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The Toxic Effects of Phorate Exposed for Ten Minutes Before Fertilization on Egg Cell of Sedentary Polychaete *Hydroideselegans* (Haswell 1883)

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ABSTRACT

In the present study, a toxicity test has been developed to examine the effectsof pesticides on fertilization and early development of marine polychaete *Hydroideselegans (H.elegans).* The pesticides are lead to pollution of the ground water, aquatic environments and also marine environment.It directly enters the food chains of the organismsand it affects the marine ecosystems. Thepesticides alter the regular functions of the marine organisms as well as physiological structure.The toxic effect of phorate onegg cell of *H. eleganswas* 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 intracellulartargets based on EC_{50} values of the present study;It indicated that phorate was toxic to the early developmental stages of *H.elegans* when the eggwerealready exposed to the same concentration of phorate for ten minutes before fertilization.The results indicate that theegg and early development stages of *H. elegansare* sensitive and toxic to phoratewhen the eggs werealready exposed to the phorate before fertilization. The sedentarypolychaete, *Hydroideselegans* can be routinely used as a test organism for eco-toxicity bioassaysexperimentsat tropical and sub- tropical regions.

Keywords: Phorate, *Hydroideselegans* embryo, Fertilization, Blastula.

INTRODUCTION

The use of pesticides can potentially pose a 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) (Komarek et al. 2010,Vijayaragavan and Vivek Raja 2019). 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 (Wightwick et al. 2008, Komarek et al. 2010). 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 (Wightwick et al. 2008,Komareket al. 2010).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 harbors and drainages. Therefore, it is essential that the bioassay techniques should be established to monitor the pollutantsthat pose a danger or hazard to

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humans and the biota (Ringwood 1992, Gopalakrishnan et al. 2008,Vijayaragavan and Vivek Raja 2019).

Phorate is a highly toxic organophosphate pesticide which is used to control the chewing insects, leafhoppers, leaf miners, 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 chainand causes several ailments. It is essential to study thatthe effects of the pesticides by using bio-organism for aquatic environmental management and monitoring Gopalakrishnanet al.2008,Vijayaragavan and Vivek Raja 2019).Most of the pesticides affect the embryo, teratogenic effects by directly or indirectly affecting cellular physiology (Calevroet al. 1998). Many cases of surface water contamination with pesticides were noticed and reported (Halderet al. 1989).

The bioassays allow the detection of the effects by measuring the biologic response of marine organisms, particularly in their early life stages (Hiset al.1999). 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. (Richardson and Martin 1994,Gopalakrishnanet al.2008). 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 (Connor 1972, Rand et al.1995). 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 species (*Mytilusedulis, Crossostreavirginice* and *C.gigas*) and gametes of echinoderm species. (*Strongylocentrotusp urpuratus, S.tranciscanusand Arabica punctata*) have been developed (ASTM 1995,Dinnelet al. 1987). 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 (Qiu et al. 2002). In contrast *H.elegans* requires 2 to 3 minutes for fertilization, 30 minutes for first cleavage and approximately 12 hours for distinguishable trochophore larva (Vijayaragavan 2009,Vijayaragavan and Vivek Raja 2018,Vijayaragavan and Vivek Raja 2019). Therefore, the advantages of developing bioassays using *H.elegans* embryos are more clear and accurate.

H. elegans (Haswell 1883), a sedentary,tubicolousserpulidpolychaeteis common in all temperate region and produces viable gametes throughout the year (Raja 1999,Sellappan 2000,Gopalakrishnan and Raja 2002). 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 (Raja 1999, Gopalakrishnanand Raja 2002,Vijayaragavan 2009, Vijayaragavan and Vivek Raja 2019). Therefore, the aim of the present study was to determine the toxic effects of phorate on early embryonic stages of *H.elegans* when the eggswerealready exposed to the same concentration of phorate for ten minutes before fertilization.

