بسم الله الرحمن الرحيم

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INFLUENCE OF AZADIRACHTIN AND ITS ANALOGUES ON SOME ORGANS AND TISSUES OF THE ADULT FRESH WATER FISH, Clarias lazera.

THESIS

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TO MY FAMILY

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CONTENTS

CHAPTER I

INTRODUCTION

INTRODUCTION

The use of chemical pesticides and insecticides has created a large number of problems which continued to affect the aquatic ecosystems and their component biota. The most common of these pesticides are organochlorine compounds which persist and accumulate in the food chain.

Several recent studies, in which aquatic organisms were exposed to sublethal concentrations of pesticides, have been accumulating a growing body of evidence on the biological effects of these chemicals. For example, cutthroat trout, exposed to sublethal concentrations of organochlorine immediately before spawning, produced offspring which experienced heavy larval mortality (Allison et al., 1964). Growth of marine bivalve molluscs was measurably reduced when they were subjected to low levels of organochlorine insecticides (Davis, 1961; Butler and Springer, 1963).

Fish accumulate pesticides from the water, build up residues and gradually store them mainly in the fat tissue. For example, Oysters exposed to concentrations of organochlorine as low as 0.1 ppb in the surrounding water, may concentrate up to 7.0 ppm in their tissues in about a month (Butler, 1966). This biological magnification shows the extent of danger of these trace pollutants in the environment which may be concentrate and can enter food wed causing a threat to the survival of fish.

Natural products offer a great potential for the development of new pesticides. Numerous trials to utilize certain natural insecticides and pesticides of plant origin, which are supposed to be non toxic, safe and less expensive, have been attempted.

Azadirachta indica (neem) and **Melia azedarach** (chinaberry) are evergreen flowering plants belonging to the family Meliaceae. **Azadirachta indica** does not grow in Egypt but **Melia azedarach** is available in this country as well as in many tropical and subtropical countries of the world. If widely advertised the awareness of this plant's popular insecticidal and pesticidal effects will enable many farmers in these countries to grow their own pesticides.

Melia azedarach is variously called chinatree, chinaberry tree, Pride of India, Pride of china, Persian lilac, Indian lilac, Paradise tree, bead tree (British Museum) and its varnaculate Egyptian name is Zanzalacht (one of the street and garden trees in Egypt).

Several Meliaceae species, including neem, chinaberry and other plants are used as repellants; antifeedants; insect growth regulators and strong fecundity reducing agents. Neem possesses insecticidal, acaricidal, repellant properties (Radwanski, 1977; Ladd **et al.**, 1978; Warthen **et al.**, 1978; Schmutterer and Rembold, 1980; Ascher and Gsell, 1981; Islam, 1984; Jacobson **et al.**, 1984; Ketkar and Ketkar, 1984; Schmutterer and Zebitz, 1984;

Saxena et al., 1985). The toxic effects of such plants have been previously discussed in different animals (Vijjan et al., 1982).

The active compound extracted from chinaberry tree, **Melia azedarach,** and the closely related **A. indica** is called azadirachtin (Butterworth and Morgan, 1971).

Over the last 15 years or so, the neem and chinaberry trees have been shown to posses active principles in their leaves, bark and fruits, these can be exploited for their medicinal, biological and biocidal properties (Schmutterer et al., 1981; Schmutterer and Ascher, 1984).

It appeares that spraying with neem or chinaberry oil is an effective measure for controlling viruses or diseases caused by bacterium -like organisms, such as the yellow shoot-disease of the citrus. Also, the timber of **Melia azedarach** is good for making furniture. Experiments have shown it to be termite -resistant (highly resistant to the Formosan subterranean termite, **Coptotermes formosanus** (Rembold et al., 1980 b).

Neem as well as chinaberry are considered beneficial in the treatment of a wide range of disorders like cough, nausea, vomiting, gonorrhoea, urinary tract infection, intestinal worm infestation, leprosy, skin disorders, rheumatism, chronic syphilitic sores and ulcers (Kirtikar and Basu, 1975). They are also used as antiseptic, anti-inflammatory and parasiticide in various infection like ringworm and scabies (Chopra et al., 1956).

From 1984 to 1986 experiments were carried out in southern china on the practical application of **Melia azedarach** L. and other promising botanical pesticides for the control of fruit and vegetable pests (Chiu, shin- Foon, 1984, 1985, 1986). The controlling effect of **M. azedarach** on insects has been extensively studied (Volkonsky, 1937; Mc Millian et al., 1969; Butterworth and Morgan, 1971; Steets, 1975; Warthen, 1979; Saxena et al., 1981 a,b; Hashem et al., 1991). However a few data was obtained from the available literature concerning the effect of the this natural pesticide on vertebrate animals (mammals, birds and fishes).

