Indian J. Applied & Pure Bio. Vol. 37(1), 115-127 (2022). **ISSN: 0970-2091**
A web of Science Journal

The toxic effects of phorate exposed for ten minutes before fertilization on sperm cell of sedentary polychaete *Hydroides elegans* (Haswell, 1883)

S. Vijayaragavan*

Indira Gandhi National Open University, Regional Centre, Cochin-682017 (India)
Email: vijayaraja100@gmail.com
***Correspondence Author:** Dr.S.Vijayaragavan
Assistant Regional Director,
Indira Gandhi National Open University, Regional Centre, Cochin-682017 (India)
Email: vijayaraja100@gmail.com

Abstract

The toxicity test has been developed to examine the effects of pesticides on fertilization and early development of marine Polychaete *Hydroides elegans (H. elegans).* The Pesticides lead to pollution of the ground water, soil, aquatic environments and also marine environment. It directly enters the food chains of the organisms and it affects the marine ecosystems. The pesticides alter the regular functions of the marine organisms as well as nervous system and physiological structure.

The toxic effect of phorate on sperm cell of *H. elegans* was examined and it was found that the rate of successful development of embryonic development decreased when the concentration of phorate increased in sea water. The results presents here, strongly suggest that the mechanism of action of the phorate probably acts on sever as intracellular targets based on EC50 values of the present study; It indicated that phorate was toxic to the early developmental stages of *H. elegans* when the sperm were already exposed to the same concentration of phorate for ten minutes before fertilization.

The results indicate that the sperm and early development stages of *H. elegans* are sensitive and toxic to phorate when the sperm were already exposed to the phorate before fertilization. The sedentary polychaete, *Hydroides elegans* can be routinely used as a test organism for eco-toxicity bioassays experiments at tropical and sub tropical regions.

The use of pesticides can potentially pose a high risk to the environment, particularly if residues persist in the soil or migrate off-site and enter waterways (*e.g.* due to spray drift, run-off)[13,19]. If this occurs it could lead to adverse impacts to the health of terrestrial and aquatic ecosystems. For instance, concerns have been raised over the long term use of copper-based fungicides, which can result in an accumulation of copper in the soil[13,31]. This in turn can have adverse effects on soil organisms (*e.g.* earthworms, microorganisms) and potentially pose a risk to the long-term fertility of the soil[13,31]. The urbanization and industrialization growth which endangers the costal eco-system and also the ecosystem which may be polluted by the discharges from specific point sources like sewage, effluents and industrial wastes etc. and also from non-point sources like harbours and drainages etc. Therefore, it is essential that the bioassay techniques should be established to monitor the pollutants that pose a danger or hazard to humans and the biota[8,21,29,30].

Phorate is a highly toxic organophosphate pesticide which is used to control the chewing insects, leafhoppers, leafminers, mites, some nematodes, rootworms in pine forests and on root insects and insects in field crops, including corn, cotton, coffee, some ornamental and herbaceous plants and bulbs. It is a relatively stable clear to yellow liquid at room temperature in appearance and it has been labeled as highly toxic- poison and it is a restricted use pesticide (RUP) which can be used for specific uses only. It is registered for half use in countries like India, US, Canada, Australia and New Zealand which is declared by Pesticide Action Network (PAN, 2001). It contributes to agricultural waste which moves up to aquatic environment during rainy season and it is transported through the food chain and causes several ailments. It is essential to study that the effects of the pesticides by using bio-organism for aquatic environmental management and pollution monitoring[8,29]. Most of the pesticides affect the embryo, teratogenic effects by directly or indirectly affecting cellular physiology[2]. Many cases of surface water contamination with pesticides were noticed and reported[10].

The bioassays allow the detection of the effects by measuring the biologic response of marine organisms, particularly in their early life stages[11]. The test species must be sensitive enough to respond to low levels of contaminants and must be available for use from either laboratory cultures or from field collection throughout the year, accordingly, biologic tests are to be ecologically relevant and easily available of species for experimentation. [8,20,30]. Although, toxicity tests conducted in the field are desirable and analyzing the developmental stages are easier to perform but only the laboratory conditions provide accurate results which are highly useful.

