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GENOTOXICITY ASSESSMENT OF WATER EXTRACT OF *OCIMUM GRATISSIMUM* L. USING THE *ALLIUM CEPA* ASSAY

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ABSTRACT: Aqueous leaf extract of *Ocimum gratissimum* was used to investigate its cytotoxic and genotoxic effects on root meristematic cells of *Allium cepa*. 1, 2.5, 5, 10 and 20% concentrations of leaf extract were used in the present study. It was found that leaf extract of *O. gratissimum* exhibits mitodepressive activity. Mitodepressive acitity was found maximum at high concentrations. Of all the concentrations used in the study, 5% concentration of leaf extract induced maximum genotoxicity leading to formation of sticky chromosome, c-metaphase, metaphasic and anaphasic disorders while 20% concentration showed binucleate cells.
Keywords: *Ocimum gratissimum*, Leaf extract, *Allium cepa*, Genotoxicity, Cytotoxicity.

INTRODUCTION

Medicinal herbs have been used in folk medicine for millennia. The world Health Organization (W.H.O.) estimates that 80% of the world population uses herbal medicines for some aspects of primary health care (Farnsworth *et al*.,[1]). Among all the medicinal plants, a member of the Lamiaceae family- *Ocimum gratissimum* L. and its essential oil have been using in the treatment of different diseases like upper respiratory tract infections, diarrhea, headache, fever, ophthalmic, skin disease and pneumonia (Correa [2], Onajobi [3] and IIori *et al* ., [4]). Nakamura *et al*., [5] and Pessoa *et al*., [6], have reported anti-bacterial and antihelmintic properties of *O. gratissimum* respectively. Previous studies have showed that the main component of essential oil of *O. gratissimum* is Eugenol (84.84%) and other components are γ-terpiene (21.9%), β-phellandrene (21.1%), limonene (11.4%) and thymol (11.2%) (Tchoumbougnang *et al*., [7]). Olivier [8] and Sainsbury and Sofowora [9] have reported that the volatile oil of *O. gratissimum* contains mostly phenols, particularly thymol and this is responsible for its antimicrobial action.
The present study intends to investigate the cytotoxic and genotoxic effects of leaf extract of *Ocimum gratissimum* L. on the root meristematic cells of *Allium cepa*.

MATERIALS AND METHODS

Collection of plant material

The leaves of *O. gratissimum* have been collected from premises of Life Sciences Department, Dibrugarh University.

Preparation of aqueous leaf extract

Fresh leaves of *Ocimum gratissimum* were air dried and ground into coarse powder. Eight hundred grams of the powdered leaves were stirred into 450ml of boiling tap water. The boiling was allowed to continue for 5 minutes. It was then filtered through a fine sieve. The leaf extract was kept frozen at 4°C and used in subsequent experiments as stock solution.

Allium cepa test

Onion bulbs were commercially obtained from new market, Dibrugarh. Before use, the loose outer scales were carefully removed and dry bottom plates were scraped away without destroying the root primordia.
Five concentrations (v/v) of the extract were prepared from the stock, viz: 1, 2.5, 5, 10 and 20% to study mitotic and genotoxic effects. Tap water was used as control. For each concentration five onion bulbs were set up and allowed to produce root in tap water for 24 hrs.

On next day the bulbs were transferred to test samples as well as control. Roots were treated in the aforesaid test samples for 48 hrs. After end of 48 hrs, the length of the roots were measured with a ruler and other morphological abnormalities were recorded. To study the genotoxic effect, after the end of 48 hrs of treatment with the aforesaid concentrations, root tips from each concentrations were fixed in aceto-alcohol (1:3) fixative for 24 hrs. After 24 hrs, root tips were transferred to 70% ethyl alcohol and stored at 4°C.

