Environment and Ecology 37 (4) : 1285—1295, October—December 2019
Website: environmentandecology.com ISSN 0970-0420

The Toxic Effects of Carbendazim on Early Embryonic Stages of Sedentary Polychaete *Hydroides elegans* (Haswell 1883)

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Received 11 June 2019; Accepted 29 July 2019; Published on 30 August 2019

ABSTRACT

The toxicity test has been performed to examine the effects of pesticides on fertilization and early development of marine Polychaete *Hydroides elegans* (*H. elegans).*Carbendazim is a widely used, broad-spectrum benzimidazole fungicide and a metabolite of benomyl. The fungicide leads to pollution of the ground water, 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 physiological structure. The toxic effect of Carbendazim on fertilization, early developmental stages of *H.elegans* was examined and it was found that the rate of successful development of embryonic development decreased when the concentration of Carbendazim increased in sea water. The results presents here, strongly suggest that the mechanism of action of the fungicide probably acts on sever as intracellular targets based on EC_{50} values of the present study ; it indicated that Carbendazim was toxic to the early developmental stages of *H. elegans*. The results indicate that the early development stages of *H. elegans* are highly sensitive to Carbendazim (fungicide). The sedentary polychaete, *Hydroides elegans* can be routinely used as a test organism for eco-toxicity bioassays experiments at tropical and sub-tropical regions.

Keywords Carbendazim, *Hydroides elegans* embryo, Fertilization, Blastula.

INTRODUCTION

The regular use of fungicides 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). 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, Komarek et al. 2010).

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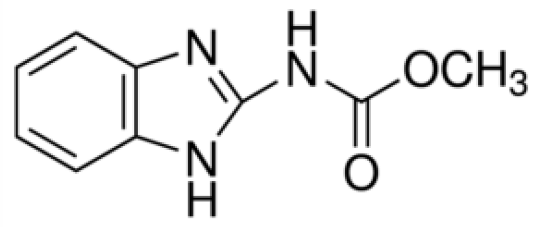

Fig. 1. Molecular structure of Carbendazim.

Fig. 2. *H. elegans* without tube.

The urbanization growth which endangers the coastal ecosystem and also the ecosystem which may be polluted by the discharges from specific point sources like sewage, effluents and industrial wastes and also from non-point sources like harbors and drainages. 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 (Ringwood 1992, Gopalakrishnan et al. 2008).

The Carbendazim is a systemic benzimidazole fungicide (Fig. 1) that was used to control fungal diseases on pulses, fruits, macadamia nuts, cucurbits, pastures, roses, timber and turf; it was also used in post -harvest storage of fruits. Carbendazim is both a metabolite and breakdown product of benomyl and a breakdown product of thiophanate-methyl in plants and the environment. Carbendazim is classified in the hazardous category of chemicals by World Health Organization. Carbendazim along with carbomyl are classified as possible human carcinogens (Goodson et al. 2015).

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 monitoring (Gopalakrishnan et al. 2008). Most of the pesticides affect the embryo, teratogenic effects by directly or indirectly affecting cellular physiology (Calevro et al. 1998). Many cases of surface water contamination with pesticides were noticed and reported (Halder et al. 1989).

The bioassays allow the detection of the effects by measuring the biologic response of marine organisms, particularly in their early life stages (His et 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, Gopalakrishnan et 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 (*Mytilus edulis, Crossostrea virginice* and *C. gigas*) and gametes of echinoderm species (*Strongylocentrotus purpuratus, S. trancis-canus* and *Arabica punctuata)* have been developed (ASTM 1995, Dinnel et 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 (Fig. 2). Furthermore, it is noted that sea urchins require 5 to 10 minutes for fertilization, 1 h for first cleavage, 24 h for blastula and gastrula and 48 h for trochophore larva (Qiu et al. 2002). In contrast *H. elegens* requires

2 to 3 minutes for fertilization, 30 minutes for first cleavage and approximately 12 h for distinguishable trochophore larva (Vijayaragavan 2009, Vijayaragavan and Vivek Raja 2018). Therefore, the advantages of developing bioassays using *H. elegans* embryos are more clear and accurate.

