the phytochemical constituents present in them. *Emblca officinalis*, is a historical plant used since ancient times against various ailments, by virtue of its active bioingredient such as flavonoids, gallic acid and vitamin C (Yokozawa *et al.*, 2007). Malachite green is actively used in many developing countries (Sandra *et al.*, 1999), even it is banned in many European countries, considering its carcinogenic, mutagenic, bioaccumulative, genotoxic properties. Its reported use in aquaculture as an effective therapeutant, against *Icthyophthirius* and *Saprolegniasis*, make it an inevitable aquaculture toxicant.

Studies have revealed MG contamination in fish exposed to it, even after months making it unfit for human consumption. Persistence of dye is by its reduction into colourless leuco form, leuco malachite green (LMG), with even higher reported toxicity (Hidayah *et al.*, 2013) and half life (Mitrowska *et al.*, 2007). Fish are sensitive organism and serve as sentinel for the ecotoxicity assessment. Furthermore these also form important constituent of aquaculture thus serve as an experimental model for toxicity bioassay.

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Behaviour is reflection of inner health and indicates any change occurring in body. It links physiology and ecology of an organism with its environment. Behaviour is both quantitative and qualitative aspect. It depicts actions at central and peripheral nervous systems and the cumulative manifestation of genetic, biochemical and physiological processes essential for living such as feeding, reproduction and predator avoidance. During present study, observations were made on any change in behavioural aspects like opercular movements, fin movements and air gulps when exposed to malachite green and *E. officinalis* extract.

Materials and methods:-

Malachite green oxalate (90% pure), triaryl methane dye was obtained from CDH chemicals, New Delhi. Healthy fingerlings of *Cyprinus carpio* (13 ± 2.11 cm and 15 ± 2.03 gm) were procured from Sultan Singh fish seed farm, Karnal, Haryana. Fish were brought in oxygenated polythene bags and transferred to glass aquarium well fitted with filters and aerators. Semi static assay was carried in 65 L (650 x 320 x 340 mm) non toxic plastic sintex tanks. Water was renewed after every 24 h to maintain the concentration of malachite green and to remove the bio-waste. Experimental assay was conducted following the guidelines of APHA (2012). 96 h LC₅₀ was determined by Probit analysis (Finney, 1980) on the basis of percentage mortality. Four groups were maintained, group I- control, group II- MG, group III- *E. officinalis* and group IV- MG + *E. officinalis*. Fish were exposed to 0.146 mg/L of MG as sublethal concentration ($1/3^{\text{rd}}$ of 96 h LC₅₀) for 15, 30 and 60 day. *Emblica* fruits were procured from university campus and ethanolic extract was prepared by Soxhlet extraction method. Feed was prepared by adding 1000 mg/kg *E. officinalis* extract in commercially available fish feed (Toya). Behavioural aspects were evaluated by observing fish (n=6) after each exposure period in all groups for 5 minutes.

Statistical analysis:-

Analysis of variance (ANOVA) followed by Tukeys multiple comparisons test was employed to compare mean differences in between the control and different exposure groups. All statistical test were performed using SPSS 18 and statistical significance ($p < 0.05$) was considered.

Results and discussion:-

Chronic toxicity bioassay showed significant effect of both the toxicant and plant extract exposure on fish behaviour (Fig. 1).

Opercular movements

Toxicity bioassay revealed significant ($p < 0.05$) increase in fish opercular movement by 66.2% after 15 day exposure, while it has fallen down to 49.27% (30th d) with subsequent lowering by 27.66% on 60th day as compared to control. Whereas *E. officinalis* only fed fish, showed insignificant change (1.57%, 9.26% and 10.80%) after 15, 30 and 60 day exposure as compared to control. Group IV with *E. officinalis* supplementation in malachite green stressed fish, depicted significant change by 47.73% (30th day) and 21.57% (60th day) as compared to toxicated group. Srivastava *et al.* (1995) reported increased opercular movements in malachite green exposed *Heteropneustes fossilis*. As observed during (30 & 60 d) exposure, the decrease in opercular movement has also been noticed in other fishes on exposure to pesticides (Okechukwu *et al.*, 2013; Sinhori *et al.*, 2014).

Fins movements

Dorsal fin movement is related with the equilibrium and balance maintained by the fish and caudal fin movement acts as steering. Dorsal fin movements have been found to be decreased to 29.27% (15d), 44.19% (30d) and 70.71% (60d) as compared to control. Whereas, amelioration group revealed non-significant recovery till 30 day, while significant attenuation was observed after 60 day supplementation, indicating need of extended feeding period requirement for the recovery.

