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DIFFERENTIAL CYTOTOXICITY OF *MELASTOMA MALABATHRICUM* L. AQUEOUS EXTRACT ON ROOT MERISTEM CELLS OF *ALLIUM CEPA* L.

***SUROCHITA BASU, RUPA MAJUMDAR**

Department of Botany, Tripura University, Tripura 799022, India

*** Address for Correspondence:** Dr. Surochita Basu, Department of Botany, Tripura University, Tripura 799022, India; Email ID : surochitabasu@yahoo.co.in, rupamajumder.shil75@gmail.com

ABSTRACT

Melastoma malabathricum L., a plant that grows well in burnt, fallow lands and accumulates the metal Aluminum, has been variously used in indigenous system of medicine in the Indo Malaysian region, due to its antioxidant, antimicrobial and anticancer activities. Nevertheless, safety and efficacy of the herbal preparations necessitates toxicity assessment. A plant based bioassay used to assess the potential toxicity of the aqueous plant extract revealed interesting outcomes. The *Allium cepa* test, a highly sensitive and reliable plant based bioassay for environmental monitoring, was employed to assess the efficacy of the aqueous plant extract; changes in mitotic activity and appearance of chromosomal aberrations were parameters to assess toxicity. The results indicate differential sensitivity and dose dependent changes in root meristem cells upon exposure to varying concentration of plant extracts at different duration. An increase in mitotic index at low concentration *vis a vis* decrease in mitotic index at higher concentration and distinct chromosomal aberrations over treatment duration indicate cell cycle intervention and DNA damage. Interestingly, at a critical concentration, rhythmic progression in mitotic activity at specific duration was noted with leaf extract. Preponderance of prophase and high MI (Mitotic Index) at lower concentrations and that of chromosomal aberration and low MI at higher concentration indicates drastic cell cycle intervention and dose dependent toxicity of the aqueous plant extract. It implies using narrow dose for effective therapeutic applications.

Keywords: *Melastoma; Allium cepa* test; Mitotic index; Chromosomal aberrations; Dose dependent toxicity; Aluminum

INTRODUCTION

Plants not only occupy a focal position in food and energy web, provide multiple benefits to human and other organisms but are also inevitable in nutrient cycling and biosphere homeostasis. *Melastoma malabathricum* L. family Melastomataceaea, occurs wild in tropical and temperate Asia and Australia, is traditionally used as medicine in Malaysia, India, Indonesia and grows luxuriantly in humid acidic soils rich in Aluminum [1] with intrinsic homeostatic mechanisms with the metal. Such plant suited to constrained environments, traditionally used by the indigenous communities in developing countries, with environmental applications in nutrient cycling, phytoremediation, emphasize further scientific investigations for its optimum utilization. *Melastoma* is variously used in folk medicine, mostly decoctions and pastes of leaves, fruits, seeds, flowers, stem and roots have been used to relieve pain, cuts, wounds, dysentery, diarrhea, leucorrhea and hemorrhoids. The pharmacologically active principles include flavonoids, tannins, triterpenes, saponins, alkaloids, and phenolic components [1]. Aqueous extracts of the plant are reported to have antimicrobial, antibacterial, antiulcer, anti-inflammatory and anti-diarrheal effects [2,3,4,5,6]. Recently methanol and ethyl acetate extracts of the plant have shown anti-diabetic, antioxidant, anti-hyperlipidemic effects in animal model and potent cytotoxicity in human cancer cell lines [7]. The plant extract has also been used in aquaculture to stimulate growth and spawning of crab [8].

Aluminium toxicity is one of the major constrains for plant growth [9], the metal causes dose dependent toxicity through oxidative stress generation and can be fatal beyond a threshold [10]. Yet several plants have an intrinsic tolerance to Aluminum, where the metal accumulates and even promotes growth [11]. *Melastoma malabathricum* L. is a hyper accumulator of Aluminum (Al) and accumulates more than 10 mg Al per g dry weight in root and mature leaf tissues. Growth enhancement of the plant in presence of the metal is possibly due to alleviation of iron toxicity, stress management and intrinsic mechanisms for Al acquisition [12,13]. Not only aluminum, but it can also accumulate heavy metals such as lead and arsenic through phytostabilization and phytoextraction, thus could be used for phytoremediation of metal contaminated soil [14].

Crude aqueous extract of *Melastoma* contains a mixture of pharmacologically active components and even metal ions (in trace) and although there are reports on therapeutic effects of aqueous extract of the plant, scientific and clinical assessment of toxicity for their safe and efficient use is scarce. Plant based bioassay are commonly used to assess the ability of an aqueous sample to cause chromosomal damage and its environmental impact for a considerable period of time [15]. The *Allium cepa test* offers a highly sensitive, inexpensive, fast, and reliable plant bioassay based on dynamics of root growth, relatively long chromosomes and low number with stable karyotype, distinct mitotic phases and chromosomal aberrations, [15, 16, 17] that shows good correlation with mammalian system [18].

Inspired by its ethnobotanical and ecological importance, the present investigation was conducted to evaluate the efficacy and toxicity of the water extract of *Melastoma malabathricum* L, growing wild in the acidic soils [19] of Tripura. Changes in mitotic activity and appearance of chromosomal aberrations in meristematic cells were endpoints to assess toxicity, using the *Allium cepa* test.

MATERIALS AND METHODS

Plant Materials: *Melastoma malabathricum* L. plants (Figure 1) were collected from Tripura [20] during flowering season and voucher submitted to Departmental herbarium. Hand cut transverse sections of stem and leaves were observed under fluorescent microscope. The leaves and stem were separated, dried, weighed, powdered and the powdered material was kept in clean, air tight, glass container for experimental use. Fresh healthy bulbs of onion (*Allium cepa* L.) were obtained from a local market; equal sized bulbs were selected and kept in small pot containing sandy soil for 2-3 days for rooting.

Experimental Procedure: Decoction of the powdered plant material (stem and leaf) in different concentration as used in traditional applications (0.25, 0.5, 0.75, 1, 1.25g) in 100ml of tap water was made and filtered for sample preparation. Tap water was used as negative control. Onion bulbs with 1-2 cm long slender, healthy roots were exposed to different concentration of extract for several hours (6, 12, 18, 24, 30, 36, 42 and 48). Each treatment was performed in triplicate and after proper treatment in all concentrations of the extracts root tips of *Allium cepa* L. were excised. The root tips were fixed in Carnoy's fixative (1:3 acetic: alcohol) overnight. Fixed root tips were rinsed in distilled water, kept in 45 % acetic acid for 5 minutes, stained with 9:1 aceto orcein: HCl solution, warmed 3-6 seconds, kept for an hour and squashed in 45 % acetic acid. Slides were prepared for cytological observation according to aceto-orcein squash technique [21]. The slides were observed and photomicrographs obtained under Zeiss Axioscope microscope. All chemicals used were of analytical grade.

