



Parthenium hysterophorus Induced Genotoxic Hazards in *Allium cepa* L.

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ABSTRACT

The paper deals with allelopathic effect of *Parthenium hysterophorus* L. extract on the meristametic cells of onion (*Allium cepa* L.). The root tips of onion were treated with different concentrations (10%, 25%, 50%, 75%, 100%) of *Parthenium* extract for 4 hrs at room temperature. The *Parthenium* extract was prepared from plant parts such as leaf, stem, inflorescence and whole plant and the treated root tips were squashed in freshly prepared 2% acetocarmine solution. The results revealed that *Parthenium* extract exerts mitotic depression causing chromosomal abnormalities such as fragments, stickiness, nuclear vacuolation, bridge, laggards and micronuclei. The mitotic abnormalities were gradually increased with increasing concentration of the extract. Percentage of abnormal cells varied from 9.58 (10% *Parthenium* stem) to 40.59 (100% *Parthenium* inflorescence). The gradation of genotoxicity of different parts of *Parthenium* were noted as inflorescence > whole plant > leaf > stem. *Parthenium* may be considered as a strong genotoxic agent. The suppression of DNA content due to the application of *Parthenium* is another potential threat to the genomic balance which may cause the deviation of normal metabolic activities from its original parental line.

INTRODUCTION

Parthenium hysterophorus L. is a noxious exotic weed of tropical America. It is reported that seeds of *Parthenium* were introduced into India with grains imported from U.S.A. or Canada posing a threat to agriculture where it has invaded crop fields. *Parthenium* has been found to be responsible for allergic contact dermatitis in humans (Lonkar 1974). It is toxic to cattle and buffaloes. This weed also suppresses plant crops around it by virtue of its rapid growth and allelopathic effects (Patil & Hedge 1988). Its allelopathic effect on *Brassica campestris* has been marked with reducing cell survival and chlorophyll content and inhibiting seed germination and growth (Kumar & Kholi 1987). The active principles were isolated by Kanchan (1975) and found to be parthenin (C₁₅H₁₀O₄), caffeic acid, and 3,4-dihydroxy cinnamic acid. Chromosomal effects of Parthenin on plant cells have also been studied by Pushpangadam et al. (1979). In recent years, allelopathy has acquired key role to study the mutagenic/genotoxic/chromotoxic /cytotoxic effects on the living organisms. Various workers reported remarkable allelopathic effect on cytotoxicity, seed germination and growth in various plants (Awasthy et al. 1995, Sinha 2003, Sinha & Kumar 2005, Kumar & Kholi 1987)

The present paper deals with the allelopathic effect of *Parthenium hysterophorus* extract on the meristametic cells of onion (*Allium cepa*). It is proved from earlier experiments that onion assay has an excellent correlation with mammalian system.

MATERIALS AND METHODS

The root tips of onion were treated with different concentrations of the *Parthenium* extract. The treated root tips were squashed to assess the chromotoxicity by detecting chromosomal abnormalities.

Preparation of *Parthenium* extract solution: The plant parts of *Parthenium*, viz., stem, leaf, inflorescence and whole plant were washed thoroughly and chopped into small pieces. 50 g of chopped parts were soaked separately in distilled water for 24 hrs and grounded followed by filtration through Whatman filter paper No. 1. The filtrate was labelled as 100% stock solution, which was further diluted to make 10%, 25% and 75% by adding desired amount of distilled water.

Squash preparation: Actively growing root tips of *Allium cepa* were obtained from bulbs of equal size placed on the mouth of flask filled with distilled water. The distilled water was changed and the bulbs were placed again in beakers containing different concentrations of *Parthenium* plant extracts along with the control. The root tips were fixed in freshly prepared 1:3 acetobutanol for 24 hrs and squashed in 2% acetocarmine to study the frequency of cell division and chromosomal abnormalities in different treatments. The cytological effects of *Parthenium* extract on onion root tip were analysed by calculating the Mitotic Index (MI) using the formula:

$$\text{Mitotic Index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

Different types of aberrations in each stage were scored and the percentage of each aberration was calculated by the formula:

$$\text{Percentage of chromosomal aberrations} = \frac{\text{Number of abnormal cells}}{\text{Total number of dividing cells}} \times 100$$

RESULTS AND DISCUSSION

In *Allium cepa* the somatic chromosome number was 16. The cytological observation revealed that *Parthenium* caused mitotic depression and wide spectrum of abnormalities such as fragment, stickiness, nuclear vacuolation, bridges, laggards and micronuclei (Table 1). The depression of mitotic index (MI) was gradually increased with increasing concentrations of extract ranging from 15.22 ± 0.32 to 5.12 ± 0.31 . The chromosomal abnormalities were almost negligible in control. The maximum abnormalities were observed in 100% inflorescence extract of *Parthenium* (40.59%), and the minimum in 10% stem extract of *Parthenium* (9.58%). The gradation of genotoxicity for MI and chromosomal abnormalities of different parts of *Parthenium* were noted as inflorescence > leaf > whole plant > stem. There has been evidence for inducing chromosomal abnormalities and mitotoxic effect of the extract of different plants such as Neem (Sinha & Kumar 2005), *Chrysanthemum* (Sinha 2003), *Lantana* (Mohanka et al. 2005). The reduction of mitotic activity seems to be a common effect of the leaf extract tested to their action (Mohanka et al. 2005).

