



**EFFECT OF THREE DIFFERENT SEAWEED LIQUID FERTILIZERS AND A
CHEMICAL LIQUID FERTILIZER ON THE GROWTH AND
HISTOPATHOLOGICAL PARAMETERS OF *EUDRILUS EUGENIAE*
(HAPLOTAXIDA: EUDRILIDAE).**

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ABSTRACT

The present study has a strong focus on the practical aspects of the effect of Seaweed liquid fertilizers (SLF) and Chemical liquid fertilizer (CLF) on the earthworms. The three different types of seaweeds such as *Caulerpa scalpelliformis* (Chlorophyta), *Cheilosporum spectabile* (Rhodophyta), *Sargassum wightii* (Phaeophyta) were taken. The earthworm *Eudrilus eugeniae* was reared in a cowdung based medium which was amended with a chemical liquid fertilizer and seaweed liquid fertilizer in two different concentrations such as 0.5% and 1% respectively. During the experiment, the growth rate of the earthworm, pH of the amended medium was analysed. The CLF sprayed earthworms showed decreased growth rate from 0th to 60th day but the SLF sprayed earthworms recorded increased growth rate. Among the three SLF, *Sargassum wightii* indicated highest growth rate 9.88 ± 0.104 in 1% concentration on the 60th day. The pH value in the CLF amended medium was 8 ± 0.1 in 0.5% concentration and 8.06 ± 0.15 in 1% concentration on 0th day and it went down to 6.4 ± 0.4 and 5.8 ± 0.2 respectively in 0.5% and 1% concentration on 60th day. In SLF amended medium, the pH value slowly moved to neutral level. The histopathological studies were carried out in the gut region of *Eudrilus eugeniae*. In 0.5% concentration of CLF, the epithelial linings were fused and a chloragogen tissue layer was observed to be granulated whereas in 1% concentration of CLF, extravillous growth in the epithelial layer, pyknotic nuclei were found in many epithelial cells and the size of the chloragogen tissue layer was reduced with the nerve cord affected severely. But in SLF incorporated earthworm, the nerve cord was seen clearly and the chloragogen tissue was well developed and it appeared similar to the control.

KEYWORDS: Seaweed liquid fertilizer, Chemical liquid fertilizer, *Eudrilus eugeniae*, Histopathology, Chloragogen tissue.

INTRODUCTION

Nowadays there is much usage of more number of chemical fertilizers to increase the productivity but it causes several damages to the ecology of the soil and their fertility. For health benefits, agricultural practices are being modified with an organic farming (Kramer *et al.*, 2006). The use of biofertilizers is to increase the plant growth & development and it is eco-friendly to the environment. The use of seaweed as manure in farming practice is very ancient and was prevalent among the Romans. Seaweeds have been used as a food for man and animals, organic fertilizers, industrial materials, production of biofuel etc. (Algo rythme, 2000). Seaweed liquid fertilizer (SLF) contains macronutrients, trace elements, organic substance like aminoacids and plant growth regulators (Verkleij, 1992). The use of seaweeds as biofertilizers in horticulture and agriculture has increased in the recent years (Dhargalkar and Pereira, 2005). At present, there is more number of chemical fertilizers in use to compensate the deficiency of nutrient in soil. Recent researches have proved that seaweed fertilizers are preferred not only due to their nitrogen, phosphorus and potash content but also because of the presence of trace elements and metabolites similar to the plant growth regulators. The cost of chemical fertilizers is

very high and sometimes it is not available in the market for which the farmers fail to apply the chemical/ inorganic fertilizers to the crop field in the optimum time. On the other hand, the organic manure is easily available to the farmers and is cost effective when compared to that of chemical fertilizers. The earthworms play a highly significant role in the soil ecosystem by participating in organic matter cycle and modifying the soil structure. They make nitrogen available for plant growth by feeding on organic matter in the soil. Earthworms play a vital role in a variety of soil and they contribute to the complex process of decomposition while affecting aeration, water transport and soil structure (Edwards and Lofty, 1969). The earthworms have the ability to enhance the soil fertility. The earthworms consume decaying matter in the soil. They let off casting which are good for plant growth. Earthworms have been studied as a readily available, easily maintainable and cheap test species for assessing chemical pollution in soil. In this present work, the earthworms are exposed to the seaweed liquid fertilizers (SLF) and chemical liquid fertilizers (CLF) amended medium. The physical parameters like the growth rate of the worms, pH of the medium and the effect in the earthworm tissues were determined by histopathological studies.

