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Cyto-genotoxicity of *Parthenium hysterophorus* Plant Extract on *Allium cepa* Plant Assay

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ABSTRACT: *Parthenium hysterophorous* also known as congress grass a well-known weed in farmer's field which caused harm to crops as well as health of farmers. Therefore, current research paper deals with the assessment of *Parthenium hysterophorus* plant extract on *Allium cepa* plant assay for their evaluation of cyto-genotoxicity potential. The effect of *Parthenium* plant extract was studied on meristematic root tips of *A. cepa* to determine the cytotoxicity in onion root tips. Root tips of onion were treated with a series of concentrations *viz*. 2.5 %, 5.0 % and 7.5 % for 24 and 48 hours. Acetocarmine staining was done to visualize the mitotic stages. Extract effect on the relative durations and concentrations of each mitotic stage as compared with control treatments. Mitotic index was decreased significantly with increasing concentration of *Parthenium* extract but as the duration of exposure of extract was increased the MI% decreased in comparison to control. The RDR (Relative Division Rate) considerably became more negative and RAR (Relative Abnormality Rate) was increased along with increasing doses of *Parthenium* extract. But RDR was more negative with 24 hours of treatment where RAR increased with prolonged treatment. Increment in the negative value of RDR was directly proportional to the severity of the mitotic inhibition. Several chromosomal aberrations were recorded i.e., early prophase, sticky metaphase, C metaphase, disturbed metaphase and anaphase, forward metaphase anaphase and telophase chromosomes, laggards in anaphase and telophase, bridges at anaphase and telophase, micronucleus at inter-phase. Thus, it was concluded that with the increasing concentration of *Parthenium* plant extract in both the durations the total percent of abnormal cells was increased followed by decrease in Mitotic index which shows that *Parthenium* plant extract have potential to cause cytotoxicity as well as genotoxicity in *A. cepa* and a threat to other agronomic crops.

Keywords: *Parthenium hysterophorus, Allium cepa,* cyto-genotoxicity, chromosomal aberrations, inter-phase, laggards, bridges.

INTRODUCTION

Parthenium hysterophorus is an annual herbaceous plant that can reach a height of 1.5 m in native range, but can grow up to 2.5 m but it is an invasive species (Navie *et al.*, 1996). The leaves are long, pale green with lower leaves that are deeply pinnately lobed, 80 mm to 200 mm long and covered with soft fine hairs. The flowers are creamy-white and form small, compact heads about 3 mm across with five corners each containing a black seed, forming multi-branched clusters. This annual herb belongs to the family Asteraceae. Only one species *Parthenium hysterophorus* L. is found in India. It was accidently introduced to India along with the PL 480 Mexican wheat seeds in the year 1954 supplied by USA under PL-480 project (Public Law 480 passed in 1954 to give food grains to developing countries) (Apurva *et al.*, 2010). *Parthenium* has declared a noxious weed through legislation and policy for management enacted by the governments of Australia, South Africa, Sri Lanka and India, and recently within the East African region (Amer, 2021; Witt *et al*., 2021). Most literatures searched showed that *Parthenium hysterophorus* (2n=34) a weed which grows at field without any special attention and traditionally people use for medicinal purpose for treating fever, diarrhoea, neurologic disorders, urinary tract infections, dysentery, malaria and as emmenagogue (Gurib-Fakim *et al*., 1996). *Parthenium hysterophorus* has been found to

be pharmacologically active as analgesic in muscular rheumatism, therapeutic for neuralgia and as vermifuge (Maishi *et al*., 1998). Besides, having such beneficial aspects it also caused allergies to the people working in the field which is *Parthenium hysterophorus* affected (Prinsloo *et al*., 2018).

Number of cyto-genotoxicity testing assays have been developed but *Allium cepa* root assay was considered as a most effective one (Bollu *et al*., 2016; Algarni, 2018; Bonciu *et al*., 2016). Several studies have been carried out to observe the genotoxicity of various noxious weeds using plants systems such as *Allium cepa, Vicia faba. Tradescantia* etc. as bioindicators and interpreting the results with animal systems. The effects of plant extracts on eukaryotic nuclei can be assessed cytologically by observing inhibition of cell growth or division, interruption of metaphase or the induction of numerical and structural chromosomal aberrations and changes among sister and other chromatids (Fiskesjo, 1985). *Allium cepa* root-tip cell tests to investigate the cytotoxic and mutagenic potential of aqueous extract of this plant considered as model test plant. *Allium* genus, especially the use of *A. cepa* (chromosome number is 2n=16) for bio-monitoring of genotoxicity is considered to be very efficient (Datta *et al*., 2018). The parameters employed in measuring related mutagenicity and genotoxicity were the alterations in the mitotic index and changes in chromosome structure and behaviour during mitosis. Therefore, in present study we tested the three different concentrations of *Parthenium* plant extract and exposed to *Allium cepa* roots for 24 and 48 hours to evaluate the cyto-genotoxicity of *Parthenium hysterophorus*.

