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# The toxic effects of Zineb (fungicide) on fertilization and early embryonic stages of sedentary polychaete *Hydroides elegans* (Haswell, 1883)

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**Abstract**

The toxicity test has been performed to examine the effects of pesticide (fungicide) on fertilization and early development of marine Polychaete *Hydroides elegans (H. elegans*). Zineb is a dithiocarbamate agricultural fungicide used to control blights, rusts, and leaf spot. It is a polymeric zinc complex, commonly applied as a powder to foliage. While exhibiting low acute toxicity, it poses hazards to skin, eyes, and respiratory systems. Its primary toxicological risk involves thyroid impairment and potential reproductive toxicity. 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 Zineb 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 Zineb 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 EC50 values of the present study; It indicated that Zineb 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 Zineb (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.

**Key words :** Zineb, *Hydroides elegans* embryo, fertilization, blastula.

**Background**

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)[14,31]. 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[14,32]. 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[14,32].

The urbanization 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 etc. Therefore, it is essential that the bioassay techniques should be established to monitor the pollutants that pose a danger or hazard to humans and the biota[8,23,31].

Zineb is a polymeric complex of zinc with a dithiocarbamate (Fig. 1) a broad-spectrum fungicide belongs to alkylenebis (dithiocarbamate) which is very effective against diseases caused by Alternaria, Pestalotiopsis, Colletotrichum, Phytophthora etc., infecting many crops. It is used on variety of fruits, vegetables, cereals and pulses.it 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. Zineb is a dithiocarbamate agricultural fungicide used to control blights, rusts, and leaf spot. It is a polymeric zinc complex, commonly applied as a powder to foliage. While exhibiting low acute toxicity, it poses hazards to skin, eyes, and respiratory systems. Its primary toxicological risk involves thyroid impairment and potential reproductive toxicity. The fungicide has been shown to have significant negative effects on beneficial root fungi - totally preventing spore germination at levels far below recommended dosage levels[6,7].

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[8]. Most of the pesticides affect the embryo, teratogenic effects by directly or indirectly affecting cellular physiology[2]. Many cases of surface water contamination with pesticides were noticed and reported.[10]

The bioassays allow the detection of the effects by measuring the biologic response of marine organisms, particularly in their early life stages[12]. The test species must be sensitive enough to respond to low levels of contaminants and must be available for use from either laboratory cultures or from field collection throughout the year, accordingly, biologic tests are to be ecologically relevant and easily available of species for experimentation[8,22,31]. Although, toxicity tests conducted in the field are desirable and analyzing the developmental stages are easier to perform but only the laboratory conditions provide accurate results

which are highly useful.

The early developmental stages of marine invertebrates have repeatedly been found to be more sensitive to environmental pollutants than their adult counter parts[3,20]. Hence, they are subjected to the toxicity tests in most of the cases. A number of early life-stage toxicity testing protocols have been developed are effectively applied for the seawater toxicity using marine species of their early embryo for example, bioassays using embryos of bivalve species (*Mytilus edulis, Crossostrea virginice* and *C. gigas*) and gametes of echinoderm species. (S*trongylocentrotus purpuratus*, *S. tranciscanus* and *Arabica punctuata*) have been developed[1,4].

It's also observed that 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 hour for first cleavage, 24 hours for blastula and gastrula and 48 hours for trochophore larva. In contrast, *H. elegens* requires 2 to 3 minutes for fertilization, 30 minutes for first cleavage and approximately 12 hours for distinguishable trochophore larva[29-31]. Therefore, the advantages of developing bioassays using *H. elegans* embryos are more clear and accurate.

