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Research Article

ALLELOPATHIC AND CYTOTOXIC EFFECTS OF AQUEOUS EXTRACTS OF *PARTHENIUM HYSTEROPHORUS* ON *CUCUMIS SATIVUS* L. VAR. SAMBAR

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ABSTRACT
Parthenium (*Parthenium hysterophorus* L.) is an invasive weed of family Asteraceae, rapidly spreading in several parts of India. The invasive nature of this weed has been attributed mainly due to its ability to germinate fast and to inhibit growth of neighboring plant species. In addition, the inhibitory allelochemics in this weed has contributed towards its acquisition of dominant status even among crop plants in various areas. On account of its extraordinary spread, dominance and its naturalisation of the country in a short span, this weed was taken to obtain qualitative as well as quantitative data of the cytotoxic effects of aqueous extracts of its root, stem, leaf and inflorescence on the root tip cells of an important vegetable crop cucumber (*Cucumis sativus* L. var. Sambar) and their allelopathic effects on the seven day old seedlings. The LD_{50} concentration for the leaf and inflorescence extracts was recorded as 26%, while 28% concentrations of both root and stem extracts proved to be $LD_{50.}$ The extracts from all parts of the weed decreased the mitotic index of the crop with increasing concentrations (5, 10, 15, 20, 25 and 30%). However the chromosomal aberrations were increased rapidly, the highest being with leaf extract (17.65%), followed by inflorescence (11.98%), root (10.99%) and stem (8.33%) at 25% concentration. Various chromosomal abnormalities like fragments, stickiness, ring chromosomes, micronuclei, laggards and bridges were observed in all extract applications. The leaves and inflorescence of *Parthenium hysterophorus* were more potent mitodepressive agents and they played a vital role in maintaining the dominance of the weed by inducing aberrations in the cell cycle of the plant. The results indicate that prevention of this weed leachates from further intrusion into crop fields becomes essential as they are highly carcinogenic.

Key Words: *Parthenium hysterophorus, Root, Stem, Leaf and Inflorescence Extracts, Cucumis sativus, Allelopathic Effects, Cytotoxicity*

INTRODUCTION
Allelochemicals are a subset of secondary metabolites, which are not required for metabolism of the allelopathic organism. These chemicals can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms, in which the negative allelopathic effects are an important part of plant defense against herbivory (Rajendiran, 2000a). The allelochemics were liberated by volatilization from aerial parts, exudation from roots, leaching from plants and their residues by rain or by decomposition of residues or upon exposure to stress or death of the tissue (Nikki and Scott, 2010). The quantity and concentration of such chemical compounds released into the environment by a species is directly responsible for the survival as well as dominance of that species and reduction or even elimination of associated plant species (Aneja *et al*., 1991; Rajendiran, 2000a; Rajendiran, 2000c; Bertholdsson, 2012). *Parthenium hysterophorus* L. belonging to the family Asteraceae is a noxious exotic weed which colonizes disturbed sites very aggressively, impacting pastures and croplands by outcompeting native species. The allelopathic effect, coupled with the absence of natural enemies like insects and diseases, is responsible for its rapid spread in its introduced ranges. Growth inhibitors like lactones and phenols are released from this plant into the soil through leaching, exudation of roots and decay of residues. These growth inhibitors suppress the growth and yield of native plants. The test plant chosen for the study is Cucumber (*Cucumis sativus* L. var. Sambar), an important and commercially

popular cucurbitaceous vegetable crop which holds a very coveted position in the vegetable market. The crop, native to India, is one of the most nutritive vegetables rich in vitamins and minerals such as phosphorus, potassium, calcium and iron. It is mainly grown for its fruits both in tropics and subtropics of the world and produces tender fruits continuously. Hence, it was thought worthwhile to estimate the influence of aqueous extracts of leaf, stem and inflorescence of the weed *Pathenium hysterophorus* L. (Asteraceae) on seedling establishment of this important vegetable crop *Cucumis sativus* L. var. Sambar.

