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Toxic Effects of Carbendazim on Sperm Cell of Sedentary Polychaete *Hydroides elegans* (Haswell 1883)

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ABSTRACT

The toxicity test has been conducted to examine the effects of fungicide on sperm cell 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 Carbendazim on sperm cell of *H. elegans* was examined and it was found that the rate of successful development of cmbryonic 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* when the sperm were already exposed to the same concentration of Carbendazim for ten minutes before fertilization. The results indicate that the sperm and early development stages of *H.elegans* are sensitive and toxic to Cabendazim (fungicide) when the sperm were already exposed to the Carbendazim 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.

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

INTRODUCTION

The 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, 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, Komarek et 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

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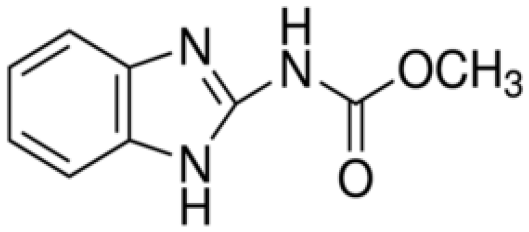

Fig. 1. Molecular structure of Carbendazim.

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 2008, Vijayaragavan and Vivek Raja 2019).

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, Vijayaragavan and Vivek Raja 2019). 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, Vijayaragavan and Vivek Raja 2019). 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 sea water 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. tranciscanus* 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. 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. elegans* 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, 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, 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, Vijayaragavan and Vivek Raja 2019). 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 (Latitued 13^0 06´N and Longitued 80^0 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 sea water. 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 ^{0}C) and pH (8.1 ±0.1). Illumination was provided in a light, dark cycle of 14 : 10 h. The polychaetes *Hydroides elegans* were kept completely immersed in sea water 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 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). The 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 sea water 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 sea water 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 sea water 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 Carbendazim on the sperm cell of *Hydroides elegan* about 0.05 ml of sperm suspension (diluted sperm) were treated with 20 ml of various concentration of Carbendazim in sea water. (1, 2, 5, 10, 15, 20 and 30 ppm) for 10 minutes before mixing untreated egg gametes. The sperm were exposed to different concentration Carbendazim 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 (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 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-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 sea water 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 0.05 ml 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-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 sea water 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 Carbendazim 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 (SPSS 1999).

Fungicide solution

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

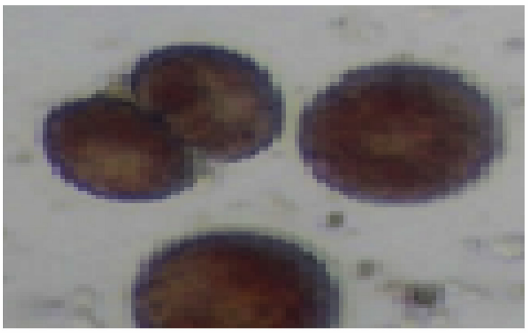

Fig. 2. FM stage and 2-cell stage.

800 mg of Carbendazim was dissolved in 2000 ml of filtered sea water in a volumetric flask to prepare 200 ppm of Carbendazim in sea water. This stock solution was stored in an amber colored bottle. From the stock solution the following concentrations of Carbendazim in sea water (1 ppm to 30 ppm) were prepared and used for the experiment.

In each experiment filtered sea water 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 1,2,5,10,15,20 and 30 ppm of Carbendazim in sea water was used for toxicity study by using embryo of *H. elegans.* Physico-chemical conditions of the experimental media were maintained at 28 $\pm 1^0$C temperature; 34±0.5 ppt salinity, 6±0.3 mgl/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. 2). The percentage of successful development of FM stage was 97.96 ± 4.93 and it decreased gradually 63.12 ± 0.53 at normal larval release stage. The cumulative time of FM stage was 5.40±0.51 minutes and the times steadily increased 299.50±0.70 minutes at larval

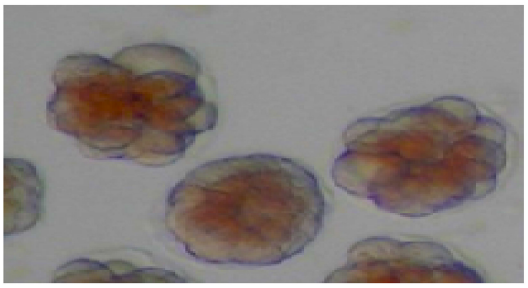

Fig. 3. Blastula stage.

release stage. The larval release stage was occurred at 5 h after the fertilization in the normal development without the Carbendazim in sea water.

Toxic effect of Carbendazim on early developmental stages of *Hydroides elegans* when the sperms were already exposed to the same concentration for ten minutes before fertilization

Fertilization membrane (FM) stage to 8-cell stage

The percentage of successful development of FM stage was 95.47 ±7.13 at 1 ppm of Carbendazim in sea water. The cumulative time of FM stage was 7.30±0.48 minutes at 1 ppm of Carbendazim and the percentage of successful development of 8-cell stage was 72.51 ±8.70 at 1 ppm of Carbendazim and it steadily decreased to 3.87 ± 5.08 at 20 ppm.