MATERIAL AND METHOD

Experimental material and design

The present investigation entitled, "Assessment of molecular markers to detect the DNA damage caused by *Parthenium* plant extract" was carried out at Post Graduate Laboratory of Department of Molecular Biology and Genetic Engineering, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.) India and Molecular work was carried out at Molecular Biology Lab (MBL) at Department of Genetics and Plant Breeding, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.) India during 2019-20. Geographically Meerut is situated between 29°01 latitude in North and 77°43 longitudes in the Eastern elevation of about 219.75 meters above mean sea level. In order to achieve the results following materials as well as methods have been used. The study was carried out by using a laboratory study to examine the cytotoxicity and genotoxicity of aqueous extract of *Parthenium hysterophorus* on *A. cepa* root tips.

Cytotoxicity and genotoxicity of *Parthenium* plant extract. The test involves the chromosomal aberration test on *Allium cepa*. The test procedure involved original form of *Allium cepa* test (Fiskesjso, 1985).

Test species and treatments. The test species included the *Allium cepa*. Onion bulbs were purchased from local market. A total of three treatments with *Parthenium* extract applied at different concentrations (2.5%, 5% and 7.5% *Parthenium* w/v plant extract).

Preparation of *Parthenium* plant extract. Fresh leaves and inflorescence of *Parthenium hysterophorus* L. were collected in their growth stage from the nearby fields around the campus of Sardar Vallabhbhai Patel University of agriculture and technology, Meerut. The aqueous extract of *Parthenium* leaves and inflorescence prepared using the protocol described by Devi *et al.* (2012). 20 gm of fresh leaves and inflorescence of *Parthenium* plant was weighed using electronic balance and grinded to fine paste in 200 ml distilled water using laboratory blender. This paste is filtered through filter paper cones. The volume of the filtrate was adjusted to 100 ml. This made 20% stock solution of *Parthenium* plant extract. Now, a series of dilutions with different strengths was prepared from this 20% stock solution using distilled water. Finally, three treatment solutions (i.e., 2.5%, 5%, 7.5%) were used to expose test plants. Distilled water was used as control. Different solutions of *Parthenium* plant extract were prepared as mentioned below:

Preparation of stock solutions. i. 2.5% aqueous extract: 7.5 ml of 20% stock solution was added and volume was made up to 60 ml using distilled water.

ii. 5% aqueous extract: 15 ml of 20% stock solution was added and volume was made up to 60 ml using distilled water.

iii. 7.5% aqueous extract: 22.5 ml of 20% stock solution was added and volume was made up to 60 ml using distilled water.

Methodology and observations. Outer scales of healthy and uniform bulbs of *Allium cepa* were removed and apices of the root primordial exposed in beaker containing tap water. The root tips of onion were treated with different concentrations of *Parthenium* extract. The treated root tips were squashed to assess the cytotoxicity by detecting chromosomal abnormalities. The procedure of cytological test was followed using the methods described by Sinha (2009); Puthanpura *et al*. (2017). Where the bulbs were allowed to grow on the mouth of flask filled with tap water. When the bulbs started rooting (1-1.5 mm length), the distilled water was changed and the bulbs were placed again on the mouth of fresh flask containing different concentrations (2.5%, 5% and 7.5%) of *Parthenium* plant extracts along with the control (only in distilled water). The flasks were maintained under laboratory conditions. The treatment was done for 24 and 48 with three replicates. The root tips of each concentrations including control were harvested after 24- and 48-hours treatment and were fixed in 1:3 carnoy's fixative (methanol: glacial acetic acid) separately for 24 hours and then stored in 70% alcohol for future analysis. From each concentration 10 root tips were collected for analysis. The conventional slide preparation method was selected in which acid hydrolysis of cellulosic cell wall was done in warmed 1N hydrochloric acid for 10-15 minutes and squashes were made in 2% acetocarmine containing 45% glacial acetic acid to visualize the dividing cell stages of mitosis.

Preparation of 2% acetocarmine stain-2 gm of carmine powder was dissolved in 100 ml of 45% glacial acetic acid by heating and stirring it on hot plate. When the solution cooled down it was filtered with 2.5 D filter paper and then 2 ml of 5% $FeCl_2$ solution was added. The solution was kept in refrigerator for 24 hours.

Preparation of 1N HCL (100 ml)-91.8 ml of distilled water was taken and 8.2 ml of conc. HCL was added in it. Mixed well and stored in a glass bottle.