MATERIALS AND METHODS

Collection of organism

H. eleganswere collected from the hulls of boats, which were in fishing operation for more than three months, berthed at Royapuram, Fish Landing Center,

Fig.1. *H. elegans* without tube

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.elegansin* 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 *Hydroideselegans* were kept completely immerzed in seawateruntil the test was initiated.

Experiment procedure

Tests were conducted in 100ml glass beakers containing 50 ml of the filtered sea water. The sex of the polychaete was distinguishable by the orange color of the female abdomen (Fig. 1) 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 polychaete (Strathman 1987). 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 egg of *Hydroideselegan* about 200 numbers of the eggs were treated with 20 ml of various concentration of phorate in sea water (0.25, 0.5, 1, 2, 5, 10, 15, 20 and 30 ppm) for 10 minutes before mixing untreated male gametes (sperm). Eggs were exposed to different concentration phorate for 10 minutes before being used for experiment. Then, added 0.5ml of untreated sperm suspension to 50 ml test solution containing treated eggs for different concentration(0.25, 0.5, 1, 2, 5,10,15,20 and 30 ppm). Fertilization observed by microscope for different embryonic stages of *H.elegans* for every 10 minutes after untreated sperm and treated 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.

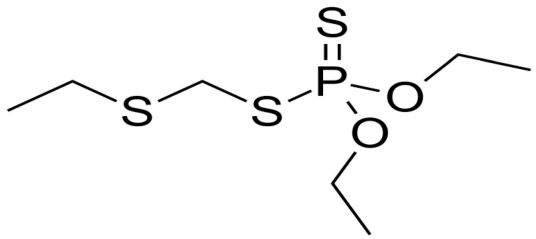

Fig.2 Molecular Structure of phorate

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 200 untreated eggs were exposed to filtered sea water for 10 minutes. At the end of the exposure periods, 0.5 ml of sperm suspension was 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 ofphorate on egg 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 (SPSS 1999).

Pesticides solution

Phorate(10% w/w), brand name: Kemfort was obtained from Keminol Enterprises, SIDCO north phase mbattur, Chennai, India (Fig. 2).

At 800 mg of phorate (10% w/w) was dissolved in 2000 ml of filtered seawater in a volumetric flask to prepare 40 ppm of phorate in seawater. This stock solution was stored in an amber colored bottle. From the stock solution the following concentrations of phorate in seawater (0.05 ppm to 40 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, 30 and 40 ppm of phorate in sea water was used for toxicity study by using embryo of *H.elegans.* Physico-chemical conditions of the experimenters were 28±1^{0}C temperature; 34±0.5 pptsalinty,6±0.3 mg1/L O_2 and 8.1±0.1pH were maintained.

RESULTS

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 98.19±0.01 and it decreased gradually 72.87±0.28at normal larval release stage. The cumulative time of FM stage was 5.40±0.72 minutes and the times steadily increased 299.40±0.28 minutes at larval release stage. The larval release stage was

occurred at 5 hour after the fertilization in the normal development without thephorate in sea water.

Toxiceffect of phorateon earlydevelopmental stages of *Hydroideselegans* when the eggs were already exposed to the same concentration for ten minutes before fertilization

Fertilization membrane (FM) stage to 8-cell stage

The percentage of success successful development of FM stage was 88.92±5.87 at 1 ppm of phorate in sea water. The cumulative time of FM stage was 11.40±0.51 minutes at 1 ppm of phorate and the percentage of successful development of 8-cell stage was 71.29±2.70 at 1 pm of phorate and it steadily decreased to 12.63±4.83 at 10 ppm.

16-cell stage to 64-cell stage

The percentage of successful development of 16-cell stage was 65.27±6.20 at 1 ppm of phorate in sea water. The cumulative time of 16-cell stage was 108.90±2.85 minutes at 1 ppm of phorate and the percentage of successful development of 64-cell stage was 55.67±6.33 at 1 ppm and it was decreased to 22.5047±6.10 at 5 ppm phoratein sea water and beyond 5 ppm, there was no development of the 64-cell stage. The cumulative time of 64-cell stage was 144.70±3.87 minutes at 1 ppm and it was increased to 175.50±3.92 minutes at 5 ppm of phoratein sea water.