Cytological observations: Different mitotic phases and distinct chromosomal aberrations were identified and recorded. Metric observations recorded include number of cells in different mitotic phases, number of aberrant cells, total number of dividing cells and number of cells in microscopic field (at 40X objective). Mitotic Index (MI), Chromosomal Aberration Rate (CAR) and Aberrant cell Frequency (AF) calculated by the following formulae:

$$MI = \frac{\text{Total number of dividing cells / microscopic field}}{\text{Total number of cells / microscopic field}} \times 100$$

$$CAR = \frac{\text{Total number of aberrant cells / microscopic field}}{\text{Total number of dividing cells / microscopic field}} \times 100$$

$$AF = \frac{\text{Number of a type of aberrant cell}}{\text{Total number of aberrant cells}} \times 100$$

Statistical Analysis: All treatments were conducted in triplicate and at least 1500 cells were scored for each concentration and duration of treatment along with control. The mean values, standard deviation and standard error of mean were calculated for each attribute and two-way ANOVA was performed at $p<0.5$ significance level.

RESULTS

The results indicate differential and dose dependent changes in *Allium cepa* upon exposure to varying concentrations of *Melastoma malabathricum* L leaf and stem extracts at different durations. Tap water was taken as negative control at all duration. The average MI for control and extract treatment were compared- average MI of control is 8.36, average MI for stem and leaf extract treated cells are 5.98 and 10.73 respectively. The maximum and minimum MI for control (8.95 at 48hrs, 7.09 at 12 hrs), treatment with stem extract (12.06 at 6hrs, 1.79 at 42 hrs) and leaf extract (21.41 at 0.25% 48hrs, 3.42 at 1% 48hrs) indicate mitopromotive effects at low concentration and treatment duration as opposed to mitoinhibitory effects at high concentration and prolonged exposure.

A mitostimulant activity concomitant with increased prophase percentage at low concentration of leaf extract treatment was noted. A general decrease in mitotic index at higher concentration and duration of treatment with stem extract was observed. Interestingly at 0.5% leaf extract treatment, a rhythmic progression in mitotic activity at 6hrs interval was noted and the distinct oscillations continued up to 36hrs. MI of cells treated with leaf extract revealed a pattern of change centered about the critical concentration (0.5%), high MI noted at 0.25% and at concentrations beyond 0.5% a trend of decrease in MI with increased exposure time (in hours) was noted (Table 1; Figure 2,3).

Chromosomal aberrations appeared with leaf extract treatment early at 6 hours and at all concentrations 0.5% onwards; with stem extract treatment aberrations appeared 0.75% onwards and after 12 hrs duration. Highest frequency of aberrant cells was observed at 0.75% of leaf extract treatment. Significant types of aberrant cells include clumped metaphase, sticky anaphase, anaphase bridge, precocious movement and fragmentation. Metaphase clumping was the most frequent type of aberration (66.67%) followed anaphase bridge (14.29%), precocious separation (9.52%) and fragmentation (9.52%) with stem extract treatment; with leaf extract treatment, metaphase clumping (60.0%) was again most frequent followed by anaphase bridge (22.9%) and sticky anaphase (17.1%). More chromosomal aberrations were observed with leaf extract treatment (Table 2, Figure 4,5).

Preponderance of prophase at lower concentrations, decrease in MI at higher concentration and treatment duration and a range of chromosomal abnormalities observed at higher concentration indicate drastic cell cycle intervention, DNA damage and dose dependent toxicity of the aqueous plant extract. Statistical analysis through ANOVA shows changes in MI to be significantly affected by treatment duration in both leaf and stem extracts.

DISCUSSION

Toxicity level of a test sample can be determined based on genotoxic endpoints such as changes in mitotic index and chromosomal aberrations [15] that are reliable parameters to identify cytotoxicity using the *Allium cepa* test [16]. Assessing these endpoints enables evaluation of action mechanism of test sample on the genetic material of the exposed organism.

In traditional practices of medicine, decoctions of plants are occasionally used for therapeutic purposes; these crude extracts contain a mixture of pharmacologically active components, minerals and even metal ions (in trace) as in the case of Al accumulator *Melastoma malabathricum*. Synergistic interaction and multifactorial effects between various phyto-components in a plant extract lend it therapeutic activity [22], subsequently purified extracts with specific metabolites are presumed to have better efficacy but occasionally fail to match the therapeutic potential of crude extract [23,24]. Crude aqueous extracts of the plant as used in folk medicine needs to be investigated [1,25] for potential toxicity before safe and efficient administration.

Significant alteration in MI of onion root meristem

cells exposed to the aqueous plant extract for specific concentration and duration of treatment as compared to the negative control indicate potential toxicity. An increase in MI (mitostimulant activity) concomitant with increased prophase percentage at low concentration and exposure time indicates delay in breakdown of nuclear envelope proteins and increased rate of cell division. Preponderance of prophase indicates inhibition of cell cycle progression at the prophase-metaphase checkpoint [26]. Decrease in MI (mitodepressive effects) at higher concentration and prolonged exposure, along with decrease in prophase percentage indicates obstruction in DNA and nucleoproteins synthesis and delay in mitotic cell cycle progression and cell cycle intervention [15, 27]. Decrease in MI and induction of chromosomal aberrations considered as reliable criteria for genotoxic activity [28].

Chromosomal aberrations are changes in chromosome structure apparently due to denaturation of protein and DNA damage. Aberrations like fragmentation, anaphase bridge indicate clastogenic effects, precocious separation indicate increased cell division rate and moderate cytotoxicity whereas abundance of stickiness and metaphase clumping indicate aneugenic effects, high cytotoxicity of the plant extract leading to irreversible cell damage and death [15,16]in the exposed tissue. Chromosomal aberrations in plants are of discernible risk to other organisms, since the genotoxic endpoint/ target is DNA damage.

High genotoxicity also relates to presence of metal ions in complex mixtures such as environmental sample [15]. Here the efficacy of safe and efficient use of aqueous extract of Al accumulating plant is under concern. The plant *Melastoma* has an essential requirement for aluminum and accumulates more than 10 mg Al per g dry weight in tissues such as roots and mature leaves. Aluminum occurs in free form, adsorbed on pectic substances, hemicelluloses, (Figure1) and as oxalates and citrates in the plant. The endodermis in stem sections and upper epidermis in leaf sections emit bright UV blue epifluorescence, probably the adsorbed Al cause metal-enhanced fluorescence-MEF of the plant cell walls [29]. Earlier studies of Al ion toxicity on *Allium cepa* reveal dose and concentration dependent moderate toxicity of the metal at 10^{-3} to 10^{-4} M [30,31], mediated through [32] oxidative stress induced DNA repair mechanism (adaptive) and cell death (toxic).

In our study, it was interesting to observe that at a critical concentration (0.5%) of aqueous leaf extract (leaves have high Al content) a rhythmic progression in mitotic activity (MI) at 6hrs interval was noted and the distinct oscillations continued up to 36hrs. Aluminum calculated to be at 0.5ppm (~10^{-4}M) in 0.5% of *Melastoma* leaf extract and it is speculated that at this concentration the metal ion Al in the water extract generates oxidative burst [33] evident from the rhythmic oscillations in MI. Oxidative stress is known to have a key role in circadian rhythm generation [34]. Transition metals play important roles in redox control, electron transport system and are involved in oxidative stress generation [35], although Al is not a transition metal, its involvement in oxidative stress is well known. Aluminum toxicity is reported to be dose dependent, initiates adaptive changes at low concentration [32], triggers ROS production, respiration inhibition, ATP depletion and beyond a threshold leads the cells to irreversible death [10]. At lower concentration (0.25%) of the leaf extract no discernible effects of metal toxicity is evident.