MATERIALS AND METHODS

The certified seeds of *Cucumis sativus* L. var. Sambar were obtained from Department of Vegetable crops, Tamil Nadu Agricultural Univeristy, Coimbatore. The fresh roots, stem, leaves and inflorescence of *Parthenium hysterophorus* L. collected from Pondicherry, were washed and ground separately in an electric grinder and the extracts were prepared in each case by boiling 10 gm of ground plant material in 100 ml of distilled water at 100^{o}C for 25 minutes. After filtration with Whatman No.1 filter paper, stock solutions were prepared.

For determining the LD_{50} concentration of the extracts three separate sets of experiments each with triplicates were conducted and the data presented in Table 1. In the first set, various concentrations of root, stem, leaf, and inflorescence extracts (25, 50, 75, and 100%) of *Parthenium hysterophorus* L. were made in distilled water. Viable seeds of *Cucumis sativus* L. var. CO 1, soaked in distilled water for 6 hours were allowed to germinate in petri plates lined with moist Whatman No.1 filter paper. Seven days old seedlings with healthy roots were treated with 5 ml of each concentration of the extracts for three days. Seedlings watered with distilled water served as control. The second treatment of different concentrations of the weed extracts (25, 30, 35, 40, 45, 50% concentrations) was given to fresh set of seedlings grown in petri plates. The third set of treatment consisted of 25, 26, 27, 28, 29, and 30% concentrations of the extracts to a new set of seven day old seedlings.

The root tips of 10 day old seedlings were highly injured after treatment with 30% concentrations of the weed extracts. Even though few seedlings survived, their root tips were unhealthy for preparing root tip squash. Hence the cytological studies with three test plants were restricted to 5, 10, 15, 20 and 25% concentrations of the weed extracts. For the cytotoxic studies root tips were excised from the control and treated seedlings (5, 10, 15, 20 and 25% concentrations of the four extracts), washed in distilled water and fixed in Carnoy's fixative for 24 hours. Root tip squash technique of Rajendiran (2005) was followed. The mitotic index in control and treated root tip cells were calculated. The prepared slides were thoroughly examined for the presence of different types of chromosomal aberrations, important stages photographed and the data recorded.

RESULTS AND DISCUSSION

In *Cucumis sativus* L. var. Sambar the root, stem, leaf and inflorescence extracts of *Parthenium hysterophorus* L. affected the process of seedling growth. The entire lot of the seedlings treated with 50, 75, and 100% concentrations in the first set died (Table 1). However the seedlings in 25% concentration of the extracts survived as the lethality was between 40 to 46.7% only. In the second set the seedlings treated with 35, 40, 45, 50% concentrations of the four extracts died completely, while in 25 and 30% concentrations the lethality ranged from 40 to 46.7% and 55 to 68.3% respectively. In the third set of experiments the LD_{50} concentration for the leaf and inflorescence extracts was recorded as 26%, while 28% concentrations of both root and stem extracts proved to be LD_{50} (Table 1). The inhibition of seedling growth was recorded maximum at the highest concentration of leaf extract treatment (Table 1). As evident from the tabulated data, differential effect of the extracts on seedling growth indicated the presence of highest concentration of inhibitory allelochemics in the leaves of the weed followed by inflorescence, stem and root. These results were in accordance with the findings of Rajendiran (2000a) in *Helianthus annuus* L. and Hridya and Rajendiran (2013) in *Cucumis sativus* L. var. CO1 seedlings.

Table 1. Lethality of the leaf, stem, root and inflorescence extracts of *Parthenium hysterophorus* L. on the 7 day old seedlings of *Cucumis sativus* L. var. CO 1 after 3 days of treatment.

