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Research Journal of Agricultural Sciences
Volume 14; Issue 05 (Sep-Oct 2023); pp 1536–1540

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Cytotoxic Leaf Aqueous Extract (LAE) Brought Ultrastructural Alterations by Dominating Mitotic Activity in Root Tip Cells of *Allium cepa* L.

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Abstract

Following experiment conducted by giving treatment of *Amaranthus viridis* L. Leaf Aqueous Extract (LAE) treatment of various concentrations i.e., 0.5%, 1%, 2% and 4% to the root tip of *Allium cepa* L. for 4 days to examine mitotic anomalies in it and mitotic index (MI) and deviations from normal chromosomal stages towards abnormal stages. After 4 days, mitotic index of root tip cells of *Allium* was assessed by means of electronic microscope. Microscopy revealed that mitotic stages deviated from normal mitoticity to abnormality after phytotoxic treatment of *Amaranthus*. This proves that *Amaranthus* possesses allelochemicals which are responsible for manifesting abnormal mitotic activity of *Allium cepa*. Also, the suppressing potentiality of this weed could be used to used prepare natural weedicide which could be beneficial in sustainable agriculture.

***Key words*:** *Allium cepa* L., *Amaranthus viridis*, Leaf aqueous extract, Mitotic activity, Root tip cells

Amaranthus viridis, Leaf Aqueous Extract (LAE), Mitotic activity, Root tip cells, Phytotoxicity, allelochemicals. Usually known as Chowlai, *Amaranthus viridis* Linn is a yearly herbaceous grasses having an upstanding, greenish stalk which extends around 60–80 cm in height, a cosmopolitan species. Several offshoots come out pedestally and the leaves are ovoid, 3–6 cm in length, 2–4 cm wider with prolong leaflets as for 5 cm. The plant has small green flowers with 3 stamens and terminal panicles with few branches [1].

Plants possess some phytochemicals which help them to establish by out competing other plant present in their nearby surroundings and *Amaranthus viridis* is such a perennial weed and part of family, Amaranthaceae [2]. It is a poisonous weed [3-6], contains a wide variety of bioactive compounds [7], which are plant secondary metabolites contain terpenoids, Saponins, Tannins, and alkaloids forming allelochemicals [8]. The current developed nations are focusing on its bioactive chemicals for various plant-based remedies such as, use of herbal remedies is prevalent for people's fundamental healthcare requirements, there is an increasing interest among the scientific community in studying chemicals with plant origins [9-10]. Since, the weed, *Amaranthus viridis* possesses phytochemicals which are naturally capable of suppressing, deteriorating the other plant growth and germination, this ability of suppression can be exploited in preparing natural suppressor called weedicide. Weedicides play vital roles in agroecosystems by controlling weeds and protecting yield production [11].

Fig 1 Representing the flowering stages of *Amaranthus viridis* L., a cosmopolitan weed

Received: 25 Aug 2023; Revised accepted: 05 Oct 2023; Published online: 10 Oct 2023

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Citation: Parveen U, Khan M, Shadab M, Quratul-Ain, Siddiqui MB. 2023. Cytotoxic Leaf Aqueous Extract (LAE) brought ultrastructural alterations by dominating mitotic activity in root tip cells of *Allium cepa* L. *Res. Jr. Agril. Sci.* 14(5): 1536-1540.

MATERIALS AND METHODS

Collection of materials

Fresh plant of *A. viridis* was collected from the campus of Aligarh Muslim University and adjoining areas of Aligarh in early winters, 2021. The different parts of the following plant were detached, washed under tap water and then shade dried to get fine powder [12] for phytotoxicity assessment to give treatment of different concentrations (25%, 50%, 100% and 200%) to get to root tip cells of onion [13-14].

Root squash technique for chromosomal anomaly and phytotoxicity monitoring of A. cepa L.