Although Al reduces crop productivity in acid soils, it plays important roles in physiological efficiency of tolerant plants, plants that accumulate it or have a requirement for it. Mechanism of aluminum dependent growth promotion in *Melastoma* is [12,13] due to accumulation of Al oxalate (as a ligand for Al detoxification and possible role in alleviation of iron toxicity) indicates metal homeostatic effects. Supporting evidences gathered from the temporal aspects of Copper homeostasis in *Arabidopsis*, the daily oscillating expression patterns of Cu deficiency marker genes COPT2 and FSD1, their patterns timed with fulfilling specific physiological demands of the plant, implicates temporal integration adapts plant development to circadian rhythms and oscillatory nutrient homeostasis for optimal plant performance under challenging environmental conditions [36,37]. Interestingly therapeutic implication of the metal is evident in animals, where aluminum citrate administration is reported to attenuate nephrotoxicity in rat [38], yet the use of Al in therapeutics remains skeptical due

to its detrimental effects on nervous system, microcytic anemia and bone disease [39]. Further research would enable future scope of Al based development of safer therapeutics.

Thus, the plant extracts exhibit dose dependent genotoxicity, in spite of the multiple therapeutic benefits it possesses. *In vivo* studies using *Melastoma* extract also lay importance on dosage range [1]. At high concentration and duration of treatment the stem and leaf extract display high levels of genotoxicity as evinced by marked decrease in MI and abundance of chromosome stickiness that may eventually lead to irreversible cell death. At lower concentration of leaf extract treatment, intervention in cell cycle progression is evident. The potential toxicity of the plant extract is partly due to metal ion toxicity, the plant accumulates high amounts of Aluminum and Al toxicity of the plant extract could be severe beyond a threshold [10]. The outcome of present investigation and supporting evidences from plant research implies that proper administration of decoctions of the plant in narrow and appropriate doses is a must for safe and efficient therapeutic applications of *Melastoma malabathricum* L. in folk medicine.

Melastoma malabathricum L. is an important plant growing in the acidic soils of Tripura with multiple benefits as traditional medicine in folklore, with scientifically validated pharmacokinetics, as a hyper accumulator of Aluminum with intrinsic homeostatic mechanisms with the metal. The plant offers prospective role in therapeutics, nutrient cycling and phytoremediation, yet with its potential dose dependent toxicity in concern, emphasizes the need of further research for its optimum utilization.

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CONFLICT OF INTEREST

The authors have no conflict of interest. R.M. performed the *Allium cepa* test and S.B. analyzed the results, designed and drafted the manuscript.

Figure 1: *Melastoma malabathricum* L : A, Plant morphology, Transverse section of stem (B) and leaf (C) showing sharp blue epifluorescence of endodermis, xylem in stem and upper epidermis in leaf with adsorbed Aluminium

Figure 2: Graphical representation of *Allium cepa* root cells Mitotic Index on exposure to *Melastoma* leaf extract at various treatment concentrations, duration with tap water control

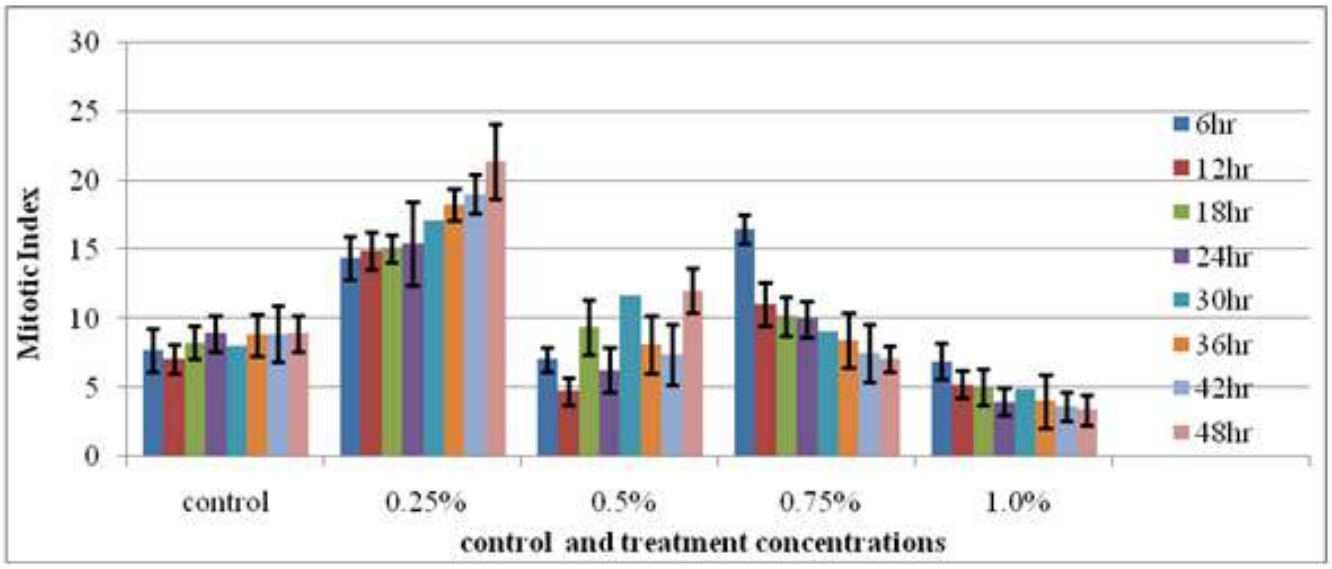


Figure 3: Graphical representation of *Allium cepa* root cells Mitotic Index on exposure to *Melastoma* stem extract at various treatment concentrations, duration with tap water control