H. elegans (Haswell 1883), a sedentary, tubicolous serpulid polychaete is 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,Gopalakrishnan and Raja 2002, Vijayaragavan 2009). Therefore, the aim of the present study was to determine the toxic effects of Carbendazim on early embryonic stages of *H. elegans.*

MATERIALS AND METHODS

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 (Latitude $13^0$06' N and Longitude 80^0 18´ E). Other sedentary animal 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 ± 1 ppt), temperature (28 ± 10 ^{0}C) and pH (8.1 ± 0.1). IIlumination was provided in a light, dark cycle of 14:10 h. 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 seawater. The sex of the polychaete was distinguishable by the orange color 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 polychaete (Strathman 1987); 5 to 10 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

About 200 eggs were introduced into each test chamber containing fresh seawater by Pasteur pipette. Then 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 50 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-cell stage, 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 various concentration of Carbendazim 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

The Carbendazim (50% w/w), brand name: Bavistin was obtained from BASF India limited, Thane, India;

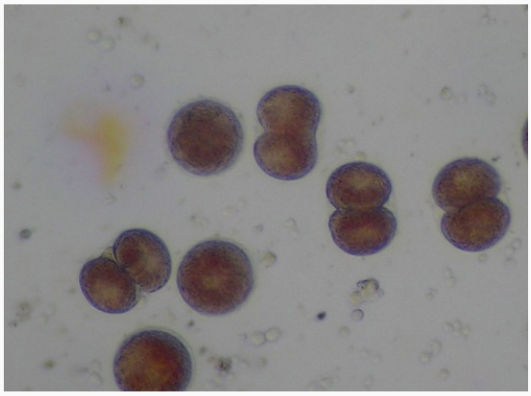

Fig. 3. FM stage and 2-cell stage.

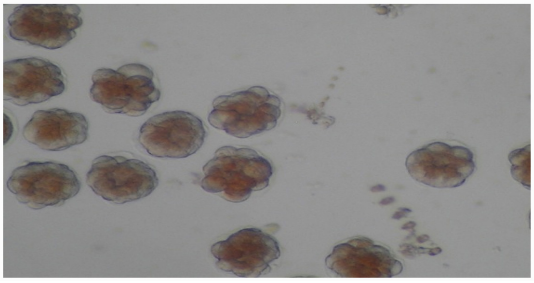

Fig. 4. Blastula stage.

800 mg of Carbendazim was dissolved in 2000 ml of filtered seawater in a volumetric flask to prepare 200 ppm of Carbendazim in seawater. This stock solution was stored in an amber colored bottle. From the stock solution the following concentrations of Carbendazim in seawater (0.05 ppm to 100 ppm) were prepared and 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 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, 15, 20, 30, 40, 50 and 100 ppm of Carbendazim in seawater was used for toxicity study by using embryo of *H. elegans.* Physico-chemical conditions of the experimental media were maintained at 28 ± 1 ^{0}C temperature, 34 ± 0.5 ppt salinity, 6 ± 0.3 mg1/L O_2 and pH 8.1 ± 0.1.

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 97.67 ± 0.57 and it decreased gradually 78.33 ± 1.00 at normal larval release stage (Figs. 4 and 5). The cumulative time of FM-stage was 6.00 ± 0.38 minutes

