INTRODUCTION

Freshwater ecosystems can be contaminated with pesticides by leaching, runoff, or direct or indirect spraying, this later occurring by action of the wind [1]. Most of the chemicals that are used as pesticides are generally toxic to many non-target species including humans, and other desirable forms of life that co-inhabitant the environment [2, 3].

During past few decades, the use of organophosphorus pesticides (Ops) has largely replaced organochlorine compounds in the agricultural activities. Ops have been widely used to control agricultural pests, but these are harmful to non-target aquatic organisms when frequently used, due to contamination of aquatic environment through run-off [4, 5]. The toxicity of organophosphates arises from their inhibition of the enzyme acetylcholinesterase (AChE E.C 3.1.1.7), which is essential for the transmission of nerve impulse and also plays a vital role in the development of neurons and network formation [6, 7, 8]. Ops are highly soluble in water and can therefore easily contaminate aquatic ecosystems, thereby increasing the exposure risk of aquatic flora and fauna [9]. Although other groups of insecticides with a shorter life and comparatively very low mammalian toxicity are available (e.g. pyrethroids), organophosphorus (OPS) compounds are still used frequently in agricultural practices. Therefore, extensive use and discharges of OPS in environment may impair biological communities and the accidental release into water sources may cause unexpected human intoxication.

Histopathological examination has been increasingly recognized as a valuable tool for the assessment of the impact of environmental pollutants on aquatic animals [10, 11, 12, 13, 14]. The fairy shrimp *Streptocephalus dichotomus* are commonly found in seasonal vernal pools. They are subjected to risks of exposure to agrochemicals especially OPs and other pollutants [15]. Hence, this study documents the possible impact of malathion and glyphosate on histological aspects of ovaries and muscle tissues of freshwater fairy shrimp, *S. dichotomus*.

MATERIAL AND METHODS

*S. dichotomus* were originally collected from the temporary ponds of Putlur, Thiruvallur District and maintained in laboratory for cyst production. Fairy shrimps were hatched from cysts by following the procedures of Ali and Dumont [16]. Toxicity tests were performed to determine the 96hrs LC<sub>50</sub> values of malathion and glyphosate and were found to be 9.1 ppm and 0.0055 ppm, respectively to the preadults of *S. dichotomus* [15]. Based on the 96hrs LC<sub>50</sub> value, a sub lethal concentration of each pesticide (1/5<sup>th</sup> of the 96hrs LC<sub>50</sub>; malathion, 2.0 ppm; glyphosate, 0.0011 ppm) was chosen for histopathological studies. Preadults of *S. dichotomus* were exposed to sub lethal concentrations of malathion and glyphosate for a period of 30 days and at the end of the experimental period treated fairy shrimps were collected and preserved in 10% buffered formalin for histological studies. Preserved whole fairy shrimps were carefully processed by conventional method [17].
They were cut into 4 to 5µm thickness by a rotary microtome, stained with haematoxylin-eosin and examined under the Labomed light microscope.

RESULTS AND DISCUSSION
Chemical pollution in aquatic ecosystems especially seasonal vernal pools is a major environmental concern. Not only does this type of pollution cause a decrease in water quality, but subsequently affects all living organisms in the system [18, 19, 20, 21]. In the present investigation, the freshwater fairy shrimp *S. dichotomus* was exposed to sub lethal concentration of malathion (2.0 ppm) and glyphosate (0.0011 ppm).

Histopathological abnormalities in ovaries may be caused by several factors, viz., ionizing radiations, electric current, parasitic infections, mechanical injuries, xenobiotic toxicants [22] and by a variety of effluents [23, 24, 25]. The results of the present study revealed that *S. dichotomus* manifested histopathological changes in ovaries and muscles. In the control, ovaries showed normal histoarchitecture of epithelial layer, follicle cells, nurse cells and oocytes whereas the fairy shrimps exposed to sub lethal concentration of malathion and glyphosate had mild destruction of epithelial layer, follicle cells, nurse cells, necrosis and degeneration of oocytes (Figures 1-3). Similar histopathological alterations were reported in different aquatic organisms exposed to different pollutants by many investigators. Reddy et al., [26] noticed destruction of epithelial layer, follicle cells, nurse cells, necrosis and degeneration of oocytes in the ovary of freshwater crab, *Barytelphusa guerini* when exposed to heavy metals. However, Sarojini et al., [27] documented the deleterious effects of cadmium chloride on the ovarian development for the same species. Tehrani et al., [28] postulated that the degree of damage in the ovaries of *Artemia urumiana* affected by carbamates pesticide was indicated by necrosis in ovarian nurse cells.