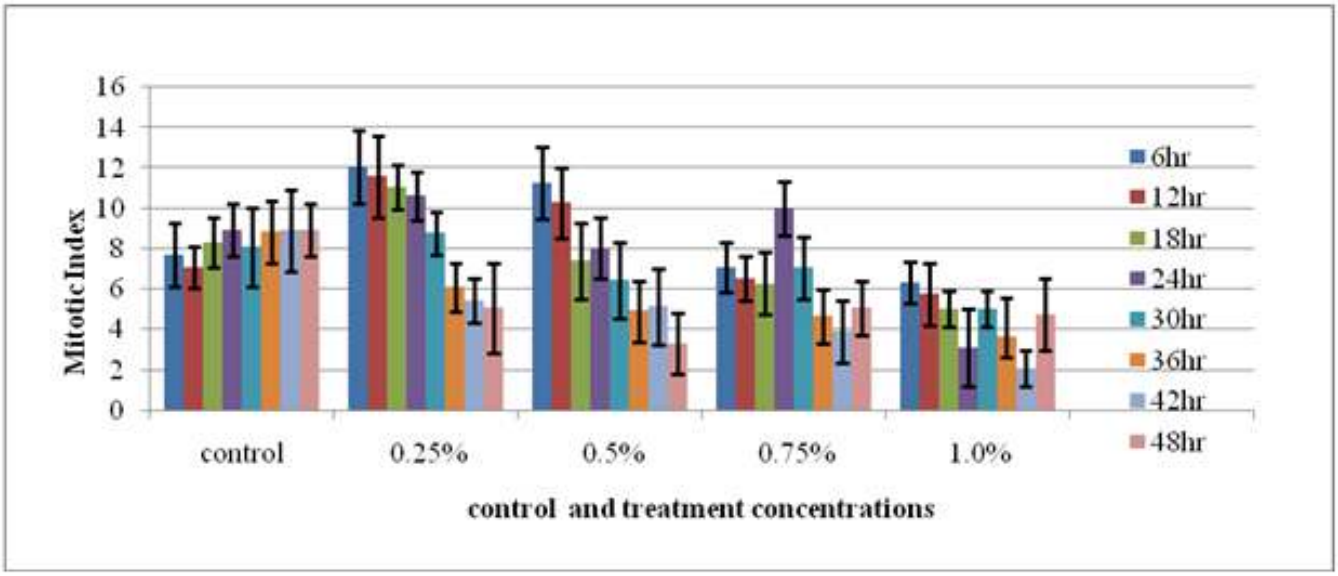


Figure 4: Mitotic phases of *Allium cepa* root cells: A, B, C-normal metaphase, anaphase, pro metaphase; D, F- Anaphase Bridge, E- Sticky Anaphase, G-clumped metaphase, H-unequal separation, I- Fragmentation. Bar =10µm

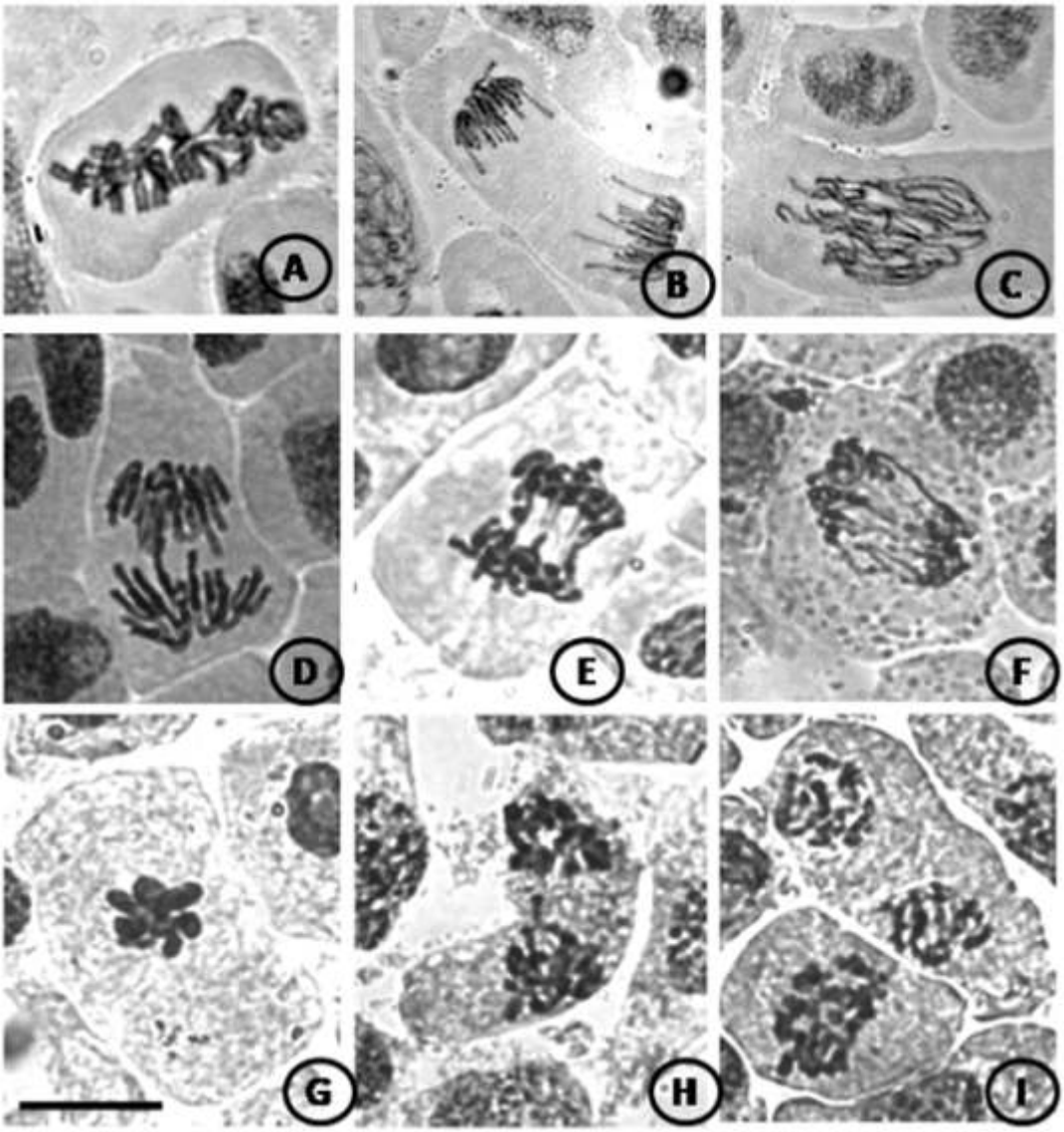


Figure 5: Aberrant cell Frequency obtained with leaf and stem extract treatment

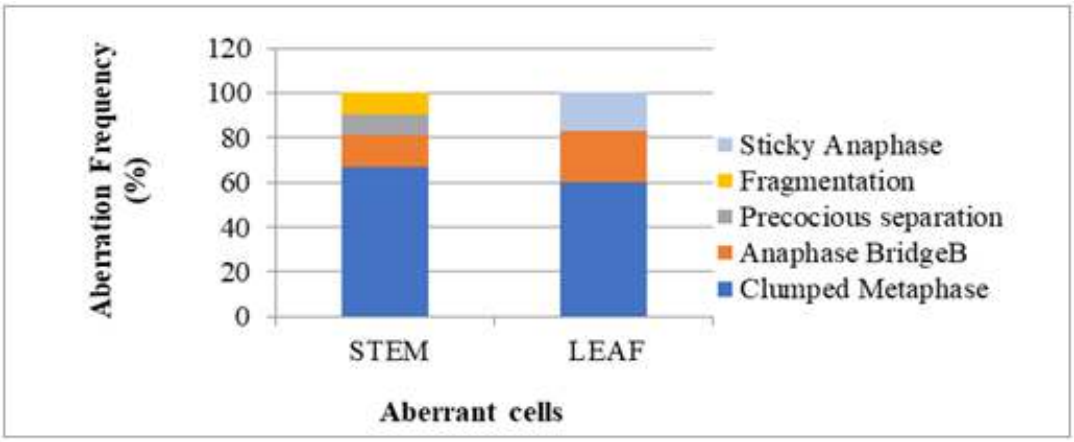


Table 1: Mitotic Indices of *Allium cepa* root meristem cells recorded after exposure to different concentration and duration of *Melastoma* leaf and stem