*H. elegans*[4], a sedentary, tubicolous serpulid polychaete is common in all temperate region and produces viable gametes throughout the year[9,18,24]. 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.[9,18,24] Therefore, the aim of the present study was to determine the toxic effects of Zineb on early embryonic stages of *H. elegans.*

*Collection of Organism :*

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

*Experiment Procedure :*

Tests were conducted in 100-ml glass beakers containing 50 ml of the filtered sea water. The sex of the polychaete was distinguishable by the orange colour of the female abdomen and creamy white of male abdomen. The eggs were visible to the naked eye. Release of gametes began almost immediately and was allowed to continue for

10 minutes, after which the animals were removed. Gamete release after removal from the calcareous tube is a stress response in polycheate[26]. Five 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*

About 200 eggs were introduced into each test chamber containing fresh sea water 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-cellstage, 4-cell stage, 8-cell stage, 16-cell stage, 32-cell stage, 64-cell stage, blastula stage, blastula rotation stage, larval release stage was observed. The experiment was repeated six times and the values were recorded (n = 6). To confirm the percentage of successful development, about 100 to 200 developing eggs at different stages were fixed in 10% neutral buffered formalin prepared in seawater and were counted on the same day. Abnormal cells were also noted at all concentrations and in each developmental stage. Nikon Photostat research microscope was used to record photomicrographs. The size of the cell at developmental stage was observed by using compound microscope. Percentage of successful development was calculated.

*Statistical Analysis :*

To test the effects of various concentration of Zineb 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,[25]).

*Pesticides solution :*

The Zineb (50% w/w), brand name: Zineb was obtained from Indofil Industries

Pvt.Ltd., Mumbai. 800 mg of Zineb was dissolved in 2000 ml of filtered seawater in a volumetric flask to prepare 200 ppm of Zineb in seawater. This stock solution was stored in an amber coloured bottle. From the stock solution the following concentrations of Zineb 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 Zineb in sea water was used for toxicity study by using embryo of *H. elegans*. Physicochemical conditions of the experimental media were maintained at 28±1°C temperature; 34±0.5 ppt salinty, 6±0.3mg1/L $O_2$ and pH 8.1±0.1.

*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 98.82±0.35 and it decreased gradually 78.22±4.47 at normal larval release stage (Fig. 4 and 5). The cumulative time of FM stage was 6.00±0.32 minutes and the times steadily increased 306.67±2.53 minutes at larval release stage. The larval release stage was occurred at 5 hour after the fertilization in the normal development without the Zineb in sea water.

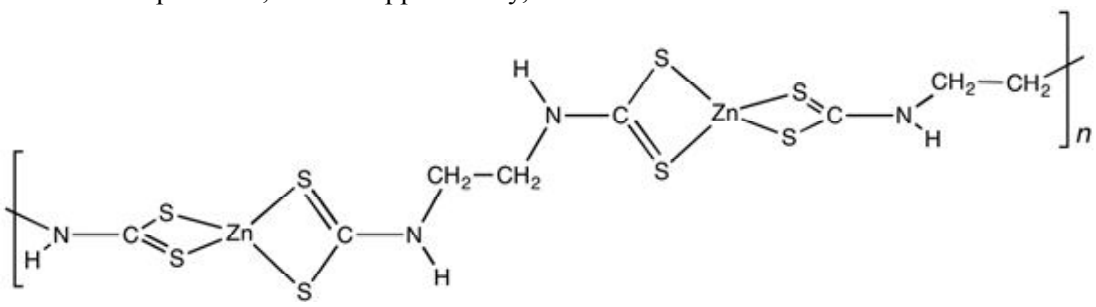


Fig.1. Molecular stucture of Zineb (Fungicide)

Fig. 2. *H. elegans* without tube

Fig. 3. FM Stage

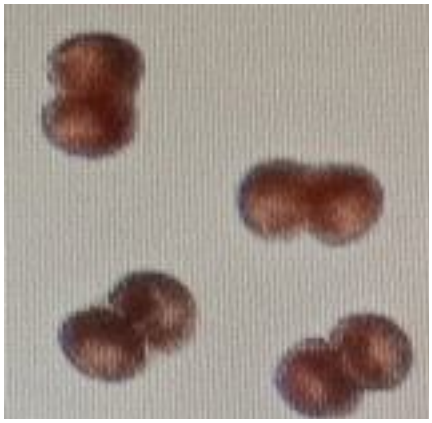

Fig. 4. Two cell stage

Fig. 5. Four cell stage

*Toxic Effect of Zineb on Early Developmental Stages of Hydroides elegans :*

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

The percentage of successful development of FM stage was 96.42±1.47 at 0.05 ppm of Zineb in sea water. The cumulative time of FM stage was 6.52±1.53 minutes at 0.05 ppm of Zineb and the percentage of successful development of 8 cell stage was 86.68±4.56 at 0.05 pm of Zineb and it steadily decreased to 3.45±3.56 at 40 ppm and beyond 40 ppm, Zineb in sea water, there was no development observed.

