CHAPTER I

INTRODUCTION

At the present, there is growing awareness for the increasing use of harmful chemicals in our food and environment. Because of synthetic agrichemicals, particularly pesticides, are widely used for the control of pest inspite of the fact that these chemicals are now causing serious environmental hazards. Botanical insecticides have gained greater importance since it is believed that natural products are ecologically sound and culturally more acceptable than synthetic ones. One such source of biological pesticide is the neem tree (Isman et al., 1990).

Neem is an evergreen flowering plant botanically known as *Azadirachta indica* A. Juss., belonging to the family Meliaceae. It is native to Indian subcontinent, this fast growing shade tree has been widely cultivated in Africa, Asia, Australia, the Carribean, Central and South America (Isman et al.,1990). In Southeast Asia this plant species occurs in Thailand, Southern Malaysia and in the drier Indonesian islands east of Java. Neem is one of the most valuable exploited of all tropical trees which has been long used in agriculture and medicine. Its bark, leaves, roots, seeds, flowers and fruits have been used as soil fertilizer, insect repellant, insecticide, animal fodder, dye, wax, fuel, lubricant, soap, mouth hygiene products and in traditional medicine (van der Nat et al., 1991).

Several chemical constituents have been isolated and characterized from neem seed. These include azadirachtin, salannin, laevorotatory and desacetylnimbin (Atal and Kapur, 1982). The more data on the chemical components of neem seeds have been recorded by Wealth of India in 1985 (Upadhyay et al., 1992). They consist of proto-meliacins, meliacins, pentanortriterpenoids, hexanortriterpenoid and variety of non-terpenoidal components such as fatty acids, sterols, phenols, flavonoids and carbohydrates. However, the major product which is used for control of a variety of agricultural insect pests is azadirachtin. Azadirachtin ($C_{35}H_{44}O_{16}$) is a limonoid of the tetranortriterpenoid type. It is the

remarkable antifeedant activity. Moreover, it has been demonstrated to strongly interfere with molting and reproduction in several species of insects (Isman et al., 1990). Many other limonoids from neem seed have also been identified and shown to inhibit bacterial and tumor cell growth and have spermicidal and antimalarial effects (Jacobson, 1989). Such the cytotoxicity of nimbolide to N1E-115 neuroblastoma (mouse) was observed from the disruption of cell membrane (Cohen et al.,1996).

Recently, the neem seed extract have been used for a commercial insecticide. This product is proposed for food crop use and tends to be more widely used, despite less information exists on its effects in the aquatic environment. The aquatic environment has suffered impact caused by the increasing number of chemicals and other anthropogenic materials or xenobiotics discharged. This may produce toxic effects on aquatic organisms such as accumulating in living organisms, especially those at the top of the food chain. Therefore, biological effect monitoring by determining the early adverse alteration is necessary for a reliable examination of bioavailable aquatic pollution. A knowledge of an alteration in a biological response, including physiology, histology, behavior as well as haematology and biochemistry is needed in order to understand the effect of toxicants on fish.

Therefore, the evaluation of haematological parameters of fish blood provides facts concerning with the physiological response of fish to changes of the external environment. Moreover, haematological variables are well known for their clinical value in prognosis and diagnosis. There are many researches about changes in haematological parameters that have been noted about the adverse effecfs of various toxic substances on some species of fish. Such the effects of tri-*n*-butyltin oxide (TBTO) on *Salmo gairdneri* Richardson and *Tilapia rendalli* Boulenger have been exhibited the increasing values of haemoglobin concentration and erythrocyte count (Chliamovith and Kuhn, 1977). Consistent with this, there has been an increase in RBCs number in blood of the flounder *Pleuronectes flesus luscus* maintained in the water with low oxygen content. In contrast, five species of fish, grey mullet *Mugil cephalus*, Borneo mullet, *Liza macrolepis*, goldspot mullet, *Liza parsia*, long whiskered catfish *Mytus gulio* and Indian mackeral *Rastrelligel kanagurta* from complex polluted waters of Visakhapatnam harbour showed significantly lower erythrocyte numbers, haematocrit, haemoglobin and thrombocyte percentage and significantly