Blastula stage to release stage

The percentage of successful development of blastula stage was 50.20±5.19 at 1 ppm of phorate in sea water and the cumulative time of blastula stage was 178.20±4.57 minutes at 1 ppm of phorate in seawater. The percentage of successful development of blastula stage was at 50.20±5.19 at 1ppmand it was decreased to 20.10±6.55 at 5 ppm phorate in sea water and beyond 5 ppm, there was no development observed. The present study the Blastula RotationStage was observed upto5 ppm and above 5ppm there was no rotation. The percentage of successful development of Release Stage was 35.44±10.59at 1ppm of phorate and it was decreased to 14.37±4.70at 5 ppm phorate in sea water and beyond 5 ppm there was no Release Stage observed.

The cumulative time of Release Stage was 347.30±0.31 minutes at 1 ppm of phoratein sea water and it increased to 381.10±2.08 minutes at 5 ppm.

The results presents here, strongly suggest that the mechanism of action of the phorate probably acts on sever as intracellulartargets based on EC_{50} values (Table1) 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 eggs were exposed to the same concentration for ten minutes before fertilization (Tables 2 and 3).

The effective concentration value (EC_{50}) referred to sensitivity towards the embryonic stages while exposed to different concentration ofphorate in sea water. The result indicated that the FM stage EC_{50} value was 5.0489 ppm which least sensitive stage of phorate in sea water and highest sensitive stage value was 0.2627 ppm at larval release stage

DISCUSSION

The sedentarypolychaetes 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 (Reish and Gerlinger 1997,Gopalakrishnanet al. 2008,Vija-

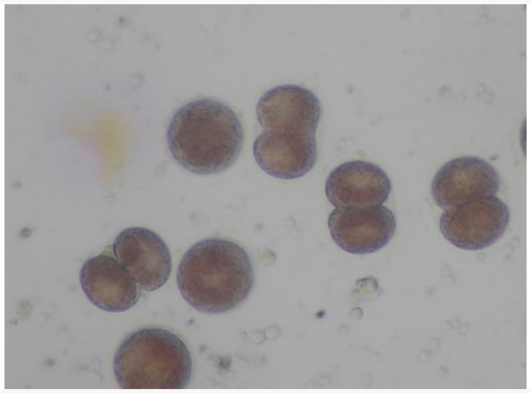

Fig 3. FM stage and 2 cell stage

Table 1 .Stage EC50values of phorate fordifferent embryonic stage of *H.elegans:* Egg exposed for tenmintues (Temp. 28±0.2°C, Salinity 34±0.1°/00, pH 8.1±0.1) stage EC_{50} values are expressed in ppm. n =Number of experiments lower and upper range of 95% confidence limits are given in parentheses.

Developmental stages	Phorate 10 minutes
FM -stage	5.0489 (.9911-74999)
2-cell stage	3.8910 (2.5608-6.3705)
3-cell stage	3.4155 (2.3007-58342)
4-cell stage	2.6336 (1.7256-4.5270)
8-cell stage	1.3084 (1.2038-3.7029)
16 cell stage	0.9484 (0.8860-27796)
32-cell stage	0.8985 (0.7440-2.1290)
64-cell stage	0.7102 (0.6478-1.4983)
Blastula stage	0.6062 (0.5320-1.1309)
Blastula start Rotation stage	0.3480 (0.3149-0.8614)
Blastula stop Rotation stage	0.3480 (0.3149-0.8614)
Relation stage	0.2627 (.2591-0.5686)

yaragavan and Vivek Raja 2019). Polychaetes are ecologically important marine organisms, making up from 30% to 80% of the total numbers of benthic fauna regardless of the ocean depth (Hutchings1998). The results revealed that the stage EC_{50} value of phorate decreased steadily from 5.0489 ppm in the FM-stage to 0.2627 ppm in the release stage. It isindicating 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 embryo 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 98.19 ± 3.39% successful in control sea water and it gradually decreased to 20.13 ± 2.34 at 20 ppm. There was no fertilization at 30 ppm. Similar trend was reported in the same species on effect of Monocrotophos, D.D.T., Chlorofyrifos, Endosulfan (Sellappan 2000). Heavymetals, (Gopalakrishnan and Raja 2002) Petroleum oils (Sellappan 2000,Vignesh 2002) phorate and carbendazim (Vijayaragavan and Vivek Raja 2018).

