

Artemia nauplii after 24 and 48 hrs using SPSS ver.13.0 IBM software [33].

3. RESULTS

The 24 and 48hrs LC₅₀ values for *Artemia* nauplii exposed to Malathion and Glyphosate were depicted in Table 1. The 24 and 48hr LC₅₀ values of Malathion to *Artemia* nauplii were 58.3 and 17.3 ppm, respectively and those for glyphosate were 0.028 and 0.019 ppm, respectively.

Table 1: The LC₅₀ values of Malathion and Glyphosate to *Artemia* nauplii after 24 and 48hrs exposure

S. No	Pesticides	LC ₅₀	
		24hr	48hr
1.	Malathion	58.3 ppm	17.3 ppm
2.	Glyphosate	0.028 ppm	0.019 ppm

An overall significant effect of Malathion and Glyphosate on the mortality of *Artemia* nauplii was revealed ($P < 0.05$; Tables 2 & 3). Different concentrations of Malathion and Glyphosate also had significant effect ($P < 0.05$).

Table 2: Comparative toxicity (mortality %) of Malathion and Glyphosate to *Artemia* nauplii after 24hrs exposure (ANOVA)

Variable	Source	Sum of Squares (mortality %)	df	Mean square	F	Sig.
Malathion Vs <i>Artemia</i> nauplii	Between groups	63.067	4	15.767	236.50	.000*
	Within groups	0.667	10	0.067	--	--
	Total	63.733	14	--	--	--
Glyphosate Vs <i>Artemia</i> nauplii	Between groups	71.067	4	17.767	133.250	.000*
	Within groups	1.333	10	0.133	--	--
	Total	72.400	14	--	--	--

Note: *Significant level is expressed at 95% confident interval ($P < 0.05$)

Table 3: Comparative toxicity (mortality %) of Malathion and Glyphosate to *Artemia* nauplii after 48hrs exposure (ANOVA)

Variable	Source	Sum of Squares (mortality %)	df	Mean square	F	Sig.
Malathion Vs <i>Artemia</i> nauplii	Between groups	71.600	4	17.900	134.250	.000*
	Within groups	1.333	10	0.133	--	--
	Total	72.933	14	--	--	--

Glyphosate Vs <i>Artemia</i> nauplii	Between groups	103.067	4	25.767	193.250	.000*
	Within groups	1.333	10	0.133	--	--
	Total	104.400	14	--	--	--

Note: *Significant level is expressed at 95% confident interval ($P < 0.05$)

4. DISCUSSION

The present study was aimed to evaluate the short-term toxicity of the two highest market selling organophosphorus pesticides namely, Malathion and Glyphosate on 24hr old (instar I) larvae of *Artemia*. The brine shrimp *Artemia* has many advantages as a marine test organism and widely used for the evaluation of different types of contaminants including pesticides and heavy metals [34, 27, 35-38, 28, 39]. It also play a main food supply for the millions of shorebirds, and also used as major food source in marine and fresh water aquaculture [38,40].

The results showed differences in sensitivity of *Artemia* against two pesticides namely Malathion and Glyphosate (Table 1), the 24 and 48hr LC₅₀ values of Malathion on *Artemia* nauplii were 58.3 and 17.3 ppm, respectively. In an earlier study, a group researcher reported 24hr LC₅₀ value of 81.5 ppm for Malathion to the freshwater anostracan, *Streptocephalus proboscoides* nauplii [41]. A group of scientists have found 24hr LC₅₀ value of 67.7 ppm for Malathion to *S. sudanicus* nauplii [42]. However, some of them reported 24hr LC₅₀ value of 6.4 ppm for Malathion to *S. proboscoides* nauplii [43]. Likewise, a researcher observed a 24hr LC₅₀ value of 24.5 ppm for Malathion to *Branchinecta sandiegonensis* [44]. Table 4 summarizes the toxicity of Malathion and Glyphosate used in earlier studies as well as in the present study to different branchiopods.