The early developmental stages of marine invertebrates have repeatedly been found to be more sensitive to environmental pollutants than their adult counter parts[3,18]. Hence, they are subjected to the toxicity tests in most of the cases. A number of early life-stage toxicity testing protocols have been developed are effectively applied for the seawater toxicity using marine species of their early embryo for example, bioassays using

embryos of bivalve species (*Mytilus edulis, Crossostrea virginice* and *C.gigas*) and gametes of echinoderm species. (S*trongylocentrotus purpuratus*, *S. tranciscanus* and *Arabica punctuata*) have been developed[1,4]. Some of the field collected organisms only produce viable gametes for certain period of the year, which limits their use in routine toxicity testing. Furthermore, it is noted that sea urchins require 5 to 10 minutes for fertilization, 1 hour for first cleavage, 24 hours for blastula and gastrula and 48 hours for trochophore larva. In contrast *H. elegens* requires 2 to 3 minutes for fertilization, 30 minutes for first cleavage and approximately 12 hours for distinguishable trochophore larva[27-30]. Therefore, the advantages of developing bioassays using *H.elegans* embryos are more clear and accurate.

H. elegans[9], a sedentary, tubicolous serpulid polychaete is common in all temperate region and produces viable gametes throughout the year[7,16,22]. The organism is widespread forming dense layers within the collection zone. It can be easily collected and amenable to laboratory holding and can be readily induced to release gametes and potential for use in routine laboratory toxicity tests[7,16,27,28,30]. Therefore, the aim of the present study was to determine the toxic effects of carbendazim on early embryonic stages of *H. elegans* when the sperm were already exposed to the same concentration of phorate for ten minutes before fertilization.

Collection of Organism :

H. elegans were collected from the hulls of boats, which were in fishing operation for more than three months, berthed at Royapuram, Fish Landing Center, Chennai, India (Lat. 13º 06' N and Long. 80º 18' E). Other sedentary animals like Lepas, Barnacles, Neries, Mytilus, Ascidians, Algae and few crustacean arthropods were also seen which were carefully removed from the collection before placing *H. elegans* in the collection chambers containing freshly collected seawater. These specimens were transported to the laboratory within an hour after collection and reared in rectangular glass tanks and acclimatized to laboratory conditions for three days. Tank holding conditions were 7-9 mg/L dissolved oxygen, salinity (34±1ppt), temperature (28±10 ºC) and pH (8.1±0.1). Illumination was provided in a light, dark cycle of 14:10 hours. The polychaetes *Hydroides elegans* were kept completely immersed in seawater until the test was initiated.

Experiment Procedure :

Tests were conducted in 100-ml glass beakers containing 50 ml of the filtered sea water. The sex of the polychaete was distinguishable by the orange colour of the female abdomen and creamy white of male abdomen. The eggs were visible to the naked eye. Release of gametes began almost immediately and was allowed to continue for 10 minutes, after which the animals were removed. Gamete release after removal from the calcareous tube is a stress response in polycheate[24]. 5 to10 male and 10 to 15 female Individuals were used per toxicity test. Two hundred eggs were used for each concentration, and 6 replicates per treatment were analyzed.

Selection of Eggs :

After complete spawning the worms

were removed from the watch glasses. The watch glass with eggs and seawater was slightly swirled or rotated in such a way that the bigger and heavier mature eggs settled in the center and the lighter and smaller eggs remained at the periphery of the watch glass. Such smaller eggs along with some seawater were decanted out. This process was repeated 5 times. By this method the eggs were also washed well. Only bigger, heavier and healthier eggs were selected for the experiment and unwanted debris was removed. Eggs were used for the experiment within 15 minutes of release.

Maintenance of Sperm :

After spawning, the worms were removed from the watch glasses. The sperm released and were kept in 10 ml of seawater till the beginning of the experiment. The sperm were used for the experiments within 5-10 minutes after release.

Experiment :

To study the effect of phorate on the sperm cell of *Hydroides elegan* about 0.05ml of sperm suspension (diluted sperm) were treated with 20 ml of various concentration of phorate in sea water. (0.25, 0.5, 1, 2, 5 10, 20 and 30 ppm) for 10 minutes before mixing untreated egg gametes. The sperm were exposed to different concentration phorate for 10 minutes before being used for experiment. Then, at the end of exposure period the untreated eggs collected from the ripe worms were added to the exposed sperm in 50 ml of test solution for different concentration (0.25, 0.5, 1, 2, 5,10, 20 and 30 ppm). Fertilization observed by microscope for different embroyonic stages of *H. elegans* for every 10 minutes after treated sperm and untreated eggs were mixed. The percentage of successful development of each developmental stages such as elevation of the fertilization membrane (FM) stage and other early embryonic stages namely 2-cell stage, 3-cellstage, 4-cell stage, 8-cell stage, 16-cell stage, 32-cell stage, 64-cell stage, blastula stage, blastula rotation stage, larval release stage was observed. The experiment was repeated six times and the values were recorded (n = 6). To confirm the percentage of successful development, about 100 to 200 developing eggs at different stages were fixed in 10% neutral buffered formalin prepared in seawater and were counted on the same day. Abnormal cells were also noted at all concentrations and in each developmental stage. Nikon Photostat research microscope was used to record photomicrographs. The size of the cell at developmental stage was observed by using compound microscope. Percentage of successful development and stage EC 50 value was calculated.

