Introduction
Pesticides have tremendous benefits to man by increasing crop protection and thereby increasing food production, and controlling the vectors of man and animal diseases. They are transported over long distances by global circulation, and through run-off, find their way into aquatic systems. At the same time the pollution of freshwater ecosystem by chemical pesticides has become one of the most critical environmental problems (Northoff and William, 2004). This causes extensive damage to the activities of the living resources of food-web due to their toxicity, persistency with half-lives of decades and tendency to accumulate in the organisms (Joseph and Raj, 2010; Joseph et al., 2010; Joseph and Raj, 2011).

Quinalphos (C₁₂H₁₅N₂O₃PS), O,O-diethyl O-quinoxalin-2-yl phosphorothioate, an ester of OP is used as insecticide and stomach poisoning (David and Kumaraswami, 1988; Hassal, 1990). It is frequently used in many countries and represents a source of toxicity to humans and vertebrate animals (Kegley et al., 2010). Dimethoate (C₅H₁₂NO₃PS₂), O,O-di- methyl S-[2-(methylamino)-2-oxoethy] dithiophosphate is a contact and systemic activity (David and Kumaraswami, 1988; Hassal, 1990). It’s degradation by esterases and amidases are very low in insects as compared with those of mammals (Rose and Hodgson, 2004).

The effects of pesticides, such as endosulfan, carbaryl, dichlorvos, lindane, chlorpyrifos, monocrotophos, carbofuran and methomyl have been studied on acute toxicity (Bhavan et al., 1997a, b, 2008; Key and Fulton, 2006; Satapornvanit et al., 2009) and biochemistry (Bhavan and Geraldine, 1997, 2000a, 1997a, b, 2008; Key and Fulton, 2006; Satapornvanit et al., 1999; Bhavan et al., 2011) of freshwater prawns. However, no data is available pertaining to quinalphos and dimethoate toxicity induced changes on the activities of the neurotransmitter, acetyl cholinesterase (AChE), the enzymatic antioxidant, catalase and metabolic enzymes, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT). The activity levels of AChE and catalase were found to be significantly (P<0.05) decreased in test prawns when compared with control, whereas, there were significant elevations in GOT and GPT levels (P<0.05). Among GOT and GPT, the impact was more on GOT than GPT. The dosage and time dependent manner of inhibition or elevation in activities of these enzymes were recorded. Among these two insecticides, quinalphos showed more impacts than dimethoate on this non-target organism.

Materials and Methods
The post larvae of freshwater prawn, M. rosenbergii were purchased from Happy Bay Aqua Nova Hatchery, Mugiayur, Marakanam Taluk, and Kancheepuram District, Tamilnadu, India. They were safely brought to the laboratory in polythene bags filled with hatchery water and well-oxygenated. They were stocked in large cement tank (6’ x 4’ x 3’) and acclimatized for 2 weeks in ground water. During which they were fed with boiled egg albumin, Artemia nauplii and commercially available scalapi crumble feed alternatively thrice a day. The excreta, unfed feed and exuvia if any were removed daily, three fourth of the water was renewed daily and adequately aerated.

Quinalphos (Ekalux EC 25) and dimethoate 30% EC (TAFGOR) were purchased from local agro service centre. Ten concentrations of each quinalphos (0.250-1.375 µgl⁻¹) and dimethoate (0.060-1.350 µgl⁻¹) were prepared by mixing in distilled water afresh on every day. Post larvae (PL) of M. rosenbergii (1.5 ±0.2 cm and 0.1 ± 0.03g) were transferred to plastic aquaria of 10 l capacity (each with 10 PL) with three fourth of the water was renewed daily and adequately aerated.

ABSTRACT
The commercially important freshwater prawn, Macrobrachium rosenbergii post larvae (PL), 1.5±0.2 cm and 0.1±0.03 g were subjected to static renewal type acute toxicity bioassays against two organophosphate insecticides, quinalphos (Ekalux EC 25) and dimethoate 30% EC (TAFGOR). The 96 hr LC₅₀ values were determined to be 0.774 µgl⁻¹ for quinalphos and 0.856 µgl⁻¹ for dimethoate. The PL were exposed to lethal (the 96 hr LC₅₀) and sub-lethal (1/2nd and 1/4th of the 96 hr LC₅₀) concentrations of these insecticides (quinalphos: 0.774, 0.384 and 0.193 µgl⁻¹; dimethoate: 0.856, 0.428, 0.214 µgl⁻¹) for a duration of 4, 8 and 12 days to study their acute and chronic impacts on whole body activities of enzymes, the neurotransmitter, acetyl cholinesterase (AChE), the antioxidant, catalase and metabolic enzymes, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT). The activity levels of AChE and catalase were found to be significantly (P<0.05) decreased in test prawns when compared with control, whereas, there were significant elevations in GOT and GPT levels (P<0.05). Among GOT and GPT, the impact was more on GOT than GPT. The dosage and time dependent manner of inhibition or elevation in activities of these enzymes were recorded. Among these two insecticides, quinalphos showed more impacts than dimethoate on this non-target organism.

