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The effect of *Parthenium* extract on cells and chromosomes in *Allium cepa* L.

[1]Puthanpura Sasidharan Sreeja*, [2]Karuppusamy Arunachalam, [1]Ramakoti Reddy V., [1]Sathyanarayanan Saikumar, [1]Muniyandi Kasipandi, [1]Sivaraj Dhivya a

[1] Department of Botany, Bharathiar University, Coimbatore – 641046, Tamil Nadu, India

[2]Department of Basic Sciences in Health, Area pharmacology, Faculty of Medicine, Federal University of Mato Grosso (UFMT), Av. Fernando Correa da Costa, no.2367, Coxipo, Cuiabá, Mato Grosso 78060-900, Brazil

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Abstract

Parthenium plants are growing everywhere in the country, from road side to crop fields. *Parthenium hysterophorus* L., native of tropical and subtropical America is the only one species found in India which is accidently introduced into India and spread everywhere. It is considered as poisonous, allergic and aggressive obnoxious weed posing a serious threat to human beings and livestock. Hence, the present was aimed to study the effect of *Parthenium* extract, obtained from its various parts (stem+leaves, root and flowers), on cells and chromosomes in root meristem of *Allium cepa*. Aquous extract from the dried and powdered plant parts was used for treatments. 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. The results observed chromosomal aberrations and reduction in mitotic index in treated *A. cepa* cells and the observation was high in flower extract treated cells. But in recovery period of 48 h, the root extract treated cells showed slight improvement in the mitotic index and reduction in the frequency of cytological aberrations. The chromosomal aberrations include chromatid breaks and clump, gaps, abnormal cells, disturbed anaphase, etc. From the results obtained it is evident that among various parts of *Parthenium*, the flowers are more harmful followed by stem+leaves and root. Therefore, the *Parthenium* plants should be irradiated completely in the surroundings of other plant systems as well as other live stocks.

1. Introduction

Parthenium hysterophorus, with diploid chromosome number 2n=34, is an aggressive weed invading all disturbed land, including farms, pastures, and roadsides. This annual herb is a native of America and Mexico consists of some 16 species (Nath, 1988) belongs to the family Asteraceae. Only one species *Parthenium hysterophorus* L. is found in India, commonly known as Bitter weed, false ragweed, fever few, Parthenium weed, Ragweed, whitetop, whitehead, congress grass or carrot grass etc; vernacular names: *Kanike ghans, Bethu ghans*, or *Padke phul*. It was accidently introduces 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) (Rao, 1956).

*Corresponding author
E-mail: sree.ps0606@gmail.com

Scientists describe it as a "poisonous, allergic and aggressive obnoxious weed posing a serious threat to human beings and livestock". *Parthenium* causes considerable yield reduction and indirect effects include poor fruiting of leguminous crops. It was reported that in *parthenium*-infested fields, *parthenium* pollen was found on *Crotalaria* and *Desmodium* (Jayachandra, 1980). *Parthenium* pollen was found to reduce the chlorophyll content probably by interference with porphyrin biosynthesis (Jayachandra, 1980). Another indirect effect is its potential role as an alternate host for crop pests functioning as an inter season reservoir or inoculum source (Robertson and Kettle, 1994). Though cattle do not eat *Parthenium*, its effects were observed on them when they walk by or graze through patches of this weed as inflamed udder and subsequently suffered from fever and rashes. It is responsible for bitter milk disease in livestock fed on grass mixed with *Parthenium* and causes both acute and chronic forms of toxicity (Narsimhan *et al.*, 1980; Ahmed *et al.*, 1987). Also ulcerations were caused both in the mouth and digestive tract. Among the humans it causes the contact dermatitis and causes asthma, bronchits, nasal-dermal, naso-bronchial types of diseases and hay fever (Lakshmi and Srinivas, 2007). Almost every part of the plant except root is reactive.

Several studies have been carried out to observe the genotoxicity of various heavy metals using plants systems such as *Allium cepa, Vicia faba. Tradescantia* etc. as bioindicators and interpreting the results with animal systems. For example, micronuclei test on *Vicia fab*a grown in contaminated soil sample from near an abandoned heavy metal processing plant showed linear dose responses to heavy metal content, which were inversely proportional to the distance from the source (Knasmuller *et al.,* 1998). *Allium cepa* being utilized as a test system by numerous workers since its high uniform germination and to have a low rate of spontaneous aberrations. Furthermore, its chromosome number is relatively low ($2n$=16) and the size of the chromosomes are relatively bigger (Kalkman, 1984; de Vries, 1990).

Therefore, the present investigation was undertaken to study the effect of *Parthenium* extract, obtained from its various parts, on cells and chromosomes in root meristem of *Allium cepa*. 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.

2. Materials and Methods

2.1. Bulbs of Onion

Bulbs of uniform size of onion (*Allium capa* L.) var. CO-30 were procured from Department of Horticulture, Tamil Nadu Agricultural University, Coimbatore.

