4. DISCUSSION

Toxicological bioassays are currently the most commonly used tests to determine the effectiveness of certain species as bioindicators and to evaluate pollution in the environment (Bustos-Obregon and Vargas, 2010). A bioassay is a test that involves living organisms to analyse substances in terms of the biological response they produce (Silva *et al.,* 2007). There is no doubt that currently ecotoxicological bioassays have become an increasingly common research topic for scientists worldwide mainly because of growing pollution of global environments.(Bustos Obregon and Vargas, 2010). *Artemia* is one of the most valuable test organisms available for ecotoxicity testing and research done so far allows us to state that it is possible to sustain several options related to *Artemia* use in toxicology and eco-toxicology. *Artemia franciscana* was chosen as the testing organism, considering the feasibility of culturing large populations using laboratory methods, facility to manage, short term results given its short life cycle, its availability, low cost and the fact that it is found worldwide.

In order to characterize *Artemia franciscana* used in the present experiment, biometrics of cyst and nauplii, morphometric and meristic characters of adult *Artemia* were studied. The cyst size and naupliar length are unique characteristics, which seem to be species specific, since they do not differ significantly in values recorded for cysts produced under laboratory culture (Abatzopoulos, *et al.,* 1998). In the present study, the diameter of the cysts was 245.15 ± 14.31µm and the length of the nauplii was 442.80 ± 20.05µm (Table 3.1). The difference in diameter of the cyst and nauplii

was due to the presence of bisexual population. Hontoria (1990) studied fourteen *A .franciscana* populations in Utah; USA found that the diameter of cysts ranged between 217 and 230µm and Galera, Zambia (Columbia) cysts with 242 and 245µm diameter respectively (Cohen *et al*, 1999). The diameter of cysts of *Artemia franciscana* harvested from Bahia de Ohuira, Salinas of Hidalgo and El Marquez, Mexico were 266.3, 386.3µm and 292 µm respectively (Castro *et al.*, 2006). The *Artemia* cysts collected from Pozos Colorados Columbian Caribbean had the diameter of 252.9 µm (Camargo *et al.*, 2005) and from Great Salt Lake, Utah, were 244.2 – 252.5 µm (Sorgeloos *et al.*, 1986). The cysts collected from Sabkhet Sijoumi, NE Tunisia with a mean diameter of 260.9 µm (Ben Naceur *et al.*, 2008), Tayrona and Kangaru Colombia Caribbean with 233.4 and 236.8 µm respectively (Camargo *et al.*, 2005). The diameter of Tibet cysts was 330 µm, which is the biggest *Artemia* cysts recorded by Abatzopoulos *et al.*, (1998).

Yu and Xin (2006) recorded the largest mean size of cysts for a population of *Artemia tibetiana* 358.78µm, from 'Gaize Lake' recently known as Lagkor Co (Zheng and Sun, 2013). Ma and Wang (2003) found that, the largest cysts of *Artemia* belonging to parthenogenetic population of Aqqikkol Lake (China) 315.8µm which was supported by Wang and Sun (2007), Wang (2003). Similarly, in the present study also the diameter of cysts of *Artemia franciscana* was 245.15 ±14.31µm which coincides with the diameter of cyst of *A.franciscana* reported by Cohen *et al.*, 1999 and Sorgeloos *et al*, (1986).

The chorion thickness of *A.franciscana* used in the present study was 8.10 ±0.07µm. The thickest chorion of *Artemia*has been reported for parthenogenetic cysts

from Chengkou Saltern with a thickness of 17.3µm (Xu, 1996). Asem and Sun (2014) indicated a chorion thickness of 6.3µm in parthenogenetic population of China. Chorion thickness values of 10.2µm (Wang and Sun, 2007; Wang,2009) and 8.7 µm (Liu *et al*., 1998) have also reported in Chinese saltern. In the present study the chorion thickness was observed 8.10 ± 0.07 which in consistent with the study of Liu *et al* (1998).

