5. SUMMARY

The brine shrimp *Artemia* is widely distributed in more than 600 coastal locations and inland waters in and around the world. The genus *Artemia* is highly adapted to live in the hypersaline waters in inland salt lakes and the evaporation ponds, channels and reservoirs of salterns. In India, *Artemia* has been recorded from the coastal saltpans from Gujarat, Didwana Lake and Sambar Lake in Rajasthan, Gulf of Kutch, Tuticorin and Kanyakumari Districts in Tamilnadu.

Artemia is a crustacean that is widely used in laboratory toxicity studies due to its small body size and short life span together with its availability from dry cysts. It has the broad tolerance capacity to environmental factors such as salinity, temperature and dissolved oxygen and *Artemia* is considered as a key species. Brine shrimp are also constituting a major food source for birds that are visiting saline ponds, which in turn constitute an effective cyst dispersal mechanism (Green *et al.,* 2005).

The purpose of pesticide usage is to eliminate undesired organisms that could damage crops of transmit diseases to animals and humans. The environment including water ecosystem is very often contaminated by low concentration of various chemical compounds of foreign origin (Benova *et al.,* 2001). The contamination of water bodies is generally due to crop treatments, farm disinfections and industrial wastes.The branchiopod crustacean *Artemia sp.* has been commonly used as larval food for fish and crustaceans in aquaculture (Varo *et al.,* 2002). The common inhabitats for this species are coastal and shallow saline water, which are usually close to agricultural areas. Insecticides commonly used in agriculture are a danger for

Artemia populations due to its toxicity, especially considering that this is a target species for its capacity of bioaccumulation. This factor is of great importance because the accumulation of the pesticide in *Artemia* tissue that could reach the aquatic trophic chain to which *Artemia* is one of the first links. Therefore acute and chronic toxicity bioassay with the microcrustacean *Artemia franciscana* was chosen as testing organisms for the organochlorine, organophosphate, pyrethroid, and carbamate was performed in the present work in order to determine whether this species is suitable for ecotoxicity testing in aquatic environments.

For the present study commercially available *Artemia franciscana* cysts were purchased from commercial laboratory. The diameter of the hydrated cysts, decapsulated cysts and the naupliar length were measured with a compound microscope equipped with a micrometer. The morphometric and meristic characteristics of the adult such as total length, abdominal length was measured (using a dissection microscope with a graph paper fixed on the platform), abdominal width, furcal length, head width, eye separation, diameter of the eye, length of the first antenna and length of the last abdominal segment of both male and female and ovisac width of female (with a compound microscope equipped with a micrometer) were measured. The number of setae on the left and right furca was counted. The cysts were hatched in natural seawater under aeration. The nauplii were removed from the hatching medium taking advantage of the positive phototactic behaviour of the larvae and were used to study the survival and growth. The pesticide effect was assessed on eight pesticides such as two organochlorine (lindane and endosulfan), two organophosphate (malathion and monocrotofos), two pyrethroids (cypermethrin and

fenvalerate), and two carbamate (Carbaryl and carbofuran). Simultaneously a control experiment was also carried out and results were monitored.

The nauplii, preadult and adult *Artemia* species were exposed separately to various concentrations of lindane, endosulfan, malathion, monocrotofos, cypermethrin, fvenvalerate, carbaryl and carbofuran and the survival was noted. From this, the LC50 values were calculated using the programme SPSS version 11.0 for windows 2001.

The individuals of *Artemia* population were reared in two sublethal concentrations of each pesticide and the survival, growth and reproductive characteristics were studied. The offspring (1^{st} generation) were also exposed to the respective sublethal concentrations of the eight pesticides and the survival, growth and reproductive characteristics were studied. In all the experiments, the *Artemia* was fed with 1% glucose twice a day and the medium was changed in four days. The findings of the present study are summarized below

Biometrics of cysts and nauplii

The mean diameter of the fully hydrated cysts of *Artemia s*p. population was 245.15 µm and the decapsualted cysts was measured 229.80µm. The mean naupliar length was 442.80µm.