2.2. *Parthenium hysterophorus L.*

Parthenium hysterophorus L. is collected from Bharathiar University campus, Coimbatore (Fig. 1(a-b)). Fresh and green plants of *Parthenium* were collected at vegetative + flowering stage by uprooting the plants. Roots, stem+leaves and flowers were separated from the plants. The plant parts were air dried in shade for

a week. Ten gram each plant part of *Parthenium hysterophorus* was ground to make powder (Fig. 2), mixed with 100 ml of 1% acetone and left for 24 h in dark at the room temperature (average during day: 25°C) for extraction and filtered to get the extract.

2.3. Treatments

Outer scales of healthy and uniform bulbs of *Allium cepa* were removed and apices of the root primordial exposed (Grant, 1982). Bulbs were allowed to germinate in sand-saw-dust mixture for 72 hours at 25 ± 1°C in dark. When the bulbs started rooting (1-1.5 mm length), the bulbs were transferred immediately to sterilized petridishes lined double with blotting paper and moistened with 10 mL of the of extracts of all the three plant parts separately (Fig. 3). Each treatment had five replicas. One treatment was run as control with distilled water only. The petridishes were maintained under laboratory conditions (room temperature 25°C at mid day, and diffused light during day) for 24 hours.

Following treatments, treated bulbs were transferred to distilled water for 0, 24 and 48 hours for recovery (Fig.4). Zero hours means the treated bulbs were fixed immediately in the fixative.

2.4. Cytological studies

For cytological studies, 2-4 root tips from each bulb (control as well as treated) were collected and the root tips were transferred to 3:1 fixative (absolute alcohol: Glacial acetic acid) for a minimum period of 24 hours. Root tips were hydrolyzed in 1N HCl at 60°C for 5-10 minutes and squashes were made in 2% acetocarmine. About 500 dividing cells for 8-10 meristems were scored for each dose for various kinds of aberrations as well as for studying mitotic index.

Data on mitotic index (%), percent cells showing chromosomal abnormalities at different stages of cell division such as chromosomal breaks, clumping of chromosomes, stickiness of chromosomes, gaps in the chromosomes, lagging chromosomes, bridges, ring chromosomes, abnormal cells, giant cells, chromatic breaks, micronuclei, disturbed anaphase, polyploid cells, multinucleate cells, binucleate cells, multipolar cells, elongated cells, vacuoles in the cells and fragments were recorded at appropriate stages.

Mitotic index (MI) was calculated using the following formula.

$$\text{MI} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100$$

The chromosomal aberrations noticed in various Parthenium extract treated treatments in the present study can be classified mainly into two groups as per the classification of Nagpal and Grover (1994) and Nagpal *et al*. (2002) depending upon the nature, they are:

(i) Clastogenic changes – which affects the individual chromosomes. Eg. Fragments, ring chromosomes, bridges and micronuclei etc.

(ii) Physiological changes – which include the effects in whole chromosome complement and cells as a whole. *Eg*. C-mitosis, despiralization, lagging chromosomes, multipolar anaphases etc.

3. Results

The effect of the plant extract obtained from root, stem+leaves and flowers from the plant *Parthenium hysterophorus* L., collected from Bharathiar University Campus, Coimbatore, was evaluated on somatic chromosomes of *Allium cepa L.* and the results of the data obtained in this regard are presented in Tables 1 and 2.

(A) Mitotic Index (%)

(B) Chromosomal aberrations (%) – (i) Clastogenic changes

(ii) Physiological changes.

The details of the results obtained in the present investigation were presented in the following text:

3.1. Mitotic index (%) (MI)

The data on number of cells (%) i.e. mitotic index, which are in dividing stage in control and in various treatments of extract treatments are presented in Table 1. Mitosis in the control root tips was perfectly normal (Fig. 5) at all the stages and exhibited a mitotic index of 14.42. From the data presented in the tables it is also evident that as a result of *Parthenium* extract treatment, the mitotic index (MI) was decreased considerably over the absolute control in all the treatments (Fig. 6). The greater reduction in MI was noticed in the treatments of flower extract followed by treatments with leaf+stem extract and then root extract treatment. The reduction in the plant extract treatment varied from 4.10 to 9.80.

AT all the concentrations, when recovery period was given, the mitotic index (MI) was increased compared to the treatments where recovery period was not given. However, the mitotic index in the recovery period treatment was less than that of the absolute control. In recovery period treatments, in all the extract treatments, the mitotic index was increased with increase in the duration of recovery period i.e. higher mitotic index was noticed in 48 hours recovery period treatments. The mitotic index in recovery period treatments varied from 4.60 to 12.28. However, the degree of recovery of mitotic index is very low in flower extract treatment, while it was higher in root extract treatment.

3.2. Chromosomal aberrations (%)

In all the mutagenic treatments, a number of mitotic anomalies were observed at different stages of mitosis, and they were represented in per cent for each treatment and the data are presented in Tables 1 & 2. The total per cent of various cytological aberrations are given in Table 1 and 2. Representative cytological features are shown in Figs. 7 to 10.

