Cytotoxicity Analysis of *Azadirachta indica* (A. Juss) *a*nd *Mimosa pudica* L. leaf extract on *Allium cepa* cells

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***Abstract- Mimosa pudica* L. and *Azadirachta indica* (A. Juss) are two medicinally important plants used in different systems of medicine. At the same time, both the plants are used as insecticide also. Therefore, an attempt was done to identify the cytotoxic effects of the plants. The leaf extract of both plants was used to study the effects on mitotic cycle by using *Allium cepa* root tip cells. Different chromosomal abnormalities like chromosome clumping, lagging chromosomes, nuclear lesions, ball metaphase, bridge formation, misorientation of chromosomes etc. were observed. Also, the rate of mitotic activity was decreasing with increase in concentration of extract. The properties exhibited by these plants could be utilised for further medical applications like cancer treatments.**

***Key words*: C- metaphase, Ball metaphase, nuclear lesions, multiple bridge formation**

INTRODUCTION

Azadirachta indica is a fast-growing tree belonging to Family Meliaceae common in tropical and semitropical regions of the world. The plant is said to possess anticancer, antidiabetic, neuroprotective, anti-inflammatory, antiviral, antibacterial, antifungal, antioxidant properties [1]. Another plant considered for study *Mimosa pudica*, belonging to subfamily Mimosaceae of family Fabaceae is a diffuse prickly undershrub. The plant is said to have antioxidant, antibacterial, diuretic, hepatoprotective, antifungal properties [8]. Along with these medicinal properties, oil extracted from seeds, bark and leaf of Azadirachta exhibits insecticidal properties and is used as herbicide, weedicide and fungicide [2]. *Mimosa pudica* leaves, stem and root in different solvents are found to produce insecticidal and repellent activities [7]. Human population is increasing in a deadly rate and the consumption rate showing a steady graph, more crops have to be raised per unit area. But the threat of insect and pest attack have always been a dilemma for farmers. The use of synthetic insecticides is only of temporary answer to the problem as the organisms can develop resistance in the subsequent generations and also the nondegradable fractions of pesticide pose a vulnerable factor in environmental imbalance. Botanical insecticides are one of the best alternatives in place of hazardous synthetic pesticides. Phytochemicals are able to induce different types of abnormalities in insects that could safely be used for insect pest control [19].

An attempt is made in the present study to identify the chromosomal anomalies caused by these plant extracts in pure nature on cells to utilise their feasibility as biopesticides in combination.

Materials and Methods

Fresh extract of leaves of *Azadirachta indica* and *Mimosa pudica* was prepared by pulverization. Different concentrations of the extract were prepared from 0.1-0.5% range of 5 solutions. Germinated bulbs of *Allium cepa* with large number of healthy roots were collected, washed thoroughly in distilled water. The peak time of mitotic activity being 9 - 10 am for mitosis, the onion bulbs are transferred to small containers with roots dipped in extracts of varying concentration. The bulbs were taken out after a time period of 6 hours and the root tips of 1-2 cm were cut off from bulbs and washed thoroughly in distilled water. It is then immediately fixed in Carnoy's fluid for one hour. After fixing, the root tips washed thoroughly in distilled water and transferred to 70% alcohol and kept under refrigeration to prevent root degradation. Mitotic squash experiments were conducted [16]. For this the root tips were washed thoroughly in distilled water, treated with 1 N HCl for 2 minutes to separate the cells during squashing. Further the root tips washed thoroughly to remove traces of HCl, stained in 2% acetocarmine for 5 minutes. After staining, the root tips were washed with 45% acetic acid for destaining, squashed and mounted for observations.

All the slides were observed under LEICA GALEN III trinocular microscope equipped with camera.

RESULTS

Cytotoxicity and severe mitotic inhibitions were observed with *Azadirachta indica* leaf extract. Aberrations such as chromosome bridges, diagonal anaphase and telophase, chromosome stickiness and misorientation of chromosomes at metaphase and anaphase, nuclear lesions, micronuclei, early movement of chromosomes were observed. The frequency of abnormalities showed slight increase with increase in the concentrations of the extract. Chromosome bridge, diagonal orientation of chromosomes at metaphase and anaphase were the most frequent abnormalities observed.