MATERIALS AND METHOD

Collection and processing of seaweeds

The three different seaweeds such as *Caulerpa scalpelliformis* (Chlorophyta), *Cheilosporum spectabile* (Rhodophyta), *Sargassum wightii* (Phaeophyta) were collected from the coastal area of Manapadu, Tuticorin district, TamilNadu. The seaweeds were hand sorted and cleaned with sea water to remove the impurities and epiphytes later it was brought to the laboratory and washed thoroughly using tap water to remove the salt on the surface of the sample. The seaweeds were spread on blotting paper to remove excess water and then shade dried for 6 days. The shade dried seaweed samples were stored in an air-tight containers covered with aluminium foil and stored at -20°C for further experimental studies.

Preparation of seaweed liquid fertilizer

To the 100gm of each powdered seaweed sample, 1 litre of water was added and the contents were boiled at 60°C for 45 minutes. The boiled liquid extract was allowed to cool at room temperature and the contents were filtered through the muslin cloth. Again it was filtered with Whatman filter paper No.41. Now the extract was a 100% seaweed liquid extract (Bhosle *et al.*, 1975). After completion of the filtration, the 100% seaweed liquid extract was diluted into two different concentrations (0.5% and 1%) with distilled water and stored at 4° C for further studies. The chemical liquid fertilizer was obtained from the local market nearer to Tirunelveli district, Tamil Nadu, India.

Experimental design

The African variety of earthworm, *Eudrilus eugeniae* was mass cultured in plastic troughs. The feed medium was prepared to a height of 0.8m with leaf litters and cow dung into which the adult clitellated worms were introduced. Water was sprinkled on alternate days to maintain the moisture content. They were maintained in triplicates. The three seaweed liquid fertilizers and chemical liquid fertilizer were taken in two concentrations (0.5% and 1%) which were diluted in 100ml of distilled water and they

were sprinkled in the experimental plastic troughs. A control tub was maintained during the study period and the experiment was carried out for 60 days.

Growth and physical parameters

The growth rate of the selected *Eudrilus eugeniae* was determined by removing the worms from the substrate and dried on the paper towels. They were then weighed fortnightly in water filled preweighed boats. The obtained growth rate was recorded. The pH of the bedding material was determined by using a digital pH meter. The pH of the compost sample was determined as per the procedure by (Chandrabose *et al.*, 1988). Fifteen grams of air dried sample was passed through a 2mm sieve and transferred to a clean 100ml beaker to which 30ml of distilled water was added. The contents were stirred intermittently and the suspension was again stirred just before taking the reading. The electrodes were immersed in a beaker containing sample and water suspension and the pH values were recorded at every 15 days interval.

Histological study

The gut region of *Eudrilus eugeniae* was cut and the tissue was taken from the two different concentrations of CLF and three different SLF amended medium at 30 days interval and subjected to histological analysis.

RESULTS AND DISCUSSION

The growth rate of the earthworms on exposure to CLF and SLF used for the present study are presented in Table 1 and Figure 3. Growth was measured in terms of mean mass per worm per gram. The three different SLF increased the growth rate of the earthworm when compared to CLF. In CLF amended medium, the growth rate was 6.376±0.55 and 7.699±0.473 in 0.5% and 1% concentration respectively on 0th day but it decreased to 3.728±0.727 in 0.5% concentration and 3.87±0.35 in 1% concentration. Among the three different SLF, *S. wightii* showed highest growth rate of earthworms. *i.e.* 9.88±0.104 in 1% concentration on 60th day.

TABLE 1: Influence of different concentrations of fertilizers on the growth of *Eudrilus eugeniae* over 60 days.