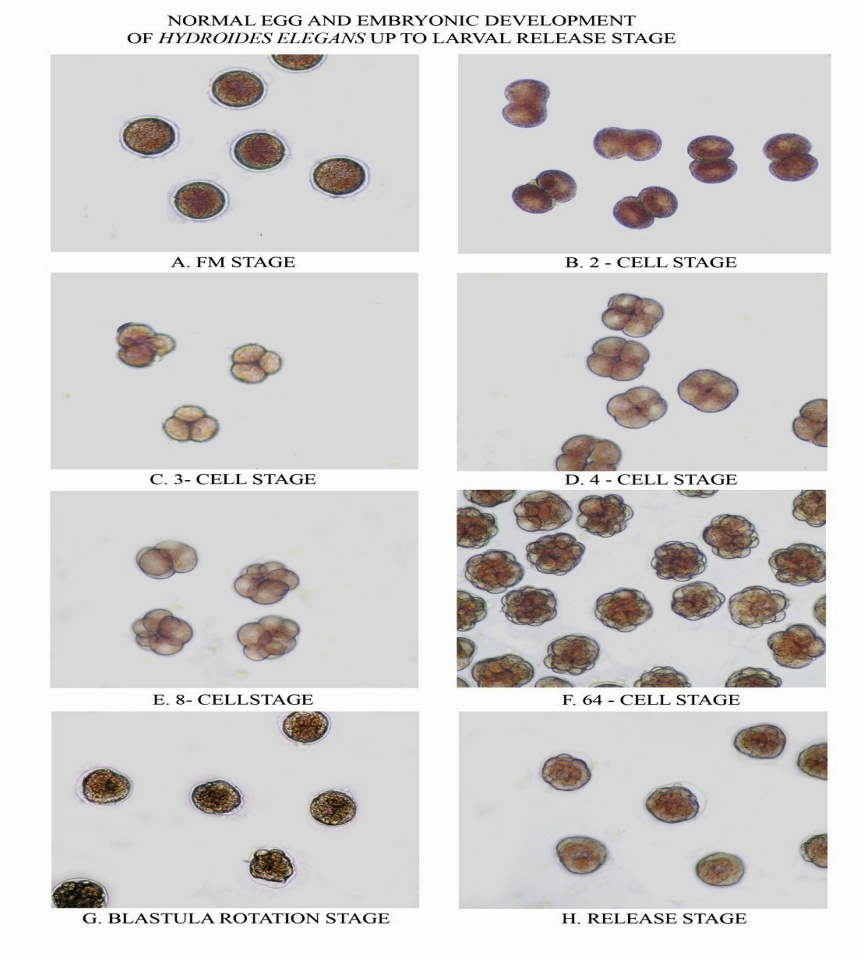

Fig. 5. Normal embryonic development of *Hydroides elegans* up to larval stage. A, FM-stage, B. 2-cell stage, C. 3- cell stage, D. 4- cell stage, E. 8-cell stage, F. 64-cell stage, G. Blastula rotation stage, H. Release stage.

and the times steadily increased 304.30 ± 2.94 minutes at larval release stage. The larval release stage occurred at 5 h after the fertilization in the normal development without the Carbendazim in seawater.

Toxic effect of Carbendazim on early developmental stages of *Hydroides elegans*

Fertilization membrane (FM) stage to 8-cell stage

The percentage of successful development of FM-stage was 96.33 ± 0.57 at 0.05 ppm of Carbendazim in seawater. The cumulative time of FM-stage was 7.50 ± 0.70 minutes at 0.05 ppm of Carbendazim and the percentage of successful development of 8-cell stage was 85.67 ± 2.51 at 0.05 ppm of Carbendazim and it steadily decreased to 3.33 ± 1.52 at 40 ppm and beyond 40 ppm, Carbendazim in seawater, there was no development observed.

16 cell stage to 64 cell stage

The percentage of successful development of 16-cell stage was 84.00 ± 1.00 at 0.05 ppm of Carbendazim in seawater. The cumulative time of 16-cell stage was 87.00 ± 2.82 minutes at 0.05 ppm of Carbendazim

and the percentage of successful development of 64-cell stage was 50.67 ± 10.60 at 1 ppm and it was decreased to 11.67 ± 1.52 at 20 ppm Carbendazim in seawater and beyond 20 ppm, there was no development of the 64-cell stage. The cumulative time of 64-cell stage was 170.50 ± 16.26 minutes at 1 ppm and it was increased to 289.00 ± 0.00 minutes at 20 ppm of Carbendazim in seawater.

Blastula stage to release stage

The percentage of successful development of Blastula stage was 76.67± 1.52 at 0.05 ppm of Carbendazim in seawater and the cumulative time of Blastula stage was 144.00 ± 4.24 minutes at 0.05 ppm of Carbendazim in seawater. The percentage of successful development of Blastula stage was 47.30 ± 10.6 at 1 ppm and it was decreased to 9.00 ± 1.00 at 20 ppm Carbendazim in seawater and beyond 20 ppm, there was no development observed.

The present study the Blastula rotation stage was observed upto 15 ppm and above 15 ppm there was no rotation. The percentage of successful development of Release stage was 68.67 ± 3.21 at 0.05 ppm pf Carbendazim and it was decreased to 11.67 ± 6.80 at 10 ppm Carbendazim in seawater and beyond 10 ppm there was to Release Stage observed. The cumulative time of Release Stage was 317.00 ± 0.00 minutes at 0.05 ppm of Carbendazim in seawater and it increased to 432.50 ± 5.33 minutes at 10 ppm.

Table 1. Comparison of stage EC_{50} values of Carbendazim for different embryonic stages of *H. elegans* (Temp 28 ± 0.2 ^{0}C, salinity 34 ± 0.1 $^0/_{00}$, pH 8.1 ± 0.1) (Stage EC_{50} values are expressed in ppm).