KEYWORDS
Prawn, quinalphos, dimethoate, AChE, catalase, GOT, GPT

Saravana Bhavan P
Department of Zoology, Bharathiar University, Coimbatore-641046, India.

Ananthi P
Department of Zoology, Bharathiar University, Coimbatore-641046, India.

Satgurunathan T
Department of Zoology, Bharathiar University, Coimbatore-641046, India.

Sowdeswari R
Department of Zoology, Bharathiar University, Coimbatore-641046, India.

Muralisankar T
Department of Zoology, Bharathiar University, Coimbatore-641046, India.

Ponmathi K
Department of Zoology, Bharathiar University, Coimbatore-641046, India.
in triplicates. The toxic water medium was renewed daily by siphoning method, causing minimum disturbance to the prawns and freshly prepared concentrations of quinalphos and dimethoate were added separately to maintain the toxic level in a steady state. During the experiment the prawns were neither fed nor aerated. The concentrations and their respective mortality percentage were subjected to computation for calculation of the median lethal concentration. The 96 h LC₅₀ value with 95% confidence limits was assessed using computerized program of Finney (1971) method of probit analysis.

Based on 96 hr LC₅₀ values of quinalphos and dimethoate, the concentrations of each for quinalphos (0.774 µg l⁻¹, 0.384 µg l⁻¹ and 0.193 µg l⁻¹) and dimethoate (0.856 mg l⁻¹, 0.428 mg l⁻¹ and 0.214 mg l⁻¹) were selected for treatment during 12 days. A common control was also maintained. Each group comprised 5 aquaria (15 l capacity) and each aquarium housed 20 PL. Sampling was done on day 4, 8 and 12. The entire quantity of medium in each aquarium was gently siphoned out daily and replaced by medium containing freshly prepared concentrations of quinalphos and dimethoate with minimal disturbance to the prawns. During the period of the experiment, the toxic medium was not aerated and the animals were fed with commercial scallop feed. The dead post larvae prawns were removed during the experiment. The activities of enzymes in post larvae prawns, such as AChE (Ellman et al., 1961), catalase (Sinha et al., 1972), and GOT and GPT (Reitman and Frankel, 1957) were assayed on 4th, 8th and 12th day of exposure by sacrificing the test PL in each group. Control prawns were similarly assayed at the same time as the test prawns. The differences between control and pesticide exposed groups were analyzed by adopting student-t test using SPSS software (version 16.0). All measurements were performed in triplicates and the results are expressed as mean ± SD of three individual observations. P<0.05 was fixed to assess the statistical significance.

Results and Discussion

The 96 hr LC₅₀ of quinalphos and dimethoate for M. rosenbergii PL was assessed to be 0.774 µg l⁻¹ and 0.856 mg l⁻¹ respectively (Tables 1 and 2). During bio-assay tests, the mortality of PL was found to be increased in response to higher concentrations of quinalphos and dimethoate (Tables 1 and 2). A comparison of the 96 hr LC₅₀ values assessed in the present study revealed that quinalphos was >1000 fold more toxic than that of dimethoate to M. rosenbergii PL (Tables 1 and 2). Therefore, it is clear that M. rosenbergii PL was more sensitive to quinalphos toxicity than that of dimethoate. The toxicity of quinalphos and dimethoate caused severe metabolic distress, which was evident from the escaping tendency of test PL from the aquaria and such behavior was based on dosage of these pesticides, which eventually leads to death of test PL.

Available literature revealed that the 96 hr LC₅₀ values report for quinalphos in a another species of freshwater prawn, Macrobrachium lamarmor (0.461 mg l⁻¹) was many more times lower than that of dimethoate to M. rosenbergii PL (Tables 1 and 2). Therefore, it is clear that M. rosenbergii PL was more sensitive to quinalphos toxicity than that of dimethoate. The toxicity of quinalphos and dimethoate caused severe metabolic distress, which was evident from the escaping tendency of test PL from the aquaria and such behavior was based on dosage of these pesticides, which eventually leads to death of test PL.