The frequency of total chromosome aberrations increased as a result of treatment with extract of Parthenium. No significant abnormalities were noticed in control plants. On other hand, high frequency of abnormalities was noticed in flower extract treatment and was closely followed by leaf+stem extract treatment and root extract treatment, respectively. The per cent abnormalities were reduced in recovery period treatments. The reduction was seen with increase in the duration of recovery. Even after 48 h of recovery period none of the treatments showed absolute lacking of any aberrations in the chromosomes as well as cells as a whole indicating

irreversible damage has already happened.

The data on the clastogenic effects observed in the form of breaks, clumping of chromosomes, stickiness of chromosomes, chromosomal gaps, laggards, bridges and ring chromosomes, abnormal cells, giant cells are presented in Table 1. From the data it is evident that these aberrations were not present in control material, while variable frequencies of chromosomal aberrations were present in all the Parthenium extract treated treatments.

Overall, in general, the frequency of these chromosomal aberrations was increased with treatements in the following order root extract treatment > leaf+stem extract treatment > flower extract treatment. Highest frequency of chromosomal aberrations was noticed in treatments where recovery period is not given. In recovery period treatments the frequency of aberrations were decreased. Maximum decrease was noticed generally in 48 h recovery period treatments. This is particularly in treatments treated with root extract. On the other hand, in flower extract and leaf+stem extract treatments, even after 48 hours of recovery period the frequency of various chromosomal aberrations were not reduced considerably. Among various clastogenic affects, the frequency of chromatid breaks and clumps / stickiness were found highest and were followed by gaps, laggards/ bridges and ring chromosomes.

The second category of chromosomal aberrations is physiological in nature. These aberrations include disturbed anaphase, abnormal cells such as diagonal distribution of metaphase, anaphase and telophase, polyploid cell, multinucleate cell, binuclete cell, multipolar cell, elongated cells, fragments and vacuoles in the nucleus. Cells with micro and meganucleoli were also noticed in many of the treated materials. The frequency data of the above abnormalities recorded in the present study are presented in the Table 2.

The data clearly indicate that the extracts of Parthenium is capable of inducing these abnormalities similar to that of clastogenic effects except in the extent of values. These aberrations were also more or less high in flower extract treatment and were less in root extract treatment. Treatments without recovery showed highest aberrations, while recovery period treatments showed slight reduction in various cytological abnormalities. Among various physiological effects, the frequency of the cells showing disturbed anaphase, abnormal cells, chromatid breaks, elongated cells found highest and were followed by giant cells, micronuclei, fragments and others. The polyploid, multipolar, binucleate and multinucleate condition was also observed particularly in flower extract treated treatments

Table 1. Effect of extracts of *P. hysterophorus* on somatic chromosomes of *Allium cepa* L.

Treatment / Control	Recovery period	Mitotic index	Chromosomal aberrations (%)							
			Breaks	Clump/ Sticky	Gaps	Laggards / bridges / fragments	Ring	Abnormal cells	Giant cells	Chromatid breaks
Control	-	14.42	-	0.01	-	-	-	-	-	-
Root extract	0h	9.80	2.83	1.28	1.04	1.23	0.78	9.10	0.42	1.00
	24h	11.46	2.26	1.05	0.90	1.02	0.44	6.27	0.28	0.82
	48h	12.28	1.64	0,98	0.68	0.78	0.32	4.14	0.14	0.46
Flower extract	0h	4.10	5.36	3.94	2.68	2.10	1.28	16.62	2.20	4.28
	24h	4.60	5.12	3.58	2.24	1.86	1.10	15.02	1.95	3.72
	48h	4,78	4.98	3.32	2.02	1.64	0.96	13.20	1.54	2.28
Leaf and stem extract	0h	5.75	5.10	3.70	2.44	1.96	1.04	13.48	1.62	2.56
	24h	5.92	4.86	3.44	2.18	1.72	0.86	11.75	1.22	1.44
	48h	6.02	4.50	3.05	1.94	1.32	0.64	9.84	0.84	0.94

Table 2. Effect of extracts of *P. hysterophorus* on somatic chromosomes of *Allium cepa* L.

Treatment / Control	Recovery period	Chromosomal aberrations (%)							
		Micro-nuclei	Disturbed anaphase	Polyploid cells	Multi-nucleate cells	Binucleae cells	Multipolar cells	Elongated cells	Vacuolated cells
Control	-	0.01	0.05	-	-	-	-	-	-
Root extract	0h	0.22	5.42	0.05	-	0.03	0.90	1.18	1.08
	24h	0.10	3.86	-	-	0.01	0.74	0.84	0.82
	48h	0.05	2.50	-	-	-	0.43	0.58	0.48

Flower extract	0h	0.92	16.26	0.28	0.10	0.38	2.32	3.88	2.85
	24h	0.72	13.46	0.10	0.06	0.24	1.88	3.20	2.14
	48h	0.46	11.98	0.04	0.02	0.19	1.40	2.85	1.90
Leaf and stem extract	0h	0,62	13.54	0.08	0.04	0.10	1.64	2.36	2.04
	24h	0,28	11.32	0.02	0.02	0.04	1.05	1.93	1.77
	48h	0.18	9.89	0.05	-	0.02	0.80	1.74	1.46