Developmental stages	EC_{50} value of Carbendazim	Developmental stages	EC_{50} values of Carbendazim
FM-stage	17.4362	32-cell stage	5.8136
2-cell stage	13.8359	64-cell stage	4.8322
3-cell stage	11.4050	Blastula stage	3.9923
4-cell stage	9.7069	Blastula start rotation stage	2.6893
8-cell stage	7.9216	Blastula stop rotation stage	2.6893
16-cell stage	6.6232	Release stage	1.8198

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 (Reish and Gerlinger 1997, Gopalakrishnan et al. 2008). Polychaetes are ecologically important marine organisms, making up from 30% to 80% of the total numbers of benthic fauna regardless of the ocean depth (Hutchings 1998).

The results presents here, strongly suggest that the mechanism of action of the pesticide probably acts on sever as intracellular targets based on EC_{50} values (Table 1) of the present study. It indicate that

Table 2. Percentage of successful development of various embryonic stages of *Hydroides elegans* in control seawater and in different concentration of carbendazim in seawater. (Temp 28 ± 0.2 ^{0}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 stagers	Percentage of successful development Concentration of Carbendazim in ppm Control	0.05	0.1	0.25	0.5	1	2	5	10	15	20	30	40	50	100
FM-stage	97.67 ±0.57	96.33 ±0.57	90.67 ±3.22	85.33 ±5.50	79.00 ±8.54	73.67 ± 11.3	69.67 ±15.6	60.60 ±11.1	55.00 ±10.4	45.67 ±7.76	33.67 ±7.76	24.00 ±2.00	18.33 ±3.21	10.00 ±2.00	N. D
2–cell stage	95.67 ±1.52	92.33 ±1.52	89.00 ±3.46	82.33 ±5.13	75.67 ±10.6	69.00 ± 13.7	66.67 ±14.5	57.33 ±12.5	50.57 ±11.8	38.33 ±4.72	29.67 ±4.93	18.33 ±4.16	11.33 ±2.8	3.33 ±0.57	N. D
3–cell stage	93.33 ±1.52	90.00 ±2.64	86.33 ±3.05	80.67 ±5.50	71.67 ±11.1	63.00 ±9.53	59.67 ±11.1	54.33 ±10.9	47.67 ±10.9	34.33 ±4.04	25.33 ±3.51	16.33 ±4.50	6.67 ±1.15	N.D	N. D
4–cell stage	92.00 ±2.00	87.33 ±2.08	84.33 ±3.05	78.33 ±5.13	68.33 ±11.3	60.00 ±8.88	57.33 ±10.7	50.33 ±10.1	45.00 ±13.0	30.00 ±3.60	22.00 ±3.0	11.33 ±4.93	5.00 ±1.73	N.D	N. D
8–cell stage	90.33 ±1.52	85.67 ±2.51	82.00 ±4.00	75.67 ±6.65	66.66 ±11.3	57.67 ±8.73	53.67 ±10.6	46.33 ±10.1	41.00 ±11.2	24.67 ±5.77	19.00 ±2.00	8.67 ±5.50	3.33 ±1.52	N.D	N. D
16–cell stage	88.67 ±2.08	84.00 ±1.00	79.00 ±3.46	72.00 ±2.00	64.67 ±11.1	54.67 ±10.4	50.67 ±8.08	40.67 ±7.76	36.67 ±9.23	22.33 ±0.57	16.67 ±0.57	5.33 ±4.16	N.D N.D	N.D N.D	N. D

Table 2. Continued.