Table-1 Comparison of Stage $EC_{50}$ values of Zineb for different embryonic stages of *H. elegans*
(Temp. 28 ± 0.2°C, Salinity 34 ± 0.1°/00, pH 8.1 ± 0.1)
(Stage $EC_{50}$ values are expressed in ppm)

| Developmental Stages | $Ec_{50}$ values of Zineb | Developmental Stages | $Ec_{50}$ values of Zineb |
|---|---|---|---|
| FM-stage | 19.6245 | 32-cell stage | 7.6425 |
| 2-cell stage | 15.2362 | 64-cell stage | 6.1532 |
| 3-cell stage | 13.4251 | Blastula stage | 5.4265 |
| 4-cell stage | 11.3542 | Blastula startrotation stage | 4.5261 |
| 8-cell stage | 9.5231 | Blastula stoprotation stage | 4.5261 |
| 16-cell stage | 8.4212 | Release stage | 1.9972 |

2. *16 cell stage to 64 cell stage :*

The percentage of successful development of 16 cell stage was 84.13±1.15 at 0.05 ppm of Zineb in sea water. The cumulative time of 16 cell stage was 87.12±3.43 minutes at 0.05 ppm of Zineb and the percentage of successful development of 64 cell stage was 50.69±3.51 at 1 ppm and it was decreased to 12.66±2.52 at 20 ppm Zineb in sea water and beyond 20 ppm, there was no development of the 64 cell stage. The cumulative time of 64 cell stage was 176.87±1.27 minutes at 1 ppm and it was increased to 292.12±1.11 minutes at 20 ppm of Zineb in sea water.

3. *Blastula Stage to Release Stage :*

The percentage of successful development of Blastula stage was 75.57±4.22 at 0.05 ppm of Zineb in sea water and the cumulative time of Blastula stage was 145.20±4.27 minutes at 0.05 ppm of Zineb in seawater. The percentage of successful development of Blastula stage was 46.35±4.20 at 1 ppm and it was decreased to 9.12±2.25 at 20 ppm Zineb in sea water 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 67.58±2.25 at 0.05 ppm of Zineb and it was decreased to 10.55±4.15 at 10 ppm Zineb in sea water and beyond 10 ppm there was no Release Stage observed. The cumulative time of Release Stage was 312.13±1.10 minutes at 0.05 ppm of Zineb in sea water and it increased to 439.44±3.31 minutes at 10 ppm.

Polychaetes are the most widely used groups of marine macro invertebrates in toxicological testing and easy in collection is undoubtedly played an important role in their selection as test animals[8,21]. Polycheates are ecologically important marine organisms, making up from 30% to 80% of the total numbers of benthic fauna regardless of the ocean depth[13].

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 Zineb 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 Zineb in sea water (Table-2 and 3).

The effective concentration value ($EC_{50}$) referred to sensitivity towards the embryonic stages while exposed to different concentration of Zineb in sea water. The result indicated that the FM stage $EC_{50}$ value was 19.6245 ppm which least sensitive stage of Zineb in sea water and highest sensitive stage value was 1.9972 ppm at larval release stage.