Observations Recorded: Cytotoxicity was determined in terms of measuring parameters as mitotic indices, mito-depressive effect of *Parthenium* extract and percentage of chromosomal abnormalities induced according to the following formulae-Mitotic index calculated using the formulae of Fiskesjo (1997):

$$\text{Mitotic index(M.I)} = \frac{\text{Number of dividing cells in the treatment}}{\text{Total number of cells observed}} \times 100$$

$$\text{Mito - depressive effect (MD\%)} = \frac{\text{M.I of control - M.I of treated cell}}{\text{M.I of control}} \times 100$$

Relative Division Rate (RDR) calculated using the formulae of Kumar and Shikha (2012):

$$\text{RDR} = \frac{\text{\% dividing cells in treated variant - \% of dividing cell in control}}{\text{100 - \%dividing cells in treated variant}} \times 100$$

Relative Abnormality Rate (RAR) or percentage of chromosomal calculated using formulae of Kumar and Shikha (2012); Akinboro and Bakare (2007) Sinha (2009):

$$\text{Relative Abnormality Rate (RAR} = \frac{\text{Number of aberrant cells}}{\text{Total number of dividing cells}} \times 100$$

Scoring and analysis for cytotoxicity. A total of 10 slides were prepared for each concentration of treatment (Murali and Panda 2010). The slides were observed under binocular light microscope using 40X objective lens. On each of 10 slides (n = 10) for each concentration and duration (24 and 48 hours), a total of 100 cells, classified into dividing cells (prophase, metaphase, anaphase and telophase) were scored as a total of 1000 cells each for the control and treatment groups. The mitotic index (M.I) was expressed as the number of dividing cells per 1000 cells scored. A dose of treatment was considered cytotoxic if the mitotic index of treated cells was ≤ ½ of the mitotic index of the water treated cells. Similarly, on each of 10 slides for each dose, a total of 100 cells were scored and examined for chromosome aberrations in all stages of mitosis (prophase, metaphase, anaphase and telophase) per treatment. The cytotoxic level was determined by the decreased rate of mitotic index.

RESULTS AND DISCUSSION

A. Cytotoxicity of aqueous extract of Parthenium plant on Allium cepa

(i) Mitotic index. From the point of view of the cytotoxicity effect induced by the two treatment durations (24 hours and 48 hours) of different concentrations of *Parthenium* plant extract to *Allium cepa*, the results indicate that the MI decreased in all variants with increased concentration (Table 1). Higher percent decrease in MI over control was notice in 24 hours duration in comparison to 48 hours duration in all the tested concentration of *Parthenium* plant extract.

At 24 hrs duration % decrease in MI over control was in a range of 58 % (2.5 % concentration) to 64 % (7.5% concentration). Whereas, this range was 33 % (2.5 % concentration) to 42 % (7.5 % concentration) at 48 hrs duration (Table 1 and Fig. 1. (a)). Fiskesjo (1995) also reported a significant decline in MI due to the effect of toxic chemicals on spindle apparatus. The MI is contemplated to be reliable for identification of cytotoxic pollutant present in the environment. This reduction in mitotic index reveals that *Parthenium* extract interferes with normal sequences of cell cycle. MI reduction may be due to the arrest of cells in G1 phase or retardation in the pace of events during S1 or S2 phases. This may cause mitotic poison which can lead to metabolic imbalance and thus, interfere the structure and synthesis of DNA resulting in change in chromosomes during cell division. Datta *et al*., (2018) also reported the same using pesticide and vermicompost treated soil with *Allium cepa* test. Bittell *et al*. (1974) reported that extracts from harmful plants seems to inhibit electron and energy transfer. The cytotoxic and all elopathic potential of *Parthenium* weed might have resulted from the release of phytotoxic substances reported by Kumar *et al.* (2006). Reduction in MI also clearly shows that potentiality of *Parthenium* extract to be useful as an anticancer agent and is able to kill the actively dividing cells. The current data shows that the *Parthenium* extract has the cytotoxic effects on *A. cepa.*

(ii) Mito-Depressive effect (MD). The Mito-Depressive effect (MD) was increasing with increase in concentration in both the treatment period (Table 1). The MD effect (%) showed that there was reduction in number of dividing cells with increase in concentration of *Parthenium* plant extract. The highest MD effect was observed in 7.5 % concentration of *Parthenium* plant extract i.e., 63.48 % and 41.44 % at 24 and 48 hours of treatment period, respectively. Whereas, least MD was observed in 2.5 % concentration of both the treatment period i.e., 57.68 % (24 hours) and 32.35 % (48 hours) (Fig. 1. (b)). As the period of treatment prolonged the Mito-Depressive effect (%) was decreased i.e., the MD in 48 hours of treatment period varied from 32.35 (2.5 %) to 41.44 (7.5 %). While, MD in 24 hours of treatment period varied from 57.68 (2.50 %) to 63.48 (7.5 %) (Fig. 1. (b)). Present study shows a clear mito-depressive properties of the *Parthenium* plant extract, which is evident from lowering of the mitotic index and

manifestation of spindle formation. Mito-inhibition by *Parthenium* plant extract has been attributed to blocking of mitotic cycle during interphase that may result from a prolonged G2 period or to the inhibition of DNA synthesis. Udengwu and Chukwujekwu (2008); Gupta *et al*. (2020) also reported the increase in mito-depressive effect using Bleaching Creams on *Allium cepa* and arsenic contaminated soil on *Vicia faba* respectively.