Table 4: Toxicity of (LC₅₀) of Malathion and Glyphosate to branchiopods

Pesticides	Test organisms	Hours (LC ₅₀)	Values	Source
Malathion	<i>S. proboscoides</i> 24hr nauplii	24hr LC ₅₀	81.5	Crisinel <i>et al.</i> (1994)
Malathion	<i>S. Sudanicus</i> 24hr nauplii	48hr LC ₅₀	67.7	Lahr <i>et al.</i> (2001)
Malathion	<i>S. proboscoides</i> 24hr nauplii	24hr LC ₅₀	6.4	Verschueren (2001)
Malathion	<i>B. sandiegonensis</i> 24hr nauplii	24hr LC ₅₀	24.5	Ripley <i>et al.</i> (2003)
Malathion	<i>S. dichotomus</i> 24hr nauplii	24hr LC ₅₀	14.1	Arun Kumar and Jawahar Ali (2014b)
Malathion	<i>S. dichotomus</i> 24hr nauplii	48hr LC ₅₀	12.3	Arun Kumar and Jawahar Ali (2014b)
Malathion	<i>Artemia</i> 24hr nauplii	48hr LC ₅₀	1.00	Sidharta (1997)
Malathion	<i>C. dubia</i>	24hr LC ₅₀	0.00318	Nelson and Roline (1998)
Malathion	<i>D. magna</i>	48hr LC ₅₀	0.003	Rassoulzadegan and Akyurtakli (2002)
Malathion	<i>Artemia</i> 24hr nauplii	24hr LC ₅₀	58.3	Present study
Malathion	<i>Artemia</i> 24hr nauplii	48hr LC ₅₀	17.3	Present study
Glyphosate	<i>T. platyurus</i> 24hr nauplii	24hr LC ₅₀	0.35	Foetman <i>et al.</i> (2000)
Glyphosate	<i>B. sandiegonensis</i> 24hr nauplii	24hr LC ₅₀	0.0118	Ripley <i>et al.</i> (2003)
Glyphosate	<i>B. thailandensis</i> 24hr nauplii	24hr LC ₅₀	0.319	Boonsoong and Bullangpoti (2012)

Glyphosate	<i>S. dichotomus</i> 24hr nauplii	24hr LC ₅₀	0.026	Arun Kumar and Jawahar Ali (2014b)
Glyphosate	<i>S. dichotomus</i> 24hr nauplii	48hr LC ₅₀	0.014	Arun Kumar and Jawahar Ali (2014b)
Glyphosate	<i>D. magna</i>	48hr LC ₅₀	42	Alberdi <i>et al.</i> (1996)
Glyphosate	<i>D. magna</i>	48hr LC ₅₀	250	Raipulis <i>et al.</i> (2009)
Glyphosate	<i>Artemia</i> 24hrs nauplii	24hr LC ₅₀	0.028	Present study
Glyphosate	<i>Artemia</i> 24hrs nauplii	48hr LC ₅₀	0.019	Present study

According to a study, the 48hr LC₅₀ value of Malathion (Technical grade) to *Artemia salina* was 1.00 ppm [45]. At the same time, two researchers observed 48hrs LC₅₀ value of was 12.3 ppm to *S. dichotomus*, which is lower than the value observed in the present study [2]. However, in other study, they documented a 24hr LC₅₀ value of 0.00318 ppm for *Ceriodaphnia dubia* [46]. The 48hrs LC₅₀ values of technical and commercial grades of Malathion to *D. magna* were 0.028 ppm and 0.003 ppm, respectively [47].