The results revealed that the stage $EC_{50}$ value of Zineb decreased steadily from 19.6245 ppm in the FM- stage to 1.9972 ppm in the release stage. It is indicating that the release stage (hatching) is more sensitive to Zineb than the earlier stages, but 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

Table-2. Percentage of Successful Development of Various Embryonic Stages of Hydroides Elegans in Control Seawater and in Different Concentration of Zineb in Seawater

(Temp. 28±0.2°C, Salinity 34±0.1°/₀₀, pH 8.1±0.1); n=6 ±=SD

| Develop-mental Stages | Control | Percentage of successful development | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentration of Zineb in ppm | | | | | |
| | | 0.05 | 0.1 | 0.25 | 0.5 | 1 | 2 |
| FM stage | 98.82±0.35 | 96.42±1.47 | 89.54±1.13 | 85.42±4.56 | 81.12±6.32 | 76.52±8.32 | 72.20±1.52 |
| 2 – cell stage | 96.67±1.32 | 94.43±2.55 | 87.12±5.25 | 82.56±5.23 | 79.22±5.62 | 73.10±5.70 | 66.52±7.56 |
| 3 – cell stage | 95.42±1.53 | 93.20±3.55 | 86.32±1.17 | 81.12±4.10 | 76.26±2.15 | 66.23±2.34 | 62.45±6.12 |
| 4 – cell stage | 94.00±2.00 | 88.45±2.35 | 83.28±4.22 | 78.18±5.12 | 65.23±7.31 | 62.10±6.42 | 60.52±3.70 |
| 8 – cell stage | 91.25±2.58 | 86.68±4.56 | 81.13±5.18 | 77.22±4.54 | 64.53±6.35 | 59.61±4.37 | 58.45±6.52 |
| 16 – cell stage | 90.56±2.10 | 84.13±1.15 | 80.15±3.56 | 75.12±3.12 | 67.26±4.14 | 56.68±6.41 | 49.31±4.32 |
| 32 – cell stage | 88.23±3.55 | 83.45±1.18 | 76.45±2.87 | 66.14±4.44 | 64.42±5.57 | 53.68±7.82 | 48.42±4.57 |
| 64 – cell stage | 86.00±5.31 | 80.35±2.26 | 75.25±4.58 | 64.35±5.17 | 56.58±2.15 | 50.69±3.51 | 45.51±5.75 |
| Blastula stage | 84.65±1.25 | 75.57±4.22 | 71.36±3.23 | 62.43±4.23 | 52.45±4.62 | 46.35±4.20 | 39.43±6.30 |
| Blastula start Rotation stage | 82.33±2.56 | 73.45±1.18 | 68.44±4.31 | 61.22±6.12 | 51.66±7.71 | 44.87±7.25 | 36.78±4.52 |
| Blastula stop rotation stage | 82.33±2.56 | 73.45±1.18 | 68.44±4.31 | 61.22±3.43 | 51.52±6.50 | 44.87±2.24 | 36.78±4.52 |
| Release stage | 78.22±4.47 | 67.58±2.25 | 63.33±5.21 | 53.23±7.52 | 43.41±6.91 | 39.69±7.51 | 34.74±3.12 |

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

| 5 | 10 | 15 | 20 | 30 | 40 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 64.43±5.12 | 56.21±8.42 | 47.52±3.78 | 38.65±7.56 | 26.42±2.10 | 18.44±2.53 | 10.12±3.12 | N.D |
| 62.14±7.52 | 54.43±6.52 | 42.43±3.76 | 32.46±3.53 | 20.25±6.25 | 12.15±1.81 | 5.42±1.46 | N.D |
| 56.44±6.73 | 49.62±5.76 | 38.57±4.52 | 26.35±6.58 | 16.33±6.53 | 8.53±1.18 | N.D | N.D |
| 54.35±8.12 | 43.00±7.10 | 35.86±2.15 | 28.52±3.15 | 14.33±4.58 | 7.22±4.76 | N.D | N.D |
| 48.52±5.21 | 41.13±5.25 | 28.43±7.88 | 23.12±4.00 | 8.56±4.52 | 3.45±3.56 | N.D | N.D |
| 44.66±4.52 | 38.77±5.12 | 24.87±2.43 | 19.47±2.56 | 7.32±5.15 | N.D | N.D | N.D |
| 38.64±4.35 | 35.35±3.26 | 20.54±1.52 | 14.22±3.00 | N.D | N.D | N.D | N.D |
| 35.33±4.13 | 27.48±2.85 | 16.15±5.43 | 12.66±2.52 | N.D | N.D | N.D | N.D |
| 28.12±4.10 | 21.32±3.42 | 13.42±2.52 | 9.12±2.25 | N.D | N.D | N.D | N.D |
| 27.12±6.12 | 18.25±6.53 | 10.78±4.56 | N.D | N.D | N.D | N.D | N.D |
| 27.12±6.12 | 16.43±2.53 | 10.78±4.56 | N.D | N.D | N.D | N.D | N.D |
| 18.31±5.15 | 10.55±4.15 | N.D | N.D | N.D | N.D | N.D | N.D |

