A review of earlier reports helps in identifying the conceptual methodological issues relevant to the present study. This would enable the researcher to collect information and subject them to sound reasoning and meaningful interpretation. Hence the relevant references in connection with the present study were reviewed and critiqued with relevant data extracted. Keeping in view the objectives of the study, following the euphoria at their ability to kill pests, many farmers indiscriminately sprayed their crop lands with excessive amount of pesticides (Veeraiah and Durga Prasad, 2001).

Since aquatic environment is the ultimate sink for all pollutants, aquatic toxicity testing has become an integral part of the process of environmental hazard and evaluation of toxic chemicals (Murty, 1986).

Hanamant Gadad and Mahabaleshwar Hegde (2014) conducted an experiment during Rabi and summer cropping season of 2012-13 at Main Agricultural Research Station, Dharwad. Among the different treatments of three biopesticides, spinosad, buprofezin and mixture compound, Spinosad, was found more effective in reducing thrips population.

Deshmukh and Dharamkar (2013) stated that sale of biopesticides touched 2.1 billion in 2012. At present, microbial insecticides are the main component of the bio-pesticide industry. The most successful microbial insecticides are the *Bacillus thuringiensis* toxins, avermectin and the spinosyns.

Ghosal *et al.* (2012) observed that during the year 2010, three days after spraying on tomato crop, the highest percentage of reduction of *Helicoverpa armigera* population was recorded in rynaxypyr followed by spinosad and indoxacarb, whereas seven days after spraying, rynaxypyr showed highest parentage of reduction of
Helicoverpa armigera population while spinosad and indoxacarb recorded decreased percent reduction of Helicoverpa armigera population than three days after spraying.

Ghosal et al. (2012) also stated the spinosad provides good control at low rates causing excitation of the insect nervous system, leading to involuntary muscle contraction, prostration with tremor, and finally paralysis. It provides good control over lepidopteran, dipteran, thysanopteran, and some coleopteran, homopteran, hymenopteran and orthopteran pest.

Christos and Eleftherohorinos (2011) studied the adverse effects of pesticide use on the environment (water, soil and air contamination from leaching, runoff, and spray drift, as well as the detrimental effects on wildlife, fish, plants, and other nontarget organisms). They also reported that many of these effects depend on the toxicity of the pesticide, the measures taken during its application, the dosage applied, the adsorption on soil colloids, the weather conditions prevailing after application, and how long the pesticide persists in the environment.

Amalendu Ghosh et al. (2010) observed that three days after spraying, the highest percentage of reduction of Helicoverpa armigera population was recorded in spinosad treatments followed by lamda-cyhalothrin, quinalphos and cypermethrin. After seven days of spraying, spinosad showed highest parentage of reduction of Helicoverpa armigera population while lamda-cyhalothrin recorded decreased percent reduction of Helicoverpa armigera population.

Saunders and Brett (1997) who applied spinosad to water, reported that very little hydrolysis occurs, and the substance can be persistent. In the absence of sunlight, half lives of spinosyn A and D are at least 200 days. In water exposed to sunlight, photodegradation occurs.
Dhawan *et al.* (2006) observed that the spinosad (45 % SC) (Spinosyn A 50% Minimum and Spinosyn D 50% Maximum) was effective for the control of bollworms complex of cotton.

Spinosad has been applied to over 200 different crops. It has been used to control caterpillars in cotton, loopers in cabbage, leafminers in various crops, leaf rollers on apples, thrips in citrus, etc. (Dow, 1997; Thompson *et al.*, 2000).

Brank (2005) observed that once spinosad is taken into the body of insects, it kills the target pest by over-stimulating its nervous system in a manner that is unique to insects. DOW asserts that this mode of action helps prevent insects from building up resistance to the compound.

Kalavathy *et al.* (2001) reported that, under sub-lethal exposure, fish body became lean towards abdomen position compared to control fish and was found under stress, but that was not fatal. Leaning of fish indicated reduced amount of dietary protein consumed by the fish under pesticide stress, which was immediately utilized and was not stored in the body weight.

Elzen (2001) observed that newer insecticides, such as spinosad, indoxacarb, and emamectin benzoate, have been shown to be relatively safe on predacious hemipterans, mites, coccinellids, lacewings and some parasitoids. Salgado (1998) established that, in insects, the mode of action of spinosad is associated with excitation of the insect’s nervous system.

Spinosad was accepted for review and registration under the U.S. Environmental Protection Agency (EPA) Reduced Risk Pesticide Program. Spinosad is classified as an organic substance by the USDA National Organic Standards Board.

Murthy (1986) stated that fish are the most often tested aquatic organisms because they are the most conspicuous as predominant and are economically
important to humans because they are linked in the food chain. Fish the non-target organisms are likely to be exposed to the potential impact of pollutants than in the terrestrial environment.

Murray and Lloyd (1997); Karar et al. (2002); and Gupta et al. (2005); Mallah and Korjo (2005) observed that Spinosad, a naturalyte compound, extracted from soil *actinomycetes, Saccharopolyspora spinosa* has proved very effective against bollworms, especially *Helicoverpa armigera*. Moreover, it is relatively safe to natural enemies also.

Sparks et al. (1995) described the biological activities of spinosad to larvae of *H. virescens* and other lepidopteran insects. Spinosad has broad spectrum nematicidal, acaricidal and insecticidal properties (Putter et al., 1981).

Nagarjuna Agrichem Limited Material Safety Data Sheet (2014) stated that the LC$_{50}$ and LD$_{50}$ values of spinosad (45% SC) were for rats $> 5000$ mg/kg and rabbits $> 2000$ mg/kg body weight. Acute inhalation for rats was $> 5.18$ mg/L. Acute oral LD$_{50}$ for Bob white quail was $> 2000$ mg/kg. Dietary LC$_{50}$ for Bobwhite quail and Mallard ducks was $> 5156$ mg/L.