Developmental stages	Percentage of successful development Concentration of Carbendazim in ppm Control	0.05	0.1	0.25	0.5	1	2	5	10	15	20	30	40	50	100
32–cell stage	86.33 ±2.30	81.33 ±1.15	76.67 ±2.88	69.17 ±8.25	61.67 ±10.6	52.67 ±10.8	47.33 ±8.08	36.66 ±7.37	30.33 ±5.68	18.67 ±1.56	14.00 ±1.00	N.D	N.D	N.D	N. D
64–cell stage	84.00 ±1.73	79.00 ±1.00	75.00 ±2.64	66.33 ±8.08	58.67 ±10.9	50.67 ±10.6	44.33 ±7.76	32.33 ±6.02	27.33 ±4.72	16.00 ±1.00	11.67 ±1.52	N.D	N.D	N.D	N. D
Blastula stage	82.67 ±2.08	76.67 ±1.52	71.67 ±3.78	64.00 ±8.18	55.67 ±10.9	47.3 ±10.6	41.67 ±7.22	27.00 ±5.00	22.33 ±4.61	14.00 ±1.00	9.00 ±1.00	N.D	N.D	N.D	N. D
Blastula start rotation stage	80.33 ±2.30	73.67 ±1.15	68.67 ±4.04	60.00 ±9.16	52.67 ±10.7	44.67 ±11.2	38.67 ±8.42	23.00 ±6.00	19.33 ±4.72	10.33 ±2.51	N.D	N.D	N.D	N.D	N. D
Blastula stop rotation stage	80.33 ±2.30	73.67 ±1.15	68.67 ±4.04	60.00 ±9.16	52.67 ±10.7	44.67 ±11.2	38.67 ±8.42	23.00 ±6.00	19.33 ±4.72	10.33 ±2.51	N.D	N.D	N.D	N.D	N. D
Release stage	78.33 ±2.30	68.67 ±3.21	63.33 ±5.50	53.33 ±11.9	44.33 ±11.8	37.67 ±10.6	31.67 ±10.1	17.33 ±7.23	11.67 ±6.80	N.D	N.D	N.D	N.D	N.D	N. D

Carbendazim 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 Carbendazim in seawater (Tables 2 and 3).

The effective concentration value (EC_{50}) referred to sensitivity towards the embryonic stages while exposed to different concentration of Carbendazim in seawater. The result indicated that the FM-stage EC_{50} value was 17.4362 ppm which least sensitive stage of Carbendazim in seawater and highest sensitive stage value was 1.8198 ppm at larval release stage.

DISCUSSION

The results revealed that the stage EC_{50} value of Carbendazim decreased steadily from 17.4362 ppm in the FM-stage to 1.8198 ppm in the release stage. It is indicating that the release stage (hatching) is more sensitive to Carbendazim than the earlier stages, but

Table 3. Cumulative times of various embryonic stages of *Hydroides elegans* in control seawater and in different concentrations of Carbendazim in seawater. N.D–No Development, Number of eggs/embryos observed in each concentration =100-150, n=Number of experiments (Temp, 28±0.2^{0}C, Salinity 34±0.1 $\%_{00}$, pH 8.1 ± 0.1) n=6, ±=SD.

Developmental stages	Times in minutes Concentration of Carbendazim in ppm Control	0.05	0.1	0.25	0.5	1	2	5
FM–stage	6.00±0.32	7.50±0.70	10.00±0.00	11.00±0.00	13.50 ±0.70	15.00±1.41	17.00±1.41	19.50±2.12
2–cell stage	31.50±2.53	34.50±0.70	38.50±0.70	42.00±1.41	46.50±0.70	52.00±1.41	54.50±2.12	59.50±2.12
3–cell stage	41.70±1.26	46.50±0.70	51.50±2.12	58.50±3.82	62.00±2.82	70.00±1.41	75.00±0.00	82.50±2.12
4–cell stage	53.30±4.53	59.00±1.41	66.00±4.24	73.00±5.67	79.50±6.36	89.50±4.95	97.00±2.82	106.50±0.70
8–cell stage	64.80±2.61	73.00±1.41	81.50±6.36	89.50±7.77	98.00±8.48	108.50±7.77	119.50±4.95	132.00±2.82
16–cell stage	76.80±3.43	87.00±2.82	97.50±7.77	107.00±11.31	117.00±12.72	130.00±11.31	144.50±12.02	157.50±6.36
32-cell stage	88.30±2.67	100.50±4.95	112.50±9.89	123.00±14.14	135.00±1.55	149.50±14.84	164.00±11.31	180.50±9.19
64–cell stage	101.10±2.35	114.00±4.24	127.00±11.30	140.00±15.55	154.00±0.00	170.50±16.26	184.50±16.26	205.00±10.66
Blastula stage	128.50±1.41	144.00±4.24	159.00±11.30	174.00±15.55	190.00±0.00	208.50±7.26	126.50±12.02	246.00±8.81
Blastula start rotation stage	156.80±3.59	173.00±1.41	191.00±11.30	208.00±15.55	226.00±8.32	245.00±14.84	266.50±10.00	288.50±7.30
Blastula stop rotation stage	247.40±7.58	258.50±2.12	273.00±16.90	286.50±12.02	298.00±9.89	310.00±12.72	326.50±16.26	340.00±4.70
Release stage	304.30±2.94	317.00±0.00	333.00±±8.72	348.00±14.14	363.50±13.43	379.00±13.55	396.00±8.79	412.00±6.40

Table 3. Continued.