Figure 1: Photomicrograph of L.S through the ovaries of control fairy shrimp showing normal epithelial layer (EL), follicle cells (FC), nurse cells (NC) and oocytes (OC). Figures 2-3: Photomicrographs of L.S through the ovaries of test fairy shrimps exposed to malathion and glyphosate, 2-3 the ovary showing mild destruction of epithelial layer (EL), follicle cells (FC), nurse cells (NC), necrosis (N) and degeneration of oocytes (DOC). Figure 4: Photomicrograph of L.S through the muscles of control fairy shrimp showing normal histoarchitecture of muscle, muscle fibers (MF) and absence of Haemorrhages (H) and Pigmented cells (PC). Figures 5-8: Photomicrographs of L.S through the muscle tissue of test fairy shrimps exposed to malathion and glyphosate, 5-8 the muscle showing degeneration (DMF), Necrosis of muscle fibers (NMF), Haemorrhages (H) and Pigmented cells (PC).
Kharat et al., [29] recorded the effects of tributyltin chloride induced histopathological insult of ovarian tissue of freshwater prawn, Macrobrachium kistensis. They reported marked damages in epithelial layer, degeneration of oocytes, vacuolization appearance in cytoplasm and nucleoplasm. Similarly, Jadhav and Sheikh [30] observed exposure and concentrated mediated changes in ovaries of freshwater crab, Barytelphusa curricularis treated with endosulfan. Likewise, Rani et al., [31] reported degenerative changes in ovaries of mud crab, Scylla olivacea when exposed to cadmium nanoparticle. Damage to the ovarian tissue may be due to the direct effects of organophosphorus pesticides on developing oocytes interfere with the enzyme system in metabolism or destroying the function of hormone that controlling the ovarian growth and leads to decline reproductive activity.

In the present study, several histopathological alterations were noticed in the muscles of S. dichotomus when exposed to sub lethal concentration of malathion and glyphosate. The pathological findings include degeneration of muscles, necroses of muscle fibers with haemorrhages and RBC like pigmented cells (Figs 4-8). As muscle tissue is the primary site of exposure, pollutants affected the muscle epidermis abruptly. Pigmented cells are prominent feature of chronic inflammatory response. The present investigation closely agreed with a similar report by Tehrani et al., [28] in the muscle tissues of Artemia urmaiana in response to carbamates pesticide resulting in degeneration, Zenkers necrosis of muscle fiber with haemorrhages and RBC like cells. Exposure of Labeo rohita to hexachlorocyclohexane was found to induce separation of muscle bundles and intracellular oedema in the muscle tissues [32]. Moreover, Fatma [33] observed degeneration of muscle bundles with aggregations of inflammatory cells and focal areas of necrosis in the muscle tissues of Tilapia zillii and Solea vulgaris exposed to heavy metal. Such observations were also made in muscle tissues of Oreochromis mossambicus on exposure to dimethoate [34]. Histopathological alterations in the muscle tissues of Heteropneustes fossilis exposed to polluted river water were also recorded by Rakhi et al., [35].

In this study, malathion and glyphosate caused histopathological alterations in S. dichotomus at the tested concentration of respective pesticides. No studies were conducted so far to document the histopathological effect of pesticides in freshwater fairy shrimp. Moreover, the findings of present study serve as “biomarkers” for assessing pesticide toxicity in the aquatic biota. The use of pesticides in various agricultural and non-agricultural landscapes should be minimized. Instead of synthetic chemicals, biological methods could be employed for controlling pests in agricultural and non-agricultural areas.

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