The naupliar length of *Artemia franciscana* of the present study was 442.80 ± 20.05µm. Kara *et al.* (2004) studied the naupliar length of *Artemia salina* from Chott Marouane 'Algeria' (428.7 µm) and Van Baller (1987) observed in Stax Tunisia (422.2 µm). *Artemia franciscana* nauplii from Kangaru 'Colombian Caribbean was 426.1 µm (Camargo *et al.*, 2005) and from San Francisco Bay USA was 428 µm (Sorgeloos *et al.*, 1986). The fresh nauplii produced from Sabkhet Sijoum, Tunisia had an average length of 436.7 µm (Ben Naceur *et al.*, 2008). The biggest size of nauplii reported so far is that of the Lagkor Co lake (Tibet, PR China) (Abatzopoulos *et al.*, 1998) and the Jingyu lake (Qinghai Tibet Plateau, PR China) (Van Stappen *et al.,* 2003) with the mean length of 667 µm and 607.1 µm respectively. Like that, in the present investigation the *Artemia franciscana* nauplii length was 442.80 ± 20.05µm which coincides with Tunisia population and it was reported by Van Baller (1987).

In *Artemia,* morphological traits have been the basis to describe species and strains, although controversy exists on the way of selecting and using the suitable morphological traits as well as on the degree of their genetic or environmental determination (Gajardo *et al.*, 1998). Analysis of variance showed statistically

significant differences. In the present study, the male morphomeric characters showed significant difference in total length, abdominal length, number of setae on the left furca, and eye separation. In females only the total length and the first antennal length showedsignificant result. The ovisac width did not show any significant differences.In both male and female the furcal length, number of setae on the right furca, head width and eye diameter did not show any significant differences.

Morphometric characters which are common between males and females measured in *Artemia* byAsem and Rastegar Pouani, (2007); Zhou *et al.*, (2003); Camargo *et al.*, (2003) Amat *et al.*, (2005). In the present study ten morphometric characters which are common to male and female were studied. The morphometric characteristics that contributed most of the discrimination between Nambian and Madagascar populations were, the length of the telson, the length from the 3rd abdominal segment to the end of the abdomen and abdominal length (Triantaphyllidis *et al.*, 1996). Very long telson and very short abdomen are the characteristics that allow the Swakopmund (Namibia) population from a group alone, far discriminated from the populations from China, Greece and Kazakhsthan (Triantaphyllidis *et al.*, 1997).

El-Bermawi *et al.* (2004) found that an Egyptian sexual strain *A. salina* was discriminated from asexual strains of Egypt mainly on the basis of three morphological characters namely total length, abdominal length and number of setae on the furca. Significant differences were demonstrated in the morphometry of the same *Artemia urmiana* strain reared at various salinities (Agh *et al.*, 2009). *Artemia urmiana* can be distinguished from the Iranian parthenogenetic populations on the

basis of 9 morphological parameters. These observations provide useful evidence for species specific. Morphological characteristics of *A. urmiana* and was supporting the findings of Triantaphyllidis *et al.* (1997).

The findings of Amat *et al.* (1995), El-Bermawi *et al.* (2004) and Agh *et al.* (2009) confirmed the importance of furcal characters as important morphometric factors for discriminating sexual and parthenogenetic populations of *Artemia* (Baxevanis *et al.*, 2005).Agh *et al.* (2009) also emphasized that some other morphometric characteristics, in particular, the distance between the compound eyes, the abdominal length and the length of the telson are important for the discrimination of sexual urmiana from asexual *Artemia* populations from Iran. According to El-Bermawi *et al.* (2004), salinity affects the length of furca and number of furca setae, also a reliable discriminating character of *Artemia* population. In the present study, the total length, abdominal length, abdominal width and furcal length of *Artemia franciscana* was higher in control than in pesticide reared ones. This view support the result of El-Bermwi *et al*. (2004), where at higher salinity, the total length, abdominal length, number of setae on the furca were reduced. In the present study also the size of the furca and the number of setae on the furca were reduced due to pesticide effect.