Days	Control	Chemical liquid fertilizer		Seaweed liquid fertilizer					
		0.5%	1%	<i>Caulerpa scalpelliformis</i>		<i>Cheilosporum spectabile</i>		<i>Sargassum wightii</i>	
				0.5%	1%	0.5%	1%	0.5%	1%
0	7.73±0.28	6.37±0.55	7.69±0.47	7.0±0.21	7.77±0.47	8.0±0.07	8.62±0.24	8.91±0.24	7.98±0.12
15	8.15±0.23	5.93±0.26	6.91±0.32	7.76±0.57	7.77±0.29	8.26±0.12	8.72±0.34	8.98±0.71	8.20±0.28
30	9.01±0.49	5.57±0.5	5.81±0.39	7.84±0.24	7.94±0.3	8.23±0.11	9.0±0.27	8.96±0.26	8.80±0.31
45	9.29±0.28	4.87±0.7	4.81±0.39	8.29±0.48	8.16±0.3	8.74±0.28	9.10±0.47	9.11±0.21	9.51±0.47
60	9.84±0.15	3.72±0.7	3.87±0.35	8.41±0.41	8.43±0.44	8.94±0.18	9.19±0.19	9.45±0.21	9.88±0.10

In the SLF, there was a gradual increase in the growth rate. (Hyman, 1940) observed that the earthworms continue to grow throughout their lives by continuous addition of segment proliferated from a growing zone just in front of the anus. According to Lofs-Holmin (1980), the sublethal effects of chemicals on the life cycle of earthworm are more readily demonstrated by the growth of juveniles and the development of sexual characteristics such as tuberculae and the clitellum. In *C. Scalpelliformis*, the

highest growth rate was 8.41±0.41 in 0.5% concentration on 60th day and 8.43±0.44 on 60th day in 1% concentration. In *C. spectabile*, the highest growth rate was 8.94±0.18 in 0.5% and it was 9.19±0.19 in 1% concentration on 60th day. Growth and reproduction of earthworm can be influenced in the laboratory by heavy metals (Malecki *et al.*, 1982) and these characteristics can also be affected under field condition.

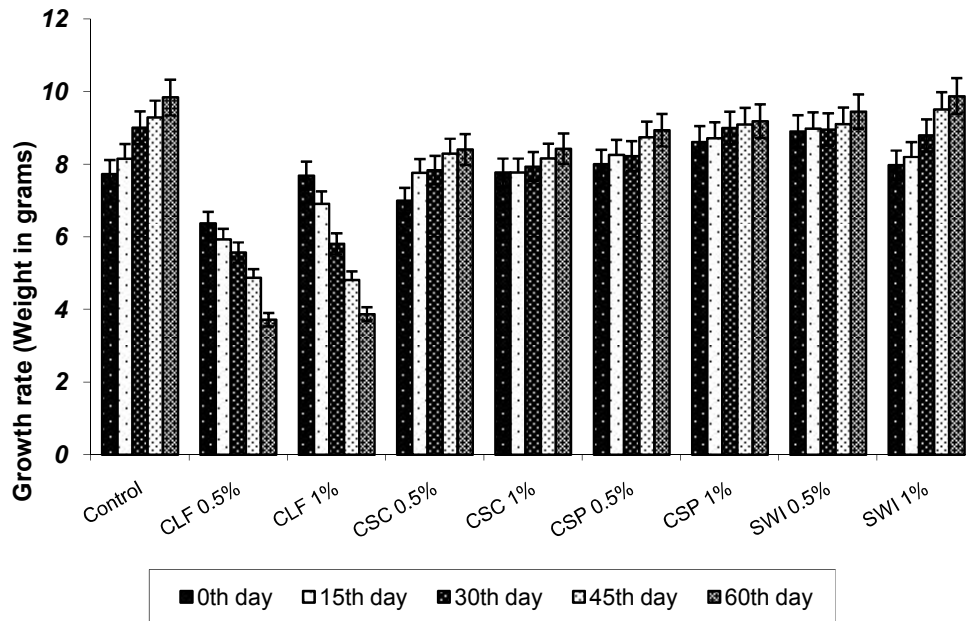


FIGURE 3: Growth rate of *Eudrilus eugeniae* over 60 days in SLF and CLF.