Table –3. Cumulative Times of Various Embryonic Stages of Hydroides Elegans in Control Seawater and in Different Concentrations of Zineb in Seawater

| Develop-mental Stages | Control | Times in minutes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentration of Zineb in ppm | | | | | |
| | | 0.05 | 0.1 | 0.25 | 0.5 | 1 | 2 |
| FM Stage | 6.00±0.32 | 6.52±1.53 | 12.21±1.12 | 13.23±1.15 | 16.10±1.76 | 17.17±2.45 | 18.21±2.42 |
| 2 Cell Stage | 33.32±4.52 | 36.41±2.76 | 36.75±2.75 | 44.25±2.46 | 49.40±2.35 | 57.32±1.46 | 59.56±3.15 |
| 3 Cell Stage | 43.52±2.26 | 48.43±2.78 | 54.43±4.12 | 60.72±5.90 | 67.20±3.86 | 73.45±1.49 | 78.72±1.51 |
| 4 Cell Stage | 53.42±4.52 | 62.25±4.52 | 66.52±5.27 | 76.10±7.58 | 89.40±6.38 | 92.41±6.91 | 100.12±2.84 |
| 8 Cell Stage | 63.82±3.65 | 73.13±2.55 | 83.51±6.38 | 92.64±7.58 | 100.15±9.45 | 110.33±3.79 | 122.56±4.97 |
| 16 Cell Stage | 76.62±2.47 | 87.12±3.43 | 99.55±6.70 | 109.30±7.31 | 119.80±1.75 | 133.54±3.34 | 147.75±8.04 |
| 32 Cell Stage | 85.41±3.55 | 102.55±3.57 | 118.51±5.72 | 129.20±8.15 | 139.25±1.57 | 155.47±1.87 | 166.31±7.35 |
| 64 Cell Stage | 106.26±2.38 | 117.11±2.26 | 129.25±7.31 | 144.35±5.46 | 158.10±0.13 | 176.87±1.27 | 189.44±6.25 |
| Blastula Stage | 128.43±2.37 | 145.20±4.27 | 162.12±6.32 | 179.52±7.52 | 194.76±5.50 | 210.62±7.25 | 129.62±1.02 |
| Blastula start Rotation stage | 156.75±3.56 | 176.21 ±1.55 | 197.25±8.35 | 210.25±8.56 | 230.35±7.36 | 248.11±4.81 | 269.33±5.71 |
| Blastula Stop Rotation stage | 248.55±7.45 | 260.52±2.15 | 276.14±4.92 | 286.43±1.01 | 300.25±8.36 | 312.51±12.33 | 329.63±6.25 |
| Release Stage | 306.67±2.53 | 312.13±1.10 | 339.25±8.76 | 351.16±7.14 | 369.46±6.43 | 382.15±4.58 | 400.25±6.75 |