Mitotic inhibition by *Parthenium* extract has been attributed to blocking of mitotic cycle during interphase which may result from a prolonged G₂ period or the inhibition of DNA synthesis. Mohandas & Grant (1972) reported similar results by the application of herbicides. The mitotic poison may cause metabolic imbalances which may interfere with the synthesis, state and structure of nucleic acid including physiological effects and structural changes in chromosomes during cell division, which may lead to mitotic delay and mitotic inhibition (Soni et al. 1982). Reduction of MI might have been achieved by the inhibition of DNA synthesis at S-phase (Sudhakar et al. 2001). Reduction in mitotic index indicates the potential of the *Parthenium* extract to arrest cell division at G₁ phase or retardation during S or G₂ phase of cell cycle. Decrease in the mitotic index as a result of treatment with a particular substance shows its capacity to arrest cell division together with its ability to kill the

Table 1: Effect of *Parthenium* extract on mitotic chromosomes of *Allium cepa* L.

Treatment Concentration (%)	Plant parts	No. of Dividing cell	% age of abnormal cells	F (%)	S (%)	N V (%)	B (%)	L (%)	Mi (%)	MI ±SE
Control		557	0.35	0.17	-	-	-	0.17	-	16.08±0.86
10 %	PS	167	9.58	2.39	4.20	0.60	1.20	1.20	-	15.22±0.32
	PL	178	10.64	2.60	3.36	0.56	1.88	0.56	1.68	14.16±0.26
	PI	259	14.71	5.02	5.02	-	0.77	1.95	1.93	13.19±0.22
	PWP	222	11.50	2.45	4.50	0.90	1.80	0.45	1.35	14.44±0.53
25 %	PS	165	15.15	3.02	6.06	1.20	3.64	0.61	0.61	13.99±0.66
	PL	180	16.09	2.77	5.00	1.77	3.78	0.55	2.22	12.52±0.52
	PI	231	22.32	6.61	6.86	0.86	3.25	2.17	2.57	11.38±0.24
	PWP	131	16.58	3.06	4.35	0.76	3.06	3.82	1.57	13.86±0.32
50%	PS	148	20.38	4.73	6.74	2.02	4.05	1.34	1.35	12.28±0.44
	PL	197	22.67	4.56	6.98	2.01	4.57	2.01	2.54	11.62±1.02
	PI	319	28.21	6.59	8.46	1.25	4.07	3.82	3.82	09.42±0.34
	PWP	151	21.22	4.98	5.30	1.32	3.99	2.64	2.99	10.16±0.32
75%	PS	184	23.81	5.44	6.51	2.17	4.89	1.63	3.17	10.32±0.54
	PL	144	27.62	6.55	6.34	0.69	6.16	4.16	3.72	08.19±0.33
	PI	164	31.77	7.82	6.71	1.83	6.17	4.05	4.65	07.16±0.52
	PWP	126	25.46	6.55	5.56	2.38	5.55	3.18	2.38	09.48±0.34
100%	PS	179	28.05	6.60	7.15	2.75	6.05	2.79	2.75	08.82±0.24
	PL	154	34.14	9.08	6.50	3.24	5.55	5.90	3.90	06.22±0.13
	PI	176	40.59	10.05	8.93	2.79	7.89	5.34	5.59	05.12±0.31
	PWP	133	31.30	7.77	7.37	3.01	6.01	3.74	3.00	06.92±0.51

F= Fragments, S = Stickiness , NV = Nuclear Vacuolation, B = Bridge, L = Laggards, Mi = Micronuclei, MI = Mitotic index, PS= *Parthenium* stem , PL= *Parthenium* leaf , PI= *Parthenium* inflorescence, PWP= *Parthenium* whole plant

actively dividing cells.

Cummins et al. (1996) reported that the proteins, which determine the duration of transition from metaphase to onwards, are concerned with the transformation of chemical energy into the mechanical work of mitosis. This transformation of energy is ceased by inhibitors such as azaserine which inhibit cell division by inhibiting the pathways of glycolysis, Kerbs’s cycle and oxidative phosphorylation.

A linear correlation between the concentration of extract and chromosomal abnormalities was recorded. Fragments may be formed due to DNA breakage by endonuclease (Grant 1978). Stickiness of chromosomes was the most common abnormality observed in the present investigation, which could be due to distribution in the cytochemically balanced reactions or due to partial dissociation of the nucleoproteins and alteration in their pattern of organization (Evans 1962). Stickiness may be considered to be a physiological effect exerted by pesticides (Grant 1982). Stickiness is a type of physical adhesion involving mainly the proteinous matrix of chromatin material. Bridges might have occurred by the union of large centric fragments at their distal ends. The chromosomes probably lost their ability to move due to localized action of *Parthenium* extract on centromere. Similar action was reported by Sreekrishna (2006). The anaphasic bridges might have been formed by unequal exchange or dicentric chromosomes, while laggards due to stickiness of chromosomal ends (Kaur & Grover 1985). The phenomenon of lagging of chromosomes can be attributed to the prometaphase movement of chromosomes accompanied by adhesion of centromere to the nuclear membrane or to the surrounding surface of the plasma membrane (Deena & Thoppil 2000). Multiplo-

lar anaphase abnormalities are caused due to inhibition of spindle formation (Amar & Ali 1983). Micronuclei might be due to aggregation of chromatin materials into masses of various number and size. Omanakumari et al. (2006) reported that micronuclei appear due to fragmentation of chromosomes by clastogenic action of monosodium glutamate. Chromosomal aberrations are considered as reliable indicators of mutational changes and are used as reliable evidence for screening the mutational activity. The reduction in MI, induction of chromosomal aberration and suppression in DNA content might be due to parthenin, caffeic acid and cinnamic acid present in the *Parthenium* extract (Kanchan 1975). The individual effect of parthenin, caffeic acid and cinnamic acid on chromosomes and DNA content needs further studies.

The present work confirmed that *Parthenium hysterophorus* extract has a strong potential for inducing chromosomal abnormalities and reducing DNA content and MI. Thus, *Parthenium* extract may be used as a potential antitumour promoting substance.

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