Changes in the earthworm densities noted in the past studies (Raw, 1962) were investigated further to determine the dose response mechanism or other important factors related to change in numbers. Ruppel and Laughlin (1977) found that the effects of herbicides of the individual level

have been assessed as qualitative decrease in numbers (Lebrun *et al.*, 1981 and De medts, 1981) gave little information relating to soil concentration of chemicals to sublethal effects such as growth, changes in egg fertility and changes in behavior.

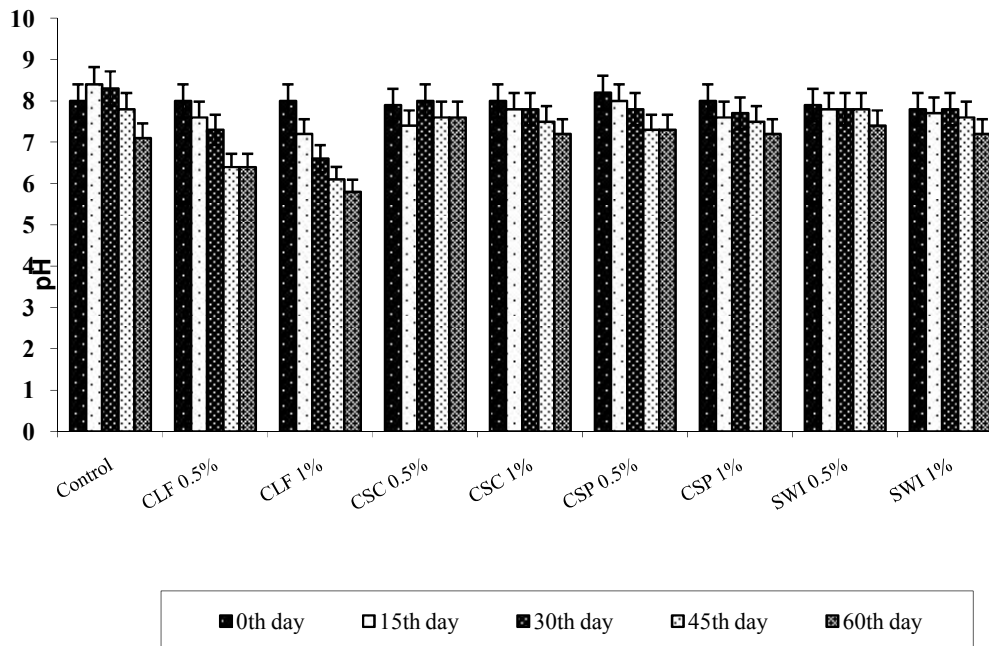


FIGURE 4: pH of the Chemical and Seaweed liquid fertilizer worked medium.

The pH values of the SLF and CLF amended medium are represented in Table 2 & Figure 4. pH of the control medium was 8 ± 0.152 on 0th day and it was decreased to

7.1 ± 0.23 on 60th day. In the CLF worked medium, the pH was 8 ± 0.1 and 8.06 ± 0.15 in 0.5% and 1% medium on 0th day which was found to decrease to 6.4 ± 0.4 and 5.8 ± 0.2 in

The growth and histopathological parameters of *Eudrilus eugeniae* on three different seaweed

0.5% and 1% concentration on 60th day respectively. Peramaki et al. (1992) have clearly demonstrated the importance of pH and soil calcium in the earthworm *Aporrectodea caliginosa* at lower pH value and confirmed the findings of Ma (1982 and 1987) who studied that heavy metal concentration in moles was closely related to the concentration in earthworms but not to the concentrations in soils. In the three different SLF amended medium, *C. scalpelliformis* have 7.9±1.08 in 0.5% concentration and 8±0.1 in 1% concentration on 0th day