Developmental stages	Times in minutes Concentration of Carbendazim in ppm 10	15	20	30	40	50	100
FM–stage	22.00±2.82	24.00±5.65	29.00±8.48	32.50±12.02	37.50±7.77	42.20±5.37	N.D
2–cell stage	65.50±6.36	71.00±8.48	79.00±11.31	87.50±8.26	96.50±12.52	105.00±9.89	N.D
3–cell stage	90.50±4.95	100.00±9.89	112.50±5.67	124.50±3.33	136.00±8.79	N.D	N.D
4–cell stage	117.00±2.82	124.00 ±18.38	147.00±1.21	163.00±2.28	177.00±6.82	N.D	N.D
8–cell stage	143.50±0.00	160.00±9.89	182.50±6.16	202.50±3.06	219.00±3.35	N.D	N.D
16–cell stage	170.00±5.65	189.00±7.07	218.50±8.99	243.00±4.84	N.D	N.D	N.D
32–cell stage	198.00±5.65	218.50±6.36	253.50±3.23	N.D.	N.D	N.D	N.D
64–cell stage	225.00±5.65	249.50±7.77	289.00±0.00	N.D	N.D	N.D	N.D
Blastula stage	268.50±4.95	297.50±10.50	339.00±4.56	N.D	N.D	N.D	N.D
Blastula start rotation stage	313.50±2.12	345.00±12.12	N.D	N.D	N.D	N.D	N.D
Blastula stop roration stage	358.50±2.57	385.50±11.52	N.D	N.D	N.D	N.D	N.D
Release stage	432.50±5.33	N.D	N.D	N.D	N.D	N.D	N.D

actually it may be due to longer exposure of embryo to the fungicide 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 Carbendazim on fertilization in *H. elegans* reveals that the success rate of fertilization decreases as the concentration of Carbendazim increases in seawater. Successful fertilization was evidenced by the elevation of fertilization membrane. Successful fertilization was 97.67 ± 0.57% successful in control seawater and it gradually decreased to 10.00 ± 2.00 at 50 ppm. There was no fertilization at 50 ppm. Similar trend was reported in the same species on effect of Monocrotophos, DDT, Chlorofyrifos, Endosulfan (Sellappan 2000). Heavy metals, (Gopalakrishnan and Raja 2002), Petroleum oils (Sellappan 2000, Vignesh 2002), Phorate (Vijayaragavan and Vivek Raja 2018).

The percentage of successful development of *H. elegans* declined as the developmental stages progressed in any given concentration of Carbendazim in seawater. In the same way abnormal development of the various developmental stages increased when the concentration of Carbendazim increase in seawater. In higher concentration the development were arrested and up normal embryo observed due to the effect of Carbendazim (Fig. 6). 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 Carbendazim increased in seawater in all the stages. It reveals that the rate of development decreases with increase in concentration of Carbendazim in seawater. Similar trend was observed by Thilagam et al. (2008).

The individual stage time of different development stages of *H. elegans,* increased except the Blastula rotation stage. At the Blastula rotation stage, Carbendazim affects the ciliary activity of the embryo. Hence, the rotation time decreases gradually when the concentration of Carbendazim 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 Carbendazim, 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). It has been already reported that the ciliary activity is essential for successful hatching in sea urchin (Okazaki 1975).