The activity of AChE was found to be significantly (P<0.05) lower in test PL on all sampling days irrespective of concentrations of quinalphos and dimethoate when compared with control (Table 3). However, maximum inhibition was seen on day-12 in 0.774 µg l⁻¹ concentration of quinalphos (58.03%) and 0.856 mg l⁻¹ concentration of dimethoate (54.40%) followed by 0.384 µg l⁻¹ and 0.193 µg l⁻¹ concentrations of quinalphos (54.14 & 49.74%) and 0.428 mg l⁻¹ and 0.214 mg l⁻¹ concentration of dimethoate (51.81 & 48.96%). Among the two pesticides, quinalphos showed maximum inhibition in AChE activity than dimethoate (quinalphos: 40.48-58.03% inhibition; dimethoate: 36.68-54.5% inhibition).

Similar inhibition in AChE activity has been reported in the grass shrimp, Palaemonetes pugio embryos exposed to OP pesticides, chlorpyrifos and malathion (Lund et al., 2000), in the marine crustacean species, Macrobrachium malcolmsonii exposed to carbaryl, dosulfan, and carbaryl (Geraldine et al., 1999; Bhavan and Geraldine, 2001, 2002), in the freshwater shrimp Paratyaus traiensis exposed to dimethoate (Kumar et al., 2010), in the clam, Ruditasates decussatus exposed to malathion (Nadji et al., 2010), in the Riceland prawn, Macrobrachium lanchesleri on exposure to chlorpyrifos (Tongbai and Damrongphol, 2011) and in freshwater fish, Streptoccephalus di-chrotomus exposed to parathion and glyphosate (Kumar and Ali, 2013). The inhibition of AChE recorded in M. rosenbergii PL indicates impairment in hydrolysis of ACh, which suggests disruption of synaptic transmission in the cholinergic system.

The activity of catalase was found to be significantly (P<0.05) lower in test PL on all sampling days irrespective of concentrations of pesticides when compared with control (Table 3). However, maximum inhibition was seen on day-12 in 0.774 µg l⁻¹ concentration of quinalphos (19.15%) and 0.856 mg l⁻¹ concentration of dimethoate (17.70%) followed by 0.428 mg l⁻¹ and 0.214 mg l⁻¹ concentration of dimethoate (16.78 & 15.07%) and 0.384 µg l⁻¹ and 0.193 µg l⁻¹ concentrations of quinalphos (15.26 & 12.36%). Among the two pesticides, quinalphos showed maximum inhibition (12.08-19.15%) of catalase activity than that of dimethoate (8.62-17.7%) in M. rosenbergii PL when exposed to quinalphos (15.26%) and 0.384 µg l⁻¹ and 0.193 µg l⁻¹ of quinalphos, and 0.428 mg l⁻¹ and 0.214 mg l⁻¹ of dimethoate) just the reverse was seen, the maximum inhibition of catalase was recorded in dimethoate (6.58-16.78%), than that of quinalphos (5.00-15.26%).

Catalase is a sensitive antioxidant biomarker against oxygen free radicals, the reactive oxygen species generated due to oxidative stress (Regoli et al., 2004; Atlı and Canlı, 2007). In the present study, due to toxicity of quinalphos and dimethoate excessive hydrogen peroxide or superoxide radical may have produced, which in turn inactivated the catalase activity (Table 3). Therefore, the protective mechanism was hampered in test PL even at lower sub lethal level of quinalphos and dimethoate (0.193µg l⁻¹ and 0.214 µg l⁻¹ respectively). OP inhibited catalase activity due to oxidative damage has also been reported in the brackish water prawn Penaeus monodon exposed to fenvalerate (Vijayavel and Balasubramanian, 2009), in the clam, Ruditasates decussatas (Nadji et al., 2010) and in freshwater fish, Labeo rohita (Thennmozhi et al., 2011) exposed to malathion.

