Summary

A study was carried out to investigate the effects of the two synthetic pyrethroids deltamethrin and permethrin on survival, growth, food consumption, biochemical constituents, enzyme activity, oxygen uptake and ultrastructural changes on gill and scale surface in the climbing perch, *Anabas testudineus*, Bloch, 1792, a non-target aquatic organism and an important food fish in south and south east Asia. The acute toxicity test revealed the 24, 48, 72 and 96 h LC$_{50}$ values of deltamethrin and permethrin for *Anabas testudineus*, which were 0.11, 0.09, 0.08, and 0.07 mg l$^{-1}$, and 2.07, 1.41, 1.02, and 0.93 mg l$^{-1}$, respectively. From the LC$_{50}$ values of the two pesticides, it could be concluded that deltamethrin was more toxic than permethrin in acute exposure of 96 h duration, although a narrowing down of this difference with longer duration of sublethal exposure could be predicted from the steeper slope of permethrin toxicity which suggested increase in its toxicity on longer exposure. Thus, the cyano-substituted deltamethrin had over an order of magnitude higher acute toxicity to *Anabas testudineus* than that of the non-cyano permethrin.

To investigate the sublethal effects of deltamethrin and permethrin on growth and food consumption of *Anabas testudineus*, fish were exposed for 11 weeks to 10% and 1% of the 96 h LC$_{50}$ of the two pesticides which were 0.007 and 0.0007 mg l$^{-1}$ for deltamethrin and 0.093 and 0.0093 mg l$^{-1}$ for permethrin. Deltamethrin treatments caused significant decrease in body weight, insignificant increase in body length along with significant increase in food consumption. Conversely, permethrin induced significant decrease in all the three parameters studied, i.e. body weight, length and food consumption. The trend of steeper slope of permethrin toxicity in acute exposure that predicted increase its toxicity on longer exposure was also
reflected in the chronic toxicity test when both length and weight increments were affected by permethrin, while deltamethrin suppressed weight increase only.

The present study also examined the effects of sublethal concentrations (1% and 10% of the 96 h LC$_{50}$) of the two synthetic pyrethroids deltamethrin and permethrin on biochemical constituents such as glycogen, protein and lactic acid contents in liver and muscle tissues of Anabas testudineus exposed for 21 days. When compared with that of the control, significant depletion of glycogen content was observed in liver tissue of fish treated with both the pesticides, while that of lactic acid was observed only in fish muscle treated with deltamethrin. Protein reduction was found significant only in muscle tissue treated with both the pesticides at their higher as well as lower concentrations. The significant decrease in glycogen content in liver tissue of Anabas testudineus in the present study indicated that liver which plays an important role in carbohydrate metabolism was significantly affected by both deltamethrin and permethrin treatments. Reduction of glycogen content in liver tissue of fish exposed to the two pesticides suggests its greater utilization to meet high energy demands during stress by the process of glycogenolysis thereby indicating near exhaustion of glycogen stores. The protein estimation results in the present study revealed that the two pesticides had significant impact on protein content in muscle tissue only which reflects poor assimilation of food and low amino acid uptake resulting in reduction of protein synthesis. Moreover, the decrease in muscle protein content in the present study seems to be correlated with the inhibition in growth of the fish as the protein required for growth was utilized in providing energy during stress. Thus, though it lacks specificity, reductions in protein concentrations could nevertheless be useful for assessing the magnitude of toxic effects of pyrethroids. Significant decrease in lactic acid content in muscle tissue on exposure to sublethal deltamethrin concentrations in the present study revealed that
these changes in lactic acid contents in fish tissue may be due to impaired function in the liver induced by deltamethrin. This also suggests the possible adaptive withdrawal of lactic acid from muscle into liver for synthesis of glycogen through the Cori Cycle. Thus, the findings of the biochemical parameters revealed that the toxicity of the cyano-substituted type II synthetic pyrethroid deltamethrin was higher than that of type I permethrin as reflected in the comparisons of their effects on glycogen, total protein and lactic acid contents in liver and muscle tissues of A. testudineus exposed for 21 days.