Artemia species generally show a size sexually dimorphic which female individuals have larger body than males (Triantaphyllidis *et al.*, 1997). A size difference between sexes can be interpreted as a mating advantage because the female carriers the male during copulation. For this reason, the female needs a large body for this mating procedure and for surviving the mating process (Asem *et al.*, 2010; Castro Mejia *et al*., 2013). In the present study also female *Artemia franciscana* had bigger

body size than the male for mating and surviving, which support the finding of Asem *et al.*,(2007; 2010); Castro Mejie *et al.*, (2013). No evidence is available regarding the pesticide effect on morphometric and meristic characters of *Artemia.*

***Artemia* Bioassay**

Short term exposure of *Artemia franciscana* larva, preadult and adult to different concentration of Organochlorine, Organophosphate, Pyrethroid and Carbamate revealed that at low concentration of each toxicant, only a few very intolerant individuals succumbed, at intermediate concentration most of them died at high concentration and only a few tolerant individuals were survived.

The 96h LC50values of Organochlorine – Lindane to *Artemia franciscana* nauplii, preadult and adult were 0.225, 0.283, and 0.566µg/L respectively and for Endosulfan the 96h LC50values were observed as 0.273, 0.565 and 0.615µg/L respectively.

The LC50Value of Organophosphate- Monocrotofos to *Artemia franciscana* nauplii, preadult and adult at 96h exposure period was 0.002, 0.006, 0.027 µg/L respectively and for Malathion it was 0.005, 0.017 and 0.049µg/L respectively. The *Artemia franciscana* nauplii, preadult and adult exposed to Pyrethroid cypermethrin at 96h showed the LC50 values such as 0.005, 0.007 and 0.014µg/L respectively and for fenvalerate the LC50 values were 0.004, 0.006 and 0.008µg/L respectively.

The LC50 values of Carbamate- Carbaryl exposed to *Artemia* nauplii, preadult and adult at 96h were 0.040, 0.065 and 0.112µg/L respectively and for Carbofuran 0.031, 0.061and 0.268µg/L respectively. From this study it is evident that *Artemia franciscana* nauplii were more sensitive to monocrotofos at 96h (0.002µg/L) than

other pesticides. *Artemia* preadult were more sensitive to monocrotofos and fenvalerate at 96h (0.006µg/L) than other pesticides, and *Artemia* adult were more sensitive to fenvalerate at 0.008µg/L than other pesticides.

Sanchez-Bayo (2006) reported that, the most toxic organochlorines to planktonic crustaceans are endrin (LC50 0.1 and 2µg/L to copepod and ostracoda respectively, but 9.1 mg/L in cladocera), endosulfan (LC50 value was from 0.9mg/L in ostracoda to 3.9mg/L in anostraca), lindane, DDD, aldrin and DDT all of which have LC50 value below 10µg/L.

Endosulfan is extremely toxic to fish and aquatic invertebrates (Sunderam *et al.*, 1992). Endosulfan residues in farmed snakehead and carp fish (*Channa striata* and *Catla catla*) revealed higher bioaccumulation factor (BAF) of log 4.5 (± 0.45) (Amaraneni, 2002).Organochlorines remain serious hazards in aquatic ecosystem even though many of them were banned for agricultural use decades ago (De Lorenzo, *et al.,* 2002). According to Sanchez-Bayo, (2006) Organophosphates are very toxic to aquatic organisms with average LC50 value ranging from 17µg/L in cladocera to 76µg/L in copepod. Anostraca species were more tolerant (LC50 8200µg/L) with parthenogenetic *Artemia* being less sensitive (Varo, *et al*.1997; Lahr, 1997; Crisnel et al. 1994). The toxic compounds to planktonic crustaceans were coumaphos and monocrotofos (0.2µg/L in cladocera) least toxic were chlorfenvinphos (5.9 µg/L) and acephate (52µg/L).