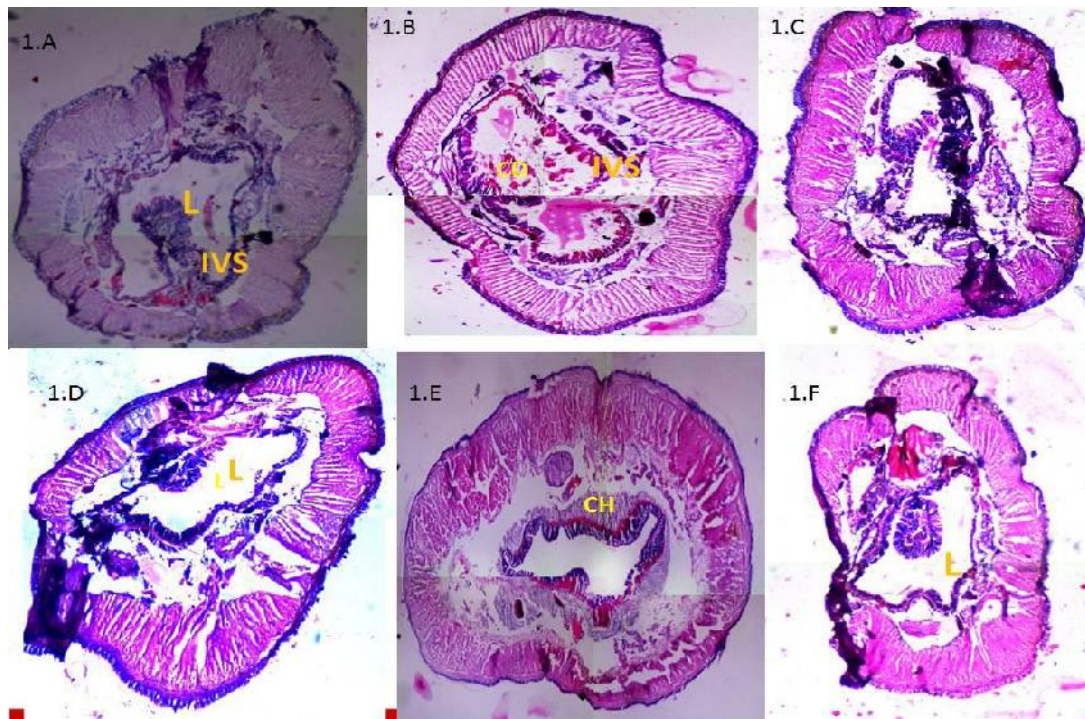
and it slowly decreased to 7.6±0.35 in 0.5% concentration and 7.2±0.11 in 1% concentration on 60th day. The pH of *C. spectabile* possessed 8.2±0.2 in 0.5% concentration and 8±0.2 in 1% concentration which slowed down to 7.3±0.1 and 7.2±0.2 in 0.5% and 1% concentrations respectively on 60th day. Whereas the pH of *S. wightii* was 7.9±0.15 and 7.86±0.11 in 0.5% and 1% concentration on 0th day which was found to decrease to 7.4±0.1 and 7.2±0.2 respectively in 0.5% and 1% concentrations.

TABLE 2: pH of the Chemical and Seaweed liquid fertilizer worked medium.

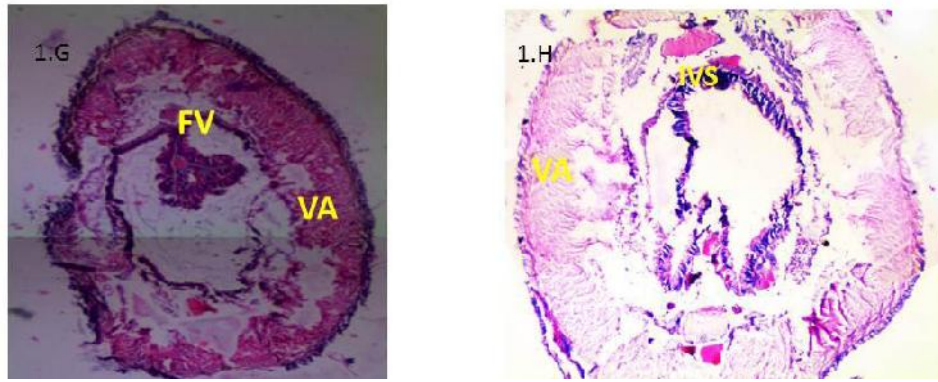
Days	Control	Chemical liquid fertilizer		Seaweed liquid fertilizer					
		0.5%	1%	<i>Caulerpa scalpelliformis</i>		<i>Cheilosporum spectabile</i>		<i>Sargassum wightii</i>	
0	8±0.15	8±0.1	8±0.15	7.9±1.08	8±0.1	8.2±0.2	8±0.2	7.9±0.15	7.8±0.11
15	8.4±0.43	7.6±0.23	7.2±0.05	7.4±0.2	7.8±0.2	8±0.2	7.6±0.2	7.8±0.11	7.7±0.15
30	8.3±0.43	7.3±0.3	6.6±0.32	8±0.2	7.8±0.2	7.8±0.1	7.7±0.1	7.8±0.1	7.8±0.15
45	7.8±0.2	6.4±0.25	6.1±0.15	7.6±0.35	7.5±0.32	7.3±0.3	7.5±0.1	7.8±0.05	7.6±0.36
60	7.1±0.23	6.4±0.4	5.8±0.2	7.6±0.35	7.2±0.1	7.3±0.1	7.2±0.2	7.4±0.1	7.2±0.2

