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Genotoxic effect of sea weed liquid fertilizer on *Allium cepa* L.**R.Anitha*, K.Mahalakshmi****Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women,****Ethiraj salai, Egmore, Chennai-600 008, (INDIA)****E-mail: anitha.rajasekaran023@gmail.com****Received: 25th March, 2013 ; Accepted: 12th June, 2013****ABSTRACT**

Seaweed Liquid fertilizers are beneficial when used in low concentration. The present study attempts to understand the genotoxic effect at higher concentrations. The studied low concentration of 10% V/V had no negative effect on the mitotic index, however abnormalities in the nucleus and chromosome was evident. Higher concentration induced abnormalities like multiple vacuolation, increase in mitotic index, condensation of cytoplasm, micronuclei formation and chromosomal aberrations. The study reveals that higher concentration reduces the mitotic index and therefore affect the growth and development of the crop plants.

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KEYWORDS

Sea weed liquid fertilizer;
 Mitotic index;
 Genotoxicity;
 Chromosomal aberrations;
Allium cepa.

Sea weeds are one of the most important marine resources of the world .In the past three decades, crude extracts from sea weeds have been shown to exhibit many bioactivities that include biostimulation, fertilizer and antimicrobial properties^[1,2]. Seaweed liquid fertilizer contains macronutrients, micronutrients, vitamins, aminoacids, appreciable quantities of plant growth regulators, like cytokinin, IAA and Gibberllins^[3]. They are reported to promote seed germination, vegetative growth and biochemical characteristics of the plants^[4-7]. The organic matter seemed to have an effect on the moisture holding capacity of the soil. The beneficial effects of the liquid fertilizer depends on the type of the seaweed that is utilized in the preparation and hence its effect on the plant as well. However despite these attributes, it has been reported to be beneficial to several plants only at lower concentrations. Positive effect of SLF on *Tagetes erectus* at lower concentration of 1.0 % was reported^[8]. Similarly a decline in the growth

of *Vigna radiata* at 2% concentration of Sargassum wightii SLF is also reported^[9-11]. SLF enhanced growth of earthworms at 1.5 % concentration^[12]. Higher concentrations of the seaweed liquid fertilizer has adverse effect on the growth and development of the plants. This study was carried out to understand the genotoxic effects of the liquid fertilizer at higher concentrations.

Young bulbs of *Allium cepa* L. were selected and germinated in sterile distilled water. After 48hrs when the root where about 1cm in length, the meristematic region of the roots were excised and fixed in Carnoyl's fixative. The root tips were teased out in to fine fragments and treated with 0.1% N HCL for 60 Seconds for separation of cells. The cells were stained with Acetocarmine, gently squashed and observed under the compound microscope and photographed. The mitotic index was calculated by the formula,

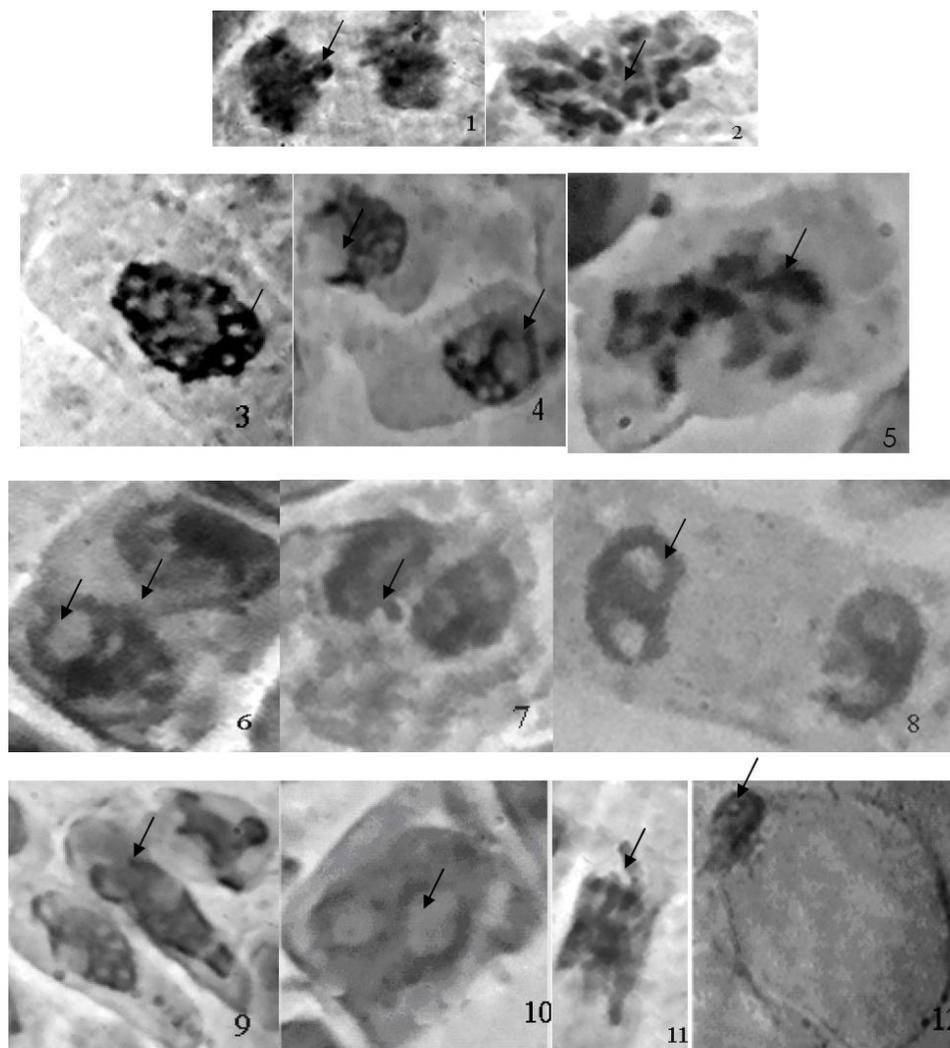
$$\text{Mitotic Index} = \frac{\text{Total Number of cells in division}}{\text{Total number of cells}} \times 100$$