Morphometry and meristic characteristics of the adult

The total length of the adult male Artemia in control and experiment varied from 7.17 ± 0.80mm to 9.68 ± 0.377mm. The abdominal length was between 3.3 ± 0.35mm and 4.81 ± 0.33. The abdominal width was between 0.768 ± 0.54mm and

0.34 ± 0.33mm and 0.57 ± 0.05mm.The total length of female *Artemia* was between 8.26 ±0.89mm and 10.48 ± 0.173mm. The abdominal length varied from 4.24 ± 0.63 to 5.255 ± 0.158mm. The abdominal width was between 0.62 ± 0.05mm and 0.77 ± 0.022mm. The furcal length was between 0.12±0.03 and 0.24±0.038mm in male and female. The head width ranged from 0.57± 0.17mm to 0.69±0.105mm in male and0.63±0.05 to 0.645±0.982mm. The eye diameter was between 0.23±0.02 to 0.30±0.028mm in male and female. The ovisac width was between 1.13±0.18 to 1.21±0.055mm.

Analysis of variance showed significant in difference total length ,abdominal length, eye separation in male *Artemia franciscana*. But only total length and firsat antennal length were significant in female *Artemia.*

Survival

The cyst was allowed to hatch in the laboratory and the survival was observed. Artemia showed better survival in control as well as in experiment. On the 30^{th} day, 52 – 58% survival was observed in all the control and experiment.

Growth

The maximum growth attained by the male and female was 9.68 and 11.081mm respectively.

Effect of pesticides

Acute toxicity and the effects of sublethal concentrations of organochlorine (lindane, endosulfan), organophosphate (malathion, monocrotofos), pyrethroid (cypermethrin, fenvalerate), carbamate (carbaryl, carbofuran) pesticidal effect on the survival, growth and reproductive characteristics were studied.

Acute toxicity

The acute toxicity for eight pesticides on *Artemia franciscana* showed higher mortality, with increase in the concentration of each pesticide as well as in the exposure period. *A.franciscana* sensitivity to chosen pesticides was found to be stage specific.Irrespective of the pesticide tested the LC50 values were low for nauplii and high for pre adult and adult *Artemia.* The LC50 values for 96h exposure of lindane to nauplii, preadult and adult *Artemia* were 0.225, 0.283 and 0.566 µg/L respectively. The 96h LC50 values of endosulfan for nauplii, preadult and adult were 0.273, 0.565, 0.515 µg/L. The 96h LC50 value of malathion for nauplii, preadult and *Artemia* were 0.002, 0.006 and 0.027 respectively. The 96h LC50 values of monocrotofos for *Artemia* nauplii, preadult and adult were 0.005, 0.017 and 0.049 µg/L respectively. The 96h LC50 values of cypermethrin for *Artemia* nauplii, preadult and adult were 0.005, 0.007 and 0.014 µg/L respectively. The 96h LC50 values of fenvalerate for *Artemia* nauplii, preadult and adult were 0.004, 0.006 and 0.008 µg/L. The 96h LC50 values of carbaryl for *Artemia* nauplii, preadult and adult were 0.040, 0.065 and 0.112 µg/L. The 96h LC50 values of carborufan for *Artemia* nauplii, preadult and adult were 0.031, 0.061 and 0.268 µg/L respectively.

Effects of sublethal concentrations of pesticides

When exposed to sublethal concentrations of lindane (0.002µg/L - 0.04µg/L), endosulfan (0.002µg/L – 0.04µg/L), malation (0.0002µg/L – 0.004µg/L), monocrotofos (0.0001µg/L- 0.002µg/L), cypermethrin (0.00025µg/L– 0.0005µg/L), fenvalerate (0.00025µg/L -0.0005µg/L), carbaryl (0.00025µg/L – 0.0005µg/L) and

carbofuran (0.0025µg/L- 0.005µg/L) separately, the pesticide exposed to *Artemia* showed lower growth and survival than the control in almost all the cases.

In most of the cases, the number of offspring per brood and the total offspring per individual were lower than that of the control. The offspring of the control showed increased survival, while in the sublethal concentrations the survival was low and the reproductive performance was also poor.

From this study, it was inferred that even very low quantities of pesticides reduced the reproductive capacity of *Artemia franciscana.* When the saltpan is contaminated with pesticides, bioaccumulation of pesticides occurs in the food chain. Also *Artemia* grown in the saltpan contaminated with pesticide may not yield good quantity and quality of cysts. Hence appropriated measures must be taken before selecting site for *Artemia* culture.

From this study it was also observed that *Artemia franciscana* is one of the most valuable test organism available for ecotoxicity testing. Its natural tolerance may be faced as an adva *Artemia franciscana* can be subjected to both field and laboratory conditions. Tolerance of *Artemia franciscana* make this genus adaptability to a great variety of testing conditions, in estuarine, marine or hypersaline environments.