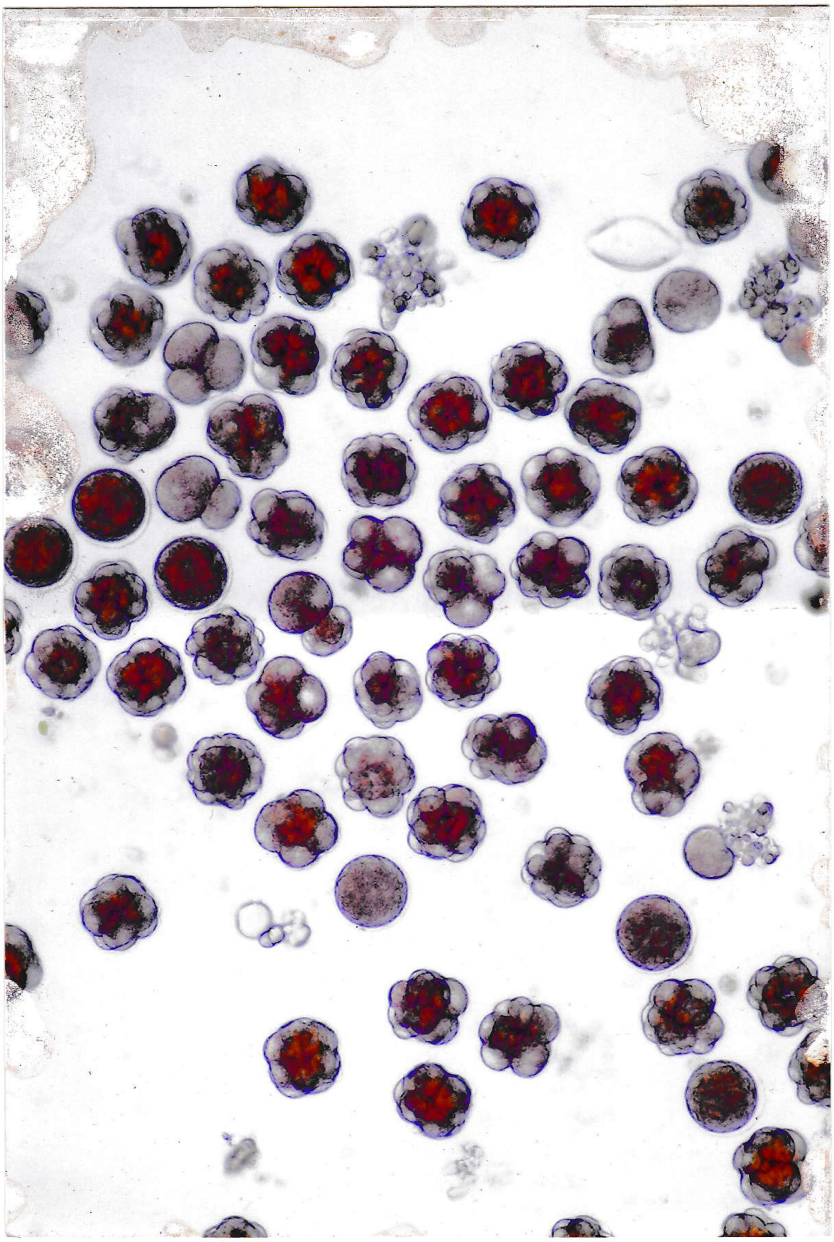

Fig. 6. Up-normal development of Embryo.

A study of Mantovani et al. (1998), revealed that animals exposed to Carbendazim in womb had serious deformities such as lack of eyes and hydrocephalus. It can disturb the development of sperm and damage testicular development in adult rats. The researchers testing the effects of Carbendazim, cultured human lymphocytes, concluded that it is obvious that Carbendazim 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 Carbendazim 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 Carbendazim in seawater. The hatching time (release time) of *H. elegans* was 304.30 ± 2.94 minutes and it gradually increases to 432.50 ±5.33 minutes 10 ppm of Carbendazim. The results may be inferred that the rate of production of hatching enzyme decreased in the presence of Carbendazim, as there was some delay in hatching up to 2 ppm of carbendazim, and the production of enzyme was reduced below the critical level or completely arrested at 10 ppm and above.

CONCLUSION

The experimental data revealed that the toxicity of Carbendazim on early embryonic stages of *Hydroides elegans* is more sensitive and its lead to abnormalities of embryos. Hence, the development stages have been arrested in high concentration of Carbendazim in seawater. It observed that the toxicity particles have inducing the abnormalities in the early 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 Carbendazim was toxic to an early embryonic stages of *H. elegans* and also leads to environmental pollutions.

ACKNOWLEDGEMENT

S. V. designed the works, performed the experiments and drafted the manuscript.

REFERENCES

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.

Calevro F, Campani S, Rangghianti M, Bucci S, Mancino G (1998) Test of toxicity and teratogenicity in biohasic vertebrates treated with heavy metals (Cr^{3+}, Al^{3+}, Cd^{2+}). Chemosphere 37 : 3011—3017.

Connor PM (1972) Acute toxicity of heavy metals to some marine larvae. Mar Poll Bull 3 : 190—1922.

Dinnel PA, Link JM, Stober OJ (1987) Improved methodology for a Seaurchin sperm cell bioassay for marine waters. Arch Environ Contam Toxic 16 : 23—32.