(iii) Relative Division Rate (RDR). The RDR values which reflect the mitotic inhibition shown in Table 1. Effect of three different concentrations (2.5 %, 5.0 % and 7.5 %) of *Parthenium* plant extract was compared with control conditions in both durations (24 hours and 48 hours) of onion root treatments. Different concentrations of *Parthenium* plant extract had negative effects on RDR. Generally, increase in negative RDR values was observed under treated conditions and a decrease in negative value was recorded as the treatment period is prolonged (48 hours). In controls of 24 and 48 hours treated cells RDR was zero.

In 24 hours of treatment period maximum negative value was observed in 7.5 % concentration of *Parthenium* plant extract i.e., -21.05 and minimum negative value was recorded in 2.5 % concentration i.e., -19.29 followed by -20.0 in 5.0 % concentration of *Parthenium* plant extract (Table 1). Similarly, in 48 hours of treatment period maximum negative value was observed in 7.5 % concentration of *Parthenium* plant extract i.e., -19.85 and minimum negative value was recorded in 2.5 % concentration i.e., -16.20 followed by -18.60 in 5.0 % concentration of *Parthenium* plant extract (Table 1). The highest negative values were observed in 24 hours treated cell which shows that maximum reduction in cell division was in 24 hours treatments as compared to 48 hours of treatments (Table 1).

Table 1 clearly indicates that the total % of dividing cells were more in controls of both the treatment period i.e., 29.3 (24 hours) 37.4 (48 hours) as compared to treated cells. Similarly, % of dividing cells were more in 48 hours of treatment period as compared to 24 hours. Among treated concentrations (2.5 %, 5.0 % and 7.5 %), the % of dividing cells more in 2.5 % concentration and reduced in 7.5 % concentration of *Parthenium* plant extract. When, the mitotic phase frequencies were compared to the control in application times, almost all treatments significantly impressed the mitotic phase frequencies. Out of the four mitotic stages (prophase, metaphase, anaphase and telophase) the percentage of dividing cells was maximum in prophase followed by metaphase and telophase. The least number of dividing cells were observed in anaphase. Increase in the negative value of RDR was directly proportional to the severity of the mitotic inhibition. Thus, it was found that along with increasing treatment concentrations of *Parthenium* plant extract the RAR increased and the mitotic index decreased which shows potential toxicity of *Parthenium* plant at their higher concentrations in this plant Thus, various concentrations of *Parthenium* induces variety of chromosomal abnormalities. Therefore, *Parthenium* plant causes chromotoxic and mito-depressive effects in plant genomes and induce various types of chromosomal aberrations and these chromosomal aberrations revealed the potential toxicity of *Parthenium* particularly at their higher concentrations in the plant *A. cepa* which is a well-known economically important plant. Similar findings reported by Kumar and Shikha (2012) using nicotinamide different concentrations on root meristems of *Cyamopsis tetragonoloba*.

(iv) Relative Abnormality Rate (RAR). The total percent of abnormal cells were zero in control treatment for 24 hours duration. Whereas a slight increase in RAR was recorded (1.2 %) in control treatment for 48 hours duration (Table 1). The total number of abnormal cells in different mitotic stages had increased with increase in concentration of extract and duration of treatment as compared to the controls (Fig. 1. (c)). The occurrence of RAR was dependent on concentrations (2.5 %, 5.0 % and 7.5 %) as well as duration of treatment (24 and 48 hours). *Allium cepa* test showed concentration and exposure time related increase in relative abnormality rate. The Fig. 1. (d) summarizes the RAR in root tip cells of *A. cepa* exposed to three different concentrations (2.5 %, 5.0 % and 7.5 %) of *Parthenium* plant extract. Out of four mitotic stages (prophase, metaphase, anaphase and telophase) the percent of abnormal cells were more in metaphase (22.8 %) followed by prophase (20.8 %) and telophase (14.5 %). The least number of abnormal cells were observed in anaphase (13.5 %).

The highest % of abnormal cells in metaphase (5.2) were found by 7.5 % concentration when given for 48 hours of treatment period (Fig. 1. (c)). While the lowest % of abnormal cells in metaphase (2.4) were reported in 5.0 % concentration for 24 hours of treatment period (Fig. 1 (c)).

No significant RAR (Relative Abnormality Rate) was observed in control condition for 24 hours and 1.2 % of chromosomal aberrations were observed in control conditions for 48 hours (Table 1).