Viswanatha et al. (2014) found that the 48 h LC$_{50}$ for neem extract in *Moina micrura* was 196.3 μg/L. The acute toxicity data were $< 6000 – 380,000$ μg/L for *D. duplex* (Goktepe and Plhak, 2002 and 2003). Glyphosate was the lowest toxic (LC$_{50}$ was 3042 μg/L) to *M. micrura*. Other values in reports were higher between 1150 – 107,000 μg/L and 30000 μg/L for *C. dubia* and *D. magna*, respectively. The LC$_{50}$ value of pesticides showed that toxicity of chlorpyrifos $> $ carbofuran $> $ malathion $> $ neem extract $> $ glyphosate. *M micrura* was susceptible to pesticides from μg/L to mg/L, with chlorpyrifos was the most toxic (LC$_{50}$ 0.08 μg/L) and glyphosate was the lowest toxic (LC$_{50}$ 3042 μg/L) to *M. micrura*. 

![nitroPDF logo](https://example.com/nitrologo.png)
Reza Davoodi (2014) conducted an experiment to determine the median lethal concentration of neem for common carp (*Cyprinus carpio*) as a non-target organism. Five test concentrations and a control were used in triplicates to determine the 72 h median lethal concentration of neem gold pesticide. With the increase of dose concentration and exposure duration, significant increase in the mortality level was noticed. The 72 h LC$_{50}$ value (with 95% confidence limits) of Neem Gold for common carp juvenile was found to be 0.09799 ml/L-1.

Nagarjuna Agrichem Limited Material Safety Data Sheet (2014) stated that the LC$_{50}$ values for different fishes Rainbow trout, Common carp, Bluegill sunfish and Japanese carp for 96 h were 30 mg/L, 5 mg/L, 5.9 mg/L and 3.5 mg/L respectively. EC values for Algae *Selenastrum capricornutum* and *Skeletonema costatum* for 96 h were 105.5 ppm and 0.2 ppm. It was highly toxic to honeybees when spinosad 45% EC sprayed directly. LD$_{50}$ and LC$_{50}$ values for 48 h for bees and worms, *Eisenia foetida* were 0.0029 µg/bee and > 1000 mg/kg soil.

Sajda *et al.* (2014) carried out static acute toxicity. The LC$_{50}$ values for rogorin for 24 h, 48 h, 72 h and 96 h were 50, 41, 33 and 27 ppm respectively. Fish exposed to lethal concentration (LC$_{50}$) of rogorin showed altered behavioral responses such as restlessness, hyperactivity and some time jerky swimming, loss of appetite, discoloration of skin and increased accumulation of mucus on gills and also on the entire body.

Rajbir Kaur and Anish Dua (2014) studied toxicity in fingerlings of *Labeo rohita* exposed to geometric concentrations (6.25 to 100%) of municipal wastewater of Tung Dhab Drain, Amritsar, India. Static, 96 h non-renewal acute toxicity tests were conducted and simultaneously behavioural and morphological observations were
noted. The 96 h LC\textsubscript{50} value along with 95% confidence limits was found to be 44.25% (38.47-50.92).

Frouzan Piri et al. (2014) observed that the susceptibility of \textit{G. pyloalis} larvae to spinosad was studied using the leaf dip method. Treatment with doses of spinosad sub-lethal concentrations (LC\textsubscript{10}, LC\textsubscript{20}, LC\textsubscript{30}, LC\textsubscript{40} of 0.026, 0.045, 0.065, 0.090 ppm, respectively) was applied. A significant difference in the effects was observed between the sub-lethal concentrations (LC\textsubscript{10}, LC\textsubscript{20}, LC\textsubscript{30}, and LC\textsubscript{40}) and the control in the content of carbohydrate and glycogen, and between the control vs. LC\textsubscript{30} and LC\textsubscript{40} in the content of protein. A significant decrease in glutathione S-transferase activity with the increase of spinosad concentration, no significant differences in the activities of \(\alpha\)- and \(\beta\)-esterases, and a significant increase in the enzyme activity of phenoloxidase were observed. Effects of LC\textsubscript{10} and LC\textsubscript{30} spinosad concentrations on some biological parameters showed that percentage of larval pupation and female fecundity significantly decreased in the concentration of LC\textsubscript{30}.

Naz and Javed (2013) observed that the mean LC\textsubscript{50} and lethal concentrations for grass carp were 56.42 ± 2.51 and 120.98 ± 7.18, while that of silver carp the same were 55.85±2.84 and 128.44±9.25 mgL\textsuperscript{-1}, respectively.

Rathod (2013) observed that when the experimental freshwater cat fishes \textit{Heteropneustes fossilis} were exposed to biopesticide, \textit{Azadiracta indica} dose treatment for varying exposure period, the natural activities of fishes got altered such as swimming ability, hyperactivity and non-directional movement. Opercular movement increased.

Ahirwar et al. (2013) observed that the data obtained from three sprays against larval population of \textit{C. acuta} and \textit{S.litura}/mrl at 24 h before and 3\textsuperscript{rd}, 7\textsuperscript{th} and 10\textsuperscript{th} days after treatments, the \textit{Bacillus thuringiensis} var. \textit{kurstaki} @ 10\textsuperscript{13} spores/ha was found
to be the most effective as it recorded the lowest larval population (3.20 larvae/mrl) followed by *Beauveria bassiana* @ $10^{13}$ spores/ha, *M. anisopliae* @ $10^{13}$ spores/ha, Spinosad 45% SC @ 73g. a.i./ha and Dipel @ 1 kg/ha.

Satyavardhan (2013) studied when the freshwater fish, *Ctenopharyngodon idella* (Valenciennes) was exposed to sub-lethal concentrations (1/10th of 24h LC$_{50}$ value) of fenvalarate and malathion for 10 days, the proteins and glycogen levels were significantly decreased over the control.

dipak Mandal *et al.* (2013) observed that the LC$_{50}$ values of flocamid (50% WG), spinosad (45% SC), dichlorvos (75% EC), chlorpyriphos (20% EC), triazophos (40% EC) and endosulfan (75% EC) were 2010, 630, 282, 226, 369 and 950 ppm respectively at 24 h after acute toxicity treatment to *Phenacoccus solenopsis* (cotton mealybug). The respective LC$_{50}$ values observed were 885, 192, 81, 65, 184 and 333 ppm, against 3rd instar nymphs at 48 hours after treatment. Based on the relative toxicity, the descending order of toxicity was chlorpyriphos (27.36) > triazophos (14.25) > dichlorvos (13.05) > endosulfan (12.22) > spinosad (3.30).