The acute toxicity studies of chloropyrifos (organophosphate) in non target aquatic organisms have been performed mostly on adult fish. Only a few investigations have been carried out with aquatic crustaceans and most of them were

conducted on larval stages. The 96h LC50 of chloropyrifos for larvae of the grass shrimp was 4.28 nmol/L (Key and Fulton, 2006). In freshwater shrimp, *Paratya australiensis*, 96h LC50 of chloropyrifos is 1.79 nmol/L (Kumar *et al.*, 2010). The 24h LC50 value of chloropyrifos for Penaeus monodon was 149.55 nmol/L (Eamkamon *et al.*, 2012) and *Artemia* salina was 9.09 nmol/L(Varo *et al.*, 2002).

Alyuruk and Cavas (2013) studied the EC50 value of herbicide Diuron of *Artemia* salina was found to be 12.01 mg/L. Gartenstein *et al.* (2006) found that the 48h LC50 for *Artemia* salina larvae when exposed to Diflubenzuren, cypermethrin and diazinon was 8.33, 4.33 and 6.88 µg/L respectively. The present result of organophosphate did not support the findings of Varo *et al* (1997) and other researchers. Taking into consideration *Artemia* is a part of zooplankton, one of the first steps in aquatic tropic chain so highly sensitive to pesticides.

The pyrethroid toxicity reported by Sanchez-Bayo (2006) indicated that their toxicity to plankton crustaceans with LC50 values ranging from 0.2µg/L for esfenvalerate to 802µg/L for permethrin, both in water fleasalso (Day, 1989). The LC50 value of esfenvalerate to caddis fly larvae was 0.05µg/L (Johnson *et al,* 2008). The short term exposure to *Daphnia magna* was 0.3µg/L (Reyanaldi and Liess, 2005).In the present study, pyrethroid, the cypermethrin 48h LC50 value for *Artemia* nauplii, preadult and adult were 0.007, 0.009 and 0.021µg/L respectively and for fenvalerate 0.005, 0.008 and 0.017µg/L respectively.

In the present result of pyrethroid did not correlate with the findings of Johnson *et al* (2008), *Artemia* is highly sensitive to other pyrethroid than other planktons. The LC50 values of carbamate - carbaryl to *Artemia* nauplii, preadult and

adult at 24h LC50 was 0.073, 0.132 and 0.205µg/L respectively and 0.046, 0.112 and 0.457 µg/L for carbofuran respectively. Sanchez-Bayo, (2006) reported that the most toxic carbamate to planktonic crustacean were carbofuran and methiocarb with the average LC50 value of 9 and 14µg/L in cladoceran respectively and least toxic were thiocarbamate, cycloate, pubulate and butylate with LC50 between 10 and 85mg/L for cladocera. Average LC50 values vary significantly between anostraca (18.7mg/L) and all other taxa(233-776µg/L).According to Tilak *et al.*(1981), the calcualted LC50 value for 96h for *Catla catla, Anabass testidineus*, *Mystus cavasius* and *Mystus vittatus* exposed to carbamate insecticide carbaryl were 6.4, 5.5, 4.6 and 2.4 ppm respectively.

According to Navid Hosseini Mansoub *et al.* (2011),*Artemia* IIinstar larvae, showed that a minimal increase of mortality rate of 0.33 ppm at 24h which was much higher than the present study in which the LC50 value for 24 h exposure of carbaryl was 0.161µg/L. The differential toxicity can be attributed to the differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion (Omitoyin *et al.*, 2006). Barahona and Sanchez-Fortun (1999) reported that increased toxicity to carbamate increases with aging of *Artemia salina*. In the present study also toxicity increases with age which coincides with the finding of Barahona and Sanchez-Fortun (1999).

