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RESEARCH ARTICLE

EFFECT OF NEEM OIL ON THE INTESTINAL TISSUE OF THE FRESH WATER CATFISH *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

The histological effect of neem oil on the intestinal tissue of the fresh water catfish *Heteropneustes fossilis* was investigated. Fishes were exposed to sub-lethal concentrations of neem *(Azadirachta indica*) oil for 24,48,72 and 96 hrs. The normal intestine of *Heteropneustes fossilis* is characterized by outer peritoneum of serus longitudinal muscle layer, circular muscle layer, submucosa and mucosa. The mucosa is produced into large number vill, but exposure to neem (*Azadirachta indica)* oil leads to degeneration of columnar epithelium and necrosis of epithelial cells, damage villi, shrinkage of submucosa and necrosis in circular muscle

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INTRODUCTION

Neem (Azadirachta indica) oil is endowed with many medicinal properties and many *Neem* oil based traditional preparations used for pesticides, fungicides and also for treating many human and livestock disease. Therefore, *Neem* is a fascinating tree; it may usher in new era of pest control, provide millions of with inexpensive medicine, cut down rate of human population growth and restore soil productivity. In India, pioneering work on the isolation and identifications of *A. indica* constituents was initiated in 1942 and been shown to contain crude fiber (11-24%), carbohydrates (48-58%), crude protein (14-18%), fat (2.3-6.9%), ash (7.7-8.5%), calcium (0.8-2.4%) and phosphours (0.13-0.24%) as well as a number of amino acids (Ascher 1993). All parts of the *A. indica* tree possess insecticidal activity but seed Karnel is the most effective. It has a multitude of pesticidal active ingredients which are together called "triterpene" more specifically "Limnoids". The four best limnoids compounds are *Azadirachtin, Salannin, Meliantrial* and *Nimbin. Azadirachtin* ($C_{35}H_{44}O_{16}$) itself is a group of compounds such as *Azadirachtin* A, B, C, D, E, F, G. *etc*. of these, *azadirachtin* – A. (Aza A) is the most plentiful and biologically active one which has shown repellent, antifeedent and insecticidal activity against a number of insect pests and it is generally. Aza A that is used for commercial insecticides (Adewole *et al.* 2002)

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In recent years, use of medicinal plants as effective alternative to synthetic pesticides and fertilizers has gained more importance because they are more effective, less expensive, biodegradable and safe for mankind and environment, than synthetic, pesticides (Singh *et al.* 1996). The use of pesticides in aquaculture systems to control fish diseases, parasites and other pests not only leads to high levels of residues in the animals but also may interfere with the maintenance of their homeostasis and thus affect their performance (Barton and Iwanma 1991, Wendelaar Benga 1997). In view of the environmental problems caused by the use of synthetic chemical and the growing need for alternative methods of pest control that minimize this damage, there had been extensive research on pest control by substances from plants (Wan *et. al.,* 1996). One of the most promising natural compounds is *Azadirachtin* (AzA), an active compound extracted from the *Neem* tree (*Azadirachta indica*), whose antiviral, antibacterial and antifungal properties have been known for several years. (Isman *et al.* 1990, Harikrishnan *et al.,* 2003). *Neem* has been used successfully in aquaculture systems to control fish predators. Dunkel and Ricilards, (1998) and Martinez , (2002) stated that aqueous extract of *Neem* leaves and other *Neem* based products have been extensively used in fish-farms as alternative for the control of fish parasites and fish by predators such as dragon-fly larvae.

MATERIALS AND METHODS

Present investigation has been carried out to study the effect of sub-lethal concentration of *Azadirachta indica* on intestine of

the freshwater Indian cat fish *Heteropneustes fossilis.* Healthy and sexually mature specimen of *Heteropneustes fossilis* measuring about 15-20 cm length and 50-100 gm in weight were selected for the experimental study. These collected fishes were maintained in glass aquaria containing tap water and acclimatized in laboratory conditions at room temperature for one week. The water of the aquarium was changed daily and fishes are fed daily with commercial fish food. Fishes are starved for 24 hours prior to the experiment and are not fed during the period of experiment (Dalela *et al.,* 1979). In this experiment, the specimens were kept in two experimental groups. Control Group and Experimental Group. Each group was exposed to sublethal concentration of the *Azadirachta indica* similar set up was also maintained as control. The animals were scarifies for optimal concentration of biopesticide (*Azadirachta indica*) for different exposure of 24, 48, 72 and 96 Hrs. For histological studies, fishes were scarified during the exposure period of 24, 48, 72 and 96 Hrs respectively. The toxicant was renewed after fixed period. The technique of microtomy is being used for the histological study purpose of intestine of the fresh water catfish *Heteropneustes fossilis.*

RESULTS

Effect of Neem oil on intestine of the fresh water catfish *Heteropneustes fossilis*

Normal intestine: The control intestine is characterized by circular muscle fibers (CMF), longitudinal uniform layer of Mucous (ULM), and submucosa (SM). The finger like projections in lumen called villi (V) can also see. The columnar epithelial (CE) consists of prismatic cells with basal nuclei containing goblet cells (GC).