Christopher Didigwu Nwani *et al.* (2013) observed that the LC$_{50}$ values (with 95 % confidence limits) of different concentrations of chloropyrifos (Termifos) in *Clarias gariepinus* were found to be 1.66 (1.40-3.30), 1.30 (1.18-1.53), 1.03 (0.98-1.11) and 0.86 (0.73-0.99) mg/L for 24, 48, 72 and 96 h exposure time, respectively, thus indicating that the pesticide is highly toxic to the fish.

Megahed *et al.* (2013) evaluated the insecticidal activities of three bioinsecticides (i.e. emamectin benzoate "Proclaim", abamectin "Romacten" and spinosad "Tracer") on the 4th larval instar of the cotton leaf worm, *Spodoptera littoralis* by leaf dipping technique as well as determining the biochemical changes in treated insects. These bioinsecticides showed immediate effects with 24 h LC$_{50}$ values
of 0.17, 0.23 and 38 ppm for emamectin benzoate (Proclaim), abamectin (Romacten) and spinosad (Tracer) respectively. Marked biochemical changes were recognized in treated insects such as reduction of ALP and AChE activities, total protein, total lipids and glucose contents. On the other hand, there were significant increases of GOT and GPT activities.

Desuky et al. (2002 and 2012) observed that malathion (organophosphorus) was the most toxic one, whereas spinosad was less toxic than the other insecticides against the tested predators, Chrysoperla carnea.

Yazhini Jagadeesana and Sheela Darcusb (2012) stated that the LC$_{50}$ value of profenofos for Catla catla was 0.6 ppm. The sub-acute concentrations were 0.015 ppm, 0.030 ppm and 0.06 ppm were chosen as the experimental concentrations.

Imtiyaz Ahmad Bhat et al., (2012) determined the toxicity of biopesticide, matrine (kethrin, Brand name) on the freshwater fish, Labeo rohita. Fishes were exposed to various concentrations of botanical insecticide, matrine for 96 h and the percent mortality was recorded. The 96 h LC$_{50}$ value determined by Finney's Probit analysis method was found to be 21.68 ppm. Behavioural patterns were observed critically during the whole experiment. The test fish exhibited erratic swimming, increased surfacing, decreased rate of opercular movement, reduced agility and inability to maintain normal posture and balance with increasing exposure time.

Veda Parimala and Uma Maheswari (2011) observed that the LC$_{50}$ values were 5.35, 225.75, 55.46, 5.85 and 6.33 ppm for deltamethrin, emamectin benzoate, spinosad, abamectin and malathion respectively in Maize (Zea mays L.) for 48 h.

Ganeshwade (2011), in a study, noted that the freshwater fish, Puntius ticto was exposed to lethal (5.012 ppm) and sub-lethal (2.50 ppm and 1.253 ppm) concentration of Dimethoate for 96 h and 60 days’ durations. Biochemical changes in
liver tissue were analyzed after the exposure period. The protein level decreased in lethal and sub-lethal exposures. Significant decrease in glycogen and slight decrease in protein were noted to both concentrations of exposure, where as increased cholesterol and ascorbic acid content.

Nikam et al. (2011) observed that the static bioassays were performed on freshwater fish, *N. botia* to evaluate the median lethal concentrations of metasystox (Oxydemeton-methyl) for 24 h, 48 h, 72 h and 96 h. The LC$_{50}$ values were 10.3, 9.131, 7.884, and 7.018 ppm after 24 h, 48 h, 72 h and 96 h respectively. He stated that the LC$_{50}$ values decreased with increase in exposure period.

Thenmozhi et al. (2011) used malathion for *in vivo* study on freshwater fish *Labeo rohita* to know its toxicity. Acute toxicity tests were conducted during certain intervals in various concentrations (5, 10, 15, 20, 25 and 30 mg/L) of malathion. While treating with malathion, the percentage of fish mortality was assessed during 24 h, 48 h, 72 h and 96 h. The lethal and sub-lethal concentration of malathion were found to be LC$_{100}$ (25 mg/L) and LC$_{0}$ (5 mg/L) respectively.

The toxicity of the carbamate insecticide carbaryl and its metabolite, 1-naphthol, to four species of fish was studied by Tilak (1981). The calculated 96 h LC$_{50}$ values of carbaryl for *Catla catla* (Ham.), *Anabas testudineus* (Bloch), *Mystus cavasius* (Ham.) and *Mystus vittatus* (Bloch) were 6.4, 5.5, 4.6 and 2.4 ppm respectively and that of 1-naphthol are 4.3, 3, 0.33 and 1.1 ppm respectively.

Shoba et al. (2011) examined the effect of sub-lethal concentration (0.00028 ppm 1/10$^{th}$ of LC$_{50}$) of the phytopesticide nimbecidine on *Sphaerodema rusticum* for 7, 14 and 21 days of exposure. A significant decline in the contents of glycogen, protein and lipid and an increase of glucose and amino acid contents of fat body, testis and seminal vesicle were observed.
Dhasarathan et al. (2000) studied about the effect of endosulfan and butachlor on the digestive enzyme and proximate composition of the fish, *Cyprinus carpio*. Bhatnagar et al. (1984) worked on rogor induced changes in alkaline phosphatase activity in few tissues of *Mystus vittatus*.

Abdel-Hafez and Abdel-Aziz (2010) cited that the LC$_{50}$ value of spinosad was 22.179 ppm after 72 h of treated larvae of cotton leaf worm, *S. littoralis* (Boisd.). Abdul Naveed et al. (2010) reported that in vivo evaluations were made to assess the pesticide activity of lihocin against freshwater fish, *Channa punctatus* and its ultimate mode of action on fish protein metabolism. Biochemical studies showed that after exposing the fish to sub-lethal dose of linocin, total protein levels significantly decreased while FAA, glutamine, alkaline phosphatases, acid phosphatases, ALAT, AAT, GDH, AMP deaminase and adenosine deaminase were significantly enhanced in the liver, brain and kidney tissues of *C. punctatus*. The alterations in all the aforementioned biochemical parameters were significantly (p < 0.05) time and dose dependent.