As shown in Table 1, the 24 and 48hr LC₅₀ values of Glyphosate to *Artemia* nauplii were 0.028 and 0.019 ppm, respectively. From these results it is obvious that Glyphosate was significantly more toxic to *Artemia* nauplii than Malathion ($P < 0.05$). A group of researchers has demonstrated that the 24hr LC₅₀ value for Glyphosate on anostracan, *Thamanocephalus platyurus* was 0.35 ppm [48]. However, some of them observed 24hr LC₅₀ value of 0.0118 ppm for Glyphosate to *B. Sandiegonensis* [44]. Whereas reported 24hr LC₅₀ value of 0.319 ppm for Glyphosate to *B. Thailandensis* nauplii. Fairy shrimp, *S. dichotomus* exposed to Glyphosate showed an LC₅₀ of 0.014 ppm, which is lower than the present study of 0.019 ppm for brine shrimp, *Artemia* in 48 hours [2]. A scientist observed that *Artemia franciscana* exposed to Glyphosate and Zinc in combination produced an increase in lethality compared to shrimp exposed only to Glyphosate and Zinc separately [39].

According to some study, the 48hr LC₅₀ value of Glyphosate was 42 ppm to *D. magna* [49]. A group of scientists has reported almost five times for the same species higher at 48hr LC₅₀ (250 ppm) [50]. A researcher also reported that fish and aquatic invertebrates were more sensitive to Glyphosate than terrestrial organisms [51]. The LC₅₀ values observed in the present investigation for *Artemia* 24hr old nauplii (instar I) exposed against Malathion and Glyphosate were comparatively lower, which might be due to differences in experimental conditions and sensitivity of the species. Toxicity of a xenobiotic is governed by many factors, such as water temperature, purity of the toxin, life stage of organism, size of the individual etc.

From the results, it was clear that *Artemia sp.* is more sensitive to the exposure of the organophosphate Glyphosate than that of Malathion. The residues of organophosphate can be easily accumulated in the tissues of *Artemia* either by direct contact or by ingestion and the secondary effects might be observable in predators of *Artemia* in aquatic environments [31]. In conclusion, the branchiopod microcrustacean *Artemia* proved to be an excellent ecotoxicological bioindicator for marine and saline environments polluted with organophosphate residues and may be considered as an important bio monitoring tool for future toxicological analysis.

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REFERENCES

- [1] Begum, G. 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn) and recovery response. *Aquatic Toxicology*, 66, 83–92.
- [2] Arun Kumar, M.S., Jawahar, A.A. 2014b. Effect of two organophosphorus pesticides on the reproductive bionomics of freshwater fairy shrimp *Streptocephalus dichotomus* (Baird, 1860)

(Crustacea: Anostraca). *International Journal of Bioassays*, 3 (09), 3307-3312.

[3] Maxwell, D.M., Brecht, K.M., Koplovitz, I., Sweeney, R.E. 2006. Acetylcholinesterase inhibition: does it explain the toxicity of organophosphorus compounds? *Archives of Toxicology*, 80, 756–760.

[4] Boelsterli, U. 2007. *Mechanistic Toxicology: The Molecular Basis of How Chemicals Disrupt Biological Targets*, 2nd, ed.; CRC Press - Taylor & Francis Group, LLC: Boca Rato.

[5] Paraoanu, L.E., Layer, P.G. 2008. Acetylcholinesterase in cell adhesion, neurite growth and network formation. *FEBS J.* 275 (4), 618-624.

[6] Arun Kumar, M.S., Jawahar, A.A. 2013. Toxic impacts of two Organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp *Streptocephalus dichotomus*. *International Journal of Pharma and Bio Sciences*, 4 (2), 966 – 972.

[7] Manna, G.K., Mukherjee, P.K. 1986. Effect of organophosphate insecticide 'Malathion' on chromosome cell division and total muscle proteins of cichlid fish, Tilapia, in: G.K. Manna, S.C. Roy (Eds.), *Proceedings of the Xth All India Congress on Cytol. Gene.*, Kalyani, West Bengal, 7-10 October, *Pers. Cytol. Genet.* 5, 225-235.