Control experiment :

About 0.05ml sperm were exposed to filtered sea water for 10 minutes. At the end of the exposure periods, eggs collected from the ripe worms were added to each test chamber and the stopwatch was switched on. After 3 minutes, about 20 ml of solution with about 200 eggs from each container was transferred to separate watch glasses and was observed under microscope at 150X magnification. The percentage of successful development of each developmental stages such as elevation of the fertilization membrane (FM) stage and other early embryonic stages namely 2-cell stage, 3-cellstage, 4-cell stage, 8-cell stage, 16-cell stage, 32-cell stage, 64-cell

stage, blastula stage, blastula rotation stage, larval release stage was observed. The experiment was repeated six times and the values were recorded (n = 6). To confirm the percentage of successful development, about 100 to 200 developing eggs at different stages were fixed in 10% neutral buffered formalin prepared in seawater and were counted on the same day. Abnormal cells were also noted at all concentrations and in each developmental stage. Nikon Photostat research microscope was used to record photomicrographs. The size of the cell at developmental stage was observed by using compound microscope. Percentage of successful development was calculated.

Statistical Analysis :

To test the effects of phorate on sperm cell, a one way analysis of variance (ANOVA) was performed for the experiments. All the above said statistical analyses were carried out by using the Software Statistical Package for Social Science[23].

Pesticides solution :

Phorate (10% w/w), brand name: Kemfort was obtained from Keminol Enterprises, SIDCO north phase Ambattur, Chennai, India.(Fig. 1) At 800mg of phorate (10% w/w) was dissolved in 2000 ml of filtered seawater in a volumetric flask to prepare 30 ppm of phorate in seawater. This stock solution was stored in an amber coloured bottle. From the stock solution the following concentrations of phorate in seawater (0.25 ppm to 30 ppm) were prepared and the stock solution was used for the experiment.

In each experiment filtered seawater was used as control solution. All glass ware were acid washed and rinsed in distilled water. Before the experiment, the experimental concentrations were chosen on the basis of preliminary trials. The concentrations were 0.25, 0.5, 1, 2, 5, 10, 20 and 30 ppm of phorate in sea water was used for toxicity study by using embryo of *Hydroides elegans*. (Fig. 2) Physicochemical conditions of the experimenters were 28±1°C temperature; 34±0.5 ppt salinty, 6±0.3mg1/L O_2 and 8.1±0.1 pH were maintained.

Normal Fertilization and Early Developmental Stages :

After the fertilization, the fertilization membrane was initiated within 3 to 5 minutes. The first cleavage was meridional and the completion of first cleavage acquired at 30 minutes after fertilization (Fig. 3). The percentage of successful development of FM stage was 91.61±2.74 and it decreased gradually 60.48±0.41 at normal larval release stage. The cumulative time of FM stage was 5.50±0.95 minutes and the times steadily increased 298.60±2.63 minutes at larval release stage. The larval release stage was occurred at 5 hour after the fertilization in the normal development without the phorate in sea water.

Toxic Effect of phorate on Early Developmental Stages of Hydroides elegans when the sperm were already exposed to the same concentration for Ten Minutes before Fertilization:

1. Fertilization Membrane (FM) stage to 8 cell stage :

The percentage of successful deve-

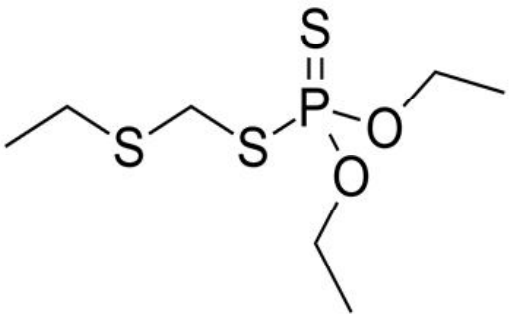

Fig.1. Molecular Structure of phorate

Fig. 2. Hydroides elegance

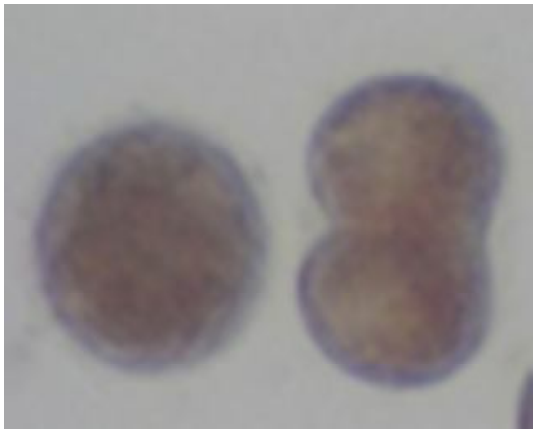

Fig. 3. FM stage and 2 cell stage

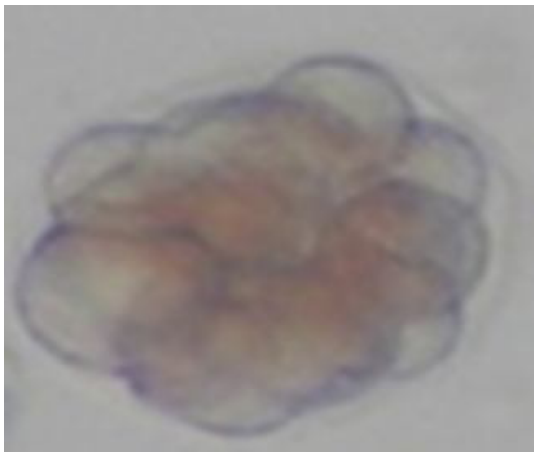

Fig. 4. Blastula Stage

lopment of FM stage was 70.42±11.9 at 1 ppm of phorate in sea water. The cumulative time of FM stage was 11.40±0.19 minutes at 1 ppm of phorate and the percentage of successful development of 8 cell stage was 48.86±8.90 at 1 pm of phorate and it steadily decreased to 10.49±2.71 at 10 ppm.

16 cell stage to 64 cell stage :

The percentage of successful development of 16 cell stage was 44.96 ±8.30 at 1 ppm of phorate in sea water. The cumulative time of 16 cell stage was 109.30 ±1.56 minutes at 1 ppm of phorate and the percentage of successful development of 64 cell stage was 39.36±6.85 at 1 ppm and it was decreased to 29.69±7.98 at 5 ppm phorate in sea water and beyond 5 ppm, there was no development of the 64 cell stage. The cumulative time of 64 cell stage was 145.10±2.02 minutes at 1 ppm and it was increased to 176.20±1.06 minutes at 5 ppm of phorate in sea water.

Table-1. Stage Ec50 Values of Phorate for Defferent Embryonic Stages of H. Elegans: Sperms Exposed for Ten, Minutes **(Temp. 28±0.2°C, Salinity 34±0.1°/$_{00}$, pH 8.1±0.1) Stage EC50 values are expressed in ppm)**

Developmental Stages	Carbendazim 10 Minutes
FM Stage	**4.9182** (3.7993-6.6309)
2 Cell Stage	**2.8352** (1.6483-5.2264)
3 Cell Stage	**2.5986** (2.0200-3.4717)
4 Cell Stage	**1.7559** (0.9775-3.3654)
8 Cell Stage	**1.2875** (0.6690-2.3364)
16 Cell Stage	**0.9283** (0.3613-2.1926)
32 Cell Stage	**0.8923 (0.6037-1.2706)**
64 Cell Stage	**0.6731** (0.4203-0.9636)
Blastula Stage	**0.4508** (0.2476-0.6615)
Blastula start Rotation stage	**0.2745** (0.1121-0.4439)
Blastula Stop Rotation stage	**0.2742** (0.1121-0.4439)
Release Stage	**0.2173** (0.0992-0.3414)

n = number of experiments Lower and upper range of 95% confidence limits are given in parenthesis

2. Blastula Stage to Release stage :

The percentage of successful development of Blastula stage was 35.09±9.07 at 1 ppm of phorate in sea water and the cumulative time of Blastula stage was 179.60±0.52 minutes at 1 ppm of phorate in seawater. The percentage of successful development of Blastula stage was at35.09±9.07 at 1ppm (Fig. 4) and it was decreased to 25.51±9.65 at 5 ppm phorate in sea water and beyond 5 ppm, there was no development observed.

The present study the Blastula Rotation Stage was observed upto 5 ppm and above 5 ppm there was no rotation. The percentage of successful development of Release Stage was 25.46±10.7 at 1 ppm of phorate and it was decreased to 13.47±2.41 at 5 ppm phorate in sea water and beyond 5 ppm there was no Release Stage observed. The cumulative time of Release Stage was 349.90±2.08 minutes at 1 ppm of phorate in sea water and it increased to 360.80±0.26 minutes at 5 ppm.

