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EFFECT OF EDTA ON SEED GERMINATION AND SEEDLING GROWTH OF *HORDEUM VULGARE L.*

Preeti Singh
Department of Botany
S. S. Khanna Girls' P.G. College,
Prayagraj (U.P.), India

Abstract: The present work was carried out to study the effect of EDTA on the seed germination and seedling growth of *Hordeum vulgare*. Seeds of barley were treated with different concentrations of EDTA (40, 80,120 and 160 mmol/l). The seed germination was recorded maximum for control, while the germination percentage decreased with the increasing concentrations of EDTA. Delayed seed germination and cytotoxic effects on cell divisions were also observed in the treated sets. The study showed that the survival and growth of the seedlings were negatively affected by the elevated EDTA concentration.

Key Words: *Hordeum vulgare*, EDTA, Mitotic index, Coleoptile.

Introduction:

There is an increasing concern about the direct or indirect potential effects of the presence of EDTA in the environment. The EDTA (Ethylenediaminetetraacetic acid) is an amino polycarboxylic acid with the formula $(CH_2N(CH_2CO_2H)_2)_2$. It is a water-soluble, white coloured solid which is generally used to bind to calcium and iron ions. The EDTA binds these ions as a hexadentate chelating agent. It is produced as several salts, especially sodium calcium EDTA ,disodium EDTA and tetrasodium EDTA. The main application of EDTA is in cleaning products and detergents based on perborates as stabilizers and in some countries, as an alternative to phosphates in detergent formulation. Presence of EDTA in soils may be due to agrochemical application or due to the disposal of products containing EDTA in garbage reservoirs. Dufkova V., (1984) studied the effect of EDTA on photosynthetic organisms and found inhibitory effects of EDTA on cellular division, chlorophyll synthesis and algal biomass production. Grcman, H., et al., (2001) reported inhibitory effects of EDTA over plants such as: absence of development of arbuscular mycorrhizae in red clover plants, necrotic lesions on leaves of Chinese cabbage and stress on soil microfauna.

Hordeum vulgare (Barley) is a member of the grass family (Poaceae). It is a self-pollinating plant with 14 chromosomes. Barley is a cereal grain that people can use in bread, beverages, stews and other dishes. *Hordeum* provides fiber, vitamins and minerals. It is used for lowering blood sugar, blood pressure and cholesterol and for promoting weight loss. Barley is also used for digestive complaints including stomach pain , diarrhoea and inflammatory bowel conditions.

The present study is an attempt to reveal the effect of EDTA on seed germination and seedling growth of *Hordeum vulgare* (Barley) plant.

Materials and Methods:

Hordeum vulgare cv. (K 287) seeds were used for experiments. Seeds of test plants were selected on the basis of uniformity in shape, size, colour & weight. Seeds were surface sterilized with 0.1% $HgCl_2$ solution and thoroughly washed with distilled water. The different concentrations of EDTA (40, 80, 120 and 160 mmol/l) were prepared. For seed germination, sterilized seeds of barley were soaked in different concentrations of EDTA solution for their whole imbibition period. Seeds soaked simultaneously in distilled water constituted the control set. Thereafter, seeds were washed thoroughly with distilled water and transferred to petridishes lined with moist filter paper and kept in dark for germination. The experiment was performed in triplicate. Percentage of seed germination in selected concentration was recorded after observing radicle emergence.

The percentage of germination was calculated from the number of seeds showing radicle emergence out of total number of seeds kept in petridishes. The growth parameters like root, leaf and coleoptile length were observed on 12^{th} day after radicle emergence. The data observed in the experiment, were statistically analyzed for the calculation of standard error. As a cytogenetic test, the MI (Mitotic Index) is extensively used both in vivo and in vitro to examine the genotoxic effects in a short time and to evaluate mutagenic effects of agents in different environments. The MI assay is used to characterize proliferating cells and to identify compounds that inhibit or induce mitotic progress. For mitotic studies root tips of germinating seeds were fixed in acetic alcohol (1:3) and squashed in 2% of acetocarmine. Number of normal and abnormal cells and types of chromosomal abnormalities were noted in all concentrations. A control set in identical condition was also managed.

Result and Discussion:

In the present study, the effect of EDTA on germination rates, lengths of root, leaf and coleoptile and Mitotic Index of barley (*Hordeum vulgare L.*) were investigated. The results obtained in the experiments are shown in table-1 & 2 and fig.–1,2,3,4 & 5. In general, EDTA treatments decreased the germination rates. The primary effect of EDTA on seed germination also showed a tendency of delay in germination in contrast to control. Increase of concentrations gradually lowered the percentage of seed germination from 81% (at lowest concentration) to 20% (at highest concentration).