16-cell stage to 64-cell stage: The percentage of successful development of 16-cell stage was 68.15 ±8.78 at 1 ppm of Carbendazim in sea water. The cumulative time of 16-cell stage was 85.00±2.55 minutes at 1 ppm of Carbendazim and the percentage of successful development of 64-cell stage was 59.83±7.10 at 1 ppm and it was decreased to 6.47 ±5.69 at 15 ppm Carbendazim in sea water and beyond 15 ppm, there was no development of the 64-cell stage. The cumulative time of 64-cell stage was 112.50±3.28 minutes at 1 ppm and it was increased to 175.40±2.64 minutes at 15 ppm of Carbendazim in sea water.

Blastula stage to release stage

The percentage of successful development of blastula stage was 56.58 ± 6.86 at 1 ppm of Carbendazim in sea water and the cumulative time of blastula stage was 142.70 ± 0.49 minutes at 1 ppm of Carbendazim in sea water. The percentage of successful development of blastula stage was at 56.58 ±6.86 at 1 ppm (Fig. 3) and it was decreased to 18.63 ± 6.01 at 10 ppm Carbendazim in sea water and beyond 10 ppm, there was no development observed.

The present study the blastula rotation stage was observed upto 10 ppm and above 10 ppm there was no rotation.The percentage of successful development of release stage was 45.92±9.44 at 1 ppm of Carbendazim and it was decreased to 10.37 ±6.87 at 10 ppm Carbendazim in sea water and beyond 10 ppm there was no release stage observed. The cumulative time of release stage was 310.80±1.57 minutes at 1 ppm of carbendazim in sea water and it increased to 365.90±1.53 minutes at 10 ppm.

The results presents here, strongly suggest that the mechanism of action of the fungicides probably

Table 1. Stage LC_{50} values of carbendazim for different embryonic stages of *H. elegans*: Sperms exposed for ten. minutes. Temperature 28±0.2^{0}C. Salinity 34±0.1‰, pH 8.1±0.1. Stage EC_{50} values are expressed in ppm. n = Number of experiments lower and upper renge of 95% confidence limits are given in parentheses.

Developmental stages	Carbendazim 10 Minutes
FM stage	9.0721 (6.225-12.5915)
2-cell stage	6.4990 (4.1349-9.7576)
3-cell stage	5.3334 (3.0780-8.8879)
4-cell stage	4.1974 (2.2786-6.8461)
8-cell stage	3.4782 (1.7064-5.7641)
16-cell stage	2.8756 (1.2014-4.9062)
32-cell stage	2.4268 (0.7130-4.5228)
64-cell stage	2.0694 (0.4286-3.9567)
Blastula stage	1.7167 (1.1031-2.3220)
Blastula start rotation stage	1.3162 (0.7594-1.8404)
Blastula stop rotation stage	1.3162 (0.7594-1.8404)
Release stage	0.9150 (0.4750-1.3305)

Table 2. Successful percentage of various embryonic stages of *H. elegans* in different concentrations of Carbendazim when the sperm were already exposed to the same concentration for ten minutes before fertilization. Temperature 28±0.2 ^{0}C, Salinity 34±0.1‰, pH 8.1±0.1; n = 6, ± = SD. ND= No development, number of eggs/embryos observed in each concentration = 100-150, n = Number of experiment.

Developmental stages	Concentration of Carbendazim in ppm								
	Control	1	2	5	10	15	20	30	40
FM stage	97.96±4.93	95.47±7.13	84.04±7.15	73.70±9.75	58.98±4.75	37.37±9.73	22.38±3.66	8.81±7.63	ND
2-cell stage	92.89±4.67	88.62±7.56	79.16±7.18	67.23±5.72	50.13±4.80	27.96±6.31	13.45±7.78	2.64±2.34	ND
3-cell stage	91.73±3.50	81.06±8.41	75.19±7.20	61.95±4.56	41.27±4.85	23.53±6.37	8.81±7.63	ND	ND
4-cell stage	87.83±3.75	76.64±8.45	70.47±8.57	56.67±6.62	33.91±2.35	18.21±8.66	5.81±7.63	ND	ND
8-cell stage	85.53±4.63	72.51±8.70	66.93±7.92	52.25±6.59	29.52±4.06	13.23±7.60	3.87±5.08	ND	ND
16-cell stage	80.55±3.70	68.15±8.78	60.42±9.84	48.42±7.57	26.86±3.81	10.89±6.52	2.64±2.34	ND	ND
32-cell stage	77.01±3.22	63.36±8.56	56.59±8.84	43.40±8.84	23.64±4.73	8.24±7.38	ND	ND	ND
64-cell stage	74.35±1.94	59.83±7.10	53.10±7.35	40.46±9.04	21.27±4.90	6.47±5.69	ND	ND	ND
Blastula stage	71.38±1.85	56.58±6.86	49.53±5.82	36.03±9.04	18.63±6.01	ND	ND	ND	ND
Blastula start rotation stage	66.38±1.01	51.53±8.37	45.70±4.82	31.90±9.48	15.68±6.06	ND	ND	ND	ND
Blastula stop rotation stage	66.38±1.01	51.53±8.37	45.70±4.82	31.90±9.48	15.68±6.06	ND	ND	ND	ND
Release stage	63.12±0.53	45.92±9.44	37.47±2.37	25.10±3.42	10.37±6.87	ND	ND	ND	ND

acts on sever as intracellular targets based on EC_{50} values (Table 1) of the present study. It indicate that 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 sea water when the sperm 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 of Carbendazim in