[8] Porte, C., Barceló, D., Tavares, T.M., Rocha, V.C., Albaiges, J. 1990. The use of the mussel watches and molecular marker concepts in studies of hydrocarbon in a tropical bay (Todos os Santos, Bahia, Brazil). *Archives of Environmental Contamination and Toxicology*, 19, 263-274.

[9] Yeh, S.P., Sung, T.G., Chang, C., Cheng, W., Kuo, C.M. 2005. Effects of an organophosphorus insecticide, Trichlorfon, on hematological parameters of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Aquaculture*, 243, 383–392.

[10] Feng, T., Li, Z.B., Guo, X.Q., Guo, J.P. 2007. Effects of Trichlorfon and Sodium dodecyl sulfate on antioxidant defense system and acetylcholinesterase of *Tilapia nilotica* in vitro. *Pesticide Biochemistry and Physiology*, 92, 107–113.

[11] Yonar, M.E., Sakin, F. 2011. Ameliorative effect of lycopene on antioxidant status in *Cyprinus carpio* during Pyrethroid Deltamethrin exposure. *Pesticide Biochemistry and Physiology*, 99, 226–231.

[12] Chang, C.C., Rahmawaty, A., Chang, Z.W. 2013. Molecular and immunological responses of the giant prawn, *Macrobrachium rosenbergii*, to the organophosphorus insecticide, Trichlorfon. *Aquatic Toxicology*, 130–131, 18–26.

[13] Lavarias, S., García, F., Crespo, R., Pedrini, N., Heras, H. 2013. Study of biochemical biomarkers in freshwater prawn *Macrobrachium borellii* (Crustacea: Palaemonidae) exposed to organophosphate Fenitrothion. *Ecotoxicology and Environmental Safety*, 96, 10–16.

[14] Sarma, S.S.S., Nandini, S. 2007. Review of recent Ecotoxicological studies on cladocerans *Journal. Environmental Science and Technology*, 41 (17), 6124-6128.

[15] Nikam, S.M., Shejule, K.B., Patil, R.B. 2011. Study of acute toxicity of Metastox on the freshwater fish *Nemacheilus botia*, from Kedrai dam in Maharashtra, India. *Biology and Medicine*, 3 (4), 13-17.

[16] Arun Kumar, M.S., Jawahar, A.A. 2014a. Histopathological changes in the Ovaries and Muscle tissues of freshwater Fairy shrimp *Streptocephalus dichotomus* (Baird, 1860), exposed to Malathion and Glyphosate. *International Journal of Bioassays*, 3 (09), 3229-3232.

[17] Krishnapriya, R., Padmaja, M. 2014. Study on individual and combined toxicity of Quinalphos and Dimethoate on certain neurological aspects of giant freshwater prawn *Macrobrachium rosenbergii* (Deman, 1879). *International Journal of Science and Research*, 4 (5), 1-5.

[18] Robinson, C.B., Samocha, T.M., Fox, Gandy, R.L., McKee, D.A. 2005. The use of inert artificial commercial food sources as replacements of traditional live food items in the culture of larval shrimp, *Farfantepenaeus aztecus*. *Aquaculture*, 245, 135-147.

[19] Tlusty, M.F., Jason, S., Goldstein, Fiore, D.R. 2005. Hatchery performance of early benthic juvenile American lobsters (*Homarus*