Table-1: Effect of EDTA on Seed Germination and Seedling Growth of *H. vulgare* cv.

Concentration of EDTA (mmol/l)	% of Seed germination	Hours taken into germination	Length of Root after 12days (cm.)	Length of Leaf after 12 days (cm.)	Length of Coleoptile after 12 days (cm.)
0 (Control)	100	46-55	17.5	13.2	5.1
40	81	48-60	12.7	10.1	3.4
80	62	50-70	10.1	7.8	2.9
120	39	62-84	6.2	4.1	1.8
160	20	70-100	4.5	2.5	1.2

The average of three triplicates ± S.E.

Table -2: Mitotic index of seeds exposed to different concentrations of EDTA.

EDTA (mmol/l)	MI (%)
0 (Control)	15.5
40	9.4
80	5.5
120	4.4
160	2.5

(MIs were determined by counting among the 250 cells for each treatment).

Fig.-1: Effect of EDTA on Seed germination of *H. Vulgare* cv.

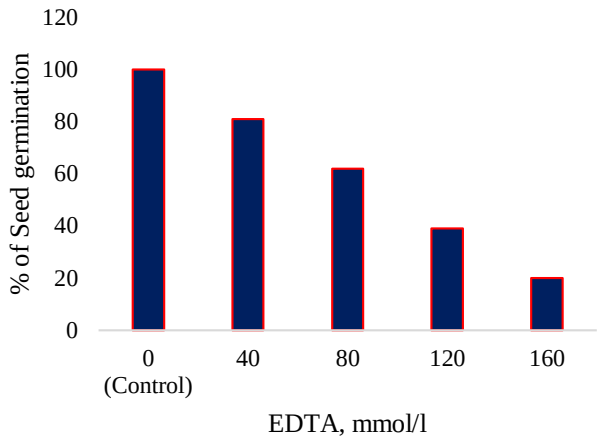


Fig.-2: Effect of EDTA on the length of Root of *H.vulgare* cv.

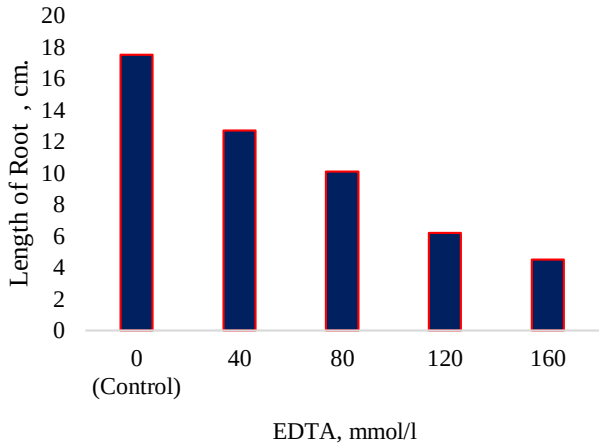


Fig.-3: Effect of EDTA on the length of Leaf of *H. vulgare* cv.

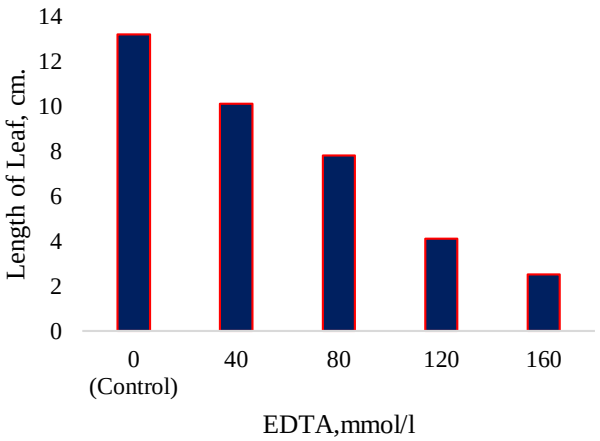


Fig.-4 : Effect of EDTA on the length of Coleoptile of *H.vulgare* cv.