Mitotic Index (Mean± SEM) at control and treatment concentration and duration with leaf extract								
Treatment	**6 hr**	**12 hr**	**18 hr**	**24 hr**	**30 hr**	**36 hr**	**42 hr**	**48 hr**
Control	7.71±1.60	7.09±1.02	8.3±1.23	8.95±1.32	8.08±1.98	8.85±1.54	8.92±2.01	8.95±1.32
0.25g/100ml	14.39±1.55	14.91±1.35	15.07±1.01	15.48±3.02	17.12±2.26	18.28±1.11	19.03±1.42	21.41±2.73
0.5g/100ml	7.06±0.92	4.76±0.96	9.4±2.00	6.29±1.61	11.75±1.69	8.14±2.07	7.4±2.18	12.03±1.61
0.75g/100ml	16.5±1.04	11.06±1.6	10.21±1.39	10.0±1.02	9.05±1.82	8.47±1.95	7.52±2.05	7.12±0.96
1 g/100ml	6.95±1.32	16.03±1.02	5.06 ±1.34	4.02±1.80	4.95±1.06	4.05±1.48	3.65±1.06	3.42±1.08
Mitotic Index (Mean± SEM) at control and treatment concentration and duration with stem extract								
Treatment	**6 hr**	**12 hr**	**18 hr**	**24 hr**	**30 hr**	**36 hr**	**42 hr**	**48 hr**
Control	7.71±1.60	7.09±1.02	8.3±1.23	8.95±1.32	8.08±1.98	8.85±1.54	8.92±2.01	8.95±1.32
0.25g/100ml	12.06±1.8	11.59±2.01	11.08±1.09	10.62±1.21	8.76±1.09	6.09±1.21	5.43±1.09	5.08±0.21
0.5g/100ml	11.27±1.8	10.28±1.76	7.39±1.87	8.06±1.51	6.43±1.87	4.92±1.51	5.14±1.87	3.29±1.01
0.75g/100ml	7.1±1.23	6.54±1.08	6.28±1.54	10.0±1.31	7.08±1.54	4.65±1.31	3.9±1.54	5.07±1.31
1 g/100ml	6.34±1.04	5.75±1.54	5.02±0.87	3.09±0.42	5.02±0.87	3.65±0.92	2.08±0.87	4.76±1.78

Table 2: Calculated values of Chromosomal Aberration Rate and Aberration Frequency for distinct aberrant cells on treatment with different

Chromosomal Aberration Rate and Aberration Frequency for distinct aberrant cells				
Aberrant cell type	**Chromosomal Aberration Rate (Mean ± SEM)**		**Aberrant cell Frequency (%)**	
	Stem	**Leaf**	**Stem**	**Leaf**
Clumped Metaphase	**3.82 ±0.41**	**2.13±0.53**	**66.67**	**60.0**
Anaphase Bridge	**3.13±0.0**	**2.07±0.22**	**14.29**	**22.9**
Sticky Anaphase	**-**	**2.01±0.0**	**-**	**17.1**
Precocious separation	**2.35±0.0**	**-**	**9.52**	**-**
Fragmentation	**1.78±0.0**	**-**	**9.52**	**-**

Supplementary table for aberrant cell types observed with stem and leaf extract treatment

Table 1:- Effect of water extract of *M.malabatricum* Leaf on Root Meristem Cell of *A.cepa* L During 6hrs of treatment

Control & experimental treatment (w/v)	Total no. of cell	Total no. of dividing cell	Mitotic Index± SD	***Percentage (%)***			
				Prophase	Metaphase	Anaphase	Telophase
Control	1788	132	7.71±1.60	12.75	2.85	1.35	0.00
500mg/200ml	1613	233	14.39±1.55	30.15	0.90	0.15	0.00
1gm/200ml	1844	129	7.06±.92	12.6	3.60	3.00	0.60
1.5gm/200ml	1891	313	16.5±1.04	21.3	14.10	9.15	2.40
2gm/200ml	1685	115	6.95±1.32	10.65	3.30	1.95	0.75
2.5gm/200ml	1932	320	16.85±1.23	25.65	12.00	6.75	4.00

Table 2:- Effect of water extract of *M.malabatricum L* Leaf on Root Meristem Cell of *A.cepa L* During 12hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1645	123	7.09±1.02	9.08	2.60	0.34	0.15
500mg/200ml	1615	240	14.91±1.35	30.15	0.90	0.15	3.60
1gm/200ml	1874	91	4.76±.96	8.25	2.85	1.95	0.45
1.5gm/200ml	1820	197	11.06±1.6	15.45	5.55	2.70	1.35
2gm/200ml	1664	110	5.25±1.02	9.30	3.25	2.83	0.65
2.5gm/200ml	1924	215	16.03±1.55	17.23	7.95	3.40	0.98

Table 3:- **Effect of water extract of M.malabatricum Leaf on Root Meristem Cell of *A.cepa* L During 18hrs of treatment**

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index+ standard deviation	***Percentage (%)***			
				Prophase	Metaphase	Anaphase	Telophase
Control	1569	134	8.30±1.23	15.90	5.50	0.34	0.15
500mg/200ml	1632	245	15.07±1.01	30.90	1.20	0.45	3.30
1gm/200ml	1895	177	9.4±2.00	9.75	7.80	6.60	2.40
1.5gm/200ml	1835	202	10.21±1.39	17.85	5.85	3.90	2.70
2gm/200ml	1675	110	5.06±1.34	5.45	3.82	2.85	0.96
2.5gm/200ml	1968	299	15.36±.78	12.75	36.15	5.55	2.85

Table 4:- Effect of water extract of *M.malabatricum L.* Leaf on Root Meristem Cell of *A.cepa L* During 24hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index+ standard deviation	***Mitotic Percent (%)***			
				Prophase	Metaphase	Anaphase	Telophase
Control	1324	139	8.95±1.32	16.45	4.87	3.21	0.23
500mg/200ml	1393	212	15.48±3.02	25.35	2.55	3.30	0.30
1gm/200ml	1533	100	6.29±1.61	7.95	3.30	2.70	0.60
1.5gm/200ml	1739	174	10.00±1.31	14.55	6.00	4.95	0.30
2gm/200ml	1565	97	4.02±1.02	5.32	3.07	1.56	0.65
2.5gm/200ml	2087	295	15.24±1.80	16.95	20.4	5.85	1.05

Table 5:- Effect of water extract of *M.malabatricum L.* Leaf on Root Meristem Cell of *A.cepa L* During 30hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1376	134	8.08±1.98	17.98	6.09	1.23	0.12
500mg/200ml	1456	250	17.12±2.26	30.75	3.00	3.45	0.15
1gm/200ml	1818	207	11.75±1.69	31.05	7.35	5.85	2.85
1.5gm/200ml	1859	169	9.05±1.82	16.95	3.75	2.85	1.80
2gm/200ml	1554	102	4.95±1.06	11.06	4.98	3.23	0.78
2.5gm/200ml	1987	262	13.22±1.07	19.05	12.45	4.80	3.30