- americanus*) fed enriched frozen adult *Artemia* diets. *Aquaculture Nutrition*, 11, 191-198.
- [20] Holme, M.H., Zeng, C., Southgate, P.C. 2006. Use of microbound diets for larval culture of the mud crab, *Scylla serrate*. *Aquaculture*, 257, 482-490.
- [21] Johnston, M.D., Johnston, D.J., Jones, C.M. 2008. Evaluation of partial replacement of live and fresh feeds with a formulated diet and the influence of weaning *Panulirus ornatus phyllosomata* onto a formulated diet during early ontogeny. *Aquaculture International*, 16, 33-47.
- [22] Gamboa-Delgado, J., Le Vay, L. 2009. *Artemia* replacement in co-feeding regimes for mysis and postlarval stages of *Litopenaeus vannamei*: nutritional contribution of inert diets to tissue growth as indicated by natural carbon stable isotopes. *Aquaculture*, 297, 128-135.
- [23] Demeny, F., Trenovszki, M.M., Varga, S., Hegyi, A., Urbanyi, B., Zarski, D., Acs, B., Miljanovic, B., Specziar, A., Muller, T. 2012. Relative efficiencies of *Artemia* nauplii, dry food and mixed food diets in intensive rearing of larval Crucian carp (*Carassius carassius* L.). *TRJAS*, 12, 691-698.
- [24] Bhavan, S.P., Kavithamani, N., Radhakrishnan, S., Muralisankar, T., Srinivasan, V., Manickam, N. 2013. Comparison of nutritional quality of sunflower oil and cod liver oil enriched with *Artemia* nauplii for assessing their efficacies on growth of the prawn *Macrobrachium rosenbergii* post larva. *International Journal of Current Science*, 7, E 67-79.
- [25] Cobo, M.L., Wouters, R., Wille, M., Sonnenholzner, S., Sorgeloos, P. 2014. Evaluation of frozen umbrella stage *Artemia* as first animal live food for *Litopenaeus vannamei* (Boone) Larvae. *Aquaculture Research*, 1-8.
- [26] Persoone, G., Vell, V.D., Steergem, M.A.V., Nayer, B. 1989. Predictive value for the laboratory test with aquatic invertebrates. Influence of experimental condition. *Aquatic Toxicology*, 14, 149-166.
- [27] Ali, J., Janseen, R.C., Persoone, G. 1991. Development of a cost-effective acute toxicity screening test to determine the toxicity of coatings on submerged structures. In: Book of abstracts. SETAC-Europe Founding Conference- Environmental sciences and sustainable development. Sheffield, U.K.
- [28] Ali, J.A., Govindaraj, A. 2005. Toxicity of antifouling paint to selected Zooplankton. *Pollution Research*, 24, 211-216.
- [29] Krishnakumar, P.K., Dineshbabu, A.P., Sasikumar, G., Bhat, G.S. 2007. Toxicity evaluation of treated refinery effluent using brine shrimp (*Artemia salina*) egg and larval bioassay. *Fishery Technology*, 44 (1), 85-92.
- [30] Varo, I., Amat, F., Navarro, J., Barreda, M., Serrano, R., Hernandez, F. 2003. Exposure of *Artemia* sp. (Crustacea) cysts to the organophosphorus pesticide Chlorpyrifos. Efficacy of the chorion as barrier. *CICTA*, Porto-Portugal.
- [31] Obregon, E.B., Vargas, A. 2010. Chronic toxicity bioassay with populations of the crustacean *Artemia salina* exposed to the organophosphate Diazinon. *Biological Research*, 43, 357-362.
- [32] Finney, D.J. 1964. *Probit Analysis*, 2nd Edn. Cambridge university press, London.
- [33] George, D., Mallery, P. 2006. *SPSS for windows Step by Step a Simple Guide and Reference 13.0 update sixth Edition* Pearson Education, Inc.
- [34] Gajbhiye, S.N., Hirota, R. 1990. Toxicity of heavy metals to brine shrimp *Artemia*. *Journal of Indian Fisheries Association*, 20, 43-50.