The percentage of successful development of *H. elegans* declined as the developmental stages progressed in any given concentration of phoratein seawater. In the same way abnormal development of the various developmental stages increased when the concentration of phorate increase in seawaterand 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 embryo 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 Thilagamet al.(2008),Vijayaragavan and Vivek Raja (2019).

In the present study that 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 phorateincreases 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 (Raja and Sellappan 1992,Sellappan 2000,Thilagam et al. 2008,Vijayaragavan and Vivek Raja 2018,Vija-

Table 2.Successful percentage of various embryonic stages of *H.elegansin* different concentrations of phorate when the eggs were already exposed to the same concentration for ten mintutes before fertilization;(Temp. 28±0.2°C, Salinity 34±0.1‰, pH 8.1±0.1); n=6 ±=SD. N.D= No Development, Number of eggs/embryos observed in each concentration =100-150, n=Number of experiments.

Developmental stages	Control	Percentage of successful development Concentration of phorate in ppm							
		0.25	0.5	1	2	5	10	20	30
FM stage	98.19	94.80	91.50	88.92	78.24	57.17	37.57	20.13	N.D
	±0.01	±3.39	±4.52	±5.87	±8.91	±10.20	±9.36	±2.34	
2– cell stage	95.94	91.37	86.67	82.77	73.67	49.80	30.70	30.70	N.D
	±0.38	±4.66	±4.21	±4.87	±8.94	±11.84	±8.90	±1.80	
3– cell stage	93.73	88.20	83.60	78.27	69.17	42.13	22.57	N.D	N.D
	±0.92	±5.56	±5.72	±4.87	±8.94	±9.34	±7.99		
4 – cell stage	91.53	84.47	79.78	74.66	63.77	36.97	18.27	N.D	N.D
	±1.01	±6.36	±4.61	±3.33	±8.56	±8.51	±7.91		
8 – cell stage	89.13	79.77	76.15	71.29	57.73	33.03	12.63	N.D	N.D
	±1.70	±6.30	±4.81	±2.70	±8.54	±9.14	±4.83		
16 – cell stage	87.23	76.44	7.70	65.27	53.5	29.17	9.00	N.D	N.D
	±1.66	±7.00	±6.34	±6.20	±10.30	±8.17	±4.60		
32 – ceal stage	85.13	73.12	67.10	61.67	49.00	25.08	8.73	N.D	N.D
	±1.64	±6.97	±4.87	±5.30	±10.37	±7.10	±1.32		
64 – cell stage	87.32	70.07	63.50	55.67	44.17	22.50	N.D	N.D	N.D
	±2.40	±7.64	±4.35	±6.33	±9.91	±6.10			
Blastula stage	79.90	67.01	58.63	50.20	39.60	20.10	N.D	N.D	N.D
	±2.79	±7.61	±5.62	5.19	±12.59	±6.55			
Blastula start	76.83	63.49	54.17	43.94	35.70	8.00	N.D	N.D	N.D
Rotation stage	±1.62	±7.62	±7.21	5.26	±10.98	±7.05			
Blastula stop	76.83	63.49	54.17	43.94	35.70	8.00	N.D	N.D	N.D
rotation stage	1.62	±7.62	±7.21	±5.26	±10.98	±7.05			
Release stage	72.82	57.10	47.51	35.44	28.80	14.37	N.D	N.D	N.D
	±0.28	±11.4	±9.21	±10.59	±8.33	±4.70			

yaragavan and Vivek Raja 2019).It has been already reported similarly in sea urchin that the ciliary activity is essential for successful hatching in sea urchin (Okazaki 1975).