24 hrs.intestine: At for 24 hours, the adjacent villis appeared covered and very close to each other. An uniform layer of mucosa (M) was seen covering all the villis, necrosis at the tip of the villi (N) and ruptured membrane (RM), ruptured submucosa (SM) and circular muscle fibers (CMF) has been observed.

48 hrs.intestine: The exposure to sublethal concentration of *Azadirachta indica* for 48 hrs, intestine of fish showed affected mucosa (AM) and submucosa layer (ASM), ruptured membrane (RM), degeneration of columnar epithelium (DCE) and necrosis at the tip of villi (N) were observed.(fig.48 hrs.intestine)

72hr intestine : The exposure to sublethal concentration of *Azadirachta indica* for 72 hrs, intestine of fish showed affected mucosa (AM) and submucosa (ASM), overlapping villi (OV), ruptured closely packed villi (RV), necrosis at the tip of villi (N) as well intervillar space.

96 hrs.intestine: The exposure to sublethal concentration of *Azadirachta indica* for 72 hrs, intestine of fish showed affected mucosa (AM) and submucosa (ASM), ruptured and closely packed villii (RV), interlocked villi (IV), excessive focal necrosis (N) with ruptured columnar cells (RCC) and vacuolization (V).

DISCUSSION

The present results revealed that exposing fish *Heteropneustes fossilis* to neem oil induced histopathological changes in the intestine. During the course of experiments, *Heteropneustes fossilis* was exposed to sub lethal concentration of neem oil for

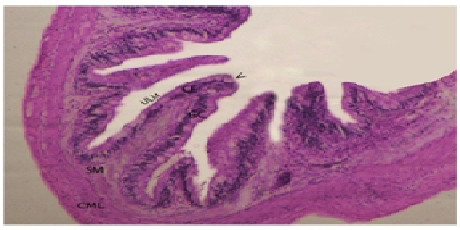

Fig - Normal Intestine

Fig - 24 hours Intstine

Fig - 48 hours Intstine

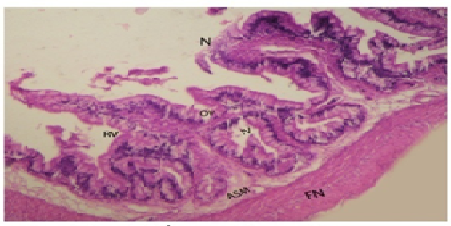

Fig - 72 hours Intestine

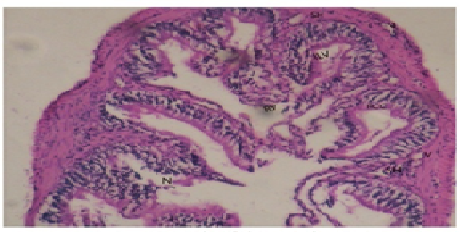

Fig - 96 HOURS INTESTINE

Figures. Effect of Neem oil on intestine of the fresh water catfish Heteropneustes fossilis

24, 48, 72 and 96 Hr respectively showed the histo-pathological alternation in intestine. The control intestine was characterized by outer peritoneum of serous longitudinal muscle layer, circular muscle layer, submucosa and mucosa. The mucosa is produced into large number villi. Exposure to biopesticide leads to degeneration of columnar epithelium and necrosis of epithelial cells, damage villi, shrinkage of submucosa and necrosis in circular muscle. The intestine is largely responsible for the breakdown of food. It also regulates the rate of emptying of the stomach via hormonal pathways. Previous studies also reported the same observations for fresh water fishes and they were also quoted that these biopesticide also interrupt the metabolic and digestive function of intestine (Anjaneyulu *et. al.,* 1998, Oti 2003, Beekage *et al.,* 2008, Velumurgan *et al.,* 2008). Similar results were observed in the intestine of *Clarias lazera* exposed to crude oil from Balayim field, Sinai, Egypt (Mazhar, et al., 1987).

Conclusion

Neem (Azadirachta indica) oil is endowed with many medicinal properties and is very beneficial plant because its products are believed to have several medicinal values. But the present study on *Heteropneustes fossilis* and previous research made on the aquatic organisms especially fresh water fishes proved that *Neem* oil is highly toxic to the fishes and also showed the hazardous effects on intestine of fresh water catfish *Heteropneustes fossilis.*

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