Expt. Set No.	Extract Concentration	Root (%)	Stem (%)	Leaf (%)	Inflorescence (%)
1	25 %	40	41.7	46.7	45
	50 %	100	100	100	100
	75 %	100	100	100	100
	100 %	100	100	100	100
2	25 %	40	43.3	46.7	45
	30 %	55	56.7	68.3	63.3
	35 %	96.7	96.7	100	100
	40 %	100	100	100	100
	45 %	100	100	100	100
	50 %	100	100	100	100
3	25 %	40	41.7	46.7	45
	26 %	43.3	46.7	50	50
	27 %	46.7	48.3	53.3	51.7
	28 %	50	50	56.7	53.3
	29 %	53.3	58.6	63.3	61.7
	30 %	55	60	68.3	63.3

The root tips of *Cucumis sativus* L. var. Sambar in control condition showed normal cell division (Plate 1, Figure 1). Mitotic index of *Cucumis sativus* L. var. Sambar followed a steady decrease with increasing concentrations of all the extract treatments (Table 2). The percentage value of mitotic index in control was 41.33% and it declined rapidly to 21.33% in both root and stem extracts, reaching the minimum of 13.66% and 20.66% after treatment with leaf and inflorescence extracts respectively in 25% concentration (Table 2; Plate 1, 2). Similar observations were reported with *Ammi majus* (Adam and rashad, 1984*)*, *Datura stramonium* (Rajendiran, 1996), *Azadirachta indica* (Rajendiran 1998a), *Catharanthus roseus* (Rajendiran, 1998b), *Lantana camara* (Rajendiran, 1999a), *Ricinus communis* (Rajendiran, 1999b), *Adhatoda vasica* (Rajendiran, 1999c), *Boerhaavia diffusa* extracts (Rajendiran, 2000b) and in *Cucumis sativus* L. var. CO1 (Hridya and Rajendiran, 2013).

Various chromosomal abnormalities accounting to seven types were induced by all the extracts of the weed in the dividing cells, which increased with increasing concentration and the largest values were recorded at the highest concentration (Table 2). The extracts of the leaves and inflorescence caused severe inhibition with more number of chromosomal aberrations (17.65 and 11.98% respectively) than the stem and root extracts (8.33 and 10.99% respectively), the least being with root extract (Table 2; Plate 1, 2). Irrigation of the weed extracts to *Cucumis sativus* L. var. Sambar seedlings altered the events of somatic cell division and caused fragmentation of chromosome (Plate 1, Figure 2), chromosome stickiness (Plate 1, Figure 3), ring chromosomes (Plate 1, Figure 4), chromosome bridges during anaphase (Plate 1, Figure 5), laggard formation (Plate 1, Figure 6) during anaphase movement, micronuclei (Plate 1, Figure 7) and precocious movement of chromosomes (Plate 1, Figure 8).

Table 2. Mitosis and chromosomal aberrations induced by *Parthenium hysterophorus* L. extracts in *Cucumis sativus* L. var. Sambar root tip cells.

Extract	Conc. (%)	Dividing cells (%)	Abnormal cells (%)	Stickiness (%)	Laggards (%)	Bridge (%)	Chromosome breakage (%)	Polyploidy (%)	Micronuclei (%)	Ring chromosomes (%)
Control		41.33	-	-	-	-	-	-	-	-
Root	5	33.66	3.00	1.33	1.00	0.67	-	-	-	-
	10	30.00	5.66	2.33	1.33	1.00	1.00	-	-	-
	15	28.00	6.33	2.33	1.67	1.33	1.00	-	-	-
	20	24.66	9.34	3.67	2.67	1.33	1.00	0.67	-	-
	25	21.33	10.99	4.67	2.33	2.33	1.33	0.33	-	-
Stem	5	32.33	2.66	1.00	1.00	0.66	-	-	-	-
	10	29.33	3.66	1.00	1.00	1.00	0.66	-	-	-
	15	26.66	6.00	1.00	1.00	1.00	1.00	0.67	0.67	0.66
	20	23.33	7.66	1.67	1.33	1.00	1.00	1.00	1.00	0.66
	25	21.33	8.33	2.00	1.00	1.33	0.67	1.00	1.00	1.33
Leaf	5	25.66	3.66	1.00	1.00	0.67	0.66	-	0.33	-
	10	23.66	6.98	1.33	1.00	1.33	1.33	1.33	0.33	0.33
	15	20.33	11.00	2.00	1.67	1.67	1.67	1.33	1.33	1.33
	20	18.00	13.33	2.67	1.67	2.67	2.33	1.00	1.66	1.33
	25	13.66	17.65	3.66	3.00	3.00	2.66	1.33	2.00	2.00
Inflorescence	5	29.33	3.33	1.00	0.67	1.33	0.33	-	-	-
	10	29.66	6.00	2.33	1.33	1.34	0.67	0.33	-	-
	15	26.00	9.63	2.66	1.66	2.33	1.33	0.66	0.66	0.33
	20	24.33	11.66	2.33	2.34	2.33	2.00	0.67	1.33	0.67
	25	20.66	11.98	2.66	2.66	2.00	2.00	1.00	1.33	0.33