To study the actively dividing cells (mitotic cells), it is really important to select a suitable crop which has large number and size of diving cells, also stains efficiently too and Onion is such a test crop with a chromosomal number of 16 [15-16]. To perform the cytotoxicity experiment further, healthy and small-sized onion bulbs were brought to the laboratory of Environment, Aligarh Muslim University, Aligarh. Onion bulbs were subjected to leaf aqueous extract (LAE) to study mitotic index and variations in various stages of the dividing cells utilizing the squash technique [12]. Onion bulbs were divided into two sets, one for control and another for treatments. *Allium* from the control set were grown in double distilled water (DDW) for 4 days until the emergence of the roots. On the day 5, the emerged-out roots from the germinated onions were treated with different concentrations 0.5, 1, 2, and 4% of the leaf aqueous extract (LAE) for a period of 24 hours while DDW to be used as control. On the day 6, when it was found that the treatment to be given to the onion bulbs was over, the root tips from the dividing region were taken out and need to be fixed in glacial acetic acid-ethyl alcohol (1:3, v/v) for another 24 hours. After the fixation of the root tips, they were washed for thrice with DDW and followed by hydrolysis with 1 N hydrochloric acid (HCl) for 1 minute at room temperature, further followed by staining with Schiff's reagent for 30 minutes and lastly followed by maceration of the two root tips on a glass slide in one drop of 40% glacial-acetic acid on a slide. The cover-slipped slides were sealed with pure nail polish. Per treatment, five replicates were maintained and the experiment was to be repeated. A bright field microscope (Olympus, model CH20i, New Delhi, India) was used to observe the various mitotic stages. MI and the chromosomal aberrations were calculated using the following formulae [17].

$$\text{Mitotic Index (MI)} = \frac{Total\ no.of\ dividing\ cells}{Total\ no.of\ cells\ counted} \times 100$$

$$\text{Mitotic abnormality} = \frac{Total\ no.of\ abnormal\ cells}{Total\ no.of\ cells\ scored} \times 100$$

Statistical Analysis

The execution of all the demonstrations had to be done in a designed haphazardly in all respects. The SPSS/PC software ver. 16 (SPSS Inc., IL) helped us to secure the facts and figures for seedling growth (root length/ shoot length and dry biomass). (Fig 3) revealing the graph signifies the standard deviation of measured readings. There are separated means of treated plants and the control at $p<0.05$ while were making differences applying DMRT [18] and ANOVA.

RSULTS AND DISCUSSION

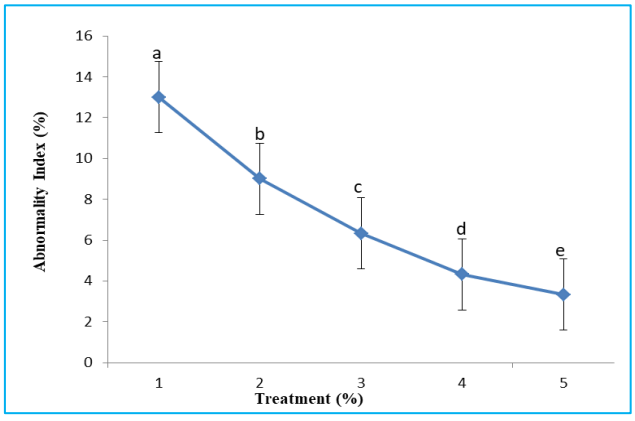
Effect of different treatments on mitotic stages and mitotic index

Allium cepa roots were treated with the different concentrations of LAE and showed abnormal mitotic stages. It was observed that normal mitotic stages were seen at control but the abnormalities of different stages of mitosis at different concentrations of the treatment and MI were concentration dependent. On increasing the concentration of LAE, there is an increase in the abnormalities of the MI of root tip cells. Maximum deviation from the normal mitotic stages to the abnormal stages is seen at 4% while the least abnormalities at 0.5% while completely normal observed in the control conditions.