For chromosomal analysis, root tips were hydrolysed in 1N HCL and 2% aceto-carmine (1:9) for 1-2 mins and kept for over night. On next day root tips were squashed in 1% aceto-carmine as proposed by Sharma and Sharma [10] and the cover slips were sealed on the slides with clear fingernail polish as suggested by Grant [11]. Five slides were prepared for each treatment and control and 1000 cells were scored per slide to study the mitotic index and aberrant cells. The slides were analysed at x 1000 magnification. The Mitotic Index(MI) was calculated as the number of dividing cells per total cells scored at each concentration. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored for each concentration of the extract (Bakare *et al*., [12]). The mitotic inhibition was obtained as follows:

$$\text{Mitotic inhibition} = \frac{(\text{MI in control} - \text{MI in treated group}) \times 100}{\text{MI in control}}$$

Statistical analysis

The SPSS 15.0 statistical package was used for the analysis. The difference between the control and the treated groups in relation to root length and root number was analysed by Students' *t-test*.

RESULTS AND DISCUSSION

The present investigation showed that, all the tested concentrations except 1% concentration of aqueous leaf extract of *O. gratissimum* inhibit root growth in comparison to control. Inhibition of root length and root number was greater with increasing concentration of leaf extract (Table-1). Root length and root number in 1% concentration was found nearly equal to the control. Normal root morphology was recorded in concentrations 1, 2.5, 5% and control. While curved root development was recorded in concentrations 10 and 20%. The roots treated in 10 and 20% concentrations appeared slightly brown in colour.

Table-1: Morphological and cytological effects of aqueous leaf extract of *O. gratissimum* on *Allium cepa* root tip cells.

Concentration (%)	Control	1	2.5	5	10	20
Av. of root length (cm) ± S.E.	5.34* ± 0.11	4.9* ± 0.07	3.02* ± 0.08	1.27* ± 0.07	1.22* ± 0.05	0.94* ± 0.08
Av. No. of root ± S.E	24.4* ± 1.61	23.6* ± 1.21	11.4* ± 0.92	7.2* ± 0.52	4.6* ± 0.46	1.8* ± 0.33
No. of dividing cell	267	245	172	82	0.0	0.0
Mitotic Index	5.34	4.9	3.44	1.64	0.0	0.0
Mitotic inhibition	0.0	8.24	35.58	69.29	100	100
Aberrant cell (%)	0.0	1.4	1.7	3.86	0.0	0.24

*Significant difference between control and treated groups using Students' t-test at 95% confidence limit with 9 degrees of freedom.

While studying the effect of leaf extract of *O. gratissimum* on MI and chromosomal alterations, no dividing cell was recorded in 10 and 20% concentrations (Table-1). This proves that leaf extract of *O.gratissimum* interfers with normal sequences of mitotic cell cycle in an inhibiting manner. It was also noticed that there was concentration dependent decrease of MI in concentrations 1, 2.5 and 5% as compared to the control. Reasons of reduction of mitotic activity might be due to blockade of G2 phases of cellular cycle, inhibition of DNA/protein synthesis etc., (Scheiderman *et al*., [13] and Turkoglu, [14]). Mitodepressive effects of some plant extracts resulting from their interaction with DNA nucleotides thus inhibiting DNA synthesis and subsequent mitotic inhibition have been reported by Mercykutly and Stephen, [15], Schulze and Kirscher, [16] and Soliman [17].

An analysis of chromosomal aberration showed (Fig-1) that leaf extract of *O. gratissimum* causes metaphasic and anaphasic disorders (Fig-2c,d and e), sticky chromosome (Fig-2b), C-metaphase. These anaphase and metaphasic disorders are indicative of disrupted kinetic of chromosomes and are generated due to qualitative and quantitative changes of chromatin kinetochore (Schneiderman *et al*., [13] and Amin, [18]), therefore may indirectly constitute a risk of aneuploidy (Maluszynska and Juchimiuk, [19]). Presence of c-metaphase indicates (Fig-2a) that leaf extract of *O. gratissimum* also interfers with spindle fibre formation. Of all the concentrations used in the study, 5% concentration of leaf extract induced maximum (Fig-1) genotoxicity. Presence of few binucleated cells were recorded in 20% concentration. This usually arises as a consequence of the inhibition of cell plate formation (Grant, [20]), which might be due to the suppression of phragmoplast formation in the early telophase (Soliman, [17]) by the alkaloids present in the extract.