The activities of GOT (aspartate aminotransferase, ASAT) and GPT (alanine aminotransferase, ALT) were found to be significantly (P<0.05) higher in test PL on all sampling days irrespective of concentrations of pesticides when compared with control (Table 3). However, maximum elevation in these enzymes activities was seen on day-12 in 0.856 mg l⁻¹ concentration of dimethoate and 0.774 µg l⁻¹ concentration of quinalphos followed by 0.428 mg l⁻¹ and 0.214 mg l⁻¹ concentration of dimethoate and 0.384 µg l⁻¹ and 0.193 µg l⁻¹ concentrations of quinalphos. Among the two pesticides, dimethoate showed maximum elevation in ASAT activity than that of quinalphos (dimethoate-GOT: 6.06-25.10%; quinalphos-GOT: 3.25-17.95%; dimethoate-GPT: 24.39-29.98%);
qlalphos-GPT: 1.29-23.29%). Similar elevation in activities of GOT and GPT has also been reported in the marine shrimp, Litopenaeus vannamei exposed to DDT, forsan, diazoin, folidal, guazion and lindane (Galindo-Reyes et al., 2000) and in the green mussel, Perna viridis exposed to copper, lead and zinc (Anand et al., 2010).

Increases or decreases in GOT and GPT activity levels are suggested as reflection of tissue damage or organ dysfunction (Olah, 1999; Rao, 2006). In the present study, the increase recorded in GOT activity suggests that an important reaction of the molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids) that G0T catalyzes was affected. Similarly, the increase in GPT indicates the fact that the test PL terribly required intensive glycogenesis to coop-up the severe energy crisis occurred due to quinalphos and dimethoate toxic stress.

In conclusion, quinalphos and dimethoate exhibited dose and time dependent responses to coop-up with toxic stress, and thus, adversely modulate the activities of AChE, catalase, G0T and GPT in M. rosenbergii. Therefore, these enzymes can be taken as biomarkers for monitoring water pollution by these pesticides in natural environment.

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Table 1: 96 h LC50 evaluation of quinalphos on M. rosenbergii PL

| Quinalphos (µgl⁻¹) | Observed Mortality | Mortality (mean) | Mortality (%) | LC50 (µgl⁻¹) | 95% confidence limit |
|-------------------|--------------------|-----------------|--------------|-------------|---------------------|
| Control           | 0.250              | 0 0 0           | 0.00         | 0.00        | --                  |
| 0.375             | 0.500              | 2 3 3           | 2.66         | 26.6        | --                  |
| 0.625             | 0.750              | 5 5 5           | 5.00         | 50.0        | 0.774               |
| 0.875             | 1.000              | 6 6 6           | 6.00         | 60.0        | 0.826               |
| 1.125             | 1.250              | 7 7 7           | 7.00         | 70.0        | 0.721               |
| 1.375             |                    |                 |              |             |                     |

T1, T2, and T3 represent triplicates of exposure each with ten numbers of M. rosenbergii PL

Table 2: 96 h LC50 evaluation of dimethoate on M. rosenbergii PL

| Dimethoate (mgl⁻¹) | Observed Mortality | Mortality (mean) | Mortality (%) | LC50 (mgl⁻¹) | 95% confidence limit |
|--------------------|--------------------|-----------------|--------------|-------------|---------------------|
| Control            | 0.060              | 0 0 0           | 0.00         | 0.00        | --                  |
| 0.150              | 0.300              | 1 1 0           | 0.66         | 33.3        | 0.856               |
| 0.450              | 0.600              | 2 3 0           | 1.66         | 16.6        | 0.891               |
| 0.900              | 1.050              | 3 4 1           | 2.66         | 26.6        | 0.822               |
| 1.200              | 1.350              | 4 4 3           | 3.66         | 36.6        | --                  |
| 1.500              |                    |                 |              |             |                     |

T1, T2, and T3 represent triplicates of exposure each with ten numbers of M. rosenbergii PL

Table 3: Activities of AChE, catalase, G0T and GPT in M. rosenbergii PL exposed to lethal and sub lethal concentrations of quinalphos and dimethoate