The results presents here, strongly suggest that the mechanism of action of the phorate probably acts on sever as intracellular targets based on EC50 values (Table-1) of the present study, It indicate that phorate was toxic to the early developmental stages of *H. elegans*. Sensitivity of pollution depends on the type of organism and the stage of development used. The results from the present study indicate that the embryos and larvae of *H. elegans* were more sensitive for phorate in sea water when the sperm were exposed to the same concentration for ten minutes before fertilization. (Table, 2 and 3).

The effective concentration value (EC_{50}) referred to sensitivity towards the embryonic stages while exposed to different concentration of phorate in sea water. The result indicated that the FM stage EC_{50} value was 4.9182 ppm which least sensitive stage of phorate in sea water and highest sensitive stage value was 0.2173 ppm at larval release stage

Table-2. Successful Percentage of Various Embryonic Stages of H. Elegans in Different Concentrations of Phorate When the Sperm Were Already Exposed to the Same Concentration for Ten Minutes Before Fertilization.
(Temp. 28±0.2°C, Salinity 34±0.1°/$_{00}$, pH 8.1±0.1); n=6 ±=SD

Developmental stages	Control	Concentration of phorate in ppm							
		0.25	0.5	1	2	5	10	20	30
FM stage	91.96 ±2.74	88.93 ±4.88	82.38 ±7.51	70.42 ±11.9	62.93 ±9.58	58.97 ±13.4	41.31 ±12.0	22.14 ±4.15	N.D
2 – cell stage	89.42 ±3.25	86.93 ±4.52	80.77 ±5.03	68.93 ±9.58	56.92 ±7.32	51.51 ±6.31	31.46 ±4.79	3.87 ±1.34	N.D
3 – cell stage	87.02 ±3.76	84.35 ±4.53	78.87 ±5.55	60.95 ±9.97	52.14 ±7.68	46.42 ±5.43	24.87 ±2.48	N.D	N.D
4 – cell stage	82.62 ±2.77	78.86 ±4.52	74.40 ±8.08	52.46 ±9.93	48.25 ±8.25	42.84 ±5.55	16.48 ±2.67	N.D	N.D
8 – cell stage	78.44 ±2.29	75.29 ±8.68	68.73 ±10.9	48.86 ±8.90	44.96 ±8.71	37.17 ±7.65	10.49 ±2.71	N.D	N.D
16 – cell stage	74.54 ±3.12	71.10 ±10.4	66.84 ±7.46	44.96 ±8.30	40.98 ±9.57	35.37 ±7.63	5.09 ±1.09	N.D	N.D
32 – cell stage	72.14 ±3.30	66.92 ±10.6	61.85 ±10.9	41.67 ±7.21	37.49 ±9.92	32.38 ±8.01	N.D	N.D	N.D
64 – cell stage	69.45 ±3.31	63.34 ±11.5	57.06 ±13.1	39.36 ±6.85	34.49 ±9.74	29.69 ±7.98	N.D	N.D	N.D
Blastula stage	66.15 ±3.12	60.65 ±11.5	48.58 ±13.0	35.09 ±9.07	30.30 ±9.35	25.51 ±9.65	N.D	N.D	N.D
Blastula start Rotation stage	63.16 ±1.77	55.87 ±13.3	39.00 ±14.3	32.40 ±9.72	26.71 ±10.0	21.61 ±9.36	N.D	N.D	N.D
Blastula stop rotation stage	63.16 ±1.77	55.87 ±13.3	39.00 ±14.3	32.40 ±9.72	26.71 ±10.0	21.61 ±9.36	N.D	N.D	N.D
Release stage	60.48 ±0.41	51.99 ±14.0	34.52 ±12.1	25.46 ±10.7	23.09 ±7.15	12.47 ±2.41	N.D	N.D	N.D

N.D= No Development, Number of eggs/embryos observed in each concentration =100-150
n=Number of experiment

Table –3. Cumulative Times of Various Embryonic Stages of Hydroides Elegans in Different Concentrations of Phorate When the Sperm Were Already Exposed to the Same Concentration for Ten Minutes Before Fertilization