The cytotoxic anomalies observed with *Mimosa pudica* extract also showed similar type of abnormalities, in addition to C-metaphase. The most frequent abnormalities observed with *Mimosa* extract were Ball metaphase, early movement of chromosomes, Diagonal metaphase and chromosomal bridges. The figures are cited below.

Discussion

The cytotoxic effect with the crude extracts showed clastogenic and nonclastogenic abnormalities. The nonclastogenic abnormalities observed were chromosome clumping, laggards, stickiness of chromosomes, disturbed metaphase and anaphase, early movement of chromosomes, ball metaphase, C- metaphase, misorientation of chromosomes, polyploidy etc. whereas major clastogenic abnormalities observed include nuclear lesions and chromosome bridge. The cytotoxic chemicals act on cells as preprophase inhibitor, mitoclastic agent or inhibitor of cell plate formation [11]. A prominent change in the number of dividing cells has been observed with increasing concentration of the extract. The change in the frequency of dividing cells is a partial proof of the degree and the kind of damage caused by a particular mutagenic agent [5].

Chromosome bridge formation was an abnormality common in all treatments. Mitotic bridge may arise due to stickiness or due to formation of dicentric chromosomes by breakage and reunion [10]; multiple bridge by fusion between broken chromosomes [21]; or due to hydration and dehydration of spindle and chromosomes [18]. Diagonal orientation of chromosomes may be due to failure and improper functioning of spindle apparatus [4]. Chromosome stickiness may be due to depolymerization of DNA [3]; stripping of protein covering of DNA in chromosomes [20]. Misorientation of chromosomes may be due to spindle interruptions caused by cytotoxic chemicals [15]. Polyploidy observed in the present study can be attributed to the inhibition of spindle mechanism [5]; lack of anaphase movement or cell plate formation [9]. Lagging chromosomes is considered to be due to abnormal spindle activity [14]. C- metaphase observed in some of the treatments can be attributed to blocking of anaphase stage by the poisonous effect of the toxic chemical with turning of cell to polyploid [13].

The extract as a stathmokinetic agent causes action on spindle preventing its normal function [17]. The present study shows several abnormalities that have been accounted by many workers in the past in different studies. From the results obtained, the potential genetic damage induced by *Azadirachta indica* and *Mimosa pudica* leaf extract even at very low concentrations could be effectively exploited for formulating potential biodegradable pesticides.

Coming to the concept of combination of these two extracts, there are reports of synergistic action of botanicals that can manifold the pesticide property. According to [6] neem products can be readily combined with many other bioagents including microbes. Recent approaches proved that “dual-attack” approach can result in higher mortality of pest than their individual effect [12]. The combination of two plant extracts, if successful can develop to a commercial bioinsecticide to aid the farming community as it can synergise the effect and overcome the shortcomings of individual extracts. The combination trials have to be conducted to produce the fruitful outcome and there are opportunities of further expansion of research in this arena for better utilization of botanicals.

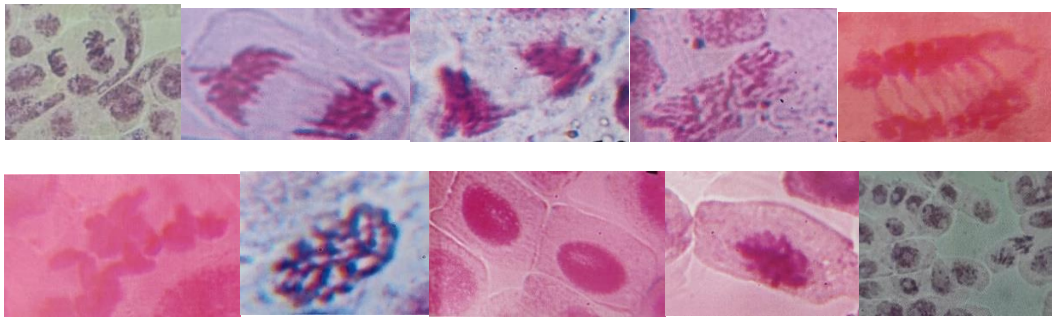

Figure Legend First row (from left to right) Early movement of chromosomes at anaphase, Micronucleus, Clumping and distorted anaphase, polyploid, multiple chromosome bridges
Second row (from left to right) C-metaphase, Ball metaphase, nuclear lesions, clumpy metaphase, diagonal orientation of chromosomes at anaphase.

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