Shailendra Kumar Singh et al. (2010) reported toxicological and biochemical alterations of cypermethrin (synthetic pyrethroids) against freshwater teleost fish, *Colisa fasciatus* at different seasons. Their result showed strong pesticidal activity in freshwater teleost fish, *Colisa fasciatus* for all the exposure periods (24 h or 96 h) in time as well as dose dependent manner. The LC$_{50}$ values decreased from 0.009 (24 h) to 0.006 ppm (96 h) in winter season (water temp. 16°C) and 0.06 (24 h) to 0.02 (96 h) in summer season (water temperature 28°C).

Marigoudar et al. (2009) conducted bioassay test to determine the acute toxicity (LC$_{50}$) of technical grade pyrethroid insecticide, cypermethrin (92.25 ppm) in freshwater indigenous carp, *Labeo rohita*. Carp fingerlings were exposed to different
concentrations (2.0 to 6.0 μg/L) of cypermethrin for 96 h. The acute toxicity value was found to be 4.0 μg/L and one fifth of LC₅₀ (0.57 μg/L) was selected for sub-acute studies. El-Aw (2008) reported that the 24 h, LC₅₀ values of emamectin benzoate and spinosad against the 4th instar larvae of Spodoptera littoralis were 0.712 and 20.02 ppm respectively and the toxicity increased to be 0.353 and 16.25 ppm at 48 h post treatment. Mondal and Kaviraj (2008) reported LC₅₀ values of 37.55 ppm and 57.54 ppm to Labeo rohita and Hypophthalmichthys molitrix, when exposed to different dilutions of jute-retting water (JRW).

The LC₅₀ of neem leaf extract for 96 h in Channa punctatus was 3.00 ppm (Farah et al., 2006), and 24 and 96 h LC₅₀ of Nerium indicum leaf extracts for Channa punctatus was 17.34 mg/L⁻¹ and 13.58 mg/L⁻¹ respectively (Tiwari and Singh, 2003). Cordero et al. (2006) found that among a group of different tested insecticides, spinosad and methoxyfenozide were relatively less toxic to natural enemies and thus they could fit well into integrated pest management programs.

Bond et al. (2004) observed that the LC₅₀ value of spinosad in Anopheles albimanus was 0.024 ppm by quadratic linear regression. Spinosad also effectively prevented breeding of Culex mosquitoes and chironomids in both trials to a degree similar to that of temephos. Thompson and Hutchins (1999) found that spinosad was relatively low toxic to mammals and birds and relatively safer to the beneficial insects. Duffie et al. (1998) tested different classes of insecticides against predators. They found that pyrethroid and organophosphorus classes were the most toxicity causing dramatic reductions in the predator numbers and carbamate was moderately toxic. While bio-insecticides, Insect Growth Regulators (IGRs), and the naturalyte, spinosad had low toxicity to predators.
Das and Mukherjee (2000) studied the acute toxicity of neem in the fingerlings of Indian major carps i.e., *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* and the 96 h LC\textsubscript{50} values were found to be 2.36, 2.04 and 2.78 ppm respectively. Hassanein et al. (2007) reported the 96 h LC\textsubscript{50} value of a neem biopesticide (trilogy) on the grass carp fish, *Ctenopharyngodon idella* was found to be 112 ppm.

Conventional toxicity tests indicated that spinosad had virtually no toxicity to birds and mammals. Spinosad was reported to be practically nontoxic to insect natural enemies such as *Orius* spp., *Chrysopa* spp. Coccinelids and the predaceous mite *Phyoseioulus persimilis* (Bret et al., 1997). Additional studies in which spinosad treated aphids were fed by coccinelids and chrysopids larvae reported no predator mortality (Schoonover and Larson, 1995).

The toxicity of tested bioinsecticides may be due to their mode of actions; emamectin affects the nervous system of arthropods by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis (MacConnell et al., 1989; Jansson and Dybas, 1998).

Spinosad has unique mode of action on the insect nervous system at the nicotinic acetylcholine receptors and it has additional effects on gamma-aminobutyric acid (GABA) and H-Glutamate receptor sites, leading to continuous activation of motor neurons and causing cessation of feeding, tremors of most muscles in the body and later on, paralysis and death (Salgado, 1997 and 1998, and Semiz et al., 2006).

Deshmukh and Pariyal (1992) reported the toxic effect of biopesticide, neemax on the *Oreochromis mossambicus* and *Gambasia species*. The contamination affected all groups of organisms in aquatic ecosystem like invertebrate and non-target aquatic biota like fishes (Singh et al., 1996).
Schroder (1992) stated that the widely used neem based biopesticides directly enter into the various water resources such as streams, river, and lakes, and may affect the non-target organisms.

Veeraiah and Durga Prasad (2001) reported that respiratory distress is one of the early symptoms of pesticide poisoning. Exposure to sub-lethal concentrations is reported to increase respiratory activity, resulting in increased ventilation and hence increased uptake of the toxicant. Organochlorines have been reported to stimulate more oxygen consumption at sub-lethal concentrations and inhibit the oxygen uptake at lethal concentrations. In the case of synthetic pyrethroids, a steady and progressive decline in ventilatory pattern and thereby decline in oxygen consumption was noticed. However, there have been exceptions to this statement and it is difficult to generalize (Murty, 1986). One of the most important manifestations of the toxic action of chemicals is the over stimulation or depression of respiratory activity (Muirhead Thompson, 1971). The respiratory potential of the organism is related to oxygen consumption of an animal being heterotrophic which is an important physiological parameter to assess the toxic stress and is valuable in metabolism in general (Jones, 1964).

Marigoudar et al. (2009) studied that behavioural patterns and oxygen consumption were studied in lethal and sub-lethal concentration of cypermethrin, Carp, Labeo rohita in toxic media exhibited irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and sinking to the bottom, which might be due to inactivation of (AChE) acetyl cholinesterase activity which results in excess accumulation of acetylcholine in the cholinergic synapses leading to hyperstimulation. They also observed variation in oxygen consumption in both lethal and sub-lethal concentrations of cypermethrin respectively. Alterations in oxygen
consumption might be due to respiratory distress as a consequence of impairment in oxidative metabolism. Fish in the sub-lethal concentration were found under stress, but that was not fatal.

Tilak et al. (2009) observed a decrease in the oxygen consumption of the fish exposed to nuvan. The depletion of the oxygen consumption was due to the disorganization of the respiratory action caused by rupture in the respiratory epithelium of the gill tissue. Oxygen consumption decreased when the time of exposure to toxicant was increased.