Table 1 Effect on Mitotic Index (MI) of *Allium cepa* L. root tip cells at control and treated with different concentrations of LAE

Treatments	Prophase cells	Metaphase cells	Anaphase cells	Telophase cells	Total dividing cells	Total cells counted	Mitotic index (MI)
Control	108±0.94^{a}	142.66±1.18^{a}	115.33±1.02^{a}	177.00±1.08^{a}	549.77±0.59^{a}	2617±70.54^{a}	20.95±0.49^{a}
0.50%	105±2.35^{b}	121.00±1.69^{b}	107±1.02^{b}	167.66±0.25^{b}	498.87±2.09^{b}	2531.67±75.29^{b}	19.78±0.53^{b}
1.0%	82.33±1.18^{c}	82.33±1.90^{c}	95.00±1.22^{c}	149.33±0.47^{c}	406.1±0.51^{c}	2464.66±58.18^{c}	16.53±0.37^{c}
2.0%	76.00±0.81^{c}	74.33±0.98^{d}	72.33±1.02^{d}	130.00±2.04^{d}	351.97±1.02^{d}	2404±48.57^{d}	14.66±0.29^{d}
4.0%	26.33±0.72^{d}	46.00±0.98^{e}	63.33±0.62^{e}	85.00±0.81^{e}	222.98±0.44^{e}	2325.67±44.78^{e}	9.56±0.16^{e}

*Different alphabets represent significant difference between varying concentrations applying DMRT



*Different symbols along a curve represent significant difference between varying concentrations applying DMRT

Fig 2 Mitotic Index of *Allium cepa* L. root tip cells at control and treated with different concentrations of LAE

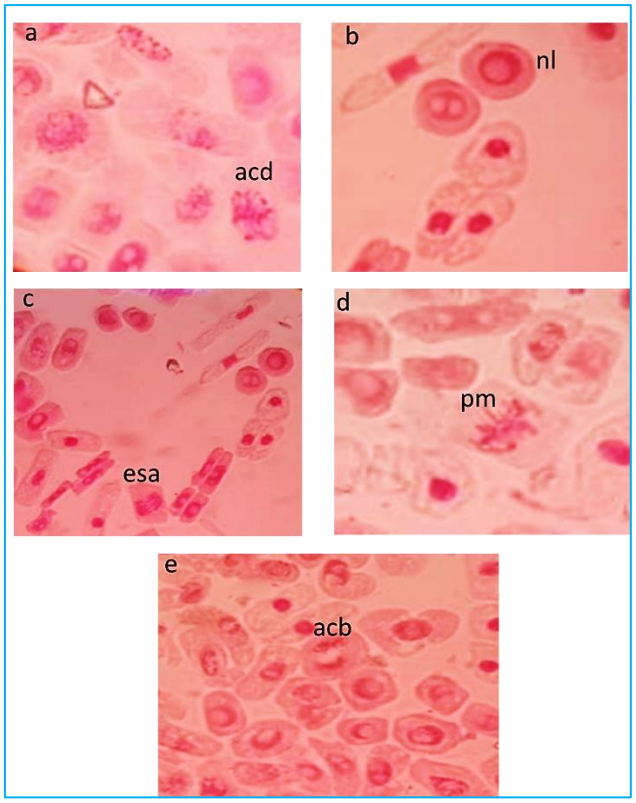
The mitotic abnormalities revealed by the treated samples show a positive correlation with the LAE concentration. The MI showed a decrease in all the samples upon exposure to extract significantly. The MI for the control was recorded to be 20.95, whereas the highest and lowest value of MI for the treated samples, was reported 19.78 and 9.56 at 0.5 and 4% respectively (Table 1, Fig 1). The result shows a progressive reduction in the number of dividing cells in the samples treated with LAE in contrast to the control.