Fig. 1 (a-b): *Parthenium* plant with full bloom and *Parthenium* plants in the filed

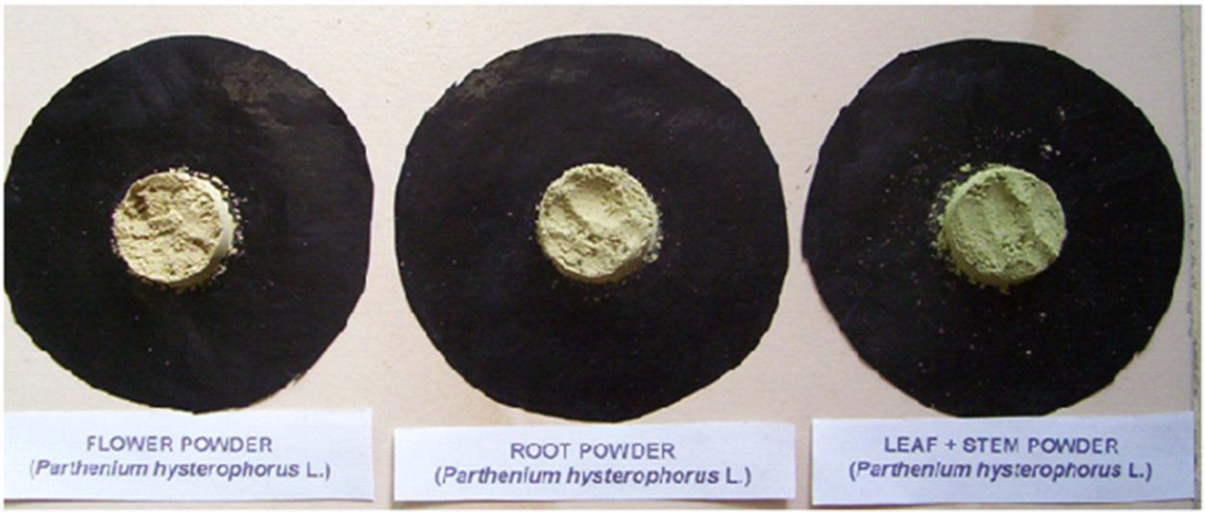

Fig. 2. Powder preparations from the flower, root, leaf+stem of ***P. hysterophorus* L.**

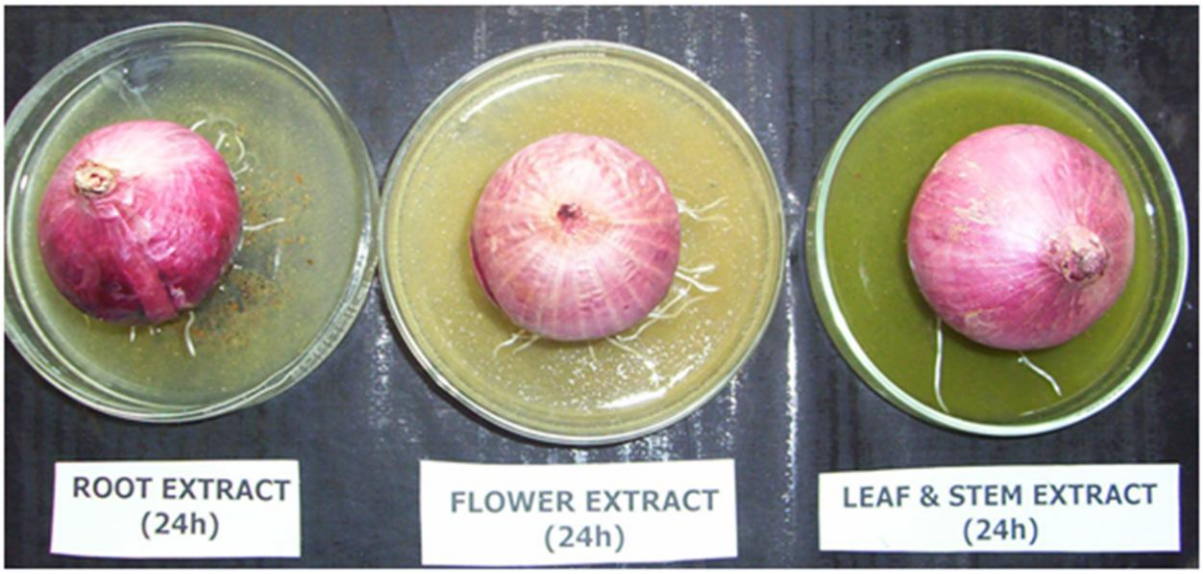

Fig. 3. *Allium cepa* L. bulbs are treated with the extracts of root, flower, leaf+stem of *P. hysterophorus* L.

Fig. 4. *Parthenium* extract (root, flower, leaf+stem) treated bulbs of *Allium cepa* were kept in water filled glass bottles for recovery periods of 24h and 48hours.