A pre-liminary pharmacological study revealed that the extracts of this group of plants acted as a spasmogen on isolated guinea pig ileum. They also increased the respiratory rate in the dog without any change in depth (Arigbabu and Don Pedro, 1971).

The effect of Meliaceae products on growth rate of lambs (Vijjan et al., 1982); rats (Vijjan and Parihar, 1983); guinea pigs and goats (Ali, 1987) were thoroughly studied.

Cardiovascular effect of the leaf extract was also studied on rabbits and guinea pigs (Emmanuel et al., 1978). The neem seed cake has been reported to exhibit toxic effects in sheep (Bhandari and Joshi, 1974; Vijjan et al., 1982), cattle (Bedi et al., 1975 b; ketkar, 1976; Pradhan, 1976; Singh et al., 1975), buffaloes (Bedi et al., 1975a) and poultry birds (Gupta et al., 1976).

The effect of aqueous suspension of the dried leaves of chinaberry, **Melia azedarach**, on some blood parameters of mice, **Mus musculus,** (Hashem and said, 1991) and of toad, **Bufo regularis,** (Hashem and Essawy, 1991) was studied. They found a significant decrease in haemoglobin content, haematocrit values, urea, cholesterol and protein.

The antifertility effect of these products was reported on mice (Deshpande et al., 1980) and female albino rats (Ramesh et al., 1986; Tewari et al., 1986).

The anti -implantation effect of the oil on rats was reported (Sinha et al., 1984a; Khare et al., 1984; Tewari et al., 1986). Also has a strong spermicidal action against rhesus monkey and human spermatozoa in vitro (Sinha et al., 1984b).

Hashem and Essawy (1991) studied the effect of aqueous leaf suspension of chinaberry tree on the gonadal activity in **Bufo regularis**. Histological and quantitative studies of the testes and ovaries showed that no significant difference against the controls were observed. They reported that there is no prominent effect of feeding water suspension of dried leaves of chinaberry on the gametogenesis in adult male and female **Bufo regularis**.

The anti-inflammatory and antipyretic activities of the neem and chinaberry products were also demonstrated in rats (Okpanyi and Ezeukwa, 1981).

According to recent studies, the Meliaceae products have no or small toxic effects on warm blooded animals, they have no oral toxicity to mammals and show no or only very moderate side effects on natural enemies of insect pests especially parasites and honey bees (Schmutterer, 1985) and other beneficial insects (Hashem and Fetyani, 1991).

Much informations were available regarding the toxic effects of these plants on birds and mammals except fish. Few investigations, however, have been performed concerning the effects of these natural products on fish.

The present study dealt with chinaberry (**Melia azedarach**) products to evaluate certain haematological, physiological and histopathological responses of fresh water catfish, **Clarias lazera**, because of the dearth of back ground information for the effect of chinaberry products on the fish. It is intended here to give an account on the changes occurring in a number of cellular constituents (erythrocytic count, haemoglobin content, haematocrit value and leucocytic count) of the adult male freshwater catfish, **Clarias lazera**.

Measurements of specific physiological changes in the non-cellular components of fish, exposed to products of this plant, may also provide a sensitive method for predicting the effects of this stressful challenge. therefore, the present study has evaluated the changes taking place in the levels of the non-cellular blood constituents namely serum total protein, glucose, total lipid, cholesterol and creatinine under this stress.

Because fluctuations in the essential metal levels may be one of the most fundamental indications of organ defects, the changes taking place in their levels have been determined.

In addition, histopathological investigations on the liver, kidney, testis and blood were also documented to assess pathological sequel that occur due to exposing of the fish to chinaberry products.

Haematology can serve as an economical model for the study of various effects caused by stresses.

A knowledge of the amount of haemoglobin in the blood of fish can give a considerable significance in understanding their ecological and physiological relationships. This parameter may indicate the presence or absence of haemolysis in the blood samples which can not be ascertained by counting erythrocyte (Wells, 1956; Hilmy **et al.**, 1987).

The haematocrit value is one of the most important of all clinical methods. Because of its simplicity and high degree of reproducibility, this parameter is most useful for the detection of anaemia (Wells, 1956). Haematocrit is extremely reliable and can be easily and rapidly performed with fish blood (Snieszko, 1960).

Blood is the first line of defence against disease because of the phagocytic properties of some of the leucocytes and antibodies of the plasma.