At 24 hours of treatment period, minimum RAR was observed at 2.5 % concentration of *Parthenium* plant extract i.e., 6.7 % and maximum RAR was observed at 7.5 % concentration of *Parthenium* plant extract i.e., 8.3 % followed by 7.4 % RAR at 5.0 % concentration of *Parthenium* plant extract (Fig. 1. (d)). Similarly, treatment period of 48 hours shows minimum RAR at 2.5 % concentration of *Parthenium* plant extract i.e., 14.4 % and maximum RAR was observed at 7.5 % concentration of *Parthenium* plant extract i.e., 17.7 % followed by 16.7 % RAR at 5.0 % concentration of *Parthenium* plant extract (Fig. 1. (d)). The RAR increased with increase in concentration of *Parthenium* plant extract and as the period of treatment prolonged. The similar results were indicated by Puthanpura *et al.* (2017); Sinha (2009) using *Parthenium* on onion. Rao *et al*. (2005) reported the increase percentage of chromosomal abnormalities as concentration increases. Kumar and Shikha (2012) also evaluated percentage of chromosomal abnormalities in terms of RAR and

reported increase in RAR value with increase in concentration. Also, Gomurgen (2005) reported the increase in aberrations with concentration increase and duration of exposure increase.

B. Genotoxicity of aqueous extract of Parthenium plant on Allium cepa

The effect of the *Parthenium hysterophorus extract* was evaluated on somatic chromosomes of *Allium cepa* L and the details of the results obtained showed genotoxicity in the present investigation which were presented below:

Types of chromosomal abnormalities: A number of chromosomal abnormalities covering all stages of mitosis were recorded after treatment with *Parthenium* plant extract (Table 2). The total percentage of these abnormalities increased gradually with increase in concentration and period of treatment. It reached a maximum value of 8.3 % and 17.7 % after treatment with 7.5 % concentration of *Parthenium* plant extract for 24 hours and 48 hours duration respectively. The value of abnormalities induced after treatment with 24 hours duration were generally low as compared with that scored in after 48 hours of treatment with all the concentrations of *Parthenium* plant extract (Table 2). Normal mitotic phases were represented in Fig. 2 (a-f).

Allium cepa test showed concentration and exposure time related increase in frequencies of chromosomal aberrations. Dhulgande *et al*. (2015) reported that the chromosomal aberrations are the good signs of deviations in the normal mechanism of the cell cycle. The somatic chromosome number in *Allium cepa* was 16. Mohandas and Grant (1972) suggested that there has been correlation between chromosomal damage and toxic effects of particular substance thus chromosomal aberrations are reliable indicators of mutagenic activities. According to Harashima and Schnittger (2010), cell division and growth of the plant body are closely related, and where growth depends upon cell proliferation. Nearly, all of the common types of known cytological aberrations have been reported in onion root tips following treatment with *Parthenium* plant aqueous extract. The following types of chromosomal abnormalities reported in the present study were given below: Late prophase (Fig. 2a), sticky metaphase (Fig. 2.b), C- metaphase (Fig. 2c), disturbed metaphase and anaphase (Fig. 2d & h), forward metaphase anaphase and telophase chromosomes (Fig. 2. e, k & o), laggards in anaphase and telophase (Fig. 2i, m & n), bridges at anaphase and telophase (Fig. 2j & l), micronucleus at interphase (Fig. 2p). The main effect of *Parthenium* plant extract was found on prophase and metaphase stages followed by telophase and anaphase abnormalities in both the treatment durations (Table 2).

Late prophase (LP) was most common aberration in all treatment durations (Fig. 2a). At metaphase most common type of aberrations was sticky metaphase (SM) (Fig. 2b), disturbed metaphase (DM) (Fig. 2.d) and forward metaphase (MF) (Fig. 2e) in both the treatments. At telophase most common type of aberration was forward telophase (TF) (Fig. 2o) in both the treatment durations (Table 2). At anaphase most common type of aberration was forward anaphase (AF) (Fig. 2k) and anaphase bridges (AB) (Fig. 2j) in 24 hours treatment duration while in 48 hours treatment period most common type of anaphase aberration was disturbed anaphase (DA) (Figure 2.h). treatment duration while in 48 hours treatment period most common type of anaphase aberration was disturbed anaphase (DA) (Fig. 2h). Rahimi *et al.* (2015) results showed that chromosomal aberrations are good indicator of assessing genotoxicity of various chemicals and toxins secreted by plants and animals.

Similar, aberrations were reported by Puthanpura *et al*. (2017) using *Parthenium* on onion; Gomurgen (2005) using potassium metasulphite and potassium nitrate food preservatives on root tips of *A. cepa* and Barroso *et al*. (2017). From the study of Olorunfemi and Ehwre (2011), chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Dutta and Ahmad (2016) reported that chromosomal abnormalities such as stickiness, bridges, and laggards are some of the indications of genotoxicity.