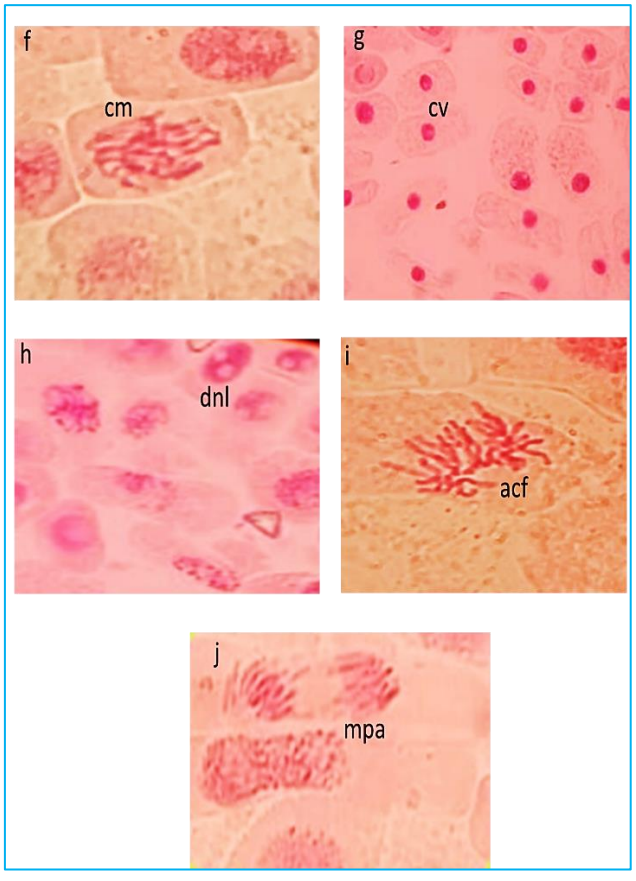
Effect of treatments on chromosomal abnormal percentage

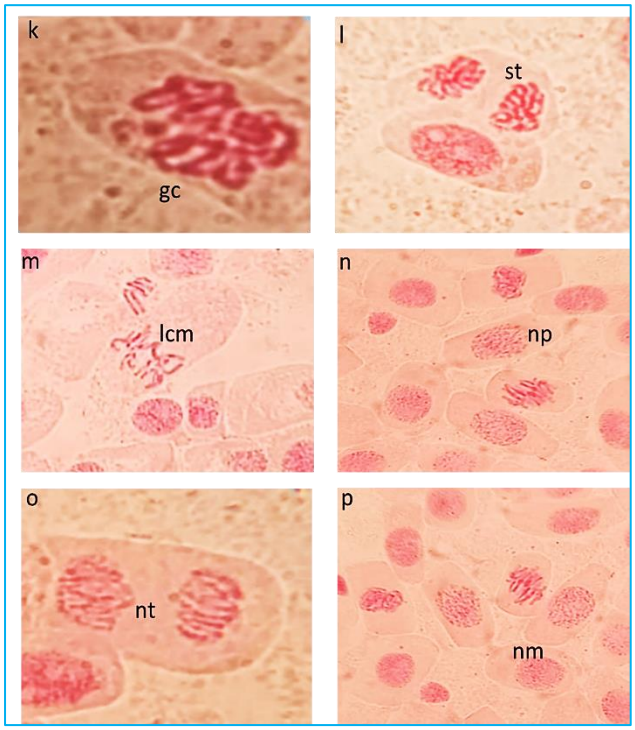
The leaf aqueous extract (LAE) treatment affected the various chromosomal stages by inducing abnormalities in them (Fig 2). It was noticed that value of anomalies was higher asa compared to the treated ones. Control samples showed normal cell division while treated sample with 4% concentration showed maximum abnormality index of 13% and the least anomalies are exhibited by 0.5% (Table 2) respectively.

Table 2 Abnormality Index secured from *Allium cepa* L. root tip cells at control and treated with different concentrations of LAE

Treatment	Abnormal cells	%age abnormality
Control	3.33±0.27[a]	3.33
0.50%	4.33±0.27[a]	4.33
1.0%	6.33±0.27[b]	6.33
2.0%	9.00±0.47[c]	9.00
4.0%	13±0.47[d]	13.0







a- acd abnormal chromosomal distribution at anaphase, **b-nl** nuclear lesion, **c-esa** equatorial separation in anaphase, **d-pm** pulverised metaphase, **e-acb** anaphase chromosome bridge, **f-cm** chained metaphase, **g-cv** cellular vacoulation, **h-dnl** double nuclear lesions in an enucleate cell, **i-acf** abnormal chromosome fragments at metaphase, **j-mpa** multipolar anaphase, **k-** gc giant cell, **l-st** stickiness, **m-lcm** lagging chromosome at metaphase, **n-**np normal prophase, **o-nt** normal telophase and **p-nm** normal metaphase