Pesticides enter into the body of fish mainly through gills and, with the onset of symptoms of poisoning, the rate of oxygen consumption increases (Holden, 1962; Premdas and Anderson, 1982; Ferguson et al., 1966a). Holden (1973) observed that one of the earliest symptoms of acute pesticide poisoning is respiratory distress. It not only serves as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also is useful to correlate the behavior of the animal. The severity of distress may lead to respiratory failure by affecting the respiratory centres of the brain or the tissue involved in breathing (‘O’ Brien, 1967).

Shivakumar and David (2004) reported that the opercular movements increased initially in all exposure periods but decreased further steadily in lethal exposure compared to sub-lethal exposure periods. Increased gill opercular movements observed initially might possibly compensate the increased physiological activities under stressful conditions.

Ajay Kumar et al. (2014) reported a significant reduction in protein content with all doses of imidacloprid and CP. There was significant decrease in acetylcholinesterase and DNA whereas significant increase with RNA was observed. However, an insignificant change in DNA and RNA at low dose of imidacloprid was
observed. The biochemical and histotoxicity induced by Imidacloprid created awareness about its judicious use by farmers/users and was of pivotal importance to have safe for next generations.

Frouzan Piri et al. (2014) observed that the sub-lethal doses of spinosad affected some biological and biochemical parameters of *G. pyloalis*. Sub-lethal concentrations of spinosad significantly inhibited larval growth, pupation, and fecundity and also affected the amount of carbohydrates, lipids, protein, and glycogen, and changed activities of detoxification enzymes and PO activity.

Binukumari and Vasanthi (2014) observed that the carbohydrate level in all organs liver, kidney, muscle and gills reduced when the fish, *L. rohita* was exposed to dimethoate (30% EC) for 24 h, 48 h and 72 h.

Alterations in physiological and biochemical parameters of toxicant treated fish recently emerged as vital indices for water quality assessment in the field of environmental toxicology (Suvetha et al., 2010).

Leena Muralidharan (2014) observed the inhibition of LDH, SDH, GOT GPT, ACP, AlP, ATP in teleost fish, *Cyprinus carpio* which was due to toxic stress of fenthion.

Jipsa et al. (2014) observed that the cypermethrin insecticide especially shakthi 10 was toxic to the fish *Tilapia mossambica* and the stress response showed by fish was dependent on concentration of pesticide and time. The protein and triglyceride levels were found to be decreased in all the exposure periods.

Lakshmanan et al. (2013) studied the impact of dichlorvos on tissue glycogen, total protein and albumen content in the selected tissues of *Oreochromis mossambicus*. In their study, when *O. mossambicus* was treated with sub lethal doses of dichlorvos for all the exposure periods, a significant decrease was observed in the
liver, kidney and muscle protein content. It was suggested that depletion of tissue total proteins after 7 days exposure period might be due to increased proteolysis thereby contributing to the availability of free amino acids. Several workers observed the decrease in protein content in liver when organisms were subjected to pesticide treatments.

Yazhini Jagadeesana (2012) observed that long term exposures of profenofos concentrations invariably showed a dose-dependent reduction in the levels of the various biochemical parameters such as protein, carbohydrate and cholesterol in the fish *Catla catla*.

El-Sheikh (2012) observed that the insecticidal, biological, biochemical and histological effects of bioagent spinosad, diple 2x (*Bacillus thuringiensis* var kurstaki) and one pyrethroid compound (cypermethrin) were evaluated on 4\(^{th}\) instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Based on the LC\(_{50}\) values, cypermethrin was the more toxic to *S. littoralis* than that of the two other compounds. Female longevity, fecundity and fertility were significantly reduced at all treatments compared to control. Furthermore, different levels of significant changes in the total protein, carbohydrate contents, phosphatasis activity and carbohydrasis were recorded. Moreover, different abnormal histological structures of ovary were noticed.

Shailendra Kumar Singh *et al.* (2010) stated that sub-lethal doses (40 and 60\% of LC\(_{50}\)) of cypermethrin after 96 h significantly alter the levels of total protein and total free amino acid in muscle and liver tissues, nucleic acids (DNA and RNA) in gonadal tissues and the activity of enzyme acetylcholinesterase (AChE), lactic dehydrogenase (LDH) and succinic dehydrogenase (SDH) in nervous tissue of the freshwater teleost fish *C. fasciatus*. 
Israel Stalin et al. (2012) reported that the maximum decrease in glycogen level was observed in liver tissue (-54.4/30 days) and minimum in intestine tissue (-0.86/60 days). The glucose content of gill, liver, and kidney showed an increase but the increase was not uniform in all the tissue.

Liu et al. (2012) studied the insecticidal activity and toxicity mechanism of spinosad on Malacosoma neustria testacea larvae by leaf membrane method and its effects on the activities of detoxifying and protective enzymes were measured 3, 6, 12, and 24 h after treatment. Their results showed that Glutathione S-Transferase activity in 4th and 5th instar larvae was first inhibited, then induced, and finally inhibited. Therefore, spinosad could effectively disrupt and interfere not only with the detoxifying and protective enzymes but also normal physiological metabolism and showed extremely high toxicity against this pest.

The effect of the biochemical composition of fishes by biopesticides was reported by Kumble and Muley (2000) and Prasad et al. (2002). The biopesticide induced effects on the physiological and biochemical changes in the fishes and other aquatic species by influencing the activities of several antioxidant enzymes (Regoli and Principato, 1995). The biopesticides also affected the genetic material (DNA, RNA) as well as total protein, total amino acids and carbohydrates.

Exposure to chemical pollutants elicited many molecular and biochemical changes in fish, which preceded cellular and systematic dysfunctions. If appropriate parameters were monitored, early warning signs of distress might be detected (Palmer, 1976). The biochemical responses of non-target organisms exposed to different pesticides were well documented (Coppage and Mathews, 1974 and Corbett, 1974). Pesticide usage for pest control has resulted in unprecedented chemical
pollution (Matsumura, 1975) affecting the non-target organisms (Pimental (1971); Ware, 1980).

Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and more often at sub-lethal levels. Incidentally, a great deal of attention has been paid to the evaluation of the hazardous effects of various pesticides on physiology of many non-target organisms by Tejendra et al. (1990), Rao et al. (1986), Sprague (1971), Sastry and Siddiqui (1984). Although the effects of pesticides on fishes are extensively studied and have also been reviewed (Holden, 1973; McKin et al., 1976; Brungs et al., 1977; Gupta, 1986 and Murty, 1986; Anita Susan, 1994; Veeraiah, 2001; Koteswara Rao, 2003; Tilak et al., 2004; Veeraiah et al., 2004; Madhavi, 2006; Sprague, 1971; Sastry and Siddiqui, 1984; Ghosh, 1986; Rao, et al.,1986; Veeraiah and Durga Prasad, 2001; Tilak, et al., 2007; Tataji, 2007; Vidyulata Devi, 2008; Wilson Raju, 2008; Ravindra Kumar, et al., 2010; Afaf et al., 2010; Satyavani et al., 2011; Aliakbar and Hedayati, 2012; Butchiram, 2012; Prasada Rao, 2012; Japamalai, 2011 and Dhillesh, 2013) yet there is a need for information from the physiological point of view. Changes in ion concentrations, organic constituents, enzyme activity and endocrinal activity as chemoregulators in fish have been attributed to pesticides (Murty, 1986).

If the toxicant is less than the lethal dose, organisms are affected by behavioral, physiological and biochemical factors. Due to stress, the changes are inevitable, leading the organisms to survive by self-defence or by detoxification (Holden, 1973; Murthy, 1986). If pesticide concentrations observed in the field are similar to those in the laboratory studies, similar effects on aquatic organisms have been observed (Eaton et al., 1985 and Crossland, 1986). Pesticide field studies conducted under natural use conditions have demonstrated that pesticide in water
concentrations may be present at relatively high levels for only a short time and then rapidly decrease (Crossland and Crosslands, 1982a; Stephenson, 1983; Clarke et al., 1986).

Ganeshwade (2011) observed a decrease in glycogen and protein levels and an increase in cholesterol and ascorbic acid content when the freshwater fish *Puntius ticto* was exposed to lethal (5.012 ppm) and sub-lethal (2.50 ppm and 1.253 ppm) concentrations of dimethoate for 96 h and 60 days. Shoba et al. (2011) examined a significant decline in the contents of glycogen, protein and lipid and an increase of glucose and amino acid contents of fat body, testis and seminal vesicle when *Sphaerodema rusticum* exposed to sub-lethal concentration (0.00028 ppm 1/10th of LC$_{50}$) of the phytopesticide nimbecidine for 7, 14 and 21 days.

Megahed et al. (2013) observed when 4$^{th}$ instar larvae of *Spodoptera littoralis* exposed to emamectin benzoate, abamectin and spinosad the highly significant decrease was noticed in glucose concentration after 24 h, 48 h and 72 h. Aboel-Ela et al. (1997) and Chitra and Reddy (2000) observed that there was reduction in glucose content of different instar larvae when they were treated with some plant extracts such as *Ammi majus*, *Apium graveolens*, *Melia azedarach* and *Vince rosea* extracts.

Ajay Kumar et al. (2014) reported that the significant reduction in protein content with all doses of imidacloprid and CP. There was significant decrease in acetylcholinesterase and DNA whereas significant increase with RNA was observed. However, an insignificant change in DNA and RNA at low dose of imidacloprid was observed. Tantarpale (2011) observed decrease in total protein in freshwater fish, *Channa straitatus* exposed to cypermetherin.

A significant decrease has been reported in the protein content of the liver and kidney in *Labeo rohita*, when exposed to fenvalerate (20% EC) (Annamani, 1986).
The protein fractions in the liver tissue are unique in appearance in the pesticide induced in *Carassius auratus* (Vaidehi *et al.*, 2013).

The decrease in total protein content in tissues of *C. mrigala* indicates active degradation of proteins under cypermethrin stress (Vasantharaja, 2012). Depletion of proteins might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery as suggested by David *et al.* (2004). These studies revealed that the cypermethrin was more toxic to common carp than the other pesticides.

Magar and Afsar Shaikh (2012) stated that the protein content in gill and liver showed decreasing trend in 96 h in treated group compared with control group. The variation in free amino acid content was due to exposed to sub-lethal i.e. (1/5th of LC<sub>50</sub> of 96 h i.e. 0.8 ppm) concentration of malathion solution. The total free amino acid contents varied from 37.31 to 42.19 in muscle, 40.29 to 46.16 in gill, 34.29 to 44.91 in liver of malathion exposed fish.

Maximum decrease in protein levels was observed in brain tissue (-40.9/30days) and minimum in liver tissue (1.6/60 days) of *Cirrhina mrigala* (Hamilton) exposed to a common organochloride pesticide lebaycid (fenthion) (Israel Stalin *et al.*, 2012). Nabih *et al.* (1990) carried out how various toxic agents affect on protein synthesis. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis were also the main causes of decreased total protein content.

Ahmed *et al.* (1993) and Rawi *et al.* (1995) reported that protein leakage during intoxication might arise from reduced body weight, conversion of protein to amino acids, degradation of protein to release energy or to the direct effect of the tested compounds on the amino acids transport of the cell.
Megahed et al. (2013) observed that when 4th instar larvae of Spodoptera littoralis (Boisd.) was exposed to emamectin benzoate (Proclaim), abamectin (Romacten) and spinosad (Tracer) after 24 h, 48 h and 72 h of treatment the total protein is significantly decreased about five times compared to that of control and that this reduction in the protein content may be due to inhibition of DNA and RNA synthesis. The decrease of the total protein in treated 4th larval instar reflects the decrease in the enzymatic activities of various enzymes.

Swarna Kumari, (2009) stated that the decline of DNA and RNA content indicates an increased catabolism of proteins which corroborates well with the decline in proteins noted in their study.

Elbarky et al. (2008) stated that the total proteins are major biochemical components necessary for an organism to develop, grow and perform its vital activities. The reduction of protein content may be due to inhibition of DNA and RNA synthesis. Shugart et al. (1992) stated that the organism functions as an integrator of exposure, accounting for abiotic and physiologic factors that modulate the dose of toxicant taken up, and the resulting magnitude of the change in DNA structure provides an estimate of the severity of exposure, hopefully in time to take preventive or remedial measures.

Nabih et al. (1990) carried out how various toxic agents affect on protein synthesis. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis were also the main causes of decreased total protein content.