Table 1: Effect of different concentrations of *Parthenium* plant extract on MI, MD, RAR and RDR on root meristems of *Allium cepa.*

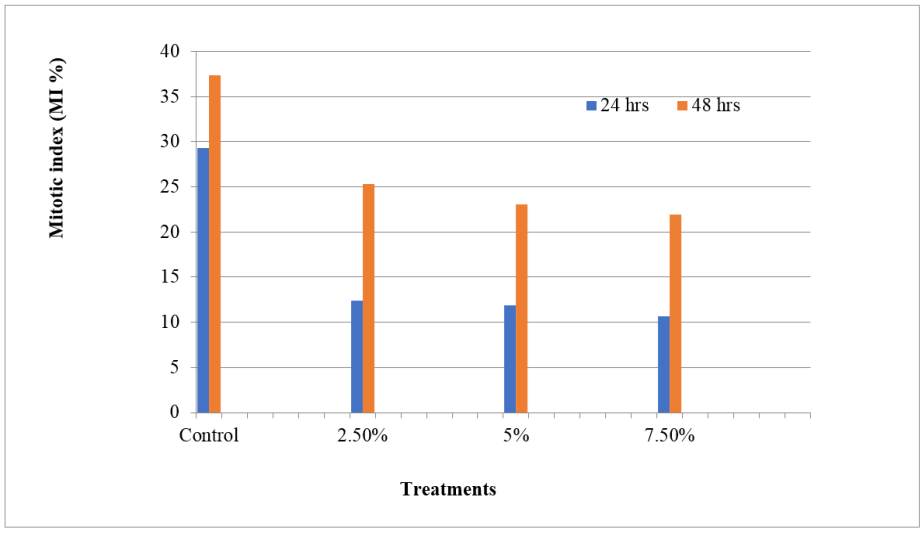
Treatments	Mitotic phase				MI%	% Decrease in MI over control	Mito-depressive effect (%)	RDR	RAR
	P (%)	M (%)	A (%)	T (%)					
24 Hrs duration									
Control	9.8	7.3	6.0	6.0	29.3	-	0	0	0
2.5 %	3.1	3.6	1.6	4.2	12.4†	58%	57.68	-19.29	6.7
5.0 %	4.0	3.0	2.2	2.7	11.9†	59%	59.39	-20.00	7.4
7.5 %	4.5	3.3	1.2	1.7	10.7†	64%	63.48	-21.05	8.3
48 Hrs duration									
Control	12.8	9.2	6.6	8.8	37.4	-	0	0	1.2
2.5 %	8.8	7.6	4.1	4.8	25.3†	33%	32.35	-16.20	14.4
5.0 %	9.0	5.4	3.7	5.0	23.1†	39%	38.24	-18.60	16.7
7.5 %	8.3	5.4	3.4	4.8	21.9†	42%	41.44	-19.85	17.7

P= Prophase M= Metaphase; A= Anaphase; T= Telophase; MI= Mitotic index; RAR= Relative Abnormality Rate; RDR= Relative Division Rate; †= Significantly toxic if MI tests≤ ½ of control

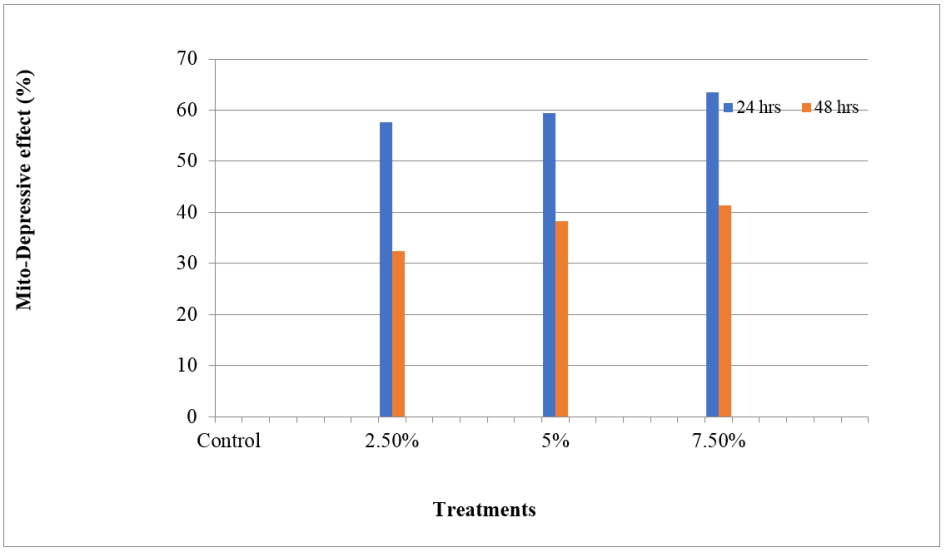
Table 2: Types and percentage of chromosomal aberrations in different mitotic phases induced by different concentrations of *Parthenium* plant extract.