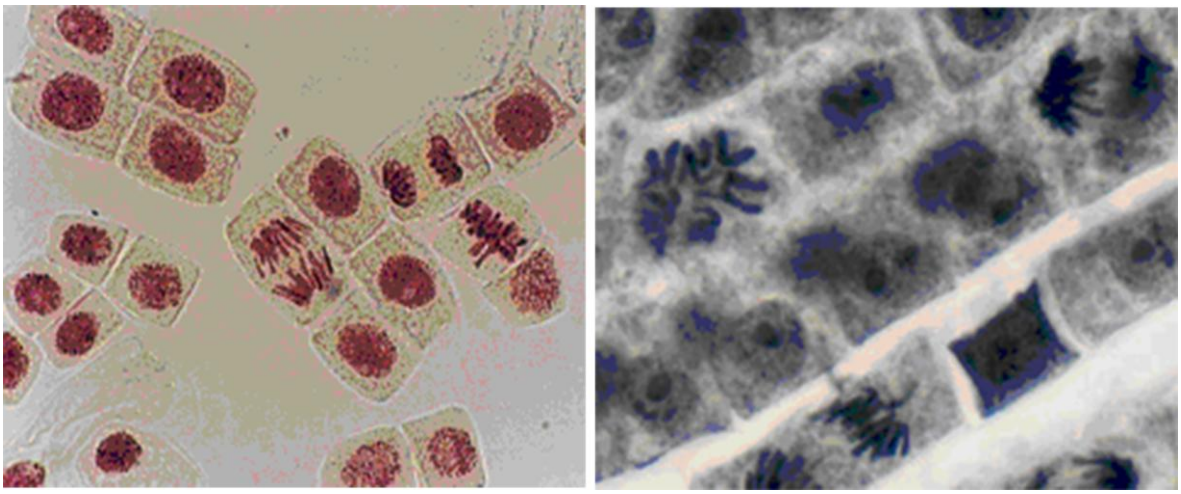

Fig. 5&6: Normal cell divisions in control of onion root tip; Reduction in cell division (MI) in *Parthenium* flower extract treated onion root tip cells.

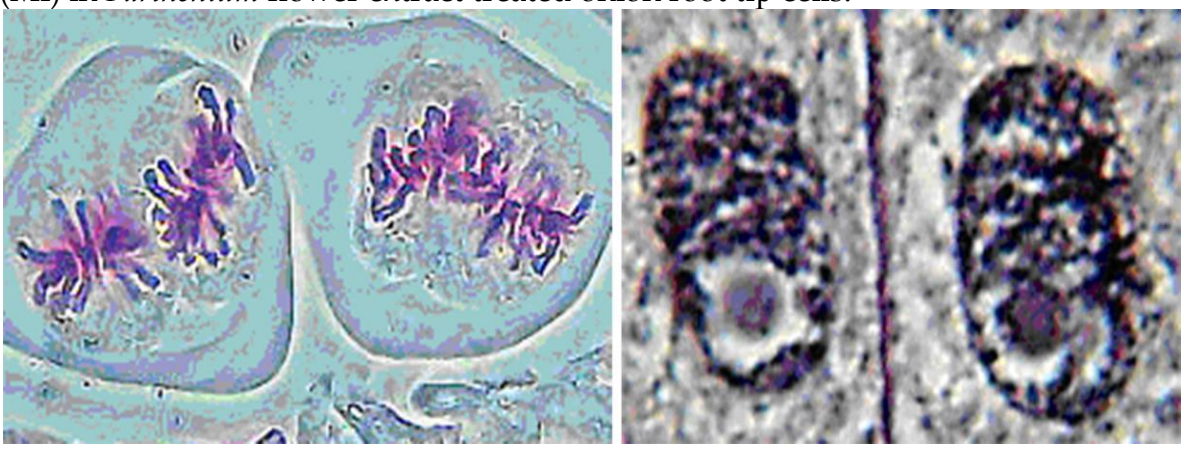

Fig.7&8: Clumping of metaphase in the cells of onion root tips treated with leaf+stem extract of *Parthenium*; Vacuoles in the cells of *Parthenium* extract treated onion root tip cells.