Fig 3 Showing different chromosomal aberrations incited by different concentrations (0.5, 1 2 and 4%) of LAE of *A. viridis* in the root tip cells of *Allium cepa*. Alterations in genetics of *Allium cepa* L. when treated with LAE: a-f 1%, g-j 2%, k–n 4%, n-p untreated control

An experimental set up to check the allelopathic capability of *Amaranthus viridis* against was conducted in In the present set up, the Mitosis of *Allium cepa* root tips was done after 4-5 days treatment of *Amaranthus viridis* leaf aqueous extract (LAE) and its mitotic index was taken, data collected showed retarding effects due to the cytotoxicity caused by allelochemicals present in it. The collected data for mitosis also showed a significant (One Way ANOVA, $P< 0.05$) decline in the mitotic indices of the extracts for all conc. (0.5, 1, 2 4%) clearly indicating the suppressive capability of the extract in mitosis (Fig 1, Table 1). Nevertheless, range of suppression of mitosis was shown to be increased with increasing the concentration of the extract i.e., from 0.5% to 4%. We can say that higher mitotic suppression was observed at 4% while the least was seen in 0.5% of LAE; though, inhibition of cell cycle observed to be significant for elevating conc. (4%) in the treatment of 24 hours. The suppression and decrease in mitotic index give the possibility of the phenomenon of cytotoxicity due to potential phytotoxins available in the leaf aqueous extract which might influencing the event of the cell cycle. We can support our experimental research with the help of some former studies [19-22]. The anomalies such as abnormal chromosomal distribution at anaphase, causing nuclear lesion, pulverized metaphase, anaphase bridging, chained metaphase, cellular vacuolation, double nuclear lesions in an enucleated cell, creating abnormal chromosomal fragments at metaphase, creating giant cell, multipolar anaphase, stickiness in chromosomes, lagged chromosomes, formation of giant cell etc. have evident the prospect that leaf aqueous extract of