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 (Mohamood and Parry 2001).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 phoratein sea water.The hatching time (release time) of *H. elegans*was 299.40 ± 0.28 minutes and it gradually increases to 381.10 ± 2.08 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 30 ppm and above

CONCLUSION

Theexperimental data revealed that the toxicity of phorate on early embryonic stages of *Hydroideselegans* is more sensitive when the *H. eleganseggs* 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

Table 3. Cumulative time of various embryonic stages of *H. elegansin* different concentrations of phorate when the egg were already exposed to the same concentration for the minutes before fertilization;(Temp. 28±0.2°C, Salinity 34±0.1°/$_{00}$, pH 8.1±0.1); n=6 ± =SD. N.D= No Development, Number of eggs/embryos observed in each concentration = 100-150, n = Number of experiments.

Developmental 1 stage	Time in minutes Concentration of phorate in ppm								
	Cotrol	0.25	0.5	1	2	5	10	20	30
FM stage	5.40 ±0.72	7.40 ±0.19	9.40 ±0.26	11.40 ±0.51	13.60 ±0.84	15.40 ±0.51	17.40 ±0.51	19.50 ±0.70	N.D
2-cell stage	30.80 ±1.02	34.60 ±0.93	39.00 ±01.35	42.60 ±0.93	46.30 ±1.78	49.70 ±0.99	53.70 ±0.69	37.90 ±1.21	N.D
3-cell stsge `	41.20 ±1.53	46.80 ±1.35	53.30 ±1.83	59.00 ±1.44	64.60 ±2.26	70.10 ±1.52	79.80 ±3.46	N.D	N.D
4-cell stage	51.60 ±2.04	59.00 ±1.77	67.60 ±2.31	75.20 ±1.86	83.20 ±3.13	90.40 ±2.08	105.800 2.04	N.D	N.D
8-cell stage	63.00 ±2.55	72.40 ±2.28	82.90 ±2.79	92.50 ±2.34	102.50 ±3.61	111.30 0.39	129.30 ±1.56	N.D	N.D
16-cell stage	73.30 ±1.03	84.70 ±2.76	97.40 ±2.49	108.90 ±2.85	121.10 ±3.45	131.70 ±4.94	151.70 ±1.07	N.D	N.D
32-cell stage	84.70 ±1.54	98.10 ±1.27	112.80 ±3.06	126.30 ±3.36	140.40 ±4.93	153.16 ±0.41	174.90 ±3.49	N.D	N.D
64-cell stage	97.10 ±4.05	112.50 ±3.78	129.50 ±4.82	144.70 ±3.87	160.70 ±0.46	175.50 ±3.92	N.D	N.D	N.D
Blastula stage	125.50 ±1.97	142.90 ±4.29	160.80 ±0.63	178.20 ±4.57	196.00 ±5.89	212.80 ±4.43	N.D	N.D	N.D
Blastula start Rotation stage	153.90 ±1.29	173.30 ±4.84	192.20 ±5.81	211.40 ±4.99	231.40 ±0.62	250.40 ±5.09	N.D	N.D	N.D
Bastala stop rotation stage	244.10 ±1.80	257.70 ±2.31	272.60 ±1.32	286.00 ±5.83	303.50 1.84	316.80 ±3.64	N.D	N.D	N.D
Release stage	299.40 ±0.28	314.10 ±3.55	331.90 ±3.83	347.30 ±0.31	366.80 ±4.32	381.10 2.18	N.D	N.D	N.D

embryo developments of *H. elegans*. Further more, the availability of *H. elegans* throughout the year which favorable and suitable for laboratory toxicity tests. The data revealed that the phoratewas sensitive andtoxic to an early embryonic stages of *H. elegans* when theeggs were already exposed to the same concentration for ten minutes before fertilization and also the pesticidesleads to severe environmental pollutions terrestrial and marine environment. Therefore, the word must understand the toxic effect of pesticide on environment and human being. Hence, the severe toxic and harmful pesticides should not be used in the agricultural practice or any other purpose around the world.

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