- [35] Varo, I., Taylor, A.C., Ferrando, M.D., Amat, F. 1997. Effect of Endosufan pesticide on the oxygen consumption rates of nauplii of different Spanish strains of *Artemia*. *Journal of Environmental Science and Health, Part B*, 32, 363-375.
- [36] Hadjispyrou, S., Kungolos, A., Anagnostopoulos, A. 2001. Toxicity, bioaccumulation, and interactive effects of Organotin, Cadmium, and Chromium on *Artemia franciscana*. *Ecotoxicology and Environmental Safety*, 49, 179-186.
- [37] Varo, I., Navarro, J.C., Amat, F., Guilhermino, L. 2002. Characterization of cholinesterases and evaluation of the inhibitory potential of Chlorpyrifos and Dichlorvos to *Artemia salina* and *Artemia parthenogenetica*. *Chemosphere*, 48, 563-569.
- [38] Brix, K.V., Cardwell, R.D., Adams, W.J. 2003. Chronic toxicity of arsenic to the Great Salt Lake brine shrimp, *Artemia franciscana*. *Ecotoxicology and Environmental Safety*, 54, 169-175.
- [39] Falis, M., Spalkova, M., Legath, J. 2014. Effects of heavy metals and pesticides on survival of *Artemia franciscana*. *Acta Veterinaria Brno*, 83, 95-99.
- [40] Bengtson, D.A., Léger, P., Sorgeloos, P. 1991. Use of *Artemia* as a food source for aquaculture. In R. A. Browne, P. Sorgeloos and C.N.A. Trotman (eds.), *Artemia Biology*, CRC Press, Boca Raton, Florida, pp. 255-285.
- [41] Crisinel, A., Delaunay, D., Rossel, J., Tarradellas, H., Meye, H., Saiah, P., Vogel, C., Delisle, Blaise, C. 1994. Cyst based ecotoxicological tests using anostracans: Comparison of two species of *Streptocephalus*. *Environmental Toxicology and Water Quality*, 9, 317-326.
- [42] Lahr, J.A., Badji, S., Marquenine, E., Schuiling, K.B., Ndour, Diallo, O., Everts, J.W. 2001. Acute toxicity of Locust insecticides to two indigenous invertebrates from sahelian temporary ponds. *Ecotoxicology and Environmental Safety*, 48, 66-75.
- [43] Verschuere, K. 2001. *Hand book of Environmental Data on organic chemicals*, fourth edition. John Wiley & sons, New York.
- [44] Ripley, B.J., Davis, K.C., Carter, B.J., Simovich, M. 2003. Toxicity of Malathion and Roundup to the San Diego fairy shrimp. *The Western Section of the Wildlife Society*, 38 (39), 13-21.
- [45] Sidharta, R.B. 1997. Effects of Malathion on Brine shrimp (*Artemia salina* L.). A preliminary lethal toxicity test. *Biota*, 11 (2), 61-66.
- [46] Nelson, S., Roline, R.A. 1998. Evaluation of sensitivity of rapid toxicity tests relative to daphnid acute lethality tests. *Bulletin of Environmental Contamination and Toxicology*, 60, 292-299.
- [47] Rassoulzadegan, M., Akyurtlakli, N. 2002. An investigation on the toxic effects of Malathion organophosphate insecticide on the *Daphnia magna* Straus, 1820 (Crustacea: Cladocera). *Turkish Journal of Zoology*, 26, 349-355.
- [48] Fochtman, P.A., Raszka, Nierzedzka. 2000. The use of conventional bioassays, microbiotests, and some "rapid" methods in the selection of an optimal test battery for the assessment of pesticides toxicity. *Environmental Toxicology*, 15, 376-384.
- [49] Alberdi, J.L., Saenz, M.E., Dimarzio, W.D., Trotorelli, M.C. 1996. Comparative Acute toxicity of two Herbicides, Paraquat and Glyphosate, to *Daphnia magna* and *D. spinulata*. *Bulletin of Environmental Contamination and Toxicology*, 57 (2), 229-235.
- [50] Raipulis, J., Toma, M.M., Balode, M. 2009. Toxicity and genotoxicity testing of roundup proceedings of the Lat. Proceedings of the National Academy of Sciences, India Section B, (63), 29-32.
- [51] Cox, C. 2000. Glyphosate factsheet. *J Pestic Reform* 108: 3. Available at: <http://www.mindfully.org/Pesticide/Roundup-Glyphosate-Factsheet-Cox.htm>.