(Temp, 28±0.2^{0}C, Salinity 34±0.1‰, pH 8.1±0.1);n=6±=SD

Developmental stages	Control	Time in minutes							
		Concentration of phorate in ppm							
		0.25	0.5	1	2	5	10	20	30
FM Stage	5.50 ±0.95	7.40 ±0.48	9.40 ±0.21	11.40 ±0.19	13.30 ±0.69	15 .40 ±0.82	17.50 ±0.76	19.40 ±0.34	N.D
2 Cell Stage	31.00 ±1.04	34.80 ±1.73	38.80 ±0.86	42.80 ±1.92	46.70 ±1.29	50.70 ±0.72	54.90 ±0.53	59.80 ±2.16	N.D
3 Cell Stage	41.30 ±1.21	47.10 ±1.63	53.20 ±1.46	59.20 ±1.75	64.10 ±1.18	71.10 ±1.52	77.30 ±1.54	N.D	N.D
4 Cell Stage	51.70 ±2.03	59.00 ±0.00	67.40 ±1.95	75.50 ±1.26	82.40 ±2.23	91.50 ±1.69	99.80 ±0.46	N.D	N.D
8 Cell Stage	63.00 ±0.00	72.10 ±1.81	82.80 ±1.46	92.90 ±2.49	101.80 ±1.79	112.90 ±2.52	125.20 ±2.57	N.D	N.D
16 Cell Stage	73.40 ±3.02	84.40 ±2.32	96.90 ±2.77	109.30 ±1.56	119.10 ±0.27	133.30 ±2.03	149.60 ±3.08	N.D	N.D
32 Cell Stage	84.90 ±1.54	97.70 ±2.81	112.30 ±3.28	126.70 ±1.68	138.50 ±3.78	153.70 ±3.54	N.D	N.D	N.D
64 Cell Stage	97.30 ±1.05	112.10 ±1.53	128.60 ±1.76	145.10 ±2.02	158.90 ±0.92	176.20 ±1.06	N.D	N.D	N.D
Blastula Stage	125.60 ±4.53	142.50 ±1.82	160.90 ±2.33	179.60 ±0.52	194.00 ±0.00	198.50 ±0.56	N.D	N.D	N.D
Blastula start Rotation stage	154.00 ±0.00	172.90 ±2.33	193.20 ±0.72	214.10 ±0.96	230.30 ±2.82	236.80 ±3.14	N.D	N.D	N.D
Blastula Stop Rotation stage	244.30 ±0.52	251.30 ±2.84	271.60 ±3.23	290.50 ±2.57	300.70 ±0.97	301.30 ±3.52	N.D	N.D	N.D
Release Stage	298.60 ±2.63	307.50 ±1.26	328.90 ±1.63	349.90 ±2.08	361.10 ±1.53	360.80 ±0.26	N.D	N.D	N.D

N.D – No Development, Number of eggs/embryos observed in each concentration = 100-150, n = number of experiments

Polychaetes are the most widely used groups of marine macro invertebrates in toxicological testing and easy in collection is undoubtedly played an important role in their selection as test animals[8,19,30]. Polycheates are ecologically important marine organisms, making up from 30% to 80% of the total numbers of benthic fauna regardless of the ocean depth[12]. The results revealed that the stage EC_{50} value of phorate decreased steadily from 9.0721 ppm in the FM- stage to 0.9150 ppm in the release stage. It is indicating that the release stage (hatching) is more sensitive to phorate than the earlier stages, but actually it may be due to longer exposure of embryo to the phorate in the seawater. This suggests that the impact of toxicity may be additive as the development progress through various stages and thus the later stages are exposed for longer duration in the test solution. The results of the present study on the effects of phorate on fertilization in *H. elegans* reveals that the success rate of fertilization decreases as the concentration of phorate increases in seawater. Successful fertilization was evidenced by the elevation of fertilization membrane. Successful fertilization was 91.61±2.74% successful in control sea water and it gradually decreased to 22.14 ± 4.15 at 20 ppm. There was no fertilization at 30 ppm. Similar trend was reported in the same species on effect of Monocrtophos, D.D.T., Chlorfyrifos, Endosulfan[22]. Heavy metals,[7] Petroleum Oils[22,26] Phorate, 29.

The percentage of successful development of *H. elegans* declined as the developmental stages progressed in any given concentration of phorate in seawater. In the same way abnormal development of the various developmental stages increased when the concentration of phorate increase in seawater and also when the sperm were already exposed to the same concentration for ten minutes before fertilization. In higher concentration the development were arrested and up normal embryo observed due to the effect of embryonic toxicity of phorate. In the present study, the cumulative time at different developmental stages of *H. elegans* from the FM- stage to the release stage (hatching) showed a gradual increase in time as the concentration of phorate increased in seawater in all the stages. It reveals that the rate of development decreases with increase in concentration of phorate in seawater. Similar trend was observed by Thilagam *et al.,*[25]; Vijayaragavan and Vivek Raja,[30] & Vijayaragavan[28].