Estimations of succinate dehydrogenase (SDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) enzyme activities in liver, muscle, brain, heart, gill and kidney tissues of Anabas testudineus after an exposure period of 21 days were also carried out. SDH level was reduced significantly in liver, muscle, heart and kidney tissues at higher (10% 96 h LC50) as well as lower (1% 96 h LC50) deltamethrin concentrations, but significant reductions in brain and gill were observed only in higher deltamethrin concentration. Significant reduction in SDH activity in permethrin treated fish was found only in higher (10% 96 h LC50 i.e. 0.093 mg l\(^{-1}\)) concentration in all the tissues examined i.e. liver, muscle, brain, heart, gill and kidney. SDH reductions in most of the examined tissues were more pronounced in deltamethrin treated fish than those treated with permethrin. The results revealed that deltamethrin was more effective even at relatively low concentrations than permethrin, which is likely to be due to the presence of a cyanide group in the former. These significant decreasing effects on SDH enzyme activity in several tissues of Anabas testudineus in the present study indicated that the two pesticides have severe impact on the respiratory enzyme disrupting the normal TCA cycle resulting in disturbances in the normal respiratory mechanism of the fish. Suppression of SDH activity also reflected the inhibition in the conversion of succinate to fumarate and also a metabolic shift from
aerobiosis to anaerobiosis. AST enzyme showed significant decrease in activity in liver and muscle tissues at both higher and lower deltamethrin concentrations, suggesting that there may be a repressive mode to counter the effects of the pesticide on the fish by disrupting fish metabolism due to inactive transamination and oxidative deamination processes, which in turn affected physiological processes at the organismic level. On the contrary, in permethrin treated fish, significant increase was obtained in brain at both higher and lower concentrations and in heart and gill tissues only at higher concentration indicating tissue impairment and enhanced protein catabolism in response to the pesticide. It was also suggestive of more conversion of alpha-amino acids into keto acids in TCA cycle so as to augment energy demand during stress. Exposure to both deltamethrin and permethrin resulted in increased or decreased ALT enzyme activity, although significant results were obtained only in muscle tissue treated with deltamethrin. Deltamethrin treatments caused decreased ALT activity suggesting inactive transamination and oxidative deamination in fish tissues thereby interrupting metabolic processes which in turn could affect the physiological processes. On the contrary, increased ALT activity occurred in most permethrin treated tissues which was an indication of amplified transamination processes. Thus, the observed depletion of protein, glycogen and lactic acid contents and changes in SDH, AST and ALT activities during deltamethrin as well as permethrin stress was likely to disrupt several important biochemical and physiological processes occurring in the fish body. Hence, these parameters could be further exploited as biomarkers of pesticide-induced stress.

Effect of sublethal concentrations of the two pyrethroids on oxygen consumption in *Anabas testudineus* was also carried out after an exposure period of 21 days. There was significant reduction in oxygen consumption in fish exposed to permethrin at both higher and lower sublethal concentrations, but in deltamethrin treatment significant reduction occurred only
at higher concentration of 0.007 mg l\(^{-1}\). It may be mentioned here that the lower sublethal concentration of deltamethrin (0.0007 mg l\(^{-1}\)) in the present study was more than one order of magnitude lower than the lower sublethal concentration of permethrin (0.0093 mg l\(^{-1}\)), the latter being higher than the higher sublethal deltamethrin concentration (0.007 mg l\(^{-1}\)). Significant reduction of oxygen consumption in pesticides exposed fish might be due to respiratory distress as a consequence of the impairment of oxidative metabolism which also appeared to be a protective measure ensuring low intake of the toxic substances. Moreover, it was an indication of disorganization of respiratory action caused by rupture in respiratory epithelium of gill tissue. Effects of both the pyrethroids on oxygen consumption and gill SDH activity increased at higher concentrations, thereby suggesting a cause-effect relationship among pesticide exposure, enzyme activity and oxygen consumption.

Scanning electron microscopy studies on gill and scale surface of *Anabas testudineus* exposed to sublethal concentrations of the two synthetic pyrethroids deltamethrin (0.035, 0.007, and 0.0007 mg l\(^{-1}\)) and permethrin (0.93, 0.093 and 0.0093 mg l\(^{-1}\)) for 21 days revealed concentration-dependent manifestations of toxic effects of both the pesticides. The deep erosive lesions on the gill lamellae exposed to deltamethrin were most likely to be due to the presence of a cyanide moiety in deltamethrin, which is a type II synthetic pyrethroid. *Permethrin*’s characteristic effect comprised oedema in both primary and secondary lamellae that might have been caused by the pesticide’s interference with Ca dynamics. The effects of deltamethrin and permethrin on gill surface ultrastructure occurred in a concentration-dependent manner, thereby establishing a cause-effect relationship among pesticide exposure and morphological anomaly. Deltamethrin exposure affected scale surface ultrastructure by causing severe deformation and fusion of two circuli in the same as well as different rows. The fusion of circuli associated with
deltamethrin toxicity was also probably caused by its cyanide moiety. Permethrin characteristically produced a thick mucous layer over the scale surface. Both the pesticides affected circuli pattern with severe breakage of circuli and loss of lepidonts present over their ridges at both higher and lower concentrations. Erythrocyte extrusions were also seen at several places over the scale surface.