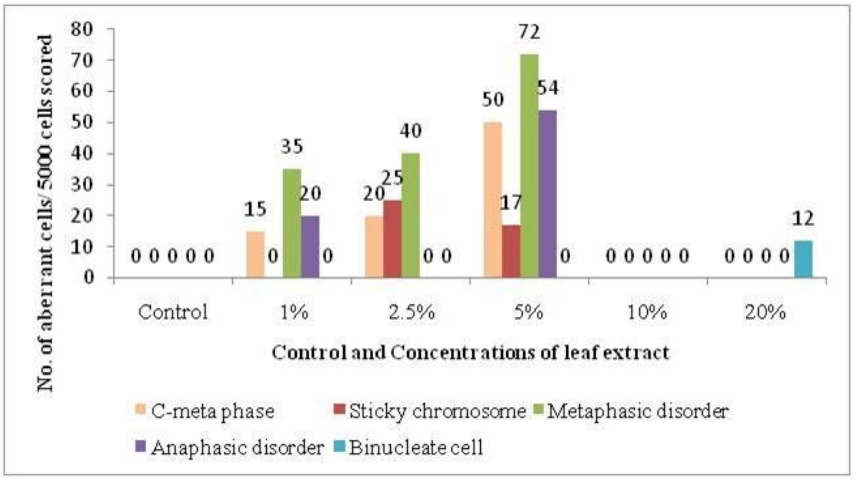

Fig-1. Number of abberant cells induced by different concentrations of aqueous leaf extract of *Ocimum gratissimum* on *Allium cepa* root meristematic cells.

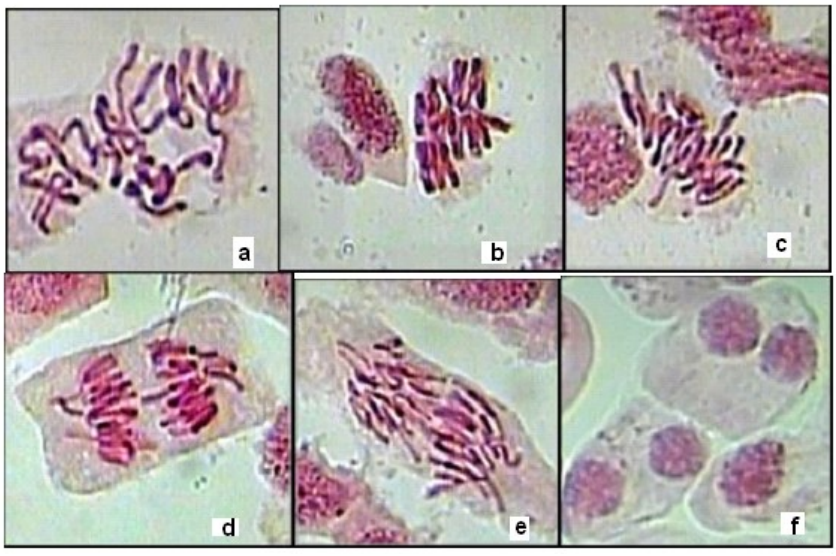

Fig-2: Aberrant cells induced in root tip cells of *Allium cepa* by water extract of *O. gratissimum*. a) C-metaphase, b) Sticky metaphase, c) Disordered metaphase, d) and e) Anaphasic disorder and f) Binucleated cells.

CONCLUSION

Finally, it can be concluded that high concentration of *O. gratissimum* leaf extract shows cytotoxic and genotoxic activities. The results of this study suggest that, although *O. gratissimum* has beneficial effects as a medicinal herb, it can cause serious problems and damage on cells when used improperly. Moreover, there is a need for a closer look at the genotoxicological effects of the tested extracts in animal test systems for human welfare.

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