Treatments	Chromosomal Aberrations types in mitotic stages in numbers																		Total abs (%)
	P abs (%)	M abs (%)						Aabs (%)						T abs (%)					
		Types of ab					Total %	Types of ab					Total %					Total %	
	EP	SM	CM	DM	MF	MB		EA	DA	AL	AB	AF		TB	TL	TF	MiNu		
24 Hrs duration																			
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.5 %	0.4	1.0 (33)	0.5 (17)	0.4 (13)	1.0 (33)	0.1 (3)	3.0	0.1 (8)	0.1 (8)	0	0.4 (33)	0.6 (50)	1.2	0	0.4 (19)	1.8 (86)	0	2.1	6.7
5.0 %	1.6	0.7 (29)	0.2 (8)	0.6 (25)	0.8 (33)	0.1 (4)	2.4	0	0.2 (12)	0.1 (6)	0.5 (29)	0.9 (53)	1.7	0.2 (13)	0.3 (19)	1.1 (69)	0	1.6	7.4
7.5 %	3.5	1.0 (36)	0	0.9 (32)	0.6 (22)	0.3 (11)	2.8	0	0.3 (38)	0	0.3 (38)	0.2 (25)	0.8	0.1 (5)	0.1 (5)	0.8 (38)	0.2 (10)	1.2	8.3
48 Hrs duration																			
Control	0.1	0.3 (60)	0	0.2 (40)	0	0	0.5	0	0.2 (40)	0	0.3 (60)	0	0.5	0.1 (100)	0	0	0	0.1	1.2
2.5%	3.9	0.9 (19)	0.8 (17)	1.3 (28)	1.5 (32)	0.2 (4)	4.7	0.5 (16)	0.6 (19)	0.2 (6)	1.4 (44)	0.5 (16)	3.2	0.7 (27)	0.6 (23)	1.3 (50)	0	2.6	14.4
5.0 %	5.6	1.0 (2)	0.9 (19)	0.9 (19)	1.6 (34)	0.3 (7)	4.7	0.5 (16)	0.6 (19)	0.2 (6)	1.5 (47)	0.4 (13)	3.2	0.5 (16)	0.3 (9)	1.4 (44)	1.0 (31)	3.2	16.7
7.5 %	5.8	1.1 (21)	1.3 (25)	0.9 (17)	1.4 (27)	0.5 (10)	5.2	0.2 (7)	0.6 (21)	0.3 (10)	1.3 (45)	0.4 (14)	2.9	0.6 (16)	0.2 (5)	1.9 (50)	1.1 (29)	3.8	17.7

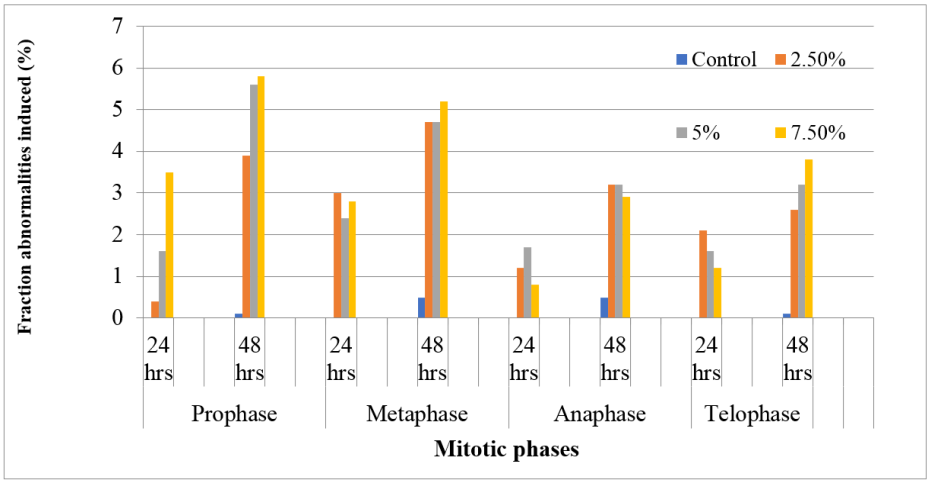
LP= Late prophase, SM= Sticky metaphase, C-M= C-metaphase, DM= Disturbed metaphase, MF= Forward metaphase, MB= Metaphase chromosome break, EA= Early anaphase, DA= Disturbed anaphase, AL= Anaphase laggards, AB= Anaphase bridges, AF= Forward anaphase,TB= Telophase bridges, TL= Telophase laggards, TF= Forward telophase, MiNu= MicroNucleus, P= Prophase, M= Metaphase,A=Anaphase, T= Telophase, Ab= Aberrations, CA= Chromosomal aberrations, %= percentage; *values in parenthesis are fraction in particular mitotic stage



