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Research Article

ALLELOPATHY AND CYTOTOXICITY OF AQUEOUS EXTRACTS OF *PARTHENIUM HYSTEROPHORUS* L. ON *ORYZA SATIVA* L. VAR. ASD-16

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ABSTRACT
Parthenium hysterophorus L. (Asteraceae) commonly known as 'white top' is a noxious exotic weed, spreading rapidly in pasture, wastelands and agricultural fields. The ability of the weed to germinate quickly and to suppress the growth of plant species in the surroundings paved way to its accelerated growth in an area. In addition, the toxic allelochemicals have contributed towards its dominance even over crop plants. On account of the extraordinary spread, dominance and its naturalisation in India in a short period of time, this weed was taken to assess the allelopathic effects and cytotoxic activities of aqueous extracts of its root, stem, leaf and inflorescence on the seven day old seedlings of important cereal crop rice (*Oryza sativa* L. var. ASD-16). The LD_{50} concentration for the leaf and inflorescence extracts was recorded as 26%, while 27% concentrations of both root and stem extracts proved to be LD_{50}. The extracts from all the parts of the weed decreased the mitotic index of rice root tips with increasing concentrations (5, 10, 15, 20 and 25 %). However the chromosomal abnormalities increased rapidly, the highest being with leaf extract (25.14%), followed by inflorescence (22.42%), stem (17.41%) and root (15.11%) at 25% concentration. Seven types of chromosomal aberrations *viz.*, fragments, stickiness, micronuclei, laggards and bridges were observed in all extract applications. The allelochemicals in the leaves and inflorescence of *Parthenium hysterophorus* were highly carcinogenic and served as a potent tool in eroding the chromosomes of rice. The present work cautions that the weed leachates have been proved to be dangerously mitodepressive and so the prevention of this weed from further intrusion into cultivable land is urgent.

Key Words: *Parthenium Hysterophorus, Root, Stem, Leaf and Inflorescence Extracts, Oryza Sativa, Allelopathic Effects, Cytotoxicity*

INTRODUCTION
Allelochemics are non-nutritional secondary metabolities produced by plants that have inhibitory effects on neighboring plants.
These chemicals were liberated by volatilization from aerial parts, exudation from roots, leaching from plants and their residues by rain or by decomposition of residues (Nikki and Scott, 2010). Allelochemicals have a wide mode of action and 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, spreading rapidly throughout India. Its rapid growth has been attributed mainly due to its ability to germinate fast and to inhibit growth of other associated plant species. Rice (*Oryza sativa* L. var. ASD-16) used as test plant in this study is an important and commercially popular cereal in Indian markets.
Even though several works of allelopathy have been done with various crop plants, no work on cytotoxity of allelochemics in cereals have been carried out.
Hence, it was thought worthwhile to evaluate the influence of aqueous extracts of leaf, stem and inflorescence of the weed *Pathenium hysterophorus* L. on seedling establishment and cell division of an important cereal crop, *Oryza sativa* L. var. ASD-16.

MATERIALS AND METHODS

The certified seeds of rice (*Oryza sativa* L. var. ASD-16) were obtained from Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu. 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 four extracts, three separate sets of experiments each with triplicates were carried out 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 rice (*Oryza sativa* L. var. ASD-16), 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 chosen to accommodate 25 seedlings in a petriplate for each treatment. The healthy seedlings were treated separately 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 weed 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. The root tips were excised from the control and treated seedlings (5, 10, 15, 20 and 25% concentrations of the four extracts) after three days of extract treatment, 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 in Labomed Photo Microscope and the data presented in Table 2.

RESULTS AND DISCUSSION

The root, stem, leaf and inflorescence extracts of *Parthenium hysterophorus* L. affected the process of seedling growth in *Oryza sativa* L. var. ASD-16. All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 41.66 to 46.33% in 25% concentration of all the extracts (Table 1). In the second set the whole lot of seedlings treated with 35, 40, 45, 50% concentrations of the four extracts died, while in 25 and 30% concentrations the lethality was 41.66 to 68.7% respectively. In the third set of experiments the LD_{50} concentration for the leaf and inflorescence extracts was recorded as 26%, while 27% concentrations of root and stem extracts proved to be LD_{50} (Table 1). The maximum inhibition of seedling growth was recorded at the highest concentration of leaf extract treatment. From the collected data it is evident that differential effect of the extracts on seedling growth revealed the presence of highest concentration of inhibitory allelochemics in the leaves of the weed followed by inflorescence, stem and root. Rajendiran (2000a) in *Helianthus annuus* L. seedlings and Hridya and Rajendiran (2013) in C*ucumis sativus* L. have reported similar inhibitions.