Table 6:- Effect of water extract of *M.malabatricum* L. Leaf on Root Meristem Cell of *A.cepa L* during 36hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1432	176	8.85±1.54	23.98	6.43	4.21	1.09
500mg/200ml	1441	259	18.28±1.11	32.55	3.00	3.15	0.00
1gm/200ml	1958	156	8.14±2.07	9.00	6.60	6.15	0.75
1.5gm/200ml	1855	162	8.47±2.0	14.4	3.90	3.30	2.70
2gm/200ml	1743	98	4.05±1.95	8.40	3.75	1.80	0.90
2.5gm/200ml	1983	241	12.10±1.48	22.95	7.80	3.45	1.95

Table 7: Effect of water extract of *M.malabatricum L.* Leaf on Root Meristem Cell of *A.cepa L* during 42hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1432	145	8.92±2.01	23.09	5.01	2.12	0.54
500mg/200ml	1446	274	19.03±1.42	33.3	3.30	3.60	0.75
1gm/200ml	1831	137	7.40±2.18	11.7	3.75	4.50	1.35
1.5gm/200ml	1831	139	7.52±2.05	11.7	4.50	3.90	1.35
2gm/200ml	1745	87	3.65±1.06	13.05	6.75	2.25	0.90
2.5gm/200ml	1796	204	11.19±1.01	18.3	6.75	3.45	2.10

Table 8: Effect of water extract of M.malabatricum Leaf on Root Meristem Cell of A.cepa L during 48hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1432	143	8.95±1.32	24.12	11.09	2.30	1.09
500mg/200ml	1348	287	21.41±2.73	22.2	8.40	11.40	0.75
1gm/200ml	1812	219	12.03±1.61	10.8	8.70	9.90	3.45
1.5gm/200ml	1534	114	7.15±0.96	7.80	4.80	3.30	1.20
2gm/200ml	1822	75	3.42±1.08	5.40	3.30	1.80	0.75
2.5gm/200ml	1859	174	9.29±1.79	13.8	5.85	3.90	2.55

Table 9: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of *A.cepa L* during 6hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)				Remarks
				Prophase	Metaphase	Anaphase	Telophase	
Control	1788	132	7.71±1.60	12.75	2.85	1.35	0.00	
500mg/200ml	1365	223	12.06±1.87	22.3	3.45	8.95	1.08	
1gm/200ml	1746	198	11.27±1.83	18.9	4.50	5.25	0.00	
1.5gm/200ml	2035	113	7.10±1.23	8.70	5.40	0.00	1.20	
2gm/200ml	1845	97	6.34±1.04	5.43	7.47	2.76	2.12	
2.5gm/200ml	1758	76	4.21±1.76	3.89	5.23	0.98	2.32	

Table 10: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of *A.cepa L* during 12hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)				Remarks
				Prophase	Metaphase	Anaphase	Telophase	
Control	1645	123	7.09±1.02	9.08	2.60	0.34	0.15	
500mg/200ml	1743	205	11.59±2.01	19.32	6.76	3.05	4.37	
1gm/200ml	1835	176	10.28±1.76	10.26	6.08	2.08	2.32	
1.5gm/200ml	1894	118	6.54±1.08	6.15	5.27	3.56	1.65	
2gm/200ml	1965	76	5.75±1.54	4.53	3.09	1.78	0.00	
2.5gm/200ml	1739	57	3.08±1.04	2.82	1.08	1.95	0.85	

Table11: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of *A.cepa L* during 18hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1569	134	8.30±1.23	15.90	5.50	0.34	0.15
500mg/200ml	1875	197	11.08±1.09	16.87	6.24	5.12	2.76
1gm/200ml	1966	146	7.39±1.87	14.25	3.45	2.85	1.65
1.5gm/200ml	1982	112	6.28±1.54	12.87	4.56	2.14	1.12
2gm/200ml	1846	85	5.02±.87	9.67	2.78	1.65	0.00
2.5gm/200ml	1893	67	3.92±.76	3.54	0.93	0.00	0.69

Table 12: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of *A.cepa L* during 24hrshrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1324	139	8.95±1.32	16.45	4.87	3.21	0.23
500mg/200ml	1987	198	10.62±1.21	9.76	10.98	6.89	1.65
1gm/200ml	1966	161	8.06±1.51	10.8	9.00	5.25	0.15
1.5gm/200ml	1739	174	10.00±1.31	15.0	6.00	4.95	0.00
2gm/200ml	1765	114	3.09±1.92	9.23	3.65	1.87	0.34
2.5gm/200ml	1874	97	2.27±1.42	5.98	1.13	0.00	0.98

Table 13: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of *A.cepa L* during 30hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1376	134	8.08±1.98	17.98	6.09	1.23	0.12
500mg/200ml	1875	145	8.76±1.09	10.87	6.24	5.12	2.76
1gm/200ml	1966	123	6.43±1.87	12.25	3.45	2.85	1.65
1.5gm/200ml	1982	135	7.08±1.54	9.87	4.56	2.14	1.12
2gm/200ml	1846	117	5.02±.87	6.67	2.78	1.65	0.86
2.5gm/200ml	1893	97	4.94±.76	3.54	0.00	0.12	0.69

Table 14: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of A.cepa L during 36hrs of treatment

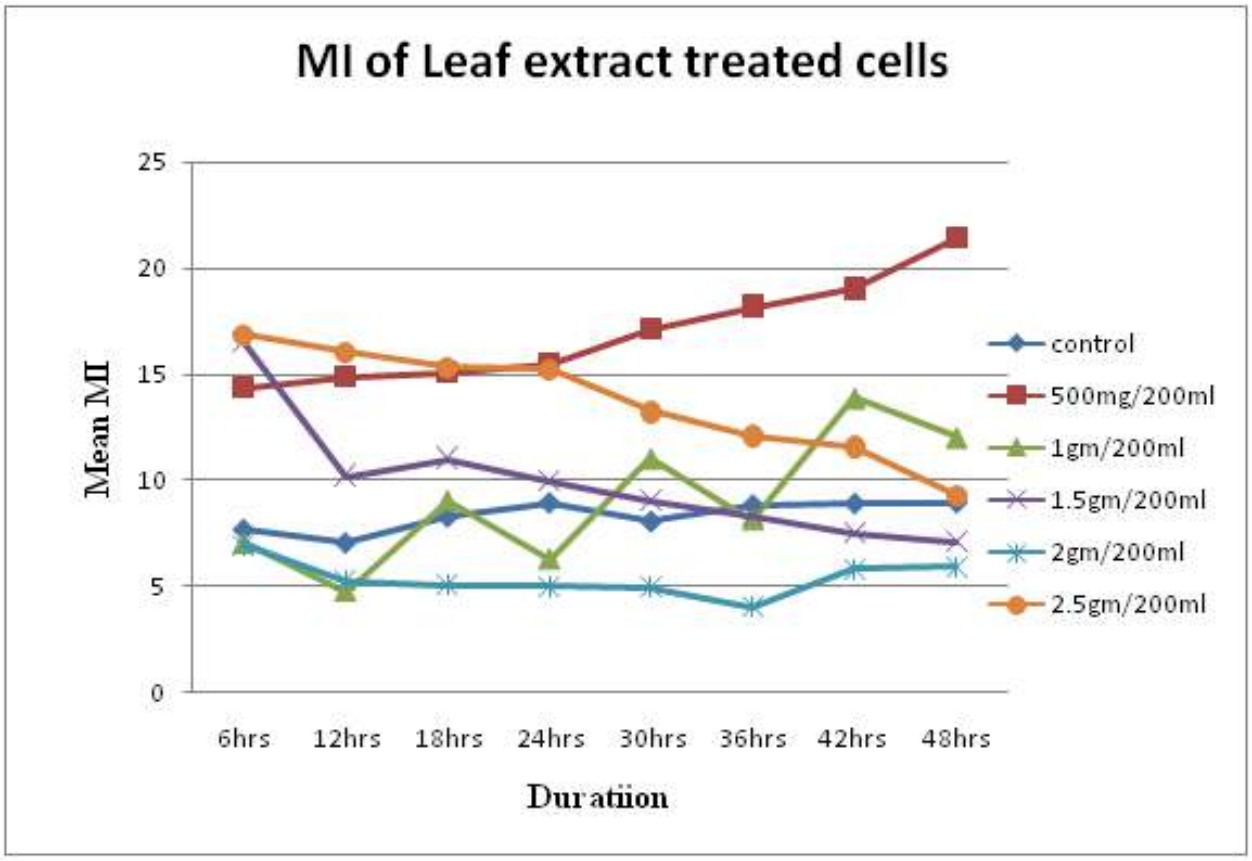
Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1432	176	8.85±1.54	23.98	6.43	4.21	1.09
500mg/200ml	1987	132	6.09±1.21	9.76	8.98	5.89	1.65
1gm/200ml	1966	109	4.92±1.51	10.8	7.0	4.25	0.15
1.5gm/200ml	1739	115	4.65±1.31	15.0	6.00	4.95	0.00
2gm/200ml	1765	96	3.65±1.92	9.23	3.65	1.87	0.34
2.5gm/200ml	1874	76	2.78±1.42	5.98	1.13	0.59	0.98