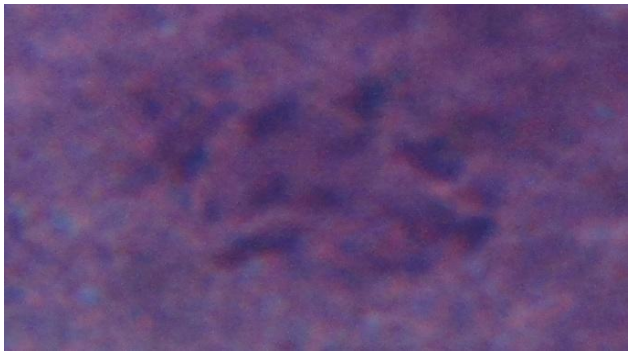

Figure 1: Normal somatic metaphase (2n=14)

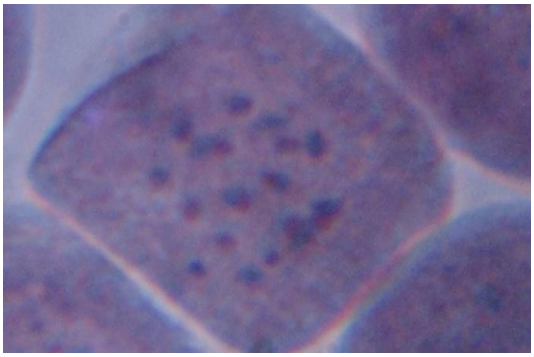

Figure 2: Chromosome fragments

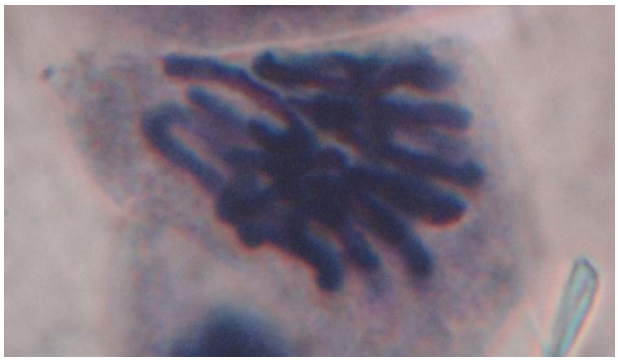

Figure 3: Stickiness of chromosomes

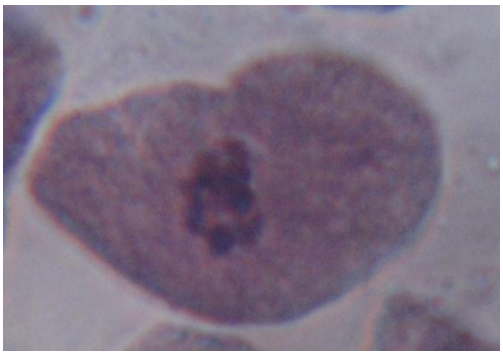

Figure 4: Ring chromosomes

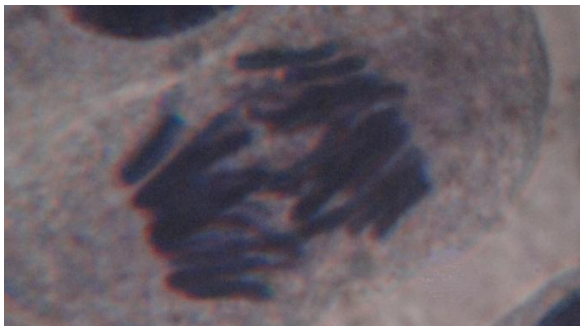

Figure 5: Anaphasic bridges