In control condition, the root tips of *Oryza sativa* L. var. ASD-16 showed normal cell division (Plate 1, Figure 1). Mitotic index of *Oryza sativa* L. var. ASD-16 showed a steady decease with increasing concentrations of all the extracts (Table 2). The percentage value of mitotic index in control was 34.44% and after treatment with root, stem, leaf and inflorescence extracts it declined rapidly with the increase in concentrations. The least values of 15.23%, 13.87%, 8.71% and 10.84% were recorded after treatment with root, stem, leaf and inflorescence extracts respectively in 25% concentration (Table 2; Plate 1, 2). Similar observations were reported in *Ammi majus* (Adam and Rashad, 1984*), Datura stramonium* (Rajendiran, 1996), *Azadirachata 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* (Hridya and Rajendiran, 2013).

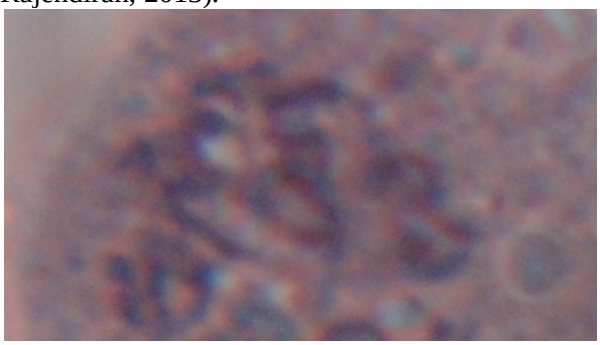

Figure 1: Normal somatic metaphase (2n=12)