| Enzyme | Day | Control | Lethal and sub lethal concentrations of pesticide | Quinalphos (µgl⁻¹) | Dimethoate (mgl⁻¹) |
|--------|-----|---------|-----------------------------------------------|-------------------|-------------------|
| AChE   | 4   | 3.68±0.16 | 4.46 (10.06) | 2.04±0.08 (44.54) | 2.19±0.07 (46.31) | 2.17±0.16 (40.03) |
| Catalase | 8   | 3.77±0.05 | 53.31 (12.08) | 1.97±0.23 (47.74) | 2.00±0.13 (46.94) | 2.01±0.04 (46.68) |
|        | 12  | 3.86±0.13 | 58.03 (10.8) | 1.62±0.12 (54.14) | 1.77±0.16 (49.74) | 1.76±0.19 (54.40) |
|        | 4   | 36.74±0.36 | 32.30±1.13 (12.08) | 34.39±0.45 (6.39) | 34.90±0.24 (5.00) | 33.57±0.69 (8.62) |
|        | 8   | 37.25±0.25 | 31.67±0.75 (14.97) | 33.49±1.16 (10.09) | 34.53±0.50 (7.30) | 31.72±1.06 (14.84) |
|        | 12  | 38.00±0.15 | 30.72±0.40 (19.15) | 32.20±0.82 (15.26) | 33.30±0.57 (12.36) | 31.27±1.0 (17.7) |
|        | 4   | 36.74±0.36 | 32.30±1.13 (12.08) | 34.39±0.45 (6.39) | 34.90±0.24 (5.00) | 33.57±0.69 (8.62) |
|        | 8   | 37.25±0.25 | 31.67±0.75 (14.97) | 33.49±1.16 (10.09) | 34.53±0.50 (7.30) | 31.72±1.06 (14.84) |
|        | 12  | 38.00±0.15 | 30.72±0.40 (19.15) | 32.20±0.82 (15.26) | 33.30±0.57 (12.36) | 31.27±1.0 (17.7) |

| Enzyme | Day | Control | Lethal and sub lethal concentrations of pesticide | Quinalphos (µgl⁻¹) | Dimethoate (mgl⁻¹) |
|--------|-----|---------|-----------------------------------------------|-------------------|-------------------|
|        | 4   | 3.68±0.16 | 4.46 (10.06) | 2.04±0.08 (44.54) | 2.19±0.07 (46.31) | 2.17±0.16 (40.03) |
|        | 8   | 3.77±0.05 | 53.31 (12.08) | 1.97±0.23 (47.74) | 2.00±0.13 (46.94) | 2.01±0.04 (46.68) |
|        | 12  | 3.86±0.13 | 58.03 (10.8) | 1.62±0.12 (54.14) | 1.77±0.16 (49.74) | 1.76±0.19 (54.40) |
|        | 4   | 36.74±0.36 | 32.30±1.13 (12.08) | 34.39±0.45 (6.39) | 34.90±0.24 (5.00) | 33.57±0.69 (8.62) |
|        | 8   | 37.25±0.25 | 31.67±0.75 (14.97) | 33.49±1.16 (10.09) | 34.53±0.50 (7.30) | 31.72±1.06 (14.84) |
|        | 12  | 38.00±0.15 | 30.72±0.40 (19.15) | 32.20±0.82 (15.26) | 33.30±0.57 (12.36) | 31.27±1.0 (17.7) |
Each value is mean ± SD of 3 individual observations. Values in parentheses are % increase (↑) / decrease (↓).

Values are significant at P < 0.05.

NS, Not significantly significant.

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GPT (IU l⁻¹)

| 4 | 26.72±0.57 | 29.15±1.02 | 28.38±0.77 | 27.59±0.59 | 28.4±0.21 | 30.22±0.71 | 31.20±0.53 | 113.09 | 28.34±0.07 | 6.06 | 29.70±0.60 | 23.17±0.11 | 23.24±0.21 | 27.70±0.60 |
| 8 | 27.70±0.60 | 30.4±0.71 | 29.37±0.82 | 30.4±0.52 | 32.33±0.52 | 31.63±0.83 | 14.18 | 29.44±0.62 | 2.28 |
| 12 | 27.45±0.54 | 32.4±0.89 | 29.0±0.39 | 34.4±0.75 | 33.66±1.18 | 30.83±0.87 | 12.32 |

GOT (IU l⁻¹)

| 4 | 23.17±0.11 | 26.59±0.98 | 24.40±0.20 | 23.47±0.81 | 28.44±0.15 | 26.40±0.28 | 24.39±0.38 | 15.26 |
| 8 | 23.24±0.21 | 26.76±1.01 | 25.46±0.68 | 24.54±0.75 | 29.6±0.13 | 27.23±0.13 | 25.65±0.43 | 10.37 |
| 12 | 23.31±0.17 | 27.84±1.09 | 26.37±0.27 | 25.48±0.41 | 30.3±0.11 | 28.54±0.27 | 26.62±0.18 | 14.19 |