The individual stage time of different development stages of *H. elegans*, increased except the blastula rotation stage. At the blastula rotation stage, phorate affects the ciliary activity of the embryo. Hence, the rotation time decreases gradually when the concentration of phorate increases in the seawater. This decrease in rotation time cannot be considered as an increase in the rate of development. In this stage (Blastula stop rotation stage), decrease in rotation time may be considered as decrease in rate of development. Hence, it may be inferred in that in blastula stop rotation stage also the rate of development decreases with increase in the concentration of phorate, the similar trend was observed for various heavy metals and pesticides[17,22,25,29,30]. It has been already reported that the ciliary activity is essential for successful hatching in sea urchin[15].

The researchers testing the effects of pesticides cultured human lymphocytes, concluded that it is obvious that pesticides is a potent aneugen (affects the number of chromosomes), even at low exposures[14]. In *H. elegans* the reduction in the rotation time in the presence of phorate suggests that the metabolic activity is reduced, as the quantity of the hatching enzyme released in the final stages of embryonic development may decrease or the secretion process slowed down. The decrease/delay in the production of hatching enzyme may be ascertained from the increased hatching time of *H. elegans* in the presence of phorate in sea water. The hatching time (release time) of *H. elelgans* was 298.60 ± 2.63 minutes and it gradually increases to 360.80 ± 0.26 minutes at 5 ppm of phorate. The results may be inferred that the rate of production of hatching enzyme decreased in the presence of phorate, as there was some delay in hatching up to 2 ppm of phorate, and the production of enzyme was reduced below the critical level or completely arrested at 10 ppm and above.

The experimental data reveled that the toxicity of phorate on early embryonic stages of *Hydroides elegans* is more sensitive when the *H.elegans* sperm were already exposed to the same concentration for 10 minutes before fertilization and its lead to abnormalities of embryos. Hence, the development stages have been arrested in high concentration of phorate in sea water. It observed that the toxicity particles have inducing the abnormalities in the early embryo developments of *H. elelgans*. Further more, the availability of *H. elelgans* throughout the year which favorable and suitable for laboratory toxicity tests. The data reveled that the phorate was sensitive and toxic to an early embryonic stages of *H. elelgans* when the sperm were already exposed to the same concentration for ten minutes before fertilization and also the pesticides leads to environmental pollutions including marine environment. Its also genetic mutant for living organism and humans while exposure of pesticides for certain periods. The world must be understand the toxic effects of pesticides and also be free from harmful pesticides usages in agriculture fields and other purpose.

Declarations:

Acknowledgements

Abbreviations repositions:

Not applicable in this section

Ethics approval and consent to publication

The author declares that do not need ethics approval regarding the work on the marine polychaete worm *H. elelgans*.

Consent for publication

Author declares that the consent has been given for publication of the manuscripts.

Availability of Data and Materials

Please contact author for the data on reasonable request.

Competing Interests

The author declares no competing Interests.

Funding

The author declares that there are no funding sources.

Author Contributions

SV designed the works, performed the experiments, and drafted the manuscript.

The author thankful to Dr. P. Vivek Raja (Former HOD Zoology) Presidency College, Chennai for his valuable Guidance to

carried out this research work.

Author Details

1. Dr.S.Vijayaragavan, Assistant Regional Director, Indira Gandhi National Open University, Regional Centre, Cochin Kerala, India-682017.

References :