higher mean cell volume (MCV), leukocyte numbers and lymphocyte percentage, compared with the control (Rao et al., 1990). As reduction in lymphocyte numbers have been initially noted in goldfish *Carassius auratus*, exposed to sublethal levels of cadmiun (Murad and Houston, 1988). While catfish *Heteropneustes fossilis* exposed to malachite green has also been observed significant decrease in the serum calcium and protein levels (Srivastava et al., 1995). The roach *Rutilus rutilus* L. exposed to bleached kraft pulp and paper mill effluent was found a significant increase of blood glucose level as well as a significant decrease of leukocrit and total plasma protein. Moreover, a decrease in a transaminase activity (glutamic pyruvic transaminase or GPT) has also been noted (Jeney et al., 1996). Also, an increased plasma glucose level was studied in rainbow trout *Oncorhynchus mykiss* exposed to mercury chloride and methylmercury (Bleau et al., 1996).

The toxicological study of neem on haematological monitoring have been investigated in some species of vertebrates too, for example, Murrah buffaloes fed neem seed cake showed the higher serum protein values (Gangopadhyay et al.,1979). However, there was no significant difference in the values of blood glucose, haemoglobin content and urea nitrogen in lambs fed neem cake (Vijjan et al., 1982). In Brown Hisex chicks fed neem leaf extract were also reported the changes in the values of erythrocyte count, haemoglobin concentration, pack cell volume, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). Furthermore, the main clinicopathological changes were the increased in lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP) activities and uric acid and bilirubin concentrations and decreased in the total protein levels in serum (Ibrahim et al., 1992). Corresponding, the increasing quantity of the enzymes (GPT, GOT and ALP) have been noted in rabbits given neem leaf extract (Akah et al., 1992). The toxicity of neem extract on aquatic organisms have also been recorded in snails *Biomphalaria pferifferi, Bulnus truncatus* and *Lymnaea natalensis* (Osuala and Okwuosa, 1993). Moreover, neem extract was found to have the molluscicidal property against the snails *Lymnaea acuminata* and *Indoplanorbis exustus* (Singh et al., 1996). The toxicity of this extract have also been observed in juvenile Pacific Northwest salmon (Wan et al., 1996). Thus, the aim of this study was to evaluate the haematological alteration of Nile tilapia *Oreochromis niloticus* after long term low level exposure to neem *Azadirachta indica* seed extract. Since there was a few

studies about toxicity of this extract on fish especially in tilapia which is an economic species and widely cultured in many agricultural areas of Thailand. Now it is promoted to culture in rice fields because of its fast growth, ease of breeding, efficient use of natural feeds, resistance to diseases, tolerance to a variety of environmental conditions and good taste. Furthermore, it is able to routine maintenance in the laboratory.

Objectives

1. To evaluate the haematological alterations of Nile tilapia *Oreochromis niloticus* after long- term low levels exposure to neem *Azadirachta indica* seed extract.
2. To determine acute toxicity of neem *Azadirachta indica* seed extract at 96 hr.

Anticipated benefits

1. To provide useful data on haematological alterations of Nile tilapia *Oreochromis niloticus* after long-term low levels exposure to neem *Azadirachta indica* seed extract.
2. The acute toxicity value of neem *Azadirachta indica* seed extract is very useful for the toxicity study on freshwater fish in agricultural areas.
3. To consider the suitability of using neem *Azadirachta indica* seed extract for pest control.

Scope of the study

The scope of this study focuses on the effects of neem *Azadirachta indica* seed extract on haematological alterations of Nile tilapia *Oreochromis niloticus* after long-term low level exposure. The data of acute toxicity value of this extract is estimated before starting long-term study. The haematological data, including total erythrocyte and leucocyte counts, differential leucocyte count, haematocrit value and blood glucose levels are collected from control and treated fish monthly until the seventh month of experiment. Moreover, the data of the enzymes monitoring the hepatic dysfunction, ALP (alkaline phosphatase), GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase) are also studied.