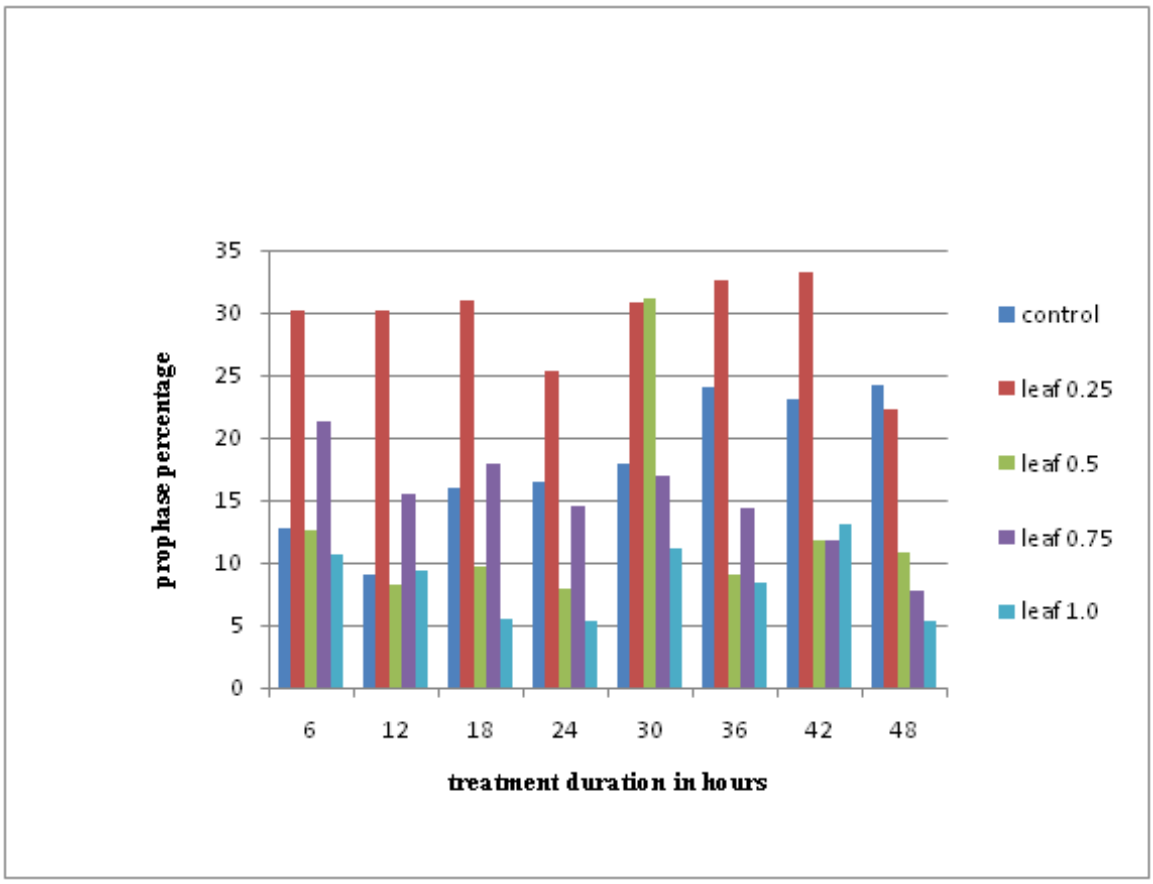
Table 15: Effect of water extract of *M.malabatricum L* stem on Root Meristem Cell of *A.cepa L* During 42hrs of treatment

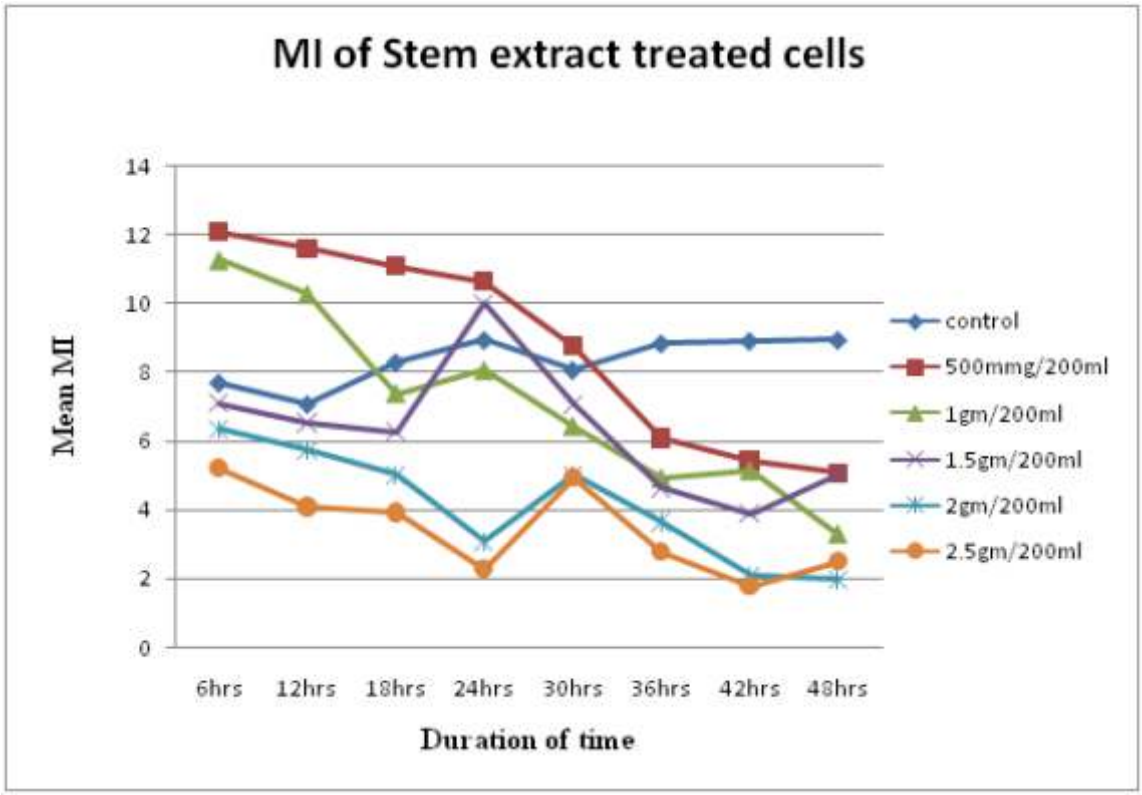
Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1432	145	8.92±2.01	23.09	5.01	2.12	0.54
500mg/200ml	1875	113	5.43±1.09	12.87	6.24	5.12	2.76
1gm/200ml	1966	121	5.14±1.87	11.25	3.45	2.85	1.65
1.5gm/200ml	1982	96	3.90±1.54	10.87	4.56	2.14	1.12
2gm/200ml	1846	85	2.08±.87	6.67	2.78	1.65	0.86
2.5gm/200ml	1893	67	1.79±.76	3.54	0.93	0.00	0.69