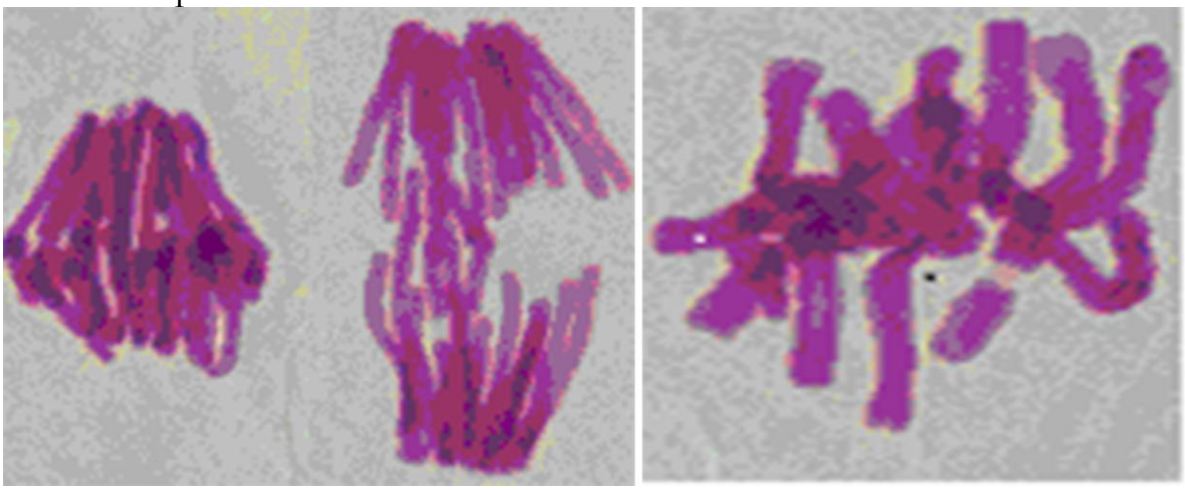

Fig. 9&10: Chromosome bridges in the anaphase of onion root tip cells treated with flower extract of *Parthenium;* Chromosome break in the somatic chromosome of onion root tip treated with the extract of *Parthenium* leaf+stem

4. Discussion

Parthenium is a curse for the bio-diversity. Chemical analysis has indicated that all the plant parts including trichomes and pollen contain toxins called sesquiterpene lactones. The major component of these toxins being parthenin and other phenolic acids such as caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid and p-anisic acid are lethal to humans and animals. In addition to health hazards a lot of available data also highlights its impact on agriculture as well as natural ecosystems (Chippendale and Panetta, 1994; Evans, 1997). Parthenium was accidentally introduced in India in around 1956 and has since spread over most part of the country.

4.1. Mitotic index (MI)

In the present study, the mitotic index decreased in all *Parthenium* extract treated treatments over control. The reduction was noticed in all the treatments irrespective of the nature and origin (root or stem+leaf or flower) of plant extract. This may indicate that *Parthenium* extract interferes with normal sequences of cell cycle to reduce the number of cells starting to divide at interphase to enter in the prophase stage. The reduction in MI may be due to the arrest of cells in G1 phase or retardation in the pace of events during S1 or S2 phases. Mitotic inhibition might be due to mitotic poison which may cause metabolic imbalance and thus interfere the synthesis and structure of DNA resulting physiological effects and structural change in chromosomes during division. Inhibition of cell division is mainly due to stoppage of synthesis of DNA, RNA, protein and energy (Sudhakar et al., 2001). Extracts from certain harmful plants seems to inhibit electron and energy transfer (Brittel et al., 1974), therefore it may be one of the factor for mitostatic effect observed the present study. Grover and Mittal (1984) suggested that the reduction in MI is due to disturbance causes by the agent in the internal milieu of the cell during interphase. The inhibitory effect indicates the action of plant extract on the metabolism of the interphase nucleus even after appropriate periods of recovery.

Reduction in MI also clearly indicates the potentiality of *Parthenium* extract to be useful as an anticancer chemotherapeutic agent.

Recent studies made by Maharjan *et al.* (2007) demonstrated that leaf aqueous extracts of *Parthenium hysterophorus* exhibited significant inhibitory effects on seed germination and seedling growth of all test species (three cereal crops, three crucifer vegetables, and two Asteraceae species. Earlier workers have also reported that foliar leachates of *Parthenium hysterophorus* reduced root and shoot elongation of *Oryza sativa* and wheat (Singh and Sangeeta 1991), maize and soya beans (Bhatt *et al.,* 1994) as well as some common Australian pasture grasses (Adkins and Sowerby, 1996). This indicates the availability of the inhibitory chemicals in higher concentration in various plant parts of the plant particularly in flowers, leaves and stem.

Tefera (2002) also reported that 10% leaf aqueous extract of *Parthenium hysterophorus* resulted in complete

failure of seed germination in *Eragostis tef*. In many other crops also Parthenium reduced seed germination considerably (Oudhia, 2000; Nagaraja *et al.,* 1998). A reduction in seed germination of wheat by 80-90% due to soaking of its seeds in stem aqueous extract of *Parthenium hysterophorus* for 20 and 40 h has been reported by Rajan (1973). Srivastava *et al.* (1985) revealed that aqueous extracts of leaves and inflorescences inhibited the germination and seedling growth of barley, wheat and peas.

4.2. Chromosomal aberrations (%)

Chromosomal aberrations have been considered as reliable indicators of mutagenic activity (Mohandas and Grant, 1972), and there have been evidences for a correlation between chromosomal damage and toxic effects of a particular substance. Since many chromotoxic chemicals can also cause point mutation, the value of mitotoxic and chromotoxic data are increasing than ever. In an earlier study, Mehta *et al.* (1995) noticed that due to Parthenium extract treatment in radish the pollen sterility increased upto 87%. They found that frequency of abnormal sporoids was directly related to the treatment dose. Mehta and Mishra (1999) further observed that after treatment with 10, 20, 30 and 40% *Parthenium* extract there was a gradual increase in the percentage of chromosome abnormalities with increasing doses both at metaphase I and anaphase I of meiosis in radish. All these results indicate the adverse affect of *Parthenium*.