The histological studies on the gut of *Eudrilus eugeniae* revealed that the inner epithelial layer followed by a circular and longitudinal muscle layer and the peritoneum which was modified clearly as a chloragogen layer. The histological observation is shown in Figure 1 and 2. After 30 days of exposure in CLF of 0.5% concentration, the epithelial linings lost their compactness and well defined

structure. The number of increased foldings with fused villi was observed and in 1% concentration of CLF, the chloragogen tissue was granulated and on exposure of SLF on 30 days, the epithelial cells were found to be normal with a prominent nucleus and a well defined chloragogen tissue layer.

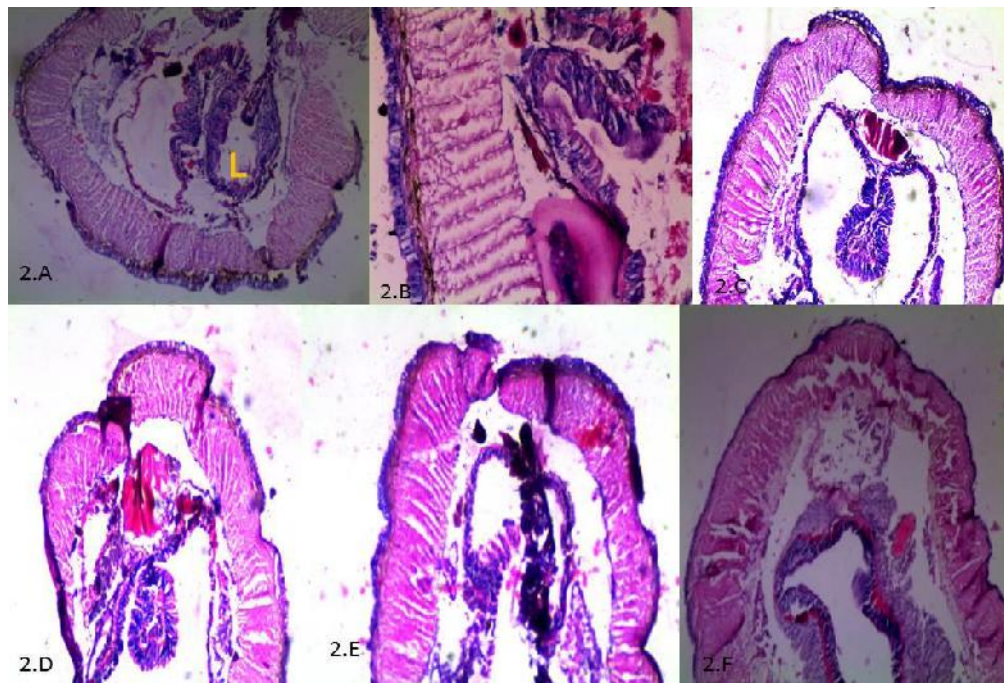


CLF treated worm on 30th day

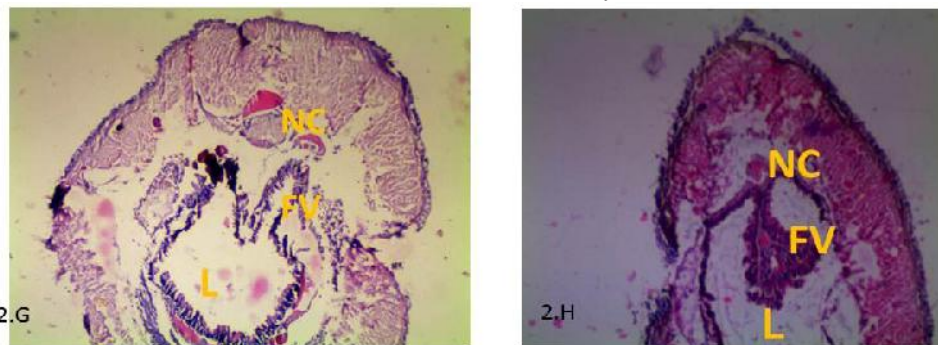


1A-0.5% *S. wightii*, 1B- 1% *S. wightii*, 1C- 0.5% *C. scalpelliformis*, 1D-1% *C. scalpelliformis*, 1E-0.5% *C. spectabile*, 1F- 1% *C. spectabile*, 1G- 0.5% CLF, 1H- 1% CLF.
 NC- Nerve cord, VA- Vacuole, FV- Fused villi, IVS- Inter villi space, L- Lumen, CH- Chloragogen tissue

FIGURE 1: SLF treated worm on 30th day



CLF treated worm on 60th day



2A-0.5% *S. wightii*, 2B- 1% *S. wightii*, 2C- 0.5% *C. scalpelliformis*, 2D-1% *C. scalpelliformis*, 2E-0.5% *C. spectabile*, 2F- 1% *C. spectabile*, 2G- 0.5% CLF, 2H- 1% CLF.
 NC- Nerve cord, L- Lumen, FV- Fused villi,

FIGURE 2: SLF treated worm on 60th day

In most group of animals, histopathological changes have been shown to cause tissue damage and it can be taken as a marker of toxicants exposure and in certain case provide precise information about the toxicant causing such damages (Hinton *et al.*, 1992). (Gupta and Sundaraman 1988) have found that carbaryl induced changes in chloragocytes of *Pheretima posthuma* where the nuclei became swollen and rounded because the effect of carbaryl toxication. Morgan *et al.