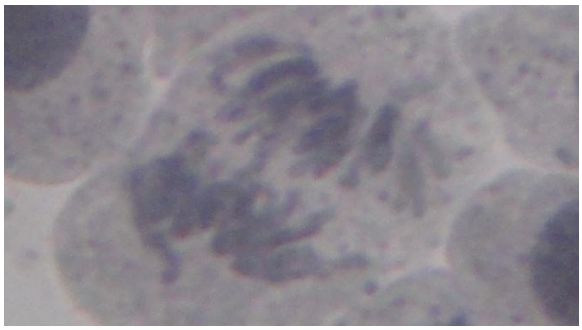

Figure 6: Laggard formation

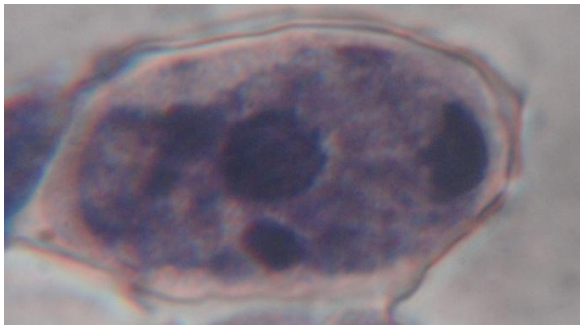

Figure 7: Micronuclei

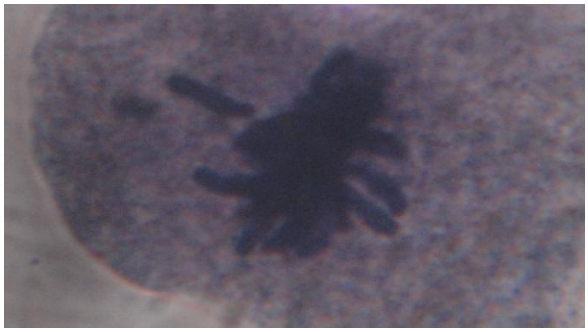

Figure 8: Precocious movement

Plate 1: Somatic metaphase and chromosomal abnormalities induced by *Parthenium hysterophorus* L. extracts in the root tip cells of *Cucumis sativus* L. var. Sambar (1000x)