Amaranthus viridi is capable of affecting formation of mitotic spindle, spindle fibre assemblage and chromatin synthesis by powering just about every single stage of cell cycle (Fig 2). Most common aneuploidy induced by phytochemical (LAE) like elongated cells, sickle-shaped cells, sticky chromosomes and chromosome bridges were the bulk. Nuclear lesions in anaphase and telophase nuclei as a result of inhibition in S phase were more common [23]. Abnormal chromosomal distribution at anaphase, nuclear lesion, equatorial separation in anaphase, pulverized metaphase, anaphase chromosomal bridging, chained metaphase were the affected stages of mitotic cycle determined at 1% LAE, cellular vacuolation, double nuclear lesions in an enucleated cell, abnormal chromosomal fragments at metaphase, formation of giant cell at 2% LAE, multipolar anaphase, stickiness in chromosomes, lagged chromosomes at metaphase at 4% LAE probably as a consequence of inhibition of formation of spindle and cytokinesis or possibly because of defects in segregation of sister chromatids at anaphase, disrupted roles of the cytoskeleton during interphase generated by allelotoxins existing in the LAE. Intense stickiness of chromosomes observed at metaphase, anaphase as well for 4% conc. originated as a consequence of the malfunctioned chromatin which resulted in proteinaceous matrix defects. Chromosomal stickiness is thought about to be irreversible thus, give rise to apoptosis thereby, credited of beholding mitotic index set down [19], [24]. Bridge formation at metaphase, anaphase and telophase would constantly discover at 1% LAE perhaps because of the translocated dicentric chromosomes causing uneven exchanges and mutations in chromosome at structural level [23]. Even so, 0.5% LAE being lesser toxic might be due to lesser allelopathic concentration ceased to give rise to a bit of apparent anomaly. Thereupon, from the observed results it can be inferred that elevated concentrations were more proficient for inhibition of cell division. Additionally, from further studies assessment of these abnormalities have also been done to examine several chemicals, radiations, and extracts [25-28]. Besides, observation and discovering of the refashioning of root tip cells of onion on treatment with LAE is in assessment of [19] as well. Performing GC-MS analysis alongside, more than 30 volatile allelochemicals from the phenols were identified by preparing methanolic leaves aqueous extract of *A. viridis*. The allelochemicals existing in the leaf extract of *A. viridis* describes these for executing an indispensable role in recounting phytotoxic nature of the selected weed. Anyhow, Phenols may not be the chief components in allelopathic interactions in plants thus, are not necessary to be a part of principal roles in plants and are thought to be formed as a part of pathways of secondary metabolites. Gulzar *et al*. [19], Gulzar and Siddiqui [29] approved the effect of phenolic acid phytochemicals in the ultrastructural and cytological aberrations in *Cassia sophera* L. and *Allium cepa* L. It has been reported that there are diverse derivatives of calix-4-arene known to give rise to chromosomal anomalies in root meristematic cells of *Allium cepa* and micronuclei (MN) in normochromatic erythrocytes (NCE) of Balb/c mice as noted down by Banti and Hadjikakou [30]. Likewise, in root elongation and cell division of plants and causing pressing distortions in the physiological roles such as uptake of nutrients, permeability of membrane, synthesis of protein, photosynthesis, respiration, balancing of hormone, activities and inhibition of enzymes also, the other phenolic derivatives were involved, Consequently, straightaway impacting their germination [31]. However, it is further needed to pick out every volatile component possessed by the leaf of *Amaranthus* concerning the supremacy of each and every separate component, also various mixtures to endorse their toxicity at the individual level and examine for feasible collective allelopathic impact. To broaden the scope for organic farming, like a concern is required to be set forth to a greater extent to advance ecological defoliants to kill weeds and additional plants.

Various alterations exhibited from the perceived anatomy of *Allium cepa* root tip cells accompanying the disruption in the inclination of the nucleus on treating with distinct concentrations of LAE on the contrary to control. A positive correlation with the different LAE concentration was shown after the treated samples disclosed the mitotic anomalies and disruptions. The Mitotic Index exhibited a significant decline in every single sample while subjecting to extract. The Mitotic Index have been accounted to be 20.95 for the control, while the maximal and minimal Mitotic Index data of the LAE nursed samples were 19.78 and 9.56, reported for 0.5 and 4% correspondingly (Fig 2). The outcome showed a gradual depletion for the counting of dividing cell samples treated with LAE conversely to the control. Control samples exhibited a normal mitotic cycle with the systematic organization of 16 chromosomes at metaphase separating ideally to get 16:16, as well as during anaphase and normal telophase. On the contrary, different chromosomal disparities were made out in the cells after treating with various concentrations of LAE (4, 2, 1 and 0.5%). The recurring anomalies detected were sticky chromosomes at metaphase and abnormal chromosomal distribution, multipolar anaphase, equatorial separation and chromosomal bridge at anaphase, double nuclear lesions in an enucleate cell, nuclear lesion, giant cell, cellular vacuolation, abnormal chromosome fragments, lagging chromosome, stickiness at metaphase, pulverized, chained metaphase as well (Fig 2).

CONCLUSION

From this experiment, it can interpret that *Amaranthus viridi* produces natural allelochemicals which play important role in affecting the cell division of *Allium cepa* root tip cells when treated with its various conc. The chromosomal anomalies and reduced mitotic index have also confirmed its rich phytochemistry which may prove to be beneficial for the environment by preparing natural weedicide to use in agricultural fields for protecting annual crop yield and land fertility thus creating green biosphere in a sustainable manner.

Acknowledgement

Authors are thankful to Aligarh Muslim University for this project and special thanks to University Grant Commission (UGC) for supporting financially.

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