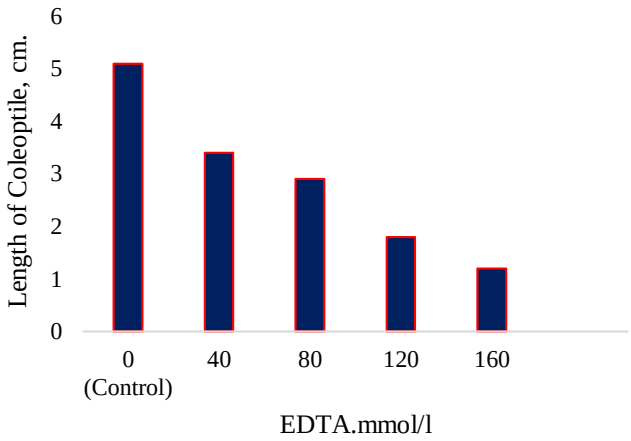
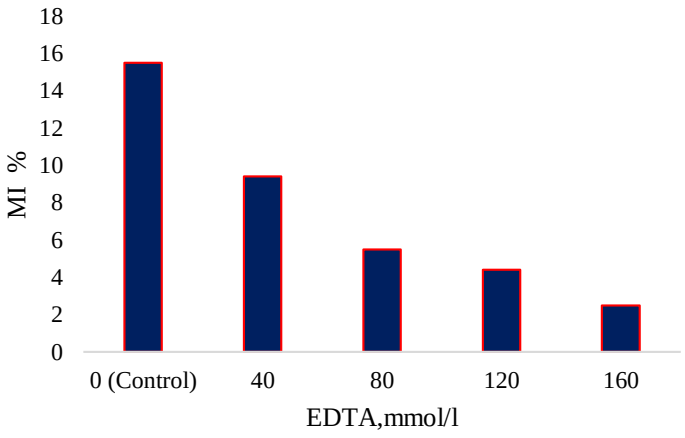


Fig. -5: Effect of EDTA on Mitotic Index of *H.vulgare* cv.



The increasing concentrations of EDTA affected the seedling growth. In particular, the root length decreased with the increasing concentrations of EDTA. The root length at different concentrations of EDTA such as 40 mmol/l, 80 mmol/l, 120 mmol/l and 160 mmol/l were 12.7 cm, 10.1 cm, 6.2 cm and 4.5 cm respectively. EDTA affected leaf length also in all treatments. The leaf length was 10.1 cm, 7.8 cm, 4.1 cm and 2.5 cm for 40 mmol/l, 80 mmol/l, 120 mmol/l and 160 mmol/l respectively. Similarly, EDTA also affected the coleoptile length. The lowest coleoptile length observed was 1.2 cm with 160 mmol/l EDTA treatment. Similar Inhibitory effect of EDTA on seed germination and growth of plants, especially at higher concentrations was reported by Ilbas A. I., et.al. (2006), Greger M. et.al. (1986, 1987). Decrease in percentage

of seed germination and delayed emergence of seedling with increasing concentrations may be attributed to the presence of certain inhibitory or toxic substances which might disturb the metabolic activity (Singh D., et.al., (2017), hence delayed emergence of seedlings. Decline in percentage of seed germination with increasing concentration clearly indicates the presence of more toxic substances in higher concentrations than that of lower concentration. These toxic substances damage some of enzyme system involved in the metabolism and repair mechanism, thus directly affects the cell division and cell elongation, hence retardation in the growth of seedlings. Present observations on seedling growth are comparable with the earlier findings reported by Dubey R.C., et. al. (1987) and Prasad, P.R., et.al, (1981).

In the present study, the increasing concentrations of EDTA apparently decreased MI (Mitotic Index). MIs of the roots of *Hordeum* were 2.5%-9.4 % in the treated sets (Table- 2). The highest MI was 15.5% in the control group and the lowest was 2.5% in the 160 mmol/l concentration. When EDTA concentrations were increased, the numbers of mitotically dividing cells significantly decreased. Chance in the proportion of nuclei undergoing division is partial index of the degree and kind of effect of the given treatment. Increase of prophase at lower concentration and significant decrease in metaphase and anaphase, inhibition of anaphase at middle concentrations and complete inhibition of cell division in higher concentration indicate that lower concentration prevents cell cycle at the end of prophase, while middle concentration breaks the spindle fibers and higher concentration prevent the entry of cell into mitosis. This finding clearly indicates that the lower concentrated solution has less amount of toxic substances while middle concentrated solution has more toxic substances. At higher concentration these toxic substances almost completely check the mitotic division. The inhibitory effects of EDTA concentrations on the mitotic index indicate that EDTA can have genotoxic and mutagenic effects on barley seedlings. Fishbein L., et al., (1970) reported that EDTA decreased mitotic division via chromosome aberrations in the bean and barley. They also reported the occurrence of mitotic abnormality in onion root cells. Redei, G.P., (1969) reported that 20 and 50 mmol/l dosages of EDTA for 12 h. at 25 °C increased mutation frequency in *Arabidopsis thaliana*. Similar effects have also been reported by Ananthaswamy, et.al.,1971, Soyfer N.V., (1983), Yuan H.Y., et.al. (1993) and Srivastava S., et.al. (2011).

The present investigation clearly indicates that EDTA has enough chance to induce genetic disturbances. Hence, it needs further research in order to have a better understanding of the mechanism of the mutagenic effect of EDTA.

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