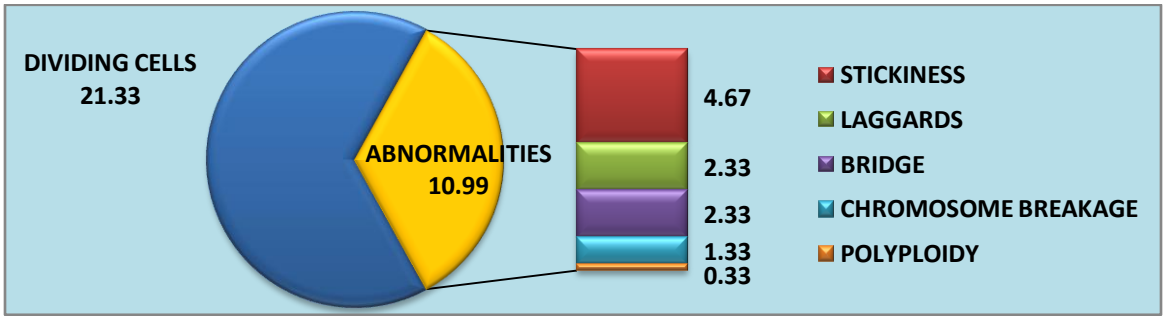

Figure 1: Root extract

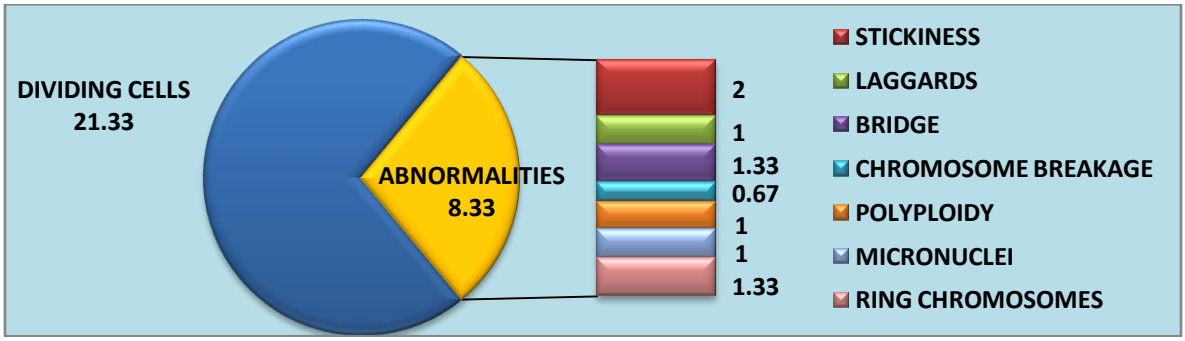

Figure 2: Stem extract

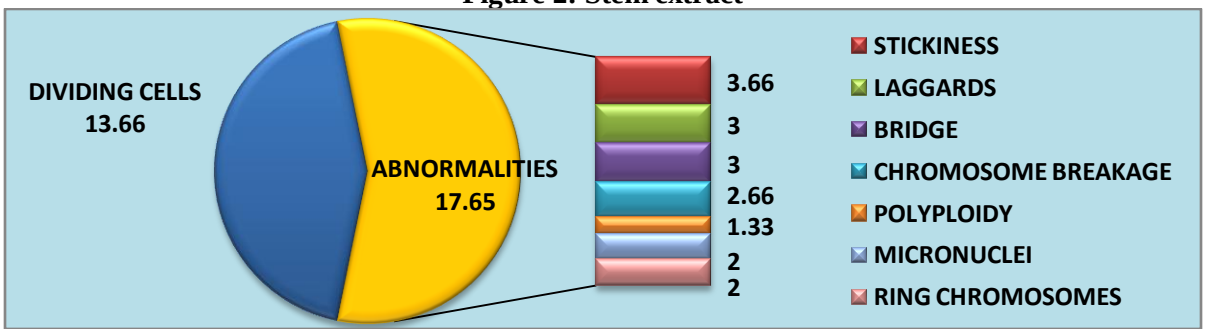

Figure 3: Leaf extract

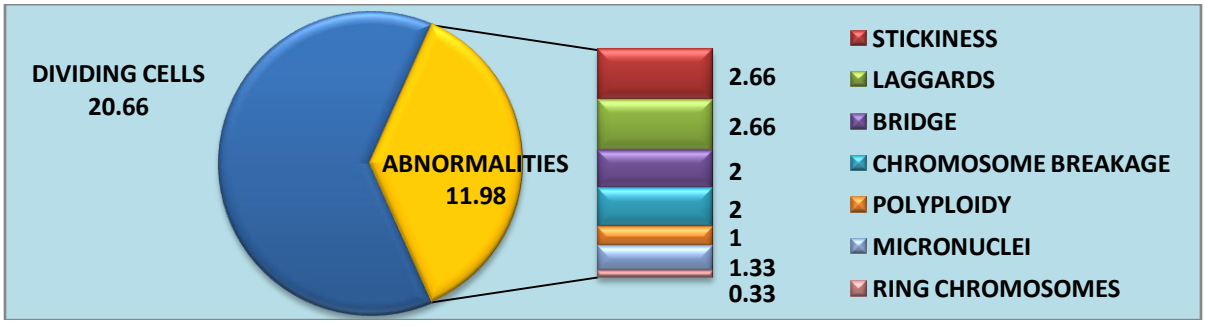

Figure 4: Inflorescence extract

Plate 2: Mitotic divisions, chromosomal abnormalities and their types induced by 25% concentrations of weed extracts in *Cucumis sativus* L. var. Sambar