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

| 5 | 10 | 15 | 20 | 30 | 40 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 19.50±3.12 | 25.21±2.86 | 26.10±6.66 | 30.40±7.45 | 38.50±8.01 | 39.70±5.76 | 48.60±5.38 | N.D |
| 62.53±3.12 | 69.54±5.37 | 74.02±7.48 | 80.43±6.37 | 89.30±7.26 | 100.75±7.56 | 108.50±6.70 | N.D |
| 85.30±4.15 | 93.62±4.86 | 101.15±7.91 | 116.50±4.65 | 127.35±3.36 | 139.13±8.75 | N.D | N.D |
| 108.20±1.75 | 120.11±3.81 | 127.30±5.38 | 150.12±1.27 | 169.52±2.30 | 179.42±7.43 | N.D | N.D |
| 136.12±6.84 | 146.57±1.13 | 166.30±5.86 | 188.61±6.18 | 208.55±3.06 | 225.12±5.13 | N.D | N.D |
| 159.45±5.37 | 176.63±7.69 | 19 2±6.01 | 220.75±8.90 | 252.13±5.75 | N.D | N.D | N.D |
| 183.55±4.12 | 200.13±5.69 | 220.55±5.31 | 258.57±3.27 | N.D | N.D | N.D | N.D |
| 208.13±1.64 | 227.43±5.66 | 251.710±6.71 | 292.12±1.11 | N.D | N.D | N.D | N.D |
| 250.11±7.81 | 270.55±5.91 | 300.53±8.61 | 345.13±4.75 | N.D | N.D | N.D | N.D |
| 290.53±3.32 | 316.56±3.12 | 347.13±7.70 | N.D | N.D | N.D | N.D | N.D |
| 343.21±5.71 | 360.58±2.56 | 389.55±6.52 | N.D | N.D | N.D | N.D | N.D |
| 418.13±7.45 | 439.44±3.31 | N.D | N.D | N.D | N.D | N.D | N.D |

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 Zineb on fertilization in *H. elegans* reveals that the success rate of fertilization decreases as the concentration of Zineb increases in seawater. Successful fertilization was evidenced by the elevation of fertilization membrane. Successful fertilization was 98:82±0.35% successful in control sea water and it gradually decreased to 10.12 ± 3.12 at 50 ppm. There was no fertilization at 50 ppm. Similar trend was reported in the same species on effect of Monocrtophos, D.D.T., Chlorfyrifos, Endosulfan[24]. Heavy metals,[9] Petroleum Oils[24,28] Phorate[30,31].

The percentage of successful development of *H. elegans* declined as the developmental stages progressed in any given concentration of Zineb in seawater. In the same way abnormal development of the various developmental stages increased when the concentration of Zineb increase in seawater. In higher concentration the development were arrested and up normal embryo observed due to the effect of Zineb. 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 Zineb increased in seawater in all the stages. It reveals that the rate of development decreases with increase in concentration of Zineb in seawater. Similar trend was observed by Thilagam *et al.,*[27] and Vijayaragavan[31].

The individual stage time of different development stages of *H. elegans*, increased except the blastula rotation stage. At the blastula rotation stage, Zineb affects the ciliary activity of the embryo. Hence, the rotation time decreases gradually when the concentration of Zineb 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 Zineb, the similar trend was observed for various heavy metals and pesticides[19,24,27,30,31]. It has been already reported that the ciliary activity is essential for successful hatching in sea urchin[17].

A study of Mantovani, *et al.,*[15], revealed that animals exposed to fungicide 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 fungicide in cultured human lymphocytes, concluded that it is obvious that the fungicide is a potent aneugen (affects the number of chromosomes), even at low exposures[16]. In *H. elegans* the reduction in the rotation time in the presence of Zineb 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 Zineb in sea water. The hatching time (release time) of *H. elelgans* was 306.67 ± 2.53 minutes and it gradually increases to

439.44 ± 3.31 minutes at 10 ppm of Zineb. The results may be inferred that the rate of production of hatching enzyme decreased in the presence of Zineb, as there was some delay in hatching up to 2 ppm of Zineb, and the production of enzyme was reduced below the critical level or completely arrested at 10 ppm and above.

The experimental data revealed that the toxicity of Zineb 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 Zineb in sea water. It observed that the toxicity particles have inducing the abnormalities in the early embryo developments of *H. elelgans*. Furthermore, the availability of *H. elelgans* throughout the years which is favorable and also suitable for laboratory toxicity tests. The data revealed that the Zineb was toxic to early embryonic stages of *H. elelgans* and also leads to environmental pollutions.

**Declarations:**

**Abbreviations repositions:**

Not applicable in this section

**Ethics approval and consent to publication**

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

**Consent for publication**

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

**Availability of Data and Materials**

Please contact author for the data on reasonable request.

**Competing Interests**

The author declares no competing Interests.

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

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