Ksheerasagar (2006) observed a significant increase in LDH activity, a significant decrease in Na⁺K⁺ ATPase, Mg²⁺ATPase, Ca²⁺ ATPase and no significant change in SDH, ASAT, ALAT, ACP activity in female mice after exposure to
carbosulfan for 20 days in liver. Carbosulfan caused significant decrease in SDH, Na\(^+\) K\(^+\) ATPase, Mg\(^++\) ATPase, Ca\(^++\) ATPase, ACP activity, whereas LDH, ASAT, ALAT, ALP activities were increased significantly in the liver of male and female mice for 30 days.

Ender et al. (2005) reported that the diet with high level of bioinsecticides significantly increased the activities of transaminases. Megahed et al. (2013) stated that there were highly significant increases in glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities after treatment 24 h, 48 h and 72 h with bioinsecticides emamectin benzoate, abamectin and spinosad. Transaminase levels were affected by the hormonal control of protein synthesis and neurosecretory hormones which involved in the regulation of transaminase levels (Etebari et al., 2005).

Abdel-Hafez et al. (1988) found that in the cotton leaf worm, Spodoptera littoralis the changes in GOT and GPT activities were in a harmony with those changes of proteins. Azmi et al. (1998) observed that the transaminases (GPT and GOT) help in the production of energy, serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions.

Tufail (1991) attributed the different responses of transaminase activities to the differences in the insect strains. The highest concentration of spinetoram insecticide was responsible for the dramatic enhanced activity of GOT.

Changes in ALP activities after treatment with bioinsecticide, azadirachtin indicated that changes in the physiological balance of the midgut affected other enzymes (Ayyangar and Rao, 1990 and Kamel et al., 2010).
Alkaline phosphatase is a brush border membrane marker enzyme and is especially active in tissues with active membrane transport, such as intestinal epithelial cells and malpighian tubules (Ferreira and Terra, 1980).

Sohail Ahmed (2004) observed that the activity of acid and alkaline phosphatases in cypermethrin treated beetles was significantly increased after 24 h exposure as compared to control beetles, which had non-significant difference with bifenthrin treated beetles.

Leena Muralidharan, (2014) stated that Acid phosphatase is a lysosomal enzyme associated with growth differentiation and the lysis of cells. There was a decrease in acid phosphatase activity in muscle and liver of fish *Cyprinus carpio* treated with fenthion.


According to Gaikwad (1981) reduction in acid phosphatase activity was due to unbalanced catabolism of enzyme protein. Verma *et al.* (1982) reported that acid and alkaline phosphatase activity increased in lethal and decreased in sub-lethal concentrations of thiodan and rogor when *Channa gachua* was exposed to them.

Novikoff (1961) reported that the rise of ACP activity was probably related to the cellular damage. The increased ACP activity resulted from enhanced enzyme turn over under pesticide stress. Joshi and Desai (1981) examined chronic effects of monocrotophos on the ACP and ALP activities in the liver and kidney of *Tilapia mossambica* and found an increased activity in the organs.
Megahed et al. (2013) observed that acetylcholinesterase activities in the head of treated 4th instar larvae of *Spodoptera littoralis* with emamectin benzoate, abamectin and spinosad after 24 h, 48 h and 72 h were clearly reduced compared with the control. The change percentage of AChE activity reached its maximum level at 48 h, while the minimum level was obtained after 72 h. The usual activity of AChE in normal larvae tended to increase gradually by the progress in larval development and growth.

Abdel-Mageed and Elgohary (2006) observed a decrease in AChE activity due to exposure to spinosad. AChE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine (ACh) in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides (Salgado et al., 1998).

Koteswara Rao (2003) observed that the control fish behaved in natural manner i.e. they were active with their well coordinated movements. They were alert at the slightest disturbance, but in the toxic environment, fish exhibited irregular, erratic and darting swimming movements, and loss of equilibrium which was due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyper stimulation.

Hashem et al. (1978) reported that AChE was markedly inhibited in treated *Spodoptera littoralis* larvae than control, and that the activity of AChE was normally associated with larval development and growth after treatment with LC$_{50}$ of bioinsecticides. Although substantial reductions in the activity of AChE of the brain of fish have not been fatal, the effect of this condition on activities as feeding, reproduction and relationships prey-predator is not known (EPA, 1986).

Saravana Bhavan and Geraldine (2009) observed that the decrease was most pronounced in prawns exposed to 15.47 Hg l-1 of carbaryl (12.4% in the
hepatopancreas and 18.9% in the gills). In prawns exposed to 7.73 Hg l-1 of carbaryl, the decrease in soluble protein levels was 8.4% in the hepatopancreas and 15.3% in the gills. In the case of 5.15 Hg l-1 of carbaryl, the decrease was 6.0% in the hepatopancreas and 10.3% in the gills. Among the two tissues tested, the gills exhibited more changes in concentration of buffer soluble protein than hepatopancreas. The decline in buffer soluble protein, noted in tissues of test prawns, was further evident from the quite obvious decline recorded in the staining intensity of various polypeptide bands of molecular mass between 150-10 kDa resolved in the hepatopancreas and gills.

Javed et al. (2009) reported that occupational exposure to a complex mixture of pesticides resulted in a significant increase of DNA damage in farmers chronically exposed to pesticides in open fields. Statistically significant difference in DNA damage of exposed individuals was observed when compared with control group on the basis of comet tail length.

Rao, et al. (2007) stated that the 96 h sub-lethal concentration (96 h LC_{50}) of the butataf herbicide for Nile tilapia, Oreochromis niloticus was 0.2 ppm. The polyacrylamide gel electrophoresis of plasma protein bands revealed that the treatment with butataf herbicide caused striking variation in number, density and mobility of bands as compared to that of the control.

Shugart et al. (1992) reported that the resulting magnitude of the change in DNA structure provides an estimate of the severity of exposure and it gives a signal in time to take preventive or remedial measures.

Sajda et al. (2014) observed that the histology of the muscle and the intestine of treated fish with lethal concentration of rogorin showed various degrees of deterioration such as edema and mild lymphocyte infiltration and disjointed intestinal
layers, shortened villi respectively, when compared to control as a result of toxicity of rogorin.