Table 3. Cumulative times of various embryonic stages of *Hydroides elegans* in different concentrations of Carbendazim when the sperm were already exposed to the same concentration for ten minutes before fertilization. Temperature 28±0.2 ^{0}C, Salinity 34 ±0.1‰, pH 8.1 ±0.1; n = 6, ± = SD. ND–No development, number of eggs/embryos observed in each concentration = 100-150, n = Number of experiments.

Developmental stages	Time in minutes Concentration of Carbendazim in ppm								
	Control	1	2	5	10	15	20	30	40
FM stage	5.40±0.51	7.30±0.48	9.20±0.42	11.30±0.23	13.50±0.70	15.30±0.69	17.30±0.84	23.30±1.24	ND
2-cell stage	30.80±0.67	34.60±0.86	38.60±1.12	42.60±0.96	45.70±1.12	49.90±1.32	54.50±1.97	65.50±1.73	ND
3-cell stage	41.10±1.15	47.00±0.00	53.20±1.95	58.90±1.57	64.10±1.36	70.40±1.22	77.90±1.12	ND	ND
4-cell stage	51.30±0.57	59.30±1.71	67.60±0.67	75.10±1.99	82.40±1.12	90.90±2.27	101.50±0.79	ND	ND
8-cell stage	62.70±1.73	72.60±1.24	82.80±2.06	92.30±2.23	101.60±1.53	112.40±2.34	125.50±0.79	ND	ND
16-cell stage	73.00±0.00	85.00±2.55	97.00±0.00	108.90±2.07	120.00±0.69	132.70±1.72	148.90±1.84	ND	ND
32-cell stage	84.20±2.31	98.30±2.86	112.30±1.96	126.00±0.00	138.40±3.34	152.90±0.44	172.70±2.66	ND	ND
64-cell stage	96.50±2.19	112.50±3.28	128.60±1.42	144.20±1.38	158.70±2.85	175.40±2.64	ND	ND	ND
Blastula stage	124.90±3.32	142.70 ±0.49	171.00±0.00	178.70±2.47	195.00±0.00	213.70±3.14	ND	ND	ND
Blastula start rotation stage	153.20±1.64	173.00±0.31	203.20±3.59	213.40±1.54	232.30±1.66	ND	ND	ND	ND
Blastula stop rotation stage	243.60±1.33	253.20±2.73	285.60±1.61	289.60±3.86	302.60±2.14	ND	ND	ND	ND
Release stage	299.50±0.70	310.80±1.57	344.00±2.76	350.90±2.61	365.90±1.53	ND	ND	ND	ND

sea water. The result indicated that the FM stage EC_{50} value was 9.0721 ppm which least sensitive stage of Carbendazim in sea water and highest sensitive stage value was 0.9150 ppm at larval release stage.

DISCUSSION

Polychaetes are the most widely used groups of marine macro invertebrates in toxicological testing and easy in collection in undoubtedly played an important role in their selection as test animals (Reish and Gerlinger 1997, Gopalakrishnan et al. 2008, Vijayaragavan 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 (Hutchings 1998). The results revealed that the stage EC_{50} value of Carbendazim 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 Carbendazim than the earlier stages, but actually it may be due to longer exposure of embryo to the fungicide in the sea water. 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 sea water. Successful fertilization was evidenced by the elevation of fertilization membrane. Successful fertilization was 97.96±4.93% successful in control sea water and it gradually decreased to 8.81 ± 7.63 at 30 ppm. There was no fertilization at 40 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 sea water. In the same way abnormal development of the various developmental stages increased when the concentration of Carbendazim increase in sea water 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 Carbendazim. 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 sea water in all the stages. It reveals that the rate of development decreases with increase in concentration of Carbendazim in sea water. Similar trend was observed by Thilagam et al. (2008), Vijayaragavan and Vivek Raja (2019).

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

A study revealed that animals exposed to Carbendazim in womb had serious deformities such as lack of eyes and hydrocephalus and it can disturb the development of sperm and damage testicular development in adult rats (Mantovana et al. 1998). 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 sea water. The hatching time (release time) of *H. elegans* was 299.50 ± 0.70 minutes and it gradually increases to 365.90 ± 1.53 minutes at 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 40 ppm and above.

CONCLUSION

The experimental data revealed that the toxicity of Carbendazim 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 Carbendazim in sea water. 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 sensitive and toxic to an early embryonic stages of *H. elegans* when the sperm were already exposed to the same concentration for ten minutes before fertilization and also the fungicide leads to environmental pollutions including marine environment.

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