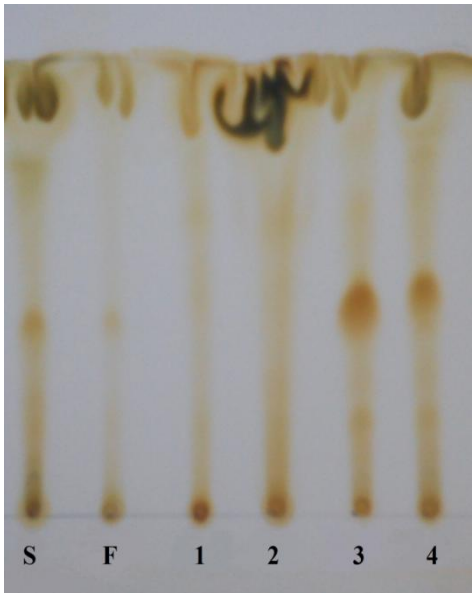

Figure 1: Stained with iodine vapour

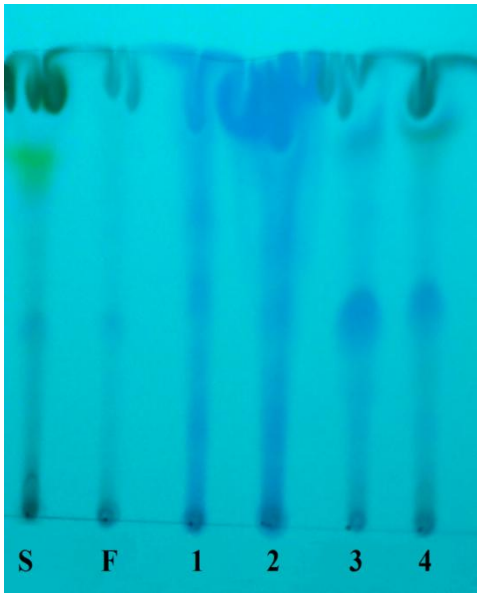

Figure 2: Observed under ultraviolet light

Plate 3: TLC of *Parthenium hysterophorus* L. root, stem, leaf and inflorescence extracts (S - Leaf stock, F- Leaf filtrate, 1 - Root, 2 - Stem, 3 - Leaf, 4 - Inflorescence extracts)

Thin layer chromatography (TLC) of all the samples resolved into two major components corresponding to Rf 0.86 and 0.43 which represented Caffeic acid and Parthenin respectively (Plate 3). The spot at Rf. 0.43 representing Parthenin was highly intense in leaf and inflorescence samples and less intense in stem. The spot appeared as evanescent in root sample. Three minor spots at Rf 0.93, 0.75, and 0.18 were identified as Ambrosin, 2-β-Hydroxycoronopilin and Dihydroxyparthenin. Ambrosin and Dihydroxyparthenin appeared in stem while 2-β-Hydroxycoronopilin and Dihydroxyparthenin resolved in leaf sample (Plate 3).

The data revealed that the leaves and inflorescence of the weed showed intensive inhibitory effects and were severely clastogenic and spindle poisoning as compared to stem and root extracts. This result correlated with TLC of leaf and inflorescence which resolved four components, while stem and root extracts resolved only two each (Plate 3). Further, the present work was supported by the report of Kanchan (1975) and Pandey (2009) that the toxins *viz.* parthenin and phenolic acids such as caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid, p-anisic acid and p-coumaric acid were maximum in the leaves of *Parthenium hysterophorus* L. followed by inflorescence, stem and roots. The allelopathic and cytotoxic studies with seedlings of *Cucumis sativus* L. var. Sambar revealed that the leaves and inflorescence of *Parthenium hysterophorus* L. proved to be potent mitodepressive agents as they played a vital role in maintaining the dominance of the weed by disturbing the cell cycle of associated plant species. Thus an immediate measure is necessary to check the weed population, as their leachates are more vulnerable in scraping away the chromosomes of crop plants.

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