Veeraiah (2001) stated that the fish, as a bio-indicator species, plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution; the effects of exposure to sub-lethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism. Veeraiah and Durga Prasad (2002) stated that Indian major carp fish, *Labeo rohita* (Hamilton) is one of the most common freshwater fishes used in toxicological studies, because it represents a number of characteristics that may make it an appropriate model. So it can be used as indicator species in biomonitoring programs.

Ecobichon (1991) observed that the aquatic vertebrates are susceptible to non-target effects, because of their relatively restricted mobility and also due to reduced pesticide dispersion leading to lengthy periods of exposure. Fishes are particularly sensitive to a wide variety of pesticide chemicals. Toxic concentrations may rise not only from excessive spillage of agricultural practices but also from several other sources. He also stated that apart from causing death of the organism either directly or due to starvation by destruction of food organisms, many pesticides have shown to affect growth rate, reproduction and behavior with the evidence of tissue damage.

Mathur *et al.* (1981) observed histo-pathological changes in the liver of bluegills exposed to pesticide, pentachlorophenol for 24 h. Sprague, (1971) underlined that lack of basic information on fish histology makes the interpretation of observed changes difficult.
Anita Susan et al. (2012) observed that the fenvalerate caused profound pathological changes under chronic exposures in the liver of *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* such as degeneration of cytoplasm in hepatocytes, atrophy, and formation of vacuoles, necrosis, and disappearance of hepatocytic wall, disintegration of lattice fibres and appearance of blood streaks among hepatocytes. All these changes indicate that fenvalerate is a highly hepatotoxic compound.

Ajay Kumar et al. (2014) observed that an increase in extent of the damages with increase in doses of imidacloprid was noticed in liver structural anatomy. The different extent of hepatocytes damage and blood sinusoids dilation, congestion within central veins and some sinusoids, necrotic debris with degenerated area and accumulation of RBC in portal vein were observed. Degeneration and necrosis of hepatocytes appeared to be due to vascular changes induced particularly in the portal vessels.

Though a number of pathological changes have been reported in fish exposed to different pesticides by Lowe (1966); Konar (1970); Jayantha Rao (1982); Jayantha Rao et al. (1985); Girija (1987); Rama Murthy (1988); Anita Sussan (1994); Vijayalakshmi (1994); Yacobu (1999); Ramana Kumari (1999); Veeraiah and Durga Prasad (2001); Tilak et al. (2001a) and Tilak et al. (2001b), spinosad-induced pathological changes in fish are relatively less. Hence no characteristic trends of pathological changes for any class of pesticides were distinct. Although major advances have been made in recent years in science, the histology and histopathology of fish and other aquatic invertebrates are still to be studied when compared with mammals (Rand and Petrocelli, 1984).

Hence, in the present study, an attempt has been made to observe possible histo-pathological changes in certain vital tissues like gill, liver and kidney of the fish,
Labeo rohita exposed to sub-lethal (1/5th of 48 h LC₅₀) concentrations of spinosad (45% SC) formulation for 24 h, 96 h and 8 days.

Kenaga (1974) reported that pesticide residues in animals may be due to contact or through food. Regardless of the source, pesticides enter the blood stream of the animals and, if stable, they are concentrated in specific tissues and excreted depending on the nature of the pesticide, reflected in terms of its chemical and physical properties. Organophosphorous pesticides which are being used for the control of agricultural pests and vector borne diseases contaminate the aquatic environment through the agricultural run off (Young and Nicholson, 1951). The prevalence of pesticides in the aquatic environment due to indiscriminate application has been monitored by using fish as biological indicators (Coppage and Mathews, 1974).

Nicholson (1967); Holden (1972) and Miles (1978) established that pesticide residues are transported to the aquatic environment either through surface runoff or through precipitation into which they get in by evaporation from crop land. The use of pesticides in agriculture may lead to the entry of small amounts of these compounds into aquatic ecosystems through a variety of potential routes including spray drift, drainage, surface runoff, or accidental spills.

Pesticide, Residues and Regulation in India (2007) reported that the highest level of DDT residues found was 2.2 mg/kg. The proportion of the samples with residues above the tolerance limit was maximum in Maharastra (74%) followed by Gujarat (70%), Andhra Pradesh (57%), Himachal Pradesh (56%) and Punjab (51%). In the remaining states, this proportion was less than 10%. Desuky et al. (2012) observed that the residual effect of methoxyfenozide recorded 86.34±3.39 8%
followed by $83.34\pm3.35$ 3% for teflubenzuron and $52.21\pm2.93$ 1% for Tracer (spinosad) in predator, *Chrysoperla carnea*.

Williams *et al.* (2003) reported that relatively rapid degradation of surface residues in the field would definitely improve the compatibility potential with natural enemies. This would likely be the case with spinosad. Puri (1998), in his study, observed that chemical pesticide residues were detected in food grains, vegetables, fruits, oils, cattle feed and fodder in most parts of the country and stated about 72 per cent of food samples in India shown the presence of pesticide residues within tolerance levels while in 28 per cent samples they were above the tolerance level as compared to 1.25 per cent globally. As a consequence, India accounted for one-third of the total pesticide poisoning cases in the world.

Veeraiah and Durga Prasad (1996) conducted a study on six drinking water pond samples of 7 villages of Guntur District, Andhra Pradesh for organochlorine residues and detected the presence of OC residues like α, γ, β HCH, DDT, DDE, DDD. They also showed that higher quantities of HCH isomers recorded were attributed to their greater water solubility, in comparison, concentrations of DDT group residues were somewhat lower reflecting their poor water solubility. The study also revealed that the long term hazard of the DDT group was much more because they were hydrophobic and were bioaccumulated to a greater extent.

Brahmaprakash Sethunathan (1987) and Gangamma and Satyanarayana (1991) reported the presence of pesticide residues in soil, water bodies, air, food materials and the bodies of living beings, and stated that pesticide residues in food in India, especially vegetables, were the highest in the world. This was mainly due to the unregulated use of pesticides. Kannan *et al.* (1995) detected residues of methly parathion, fenitrothion, malathion and endosulfan in various parts of rice plant using
Gas Liquid Chromatography and reported that these pesticide residue levels in the leaves were above environment protection agency tolerance limits even after 15 days and the same trend was reported on whole grain, dehusked grain and hay immediately after harvest.