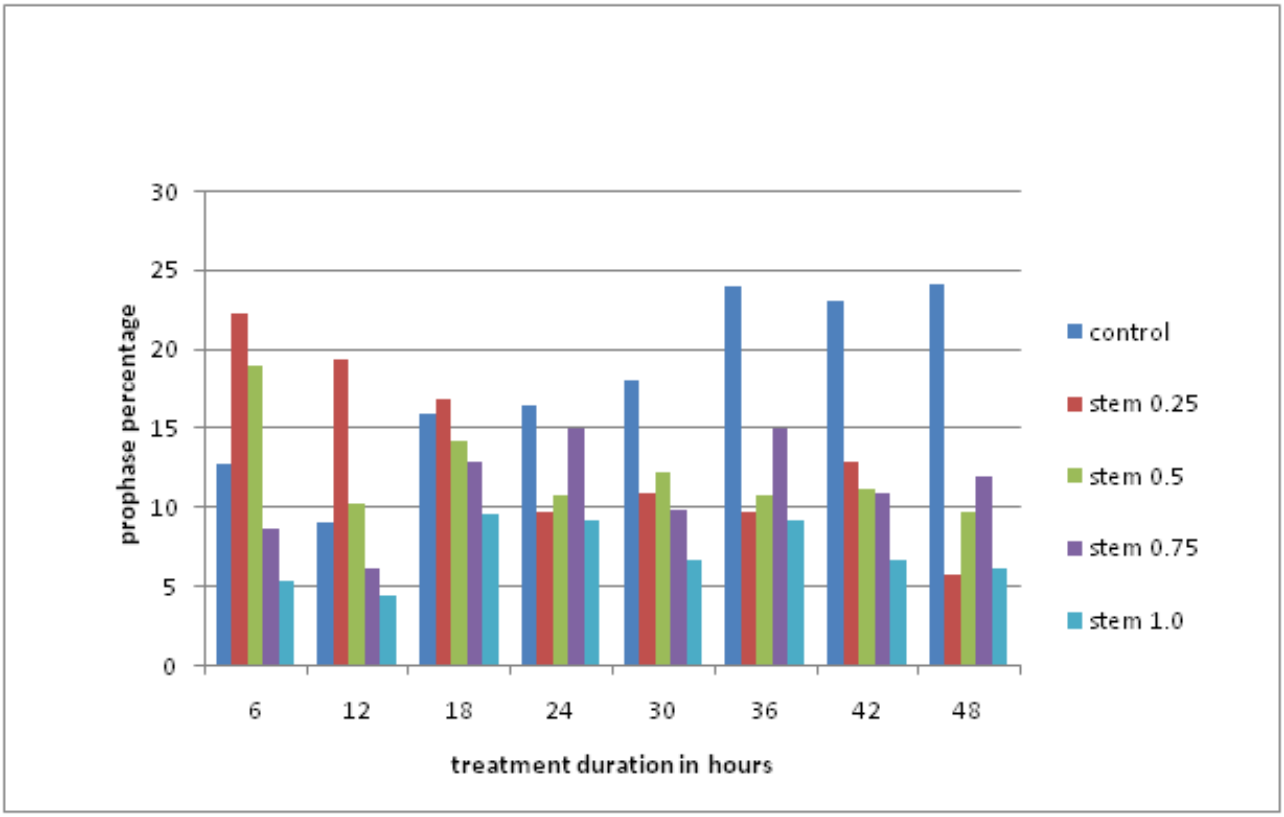
Table 16: Effect of water extract of *M.malabatricum L.* stem on Root Meristem Cell of *A.cepa L* during 48hrs of treatment

Control & experimental treatment (w/v)	Total no of cell	Total no of dividing cell	Mitotic Index± standard deviation	Percentage (%)			
				Prophase	Metaphase	Anaphase	Telophase
Control	1432	143	8.95±1.32	24.12	11.09	2.30	1.09
500mg/200ml	1987	102	5.08±2.21	5.76	10.98	6.89	1.65
1gm/200ml	1966	94	3.29±1.51	9.80	8.00	5.25	0.15
1.5gm/200ml	1739	112	5.07±1.31	12.0	6.00	4.95	0.00
2gm/200ml	1765	98	4.76±1.78	6.23	3.65	1.87	0.34
2.5gm/200ml	1874	97	2.52±1.42	5.98	1.13	0.00	0.98


MI of Leaf extract treated cells
Mean MI
25
20
15
10
5
0
6hrs
12hrs
18hrs
24hrs
30hrs
36hrs
42hrs
48hrs
Duration
control
500mg/200ml
1gm/200ml
1.5gm/200ml
2gm/200ml
2.5gm/200ml


prophase percentage
35
30
25
20
15
10
5
0
6
12
18
24
30
36
42
48
treatment duration in hours
control
leaf 0.25
leaf 0.5
leaf 0.75
leaf 1.0


MI of Stem extract treated cells
Mean MI
14
12
10
8
6
4
2
0
6hrs
12hrs
18hrs
24hrs
30hrs
36hrs
42hrs
48hrs
Duration of time
control
500mmg/200ml
1gm/200ml
1.5gm/200ml
2gm/200ml
2.5gm/200ml


prophase percentage
30
25
20
15
10
5
0
6
12
18
24
30
36
42
48
treatment duration in hours
control
stem 0.25
stem 0.5
stem 0.75
stem 1.0

Two way ANOVA performed with concentration and duration of treatment with control for leaf and stem extract

TWO WAY ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Treatment Duration	232.5843	15	15.50562	**2.28308**	0.012451	**1.836437**
Treatment Concentration	567.122	4	141.7805	**20.87606**	8.03E-11	**2.525215**
Error	407.4922	60	6.791536			
Total	1207.198	79				

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