1. ASTM (American Society for Testing and Materials), (1995). In: Standard guide for conducting acute, chronic and life cycle aquatic toxicity tests with polychaetous annelids, *ASTM, Philadelphia*, PP. 1009–1028.
2. Calevro, F., S. Campani, M. Rangghianti, S. Bucci, and G. Mancino, (1998). *Chemosphere 37:* 3011-3017.
3. Connor, P.M. (1972). *Mar. Pollut.. Bull. 3:* 190-192.
4. Dinnel. P.A., J. M. Link and O.J. Stober, (1987). *Arch. Environ. Contam. Toxic. 16:* 23-32.
5. Ecobichon, D.J. (1991). Toxic effects of pesticides, P. 565-622, In M.O. Amdur, *et al.,* eds. *Casarett and Doull's Toxicology– The basic science of poisons, 4th ed. McGraw-Hills, Inc.,* New York.
6. Goodson W.H., L. Lowe, D.O. Carpenter, M. Gilbertson, A.M. Ali and A.L. de Cerain Salsamendi *et al*. (2015) *Carcinogenesis 36:* 254–296.
7. Gopalakrishnan, S. and P.V. Raja, (2002). The effects of copper on sperm and eggs of *Hydroides elegans*. *In: proc. XX. National Symp. Reproductive Biol. Comp. Endocrinol. Bharathidasan University, Tiruchirappallai* pp 83-84 India.
8. Gopalakrishnan, S., H. Thilagam, and P. Vivek Raja (2008) *Chemosphere 71:* 515–528.
9. Haswell (1883) *Hydroides elegans* worms editorial board. World Register of Marine Species 131002.
10. Halder, P., P. Raha, A. Bhattacharya, A.K. Choudhur, and N. Adityachoudhury, (1989) *Indian J. Environmental health* pp. 52.
11. His, E., R. Beiras, and M.N.L. Seaman, (1999). The assessment of marine pollution–bioassays with bivalve embryos and larvae. In: south ward, A.J. Tyler, P.A., Young, C.M. (Eds)., *Advances in Marine Biology Academic press, Sandiego, CA*, pp. 1–178.
12. Hutchings, P., (1998). Biodiversity. *Conservation. 7:* 113.
13. Komarek, M., E. Cadkova, V. Chrastny, F. Bordas, and J.C. Bollinger, (2010). *Environment International. 36:* 138 – 151.
14. Mohmood, R., and J.M. Parry, (2001). Induction of micronuclei and chromosome non-disjunction after short –term exposure to carbendazim in cultured lymphocytes, 31st. annual meeting of EEEMS Belgium.
15. Okazaki, K. (1975), *Amer. Zool. 15:* 567-581.
16. Raja, P.V. (1999). *Adv. Ethol. 34:* 225.
17. Raja, P.V., and M. Sellappan, (1992). Fertilization and early development of a sedentary polychaete *Hydroides elegans* from Madras coast. *11th National Symposium on life sciences Madras, Abstract*, p.58.
18. Rand, G.M., P.G. Wells and L.S. Mc Carty (1995). Introduction to aquatic toxicology. In: Rand, G.M.(Eds). Fundamentals of aquatic toxicology, effects, environmental

fate and risk assessment. *Springer, Berlin*, pp. 3 – 70.

19. Reish, D.J., and T. V. Gerlinger, (1997). *Bull. Mar. Sci. 60:* 584-607.
20. Richardson B.J., M. Martin (1994). *Mar. Pollut. Bull. 28:* 138-142.
21. Ringwood, H.A., (1992). *Arch. Environ. Contam. Toxicol. 22:* 265–288.
22. Sellappan, M., (2000). Effects of oil and pesticides on fertilization and early developmental stages of sedentary polychaete, *H. elegans* (Haswell 1883) Doctoral thesis, University of Madras. PP. 1-150 India.
23. SPSS, (1999). Statistical Package for Social Sciences. *SPSS. Inc. NewYork.*
24. Strathmann, M. (1987) Reproduction and development of marine invertebrates of the northern pacific coast: Data for the study of eggs, embryos and larvae. University of Washington. *Press, Seattl, WA*, 670.
25. Thilagam, H., S. Gopalakrishnan, Vijayavel and K. P.V. Raja (2008). *Archives of Environmental. Contam. Toxicol 54*(4): 674-83.
26. Vignesh, V., (2002). Effects of petroleum oils on fertilization of marine polychaete. *Intel science Talent Discovery Fair, National Fair, I.I.T. Research project # DEL. EVI* – 045.P. 1–15.
27. Vijayaragavan S., (2009). The effect of Phorate and Carbendazim on spawning, fertilization and early developmental stages of sedentary polychaetes *Hydroides elegans* (Haswell 1883) Doctoral Thesis, University of Madras PP. 1 to 180. India.
28. Vijayaragavan S. (2021). *Environment and Ecology 38:*4 884-892.
29. Vijayaragavan S., and P.V. Vivek Raja, (2018). *The Journal of Basic and Applied Zoology 79:* 45 PP. 1-9.
30. S. Vijayaragavan S., and P.V. Vivek Raja, (2019). *Environment and Ecology 37:*4 1285-1295.
31. Wightwick, A., M. Mollah, D. Partington, and G. Allinson, (2008). *Journal of Agricultural & Food Chemistry. 56:* 2457–2464.
32. Qiu, J. W., R. Tremblay, and E. Bourget, (2002). *Mar. Ecol. Prog*. Ser. *228:* 143-152.