Formation of vacuoles in the cells seems to be a defensive mechanism since, such vacuoles will function as specific compartments for the cells so as to store harmful materials.

In the present study, the size of nucleus and nucleolus was found to be relatively small compare to control. Since, the nucleolar size is a reliable reflector of nucleolar activity and RNA synthesis (Sen and Bhattacharya, 1979) such decrease may be correlated to the mitostatic activity of the cells.

Disturbed metaphase, anaphase and telophase might have arisen due to the disturbance of spindle apparatus. Disturbances in mitotic spindle (Chauhan and Sundararaman, 1990a) and alteration in microtubule function (Chauhan and Sundararaman, 1990b) can lead to many mitotic abnormalities. In some cases, such disturbances may also be due to multipolar spindles. Diagonal orientation of chromosomes at different mitotic stages may be due to improper functioning of the spindle apparatus. Any sort of disturbance or inhibition of spindle formation may lead to disturbed metaphases and anaphases (Amer and Farah, 1979). Abraham and Rajalekshmy (1989) attributed spindle abnormalities to defective spindle formation. Therefore, in the present study *Parthenium* extract showed an adverse affect on spindle it can be considered as stathmokinetic agent (Shehab, 1979).

Presence of gaps, fragments and bridges in the chromosomes is a clear indication of occurrence of chromosomal breakages. Induction of bridges and breaks may lead to loss of genetic material (Salam *et al.,* 1993). Chromosome breakage has been reported to be induced by various metabolic inhibitors, although the relationship between chromosomal

damage and metabolic alterations remain obscure due to complexity of chromosome structure. The chromosomal breaks noticed in the form of chromatid bridges, fragments and micronuclei represent the clastogenic effects of *Parthenium*. The observations further revealed that these breakages occurred preferably near the centromere, where centromeric heterochromatin is located in *Allium* (Vosa, 1974), and is sensitive to breakage. However, the chromosome breakage is now generally attributed to unfinished or mis-repair of DNA (Evans, 1977). Similar observations were made in Cadmium chloride induced chromosomal aberrations in polytene chromosomes of *Chironomus* and lead nitrate induced somatic and germ cells of rat (Verma *et al*., 2004). Breakage followed by rejoining may repair the chromosome or may lead to various anomalies such as fragments, laggards, micronuclei etc.

The formation of small fragments can be attributed to the chromosomal breakage due to the effect of *Parthenium*.

The phenomenon of lagging chromosomes may be attributed to hindrance of chromosome movement accompanied by adhesion of the centromeres to the nuclear membrane or the surrounding surface of the plasma membrane (Barthelmese, 1957). Lagging chromosomes could also result from arrest of metaphase movement or they might have arisen either due to inactivation of spindle fibers or due to misorientation and inadequate number of spindle fibers. The results obtained supports the view of earlier workers that stress condition presumably induce molecular changes that disrupt the pairing of the chromosome homologoes (Sharma *et al*., 1988). Laggards as observed in present study have also been recorded by Jadhav and Mungikar (1998), Deena and Thoppal (2000), and Singh *et al*. (2000).

Anaphase bridge formation has been noticed in root tip cells of *Trigonella* treated with aqueous solution of leaf extract of *Ipomoea cornea* (Anis *et al*., 1999), root tip cells of pearl millet treated with fungicides bavistin and diethane M45 (Sinha and Choudhary, 1989), and root tip cells of *Trigonella* treated with pesticides (Kumar and Kumar, 2000).

In the present study stickiness of chromosomes both at metaphase and at anaphase was very significant in all *Parthenium* extract treated treatments. It clearly revealed the polymerization effect of the extract on the nucleic acid of the chromosomes. Patil and Bhat (1992) suggested that stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material. The stickiness of the chromosomes at metaphase caused inability of normal movement at anaphase. It may result in fragmentation of chromosomes from stress of anaphasic movement or in bridge formation when the chromosomes fail to separate.

Stickiness, Clumping and grouping of chromosomes can be attributed to changes in viscosity and surfaced tension of cell (Kadnykoy *et al*., 1978). The clumping and stickiness of the chromosomes is a physiological effect and is attributed to the alteration of the chromosomal proteins resulting

in the change of surface nucleo-protein configuration or improper folding of chromosome fibres (McGill *et al.*, 1974). According to Cohn (1979), stickiness and clumping of chromosomes may be suspected to be the primary effect of treated agent caused by depolymerisation of DNA or disruption of bonds between protein and nucleic acid constituents of chromosomes. Clumped chromosomes and other disturbances of the mitotic apparatus observed in the study appear to be the cytological effects of the inhibition of protein synthesis during mitosis and this suggests the possibility of *Parthenium* extract binding to DNA and thus preventing DNA from unwinding for transcription of spindle protein messenger. Chromosome stickiness leading to sticky metaphase, precocious separation of chromosomes and anaphase bridges are possibly due to the effect of *Parthenium* extract in breaking the protein moiety of nucleoprotein backbone (Patnaik *et al.*, 1984). *Parthenium* extract induced stickiness at metaphase and anaphase may be a potential source of bringing in new translocations and consequent changes in the nucleus.