Chronic Toxicity

Survival, growth and reproduction were analysed for the parental generation and for the F1 generation. Survival in the parental generation was the most sensitive end point through day 21. Between days 21 and 28 there was a decline in survival

across all concentrations resulting in no statistically significant effects at any concentrations compared to the control on day 28. After reproductive pairing on day 12, the dose response relationship at the higher test concentrations became less distinct, exhibiting higher survival in less concentration.The F1 generation appeared to acclimate to the organochlorine, organophosphate, pyrethroids and carbamate exposure was significant less sensitive, than the parental generation in terms of survival. The F1 generation growth was significantly less than the parental generation growth across all test concentrations including control is unclear (Brix *et al.*, 2003). The present study also correcter with view of Brix *et al.*,(2003). Only a very few chronic studies have been conducted with brine shrimp.

Short term esfenvalerate (0.01 to 0.1µg/L) exposure causes reduced egg production in female may fly (Beketov and Liess, 2005). Chronic exposure to fenvalerate concentrations of 0.005 µg/L increased *Daphnia galeata* mendotate longevity (Day, 1989). Continuous (21 day) exposure to 0.3 µg/L fenvalerate has a greater adverse impact on *D. magna* reproductive rate (Reynaldi and Liess, 2005). Sub-lethal concentration of pyrethroid exposures altered a number of reproductive and early developmental processes in fish, when salmon milk and eggs were exposed to cypermethrin concentrations as low as 0.1µg/L, the fertilization rate was reduced. Similarly, two pulsed exposures of 1 µg/L esfenvalerate delayed blue gill sunfish spawning (Little *et al.*, 1993). Gonad development and structure in the freshwater snake head fish, *Channa punctatus* was altered by exposure to Devicyprin (Srivastava *et al.*, 2008).

The effects of four sublethal concentrations of carbofuran (250, 500, 100 and 2000 ppm) on the reproductive capacity of freshwater snail (*Radix quadrasi*) was determined by Imelda F. Pagulayan *et al.* (1992). Results showed that incubation period is delayed and inhibited by 1000 and 2000 ppm carbofuran but not by lower concentrations. Gebhardt (1976) evaluated with brine shrimp, the effect of cadmium, copper and mercury on survival, growth and reproduction. It was conducted at 27^{o}C using a static renewal test design, Great Salt Lake water was used as the dilution water, and brine shrimp were fed the hypersaline algae *Dunaliella viridis*. Under these conditions, the onset of reproduction was considerably later and reproduces on day 29. The number of nauplii produced was not quantified in that study.But in contrast,in the present study, the control begin to reproduce on day 19 and in the experiment, the *Artemia* reproduce between 19 – 25 days. The numbers of nauplii were also quantified and it was 78 in control and it was ranged 65 - 95 in the experimental group.

Cunningham (1976) investigated the effects of the insecticide Dimilin (TH6040) on different life stages of brine shrimp under static renewal test conditions. In experiment, brine shrimp reproduction was evaluated by exposing brine shrimp pairs and monitoring the number of nauplii produced. On day 21 of the experiment, adult survival ship in the control was approximately 90% and declined to approximately 70% on the day 28. By day 40, survival had dropped below 50% and all shrimp were dead by day 80.In the present study, under the conditions of flow through, the brine shrimp completed their life cycle more quickly than those in other studies. The mortality was observed across all test concentrations between days 21 and 28 was caused by the shrimp reaching the end of their life span. This was supported by Gillespie and Stephens (1977) who suggested that the generation time of

Artemia may be less than three weeks under good conditions.The offspring in the respective sublethal concentrations had reduced reproductive performance. This was caused not only by fewer broods per female but also less offspring per brood, and this also reduced the percentage of offspring surviving to maturity (Browne, 1980c). In the present study, the offspring in the respective submethal concentrations had reduced reproductive performance, fewer broods per female and less offspring per brood, whcich support the view of Browne (1980c).