* (1992) have recognized the use of earthworm as biological monitor to determine the accumulated concentrations of pollutant in their tissue. After 60 days of exposure in CLF, the earthworms in 0.5% concentrations indicated the epithelial linings were totally obliterated and fused and development of cavitation. The nuclei were swollen with weak staining when compared to the control and in 1% concentration of CLF, the histological specimens showed the presence of extra villous growth and pyknotic nuclei were found in many epithelial cell. The size of chloragogen tissue was reduced and the nerve cord was affected severely. In SLF worked medium, tissue specimens showed the clear evidence of nerve cord in *S. wightii* and *C. scalpelliformis*. Clear chloragogen tissue layer was observed with no vacuole formation in the SLF worked worm. In the CLF exposed earthworm, the pyknotic nuclei and the enlargement of epithelial layer was visualized. A similar observation was noticed by (Morowati, 2000) on exposure of *Pheretima elongata* to herbicide glyphosate and found that the inner epithelial layer underwent necrosis with disrupted cell membrane. The bioavailability of the chemical to the earthworm can be modified dramatically by soil physical or chemical characteristics (Lanno *et al.* 2004). Reinecke *et al.* (1999) exposed *Eisenia fetida* to a sub lethal concentration of cadmium sulphate for more than ten generations to determine the extent of possible tolerance for the heavy metals. Kratz and Poh hacker (1994) studied the development of soil toxicity test systems with Lumbricides to assess sub lethal and lethal effects. (Kokta 1992) reported that sublethal effects of pesticides Benomyl and Parathion on the body weight and number of juveniles of *E. fetida*. The effect of various pesticides and heavy metals on the earthworm brings down the risk of entry of these pollutants into plant system and into sequential food chain. When worms are used for this purpose, they should be prevented from entering into food chain as they are found to concentrate very high levels of these toxins in their tissue (Ireland, 1977). Thus the present study clearly stresses that the seaweed liquid fertilizers are a boon to the agriculture and they are ecofriendly with less cost effectiveness without affecting the soil and their ecosystem. Earthworms are farmer's friend so replacement of the chemical fertilizers by the seaweed liquid fertilizers is an adaptive way for today's world.

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