Scattered and clumped metaphases are the partial and full effects respectively of a C-mitotic agent (Hadder and Wilson, 1958). Scattered chromosomes at metaphase and multipolar spindles, observed in some cells, also indicate that *Parthenium* is acting on spindle apparatus, which may ultimately lead to accumulation of polyploid cells (Grover and Malhi, 1988). Occasionally, left over centromere from previous division become active and migrate towards the newly developing spindle and so tri, tetra or pentapolar spindle with or without chromosome are formed. Similar properties have also been recorded in *Allium cepa* root tip cells treated by the extract of *Alpinia nutans* and *Pogostemon heyneaus* (Da *et al*., 1994), *Mentha rotundifolia* (Minija *et al.,* 1999), *Ocimum americanam* (Tajo and Thoppil, 1998), and *Spilanthes ciliate* (Sreeranjani and Thoppil, 2001), and other pesticides (Kumar and Kumar, 2000; Somashekar, 1988). Cytological studies in *Trigonella* treated with extract of *Paerthenium* showed many chromosomal abnormalities like stickiness, fragmentations, laggards, chromosomal bridges, multipolarity and micronuclei (Kumar *et al.,* 2004).

In the present study, few cells possessed one to many micro and meganucleoli. Micronuclei are true mutagenic aspects, which may lead to a loss of genetic material and have been regarded as an indication of the mutagenicity of their inducers (Raun *et al*., 1992). It was reported on to three micronucleoli in *Cornus* and as many as four nucleoli in *Fritillaria* (Frankle, 1937).

Micronuclei could result from the formation of nuclear membrane surrounding the laggards and chromosomal fragments. The micronuclei observed in the present study might have originated from lagging chromosomes at ana-telophase or from a chromosome fragment. They are also produced as a consequence of other anomalies such as stray chromosomes, multipolar spindles and reductional groupings. It may also arise as a result of misdivision of nucleus. In the present study the

number of micronuclei is either low or higher than that of fragments, indicating that both laggards and /or fragments (one or more) are involved in micronuclei formation. Formation of micronuclei has also been reported in other similar studies (Kumar and Kumar, 2000; Kumar and Tripathi, 2003).

Occurrence of bi-nucleate and multinucleate cells in treated cells indicating that the *Parthenium* inhibited the cell plate formation at telophase. These features might be due to suppression of phragmoplast formation in the early telophase by the extract. Therefore, neither the cell plate nor the cell wall appeared in treated cells. In a few number of cells, particularly in leaf+stem extract treatment, binucleate cells were noticed. The delay or failure of cytokinesis would account for the occurrence of such binucleate cells. The binucleate condition in grape according to Raj and Seethaiah (1969) might be due to non-disjunction of a particular chromosome. The cell receiving the extra chromosome has two nuclear organizers and potentially two nucleoli.

Polyploid cells in treated materials indicate that the *Parthenium* extract acted on the spindle fibers. Polyploid condition may occur due to non-disjunction i.e. the sister chromatids remain together instead of moving to the opposite poles. Polyploid cell formation has been noticed earlier in test systems treated with leaf extract of *Lathyrus* (Sudharson Raj and Subba Reddy, 1971), gallic acid derivatives (Sato and Tanaka, 1972), and several agrochemicals (Chand *et al*., 1991).

5. Conclusion

The reduction of mitotic index and occurrence of different degrees of chromosomal abnormalities in the somatic cells of the test system, *Allium cepa*, as a result of *Parthenium* extract treatment indicate that exposure of living cells to the *Parthenium* plants either directly or with any other medium (effluents etc) may be fatal to the genetic constitution of living systems as prolonged exposure to this plant was found mitostatic to the test system. Compounds that inhibit mitotic apparatus are regarded as mitotic poisons. The result of the present study therefore suggests that *Parthenium* plant is capable of producing numerous structural and functional alterations in mitotic cells and hence, confirm the genotoxicity of *Parthenium* extract. The present results also suggest that recovery of the cells to the normal state is not possible ever after 48 hours treatment. The present results thus indicate that prolonged exposure to *Parthenium* plants particularly with its flower leads to mitostatic. From the foregoing, it could be concluded that Parthenium plants are capable of inducing various chromosomal aberrations, inhibition of mitosis which ultimately upset the genetic architecture as well as physiological set up of a cell and consequently leads to the death of the plant. Thus it could be concluded that Parthenium plants when grown together with cultivated plant system or human beings may cause chromosomal aberrations in addition to the inhibition of mitosis, both of them ultimately upset the physiological set up of a cell and

consequently leads to either death of the plant or its aberration. Therefore, it is recommended that *Parthenium* plants should be irradiated completely in the surroundings of other plant systems as well as human beings to protect not only plants but also human beings and animals otherwise it will cause considerable damage to life.

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