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Commercially available Seaweed liquid fertilizer (SLF) available at Chennai, Tamilnadu, India was used to study the genotoxicity. Seaweed liquid fertilizer of different concentrations such as 10%, 15%, 20%, 25% and 30% were prepared with distilled water. The onion bulbs were treated with these concentration for 2 different time intervals (12 and 24 hrs). After the 12 hrs and 24 hrs of treatment the root tips were fixed in the freshly prepared fixative and the above procedure was repeated to study the Mitotic Index.

The mitotic index it was found to be 33% and the cells showed different stages of mitosis. Prophase and Anaphase were predominantly observed in normal cell division. After 12 hours treatment, 10% concentration did not enhance the mitotic index appreciably. However they showed predominant aberrations such as micronu-

clei formation and vacuolation. At 24 hours treatment a similar observation was made; however enormous increase in size of multivacuoles were evident. Earlier reports have also demonstrated in several plants^[13] that concentrations beyond 10% had adverse effects on the growth, pigmentation and biochemical constituents of the plants. At 15% concentration for 12 hours treatment, there was a reduction in the Mitotic index by 95% and formation of micronuclei and vacuolation was predominant. The decrease of mitotic index results from inhibition of DNA synthesis or metabolic activities which completely stop the cell from mitosis^[14]. The reason of inhibition of cell cycle is due to the damage of chromosome areas containing special proteins such as DNA polymerase enzyme. The lack of enzymes and proteins required for spindle apparatus to work properly can be the direct



1-Sticky anaphase with a lag, 2-Disoriented metaphase, 3-Multivacuolated nucleus, 4-Bivacuolated enlarged nucleus, 5-sticky metaphase, 6-vacuolated anaphase with a bridge, 7-Sticky anaphase with a lagging chromosome, 8-Vacuolated telophase, 9-Elongated and lobed nucleus, 10-Enlarged nucleus with vacuoles, 11-Chromosomal Lag in metaphase, 12-Condensation of nucleus.

Plate 1 : Effect of seaweed liquid fertilizer on mitosis of *Allium cepa* L. (100X)

reason. Chromosomal aberration was also observed in the metaphase. At metaphase, chromosomal lags were observed. Moreover the chromosomes were also not oriented in the equatorial plane. Anomalies such as stickiness of chromosomes in metaphase was distinctly seen. Chromosome stickiness may result from chromatin fibres sticking to each other or breaking due to inadequate condensation of these fibres, as a consequence of this movement of mitotic spindle fibres together with inner-chromosome exhibiting stickiness. It is claimed that stickiness in chromosomes is induced by chemicals regarded to be clastogenic agents^[15]. Stickiness in chromosomes is an indication of the high toxicity of the chemical substances and usually this may kill the cells with the irreversible damages. Multivacuoles and micronuclei were also observed. The size of the vacuole was proportional to the increase in the concentration.

In 24 hours treatment reduction in the mitotic index was similar to 12 hours treatment. Only the presence of vacuoles were observed with increase in duration of treatment. In general vacuolation is associated with immediate cell death due to autolysis of the cell. At 20% concentration in 12 hours treatment there was an increase in mitotic index by 23%. However although there was an increase in mitotic index, the cell showed large number of abnormalities. Binucleate cells with vacuolation was a predominate abnormality observed in the treatment. Also formation of micronuclei and chromosomal condensation, stickiness along with thickening of chromosomes in metaphase was observed^[16]. High level of mitotic index, micronuclei are formed as a result of lagging chromosomes or acentric breakages^[15]. Stickiness in chromosomes is an indication of the high toxicity of the chemical substances and usually this may kill the cells with the irreversible damages^[16] as a result it may lead to autolysis of the cell. Chromosome fragmentations, breakages and inhibition of spindle fibres result from various chemicals preventing some proteins essential for spindle apparatus. Condensation of the nuclei and micronuclei were also observed at 30%. Larger vacuolation were clearly observed. Strong vacuolization of nuclei demonstrated initiation of an irreversible process of cell death. At higher concentrations the cells were enlarged. It was also reported that SLF at concentration greater than 25% inhibited the growth and yield of *Abelmoschus esculantus*^[17] which probably is due to the above said reasons.

From the study, it is evident that although the commercial seaweed liquid fertilizer induced an increase in the mitotic index at 10% concentration, it had an adverse effect on chromosomes of *Allium cepa* and is definitely genotoxic at higher concentrations. However, it may not be the result with all the seaweed liquid fertilizers. The seaweed used in the preparation of liquid fertilizer certainly has a major role to play in the genotoxicity. Hence it is suggested that genotoxicity test needs to be carried out before being introduced into the field for crop improvement

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