Chapter 1

Introduction

In a recent year, toxicological evidences of chemicals use have been reported in a massive scale of damage to human, animals and environment. This occurred so as to maintain human themselves a high quality and enough quantities of food, health and satisfaction. In the past few decades in agriculture and farming, chemical control agents were used increasingly from day to day especially after the synthesis of DDT, a very effective organochlorine insecticide, in 1939. Therefore not until the mid-nineteenth century, pesticidal chemicals were used successfully in pest control (Ware, 1994). Unfortunately, the achievement over any kind of pests was accompanied by many unfavourable effects.

Global use of xenobiotic agents in pest management results in contamination of its residues in environment and agricultural products. Many adverse effects of them have been studied and documented. Many pesticides were banned after the establishment of US Environmental Protection Agency (USEPA) in 1970. Toxicity of organochlorine compounds using as pesticide such as DDT, lindane, dieldrin, endosulfan and nonpesticide such as PCB and its congeners were reported on different organisms including human (Hugla et al., 1996; Ungerer and Thomas, 1996; Dalsenter et al., 1996; Barry, 1996; Corrigan, French and Murrey, 1996). Using of most organochlorine pesticides including DDT, aldrin, dieldrin, endrin, toxaphene, chlordane and heptachlor were cancelled by the USEPA because of their high toxicity and long persistence in environment (Ware, 1994). Some organophosphates and carbamates were known to be lower persistent than organochlorines but even so their toxic effects on various organisms were documented (Hugla et al., 1996; Arnold et al., 1996; Wu et al., 1996; Escartin and Porte, 1996; Matsumiya et al., 1996). The organophosphorous pesticide that was banned by the USEPA due to human hazard is ethyl parathion (Ware,

1994). Pesticidal toxicants include not only these two types of chemical pesticides but also pyrethroid, a xenobiotic mimic of botanical pyrethrum. Although the synthetic pyrethroids were known to be safer for human, low mammalian toxic and less persistent, there were some reports about systemic reaction of flumethrine on human (Box and Lee, 1996) and cypermethrin action on nitrogen metabolism in fish (Philip and Rajasree, 1996).

While using of xenobiotic agents have been reduced because of environmental awareness, natural products have been introduced to use instead without previous concern about its adverse effects. In fact, natural products such as rotenone, tobacco, and pyrethrum were recorded for centuries as insect control agent. In India, neem (*Azadirachta indica* A. Juss.) have been used for more than a thousand years as an insect repellant and traditional medicine. Recently, many pharmacological and pesticidal properties of neem products were documented (Jotwani and Srivastava, 1981; Ketkar, 1982; van der Nat et al., 1991). Neem extract is one of widespread biopesticide with wide-range in mode of action, low toxicity to mammals and its active ingredients biodegrade rapidly in sunlight and within a few weeks in the soil (Olkowski, Daar and Olkowski, 1994). But some biopesticides, even if botanical products, have caused undesired side effects on nontarget organisms especially beneficial species.

There is no pesticide which is completely safe to nontarget organisms. Although biopesticide from neem are said to be safer and less persistent, they are not without risk. Accordingly, risk assessment of neem biopesticides have been conducted for weighing the benefit and loss from using of them. In this study, reproductive toxicity of neem products is focused. There are some literatures which reported effects of neem on reproductive system of insects (Thomas and Hiradhar, 1993; Williams, 1993; Sundararaj, Murugesan and Ahmed, 1995; Dhar et al., 1996; Su and Mulla, 1998), mammals and human (Riar et al., 1988; Upadhyay, Kaushic and Talwar, 1990; Sinha et al., 1984 *cited in* van der Nat et al., 1991; Upadhyay et al., 1994). Because of this, nontarget animals

such as beneficial insects, fishes, birds, other wildlife and human may be affected with this potential.

Aquatic ecosystem nowadays have been greatly contaminated with agricultural runoff. So aquatic animals such as fishes are susceptible to toxic effects of pesticide. In Thailand, biopesticide made from neem extract is widely used because it is available locally and seem to be safe to human. It has been used to control several species of insect pest including aphides in rice field (Sombatsiri, 1997). Though the neem biopesticides were believed to have very low persistence, new evidences nevertheless revealed that this is not true. Azadirachtin, an active ingredient of the biopesticide, is as persistent as the carbamates and pyrethroids in water and soil (Stark, 1997). Moreover, it is sometimes recommended to applied repeatedly, thus the amount of its residue contaminated in water is renewable and increased, the higher the residue the greater effect on nontarget fishes.

In agricultural areas there are simultaneous aquaculture. Nile tilapia (*Oreochromis niloticus* Linn.) is one of beneficial fish culturing widespread in Thailand. The fish is economically important in case of commercial fish and protein source for Thai people. Any adverse influences on growth, development and reproduction of them may impact on human health, economy and certainly on aquatic ecosystem. In addition, it is available locally, inexpensive and easy to raise in laboratory. Therefore, the tilapia is chosen as a biological model for studying the toxicological effects of neem biopesticide on reproductive system.

Female reproductive impairment is an indicator for evaluation of long term use of the biopesticide. Any reproductive injury occuring in ovaries of female fish can influence its fecundity and fertility. As a consequence, this may lead to some economic, nutritional and environmental impact. Above all, other fishes which were more sensitive than tilapia of course were susceptible to the effect.

Histological technique is a useful tool for observation of basic histology and pathological lesions occuring in the target organ after subchronic exposure to the biopesticide. This approach may provide a sensitive monitoring of the reproductive toxicity of toxicants on fish.

Objectives

1. To study basic histology of ovary of tilapia *Oreochromis niloticus* Linn.
2. To study subchronic effects of neem *Azadirachta indica* A. Juss. seed extract on female reproductive system of tilapia *Oreochromis niloticus* Linn.

Anticipated benefits

1. To obtain basic knowledge about common histology of ovary of tilapia *O. niloticus*.
2. To provide toxicological information about subchronic effects of neem *A. indica* seed extract on female reproductive system of tilapia *O. niloticus*.
3. The toxicological data of subchronic effects of neem seed extract on female tilapia can be used to determine suitability and safety of using neem seed extract as a biopesticide.