Goodson WH, Lowe L, Carpenter DO, Gilbertson M, Ali AM, de Cerain Salsamendi AL et al. (2015) Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: The challenge ahead. Carcinogenesis 36 : 254—296.

Gopalakrishnan S, Raja PV (2002) The effects of copper on sperm and eggs of *Hydroides elegans*. In: Proc XX Nat Symp Reprod Biol Comp Endocrinol Bharathidasan University, Tiruchirappalli, India, pp 83—84.

Gopalakrishnan S, Thilagam H, Vivek Raja P (2008) Comparison of heavy metal toxicity in life stages (spermiotoxicity,egg toxicity, embryotoxicity and larval toxicity) of *Hydroides elegans*. Chemosphere 71 : 515—528.

Halder P, Raha P, Bhattacharya A, Choudhury AK, Adityachoudhury N (1989) Studies on the residues of DDT and Endosulfan occurring in Ganga water . Ind J Environ Hlth pp 52.

Haswell (1883) *Hydroides elegans* worms editorial board. World Register of Marine Species 131002.

His E, Beiras R, Seaman MNL (1999) The assessment of marine pollution – bioassays with bivalve embryos and larvae. In : South Ward AJ, Tyler PA, Young CM (eds). Advances in Marine Biology Academic press. sandiego. Ca, pp 1—178.

Hutchings P (1998) Biodiversity and functioning of polychaetes in benthic sediments. Biodiv Conserv 7 : 113.

Komarek M, Cadkova E, Chrastny V, Bordas F, Bollinger JC (2010) Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environ Int 36 : 138—151.

Mantovani A, Maranghi F, Ricciardi C, Macri C, Stazi AV, Attias L, Zapponi GA (1998) Developmental toxicity of carbendazim : Comparison of no-observed-adverse-effect level and benchmarkdose approach. Food Chem Toxicol 36 : 37—45.

Mohamood R, Parry JM (2001) Induction of micronuclei and chromosome non-disjunction after short -term exposure to carbendazim in cultured lymphocytes. 31st Annual Meeting of EEEMS Belgium.

Okazaki K (1975) Spicule Formation by isolated micromeres of the sea urchin embryo. Amer Zool 15 : 567—581.

Qiu JW, Tremblay R, Bourget E (2002) Ontogenetic changes in hypo saline tolerance in the musscls *Mytilus edulis* and *M. trossulus* : Implications for distribution. Mar Ecol Prog Ser 228 : 143—152.

Raja PV (1999) *Hydroides elegans* (Haswell) an ideal organism to study pollution in seawater. 26th Int Ethol Conf Bangalore. Adv Ethol 34 : 225.

Raja PV, Sellappan M (1992) Fertilization and early development of a sedentary polychaete *Hydroides elegans* from Madras coast. 11th Nat Symp on Life Sci Madras. Abstract, pp 58.

Rand GM, Wells PG, Mc Carty LS (1995) Introduction to aquatic toxicology. In : Rand GM (ed).Fundamentals of aquatic toxicology, effects, environmental fate and risk assessment.

Springer, Berlin, pp 3—70.
Reish DJ, Gerlinger TV (1997) A review of the toxicological studies with polychaetus annelids. Bull Mar Sci 60 : 584—607.
Richardson BJ, Martin M (1994) Marine and estuarine toxicity testing : A way to go ? Additional sittings from northern and southern hemisphere perspectives. Mar Poll Bull 28 : 138—142.
Ringwood HA (1992) Comparative sensitivity of gametes and early developmental stages of a sea urchin species *(Echinometra matnei)* and a bivalve species (*Isognomon californicum*) during metal exposures. Arch Environ Contam Toxicol 22 : 265—288.
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, India. pp 1—150.
SPSS (1999) Statistical Package for Social Sciences. SPSS Inc New York.
Strathman 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, pp 670.
Thilagam H, Gopalakrishnan S, Vijayavel K, Raja PV (2008) Effluent toxicity test using developmental stages of the marine polychaete. Arch Environm Contam Toxicol 54 (4) : 674—683.
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, pp 1—15.
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, India, pp 1—180.
Vijayaragavan S, Vivek Raja PV (2018) The toxic effects of phorate on early embryonic stages of sedentary polychaete *Hydroides elegans* (Haswell 1883). J Basic and Appl Zool 79 (45) : 1—9.
Wightwick A, Mollah M, Partington D, Allinson G (2008) Copper fungicide residues in Australian vineyard soils. J Agric & Food Chem 56 : 2457—2464.