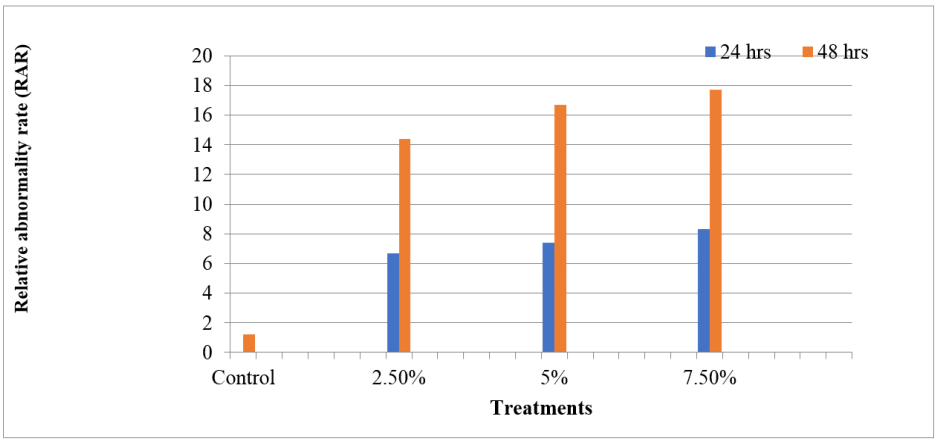
(a)



(b)



(c)



(d)

Fig. 1. Effect of different concentration (2.5 %, 5.0 % and 7.5 %) of *Parthenium* plant extract on (a) Mitotic index % (b) Mito-Depressive effect % (c) Percent abnormalities in different mitotic phases (d) Relative Abnormality Rate (RAR).

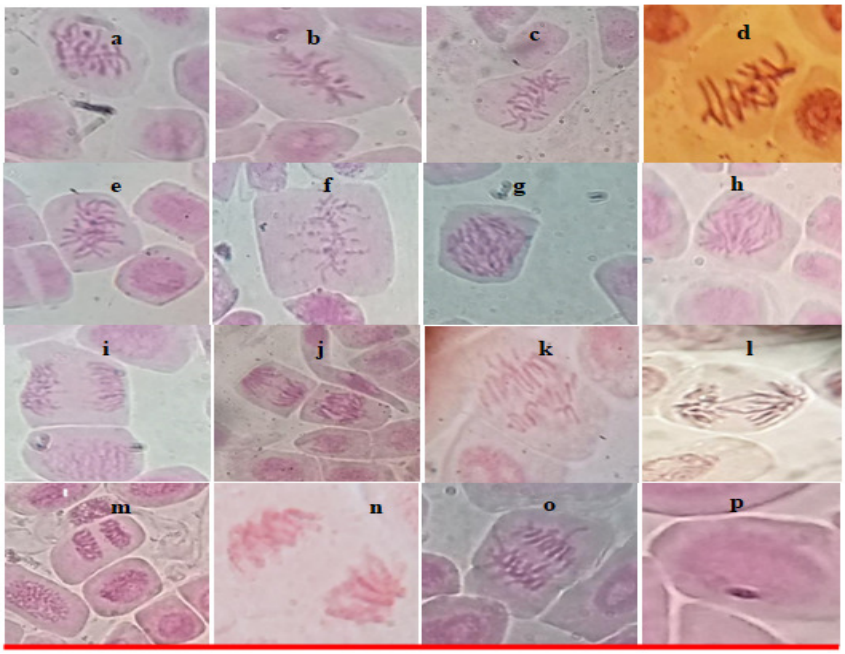


Fig. 2. A microphotographs of onion root tip types of aberrant cells induced by different concentrations of *parthenium* extract treated for 24 and 48 hours: a= late prophase, b= sticky metaphase, c= C- metaphase, d= disturbed metaphase, e= metaphase forward, f= metaphase break, g= early anaphase, h= disturbed anaphase, i= anaphase laggards, j= anaphase bridge, k= forward anaphase, l= telophase bridge, m&n= telophase laggards, o= forward telophase, p= micronuclei at interphase.

CONCLUSIONS

The result of the cytological study suggests that *Parthenium* plant is capable of producing numerous structural and functional alterations in mitotic cells and hence, confirm the cytotoxicity and genotoxicity of *Parthenium* extract. And also suggests that recovery of the cells to the normal state is not possible ever after 48-hour treatment. The present results thus, indicate that prolonged exposure to *Parthenium* plants particularly with its leaves and inflorescence leads to cyto-statics. It could be concluded that *Parthenium* plants are capable of inducing various chromosomal aberrations, inhibition of mitosis which ultimately affects the genetic architecture as well as physiological set up of a cell and consequently leads to the death of the plant.

FUTURE SCOPE

Parthenium is a serious weed in farmer's field which not only harm the animals consuming it also cause skin related problems in humans. The present assay of *Allium cepa* could be an effective assay for the assessment of harmful effect of many weeds as well as chemicals. Therefore, by assessing the cytotoxic as well genotoxic effects of various weeds and chemical a researcher could develop a diagnostic procedure accordingly.

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Conflict of Interest. None.

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