Similar declining trend has been observed in caudal fin movements. It showed insignificant ($p < 0.05$) decrease by 30.59% on 15th day, 35.70 % on 30th day on exposure to malachite green at 0.146 mg/l, while similar insignificant changes was observed at these periods. Chronic exposure (60 d) to dye resulted in significant decrease by 47.94 % as compared to control; while 38.18% amelioration was observed with respect to MG intoxication, signifying attenuating efficacy of the *E. officinalis* extract. It has been noticed that after prolonged exposure, the fish became sluggish and swimming activity decreased to a marked level and they escaped in the corners of the experimental tanks. Sluggishness, loss of equilibrium and abnormal swimming movements has also been reported by other workers (Srivastava *et al.*, 2004, Jin *et al.*, 2015).

Air gulping

Air gulping at the surface of the water is an abnormal behaviour and is indicative of severe hypoxia. The hypoxia is a state of distress and may show an escaping tendency from water. During present investigation, on exposure of the fish to the toxicant for initial days, caused insignificant increase, however continued exposure resulted in significant ($p < 0.05$) elevation by 132.42% (30 day) and 255.98 % (60 day) in air gulping compared to control. Attenuating effect of *E. officinalis* was observed with significant change by 70.89 % and 41.32 % with respect to toxicant after 30 and 60 day respectively. Indicating extended exposure period requirement for amelioration effect. The enhanced air gulping can be attributed to the hypoxic condition created by the dye, thus the fish come up to the surface to engulf air bubbles from dissolved oxygen saturated water layer. The increased surfacing and air gulping under the stress created by the toxicants has been reported in *Labeo rohita* on exposure to malathion by Patil and David (2008). Similar observations have been recorded by Bhat *et al.* (2012) and Singh *et al.* (2010).

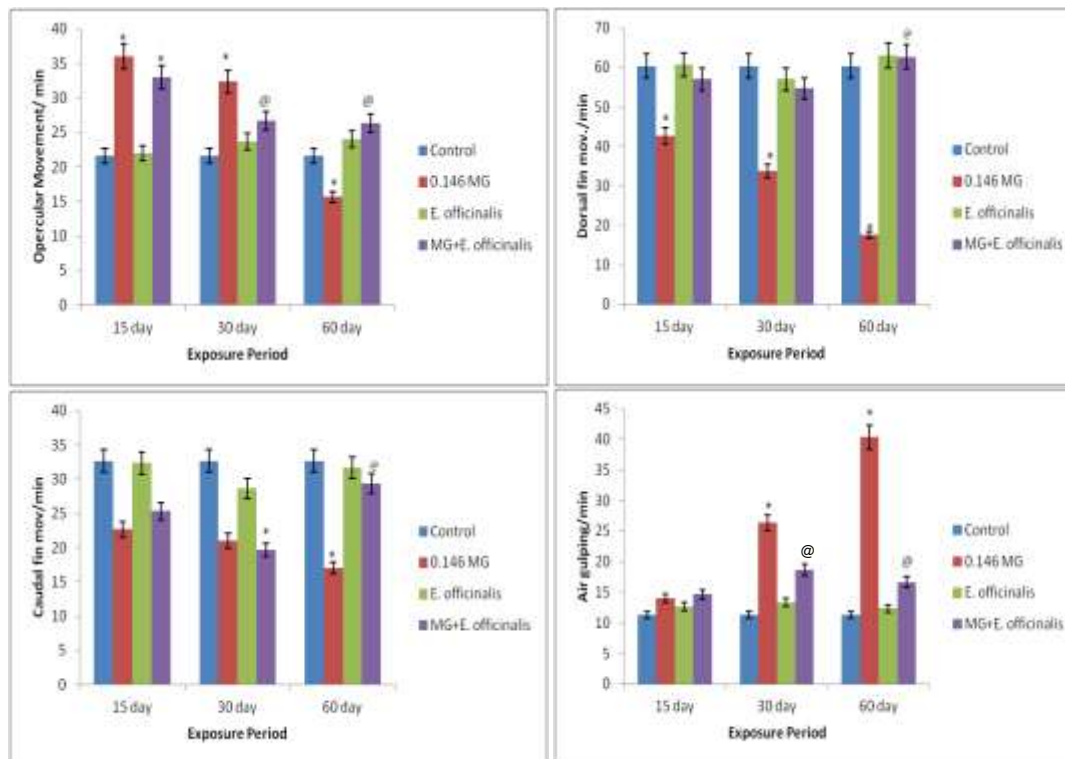


Fig. 1:- Variations in behavioural pattern in response to malachite green and *Emblica officinalis* in *Cyprinus carpio*.

Results are significant at $p < 0.05$; * indicates significant change compared to control; @ indicates significant change compared to respective MG treatment.

Conclusion:-

On account of the observations made, it could be concluded that *Emblica officinalis* supplementation does not possess any change, instead maintained fish health, thus relying its use in fish. Furthermore, it showed effectiveness against stress developed by toxicant in *Cyprinus carpio*. Thus it can be considered as diet supplement in fish inhabiting contaminated water.

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