On the basis of the present findings, it can be concluded that while deltamethrin and permethrin could be less toxic to birds and mammals, they were highly toxic to fish, and adversely affected several toxicity end-points such as survival, growth and food consumption. Sublethal concentrations of the two pesticides also affected biochemical parameters significantly such as glycogen, total protein, lactic acid, SDH, AST and ALT activities in fish tissues thereby disrupting the normal physiological processes in *Anabas testudineus*. Oxygen uptake in the fish was also affected by the two pesticides along with ultrastructural changes in fish gill and scale. Keeping in mind all the above highlighted effects of deltamethrin and permethrin in freshwater fish *Anabas testudineus*, it can be concluded that extreme care should be taken while using deltamethrin and permethrin and even other pyrethroids in agricultural activities, especially those undertaken near aquatic ecosystems so that their adverse effects on aquatic biota and ecosystems as a whole could be minimized.
Conclusion

The present study revealed the toxicities of two synthetic pyrethroids (deltamethrin- type II pyrethroid and permethrin- type I pyrethroid) on *Anabas testudineus*. The median lethal concentration (LC$_{50}$) values revealed that the 96 h LC$_{50}$ value of the type II cyano-substituted deltamethrin was more than an order of magnitude lower than the corresponding value of the type I non-cyano-substituted permethrin. On exposure to their relative sublethal concentrations (10% and 1% 96 h LC$_{50}$) values, the fish showed inhibition in growth, while food consumption pattern was also altered. They also caused significant alterations in concentrations of biochemical constituents like glycogen, total protein and lactic acid in liver and muscle tissues, as well as enzyme activities of succinate dehydrogenase, aspartate transaminase and alanine transaminase in liver, muscle, brain, heart, gill and kidney tissues of *Anabas testudineus*. In all these experiments, deltamethrin was found to be toxic at a concentration that was over an order of magnitude lower than the effective concentration of permethrin. The concentration of 0.7 ppb at which deltamethrin remained toxic was comparable to that of this pesticide recorded in rain water, surface and ground water, soil, and even honey and vegetables from different parts of India. Hence, this concentration may be considered environmentally relevant. Besides, oxygen uptake of the fish was also reduced significantly on exposure to the sublethal concentrations of the two pyrethroids. Furthermore, ultrastructural changes occurred on gill and scale surfaces of the fish as revealed by scanning electron microscopic studies on exposure to lethal and sublethal concentrations of the two pyrethroids.

Keeping in mind the possible effects of synthetic pyrethroids in general and deltamethrin and permethrin in particular to the environment, the government needs to take some initiative with a policy to minimize the use of pesticides consistently with effective pest control through
integrated pest management (IPM). People should be made aware of the toxicities of pesticides particularly synthetic pyrethroids on various life forms. Since there is a possibility of these types of pesticides reaching the humans through food chain contamination, careful attention should be paid while applying these chemicals in agricultural operations so that their effects on aquatic organisms, especially fish are minimized.
Recommendations

Excessive use of pesticides including synthetic pyrethroids caused widespread contamination of aquatic ecosystem thereby affecting aquatic species including fish to a great extent. Since the effects of these pesticides could reach humans through food chain contaminations, the following recommendations are made:

1. Special attention needs to be paid while applying these pesticides in agricultural operations nearby aquatic ecosystems so that agricultural runoff do not reach aquatic ecosystem by providing proper drainage system.

2. It is necessary to find out all the possible toxic effects of pesticides using different toxicological endpoints or parameters up to the molecular level.

3. It is also necessary to find out the recovery period or the recuperation potential of the affected fish regarding several toxicological endpoints so that minimum residual effects reach humans through food chain contamination.

4. Precautions should be taken to prevent leaching of pesticides via agricultural runoff into water bodies.

5. People must be made aware of the safe use techniques, storage mechanisms and particularly the effects of pesticides on the environment and various life forms through print, electronic and social media.

6. As an alternative means and also to reduce the pesticide residues in the environment, people particularly farmers and cultivators should be made aware of the usefulness of
organic farming, applications of bio-pesticides or plant based pesticides in agricultural activities.

7. Adoption of integrated pest management (IPM) practices needs to be given serious consideration by users and policy-makers.

8. Regarding use of pesticides, priority should be given to chemicals with high target selectivity, low toxicity to non-target species and rapid biodegradability in the environment.