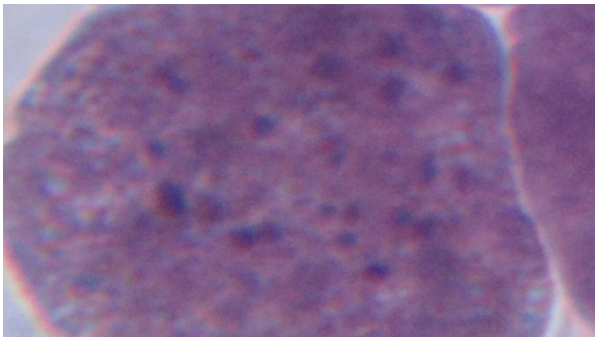

Figure 2: Chromosome fragments

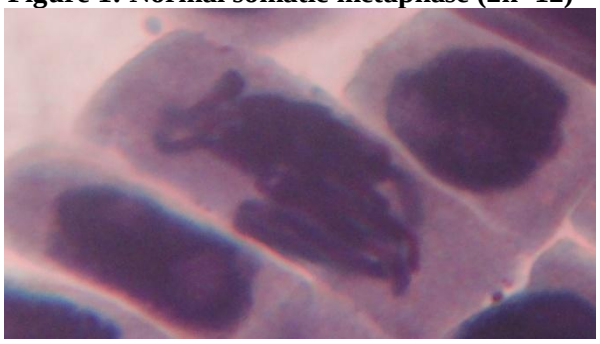

Figure 3: Stickiness of chromosome ends

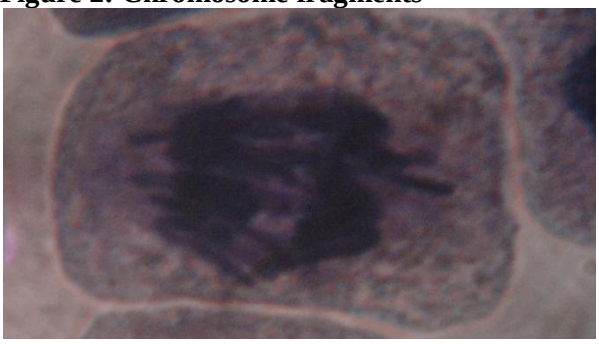

Figure 4: Anaphasic bridges

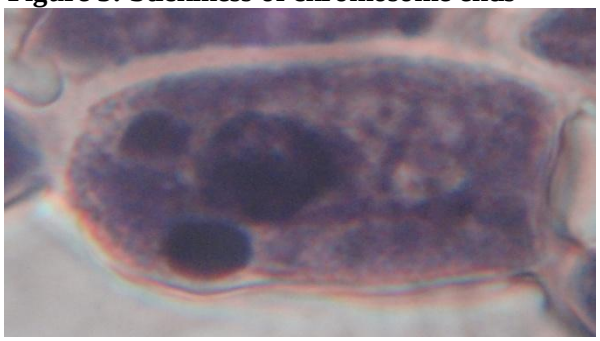

Figure 5: Micronuclei

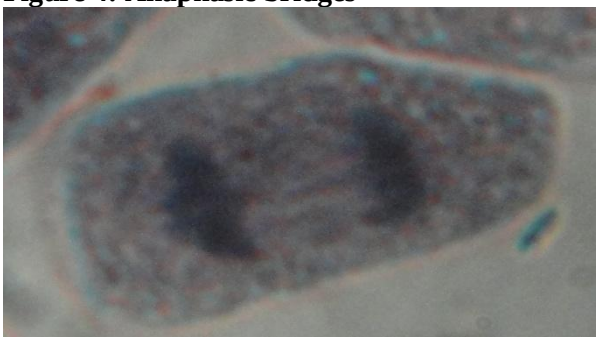

Figure 6: Laggard formation

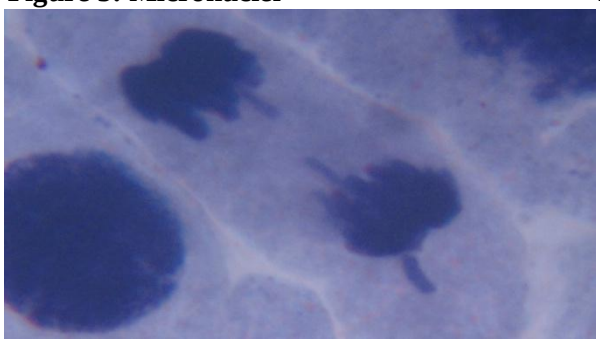

Figure 7: Precocious movement

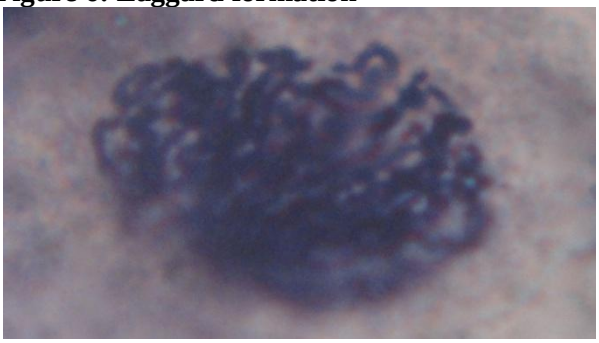

Figure 8: Polyploid cell

Plate 1: Somatic metaphase and chromosomal abnormalities induced by *Parthenium hysterophorus* L. extracts in the root tip cells of *Oryza sativa* L. ASD-16 (1000x).

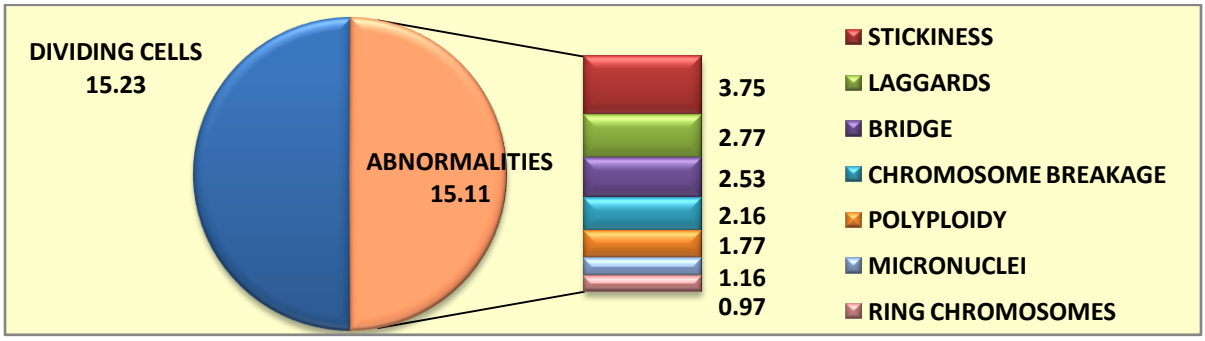

Figure 1: Root extract

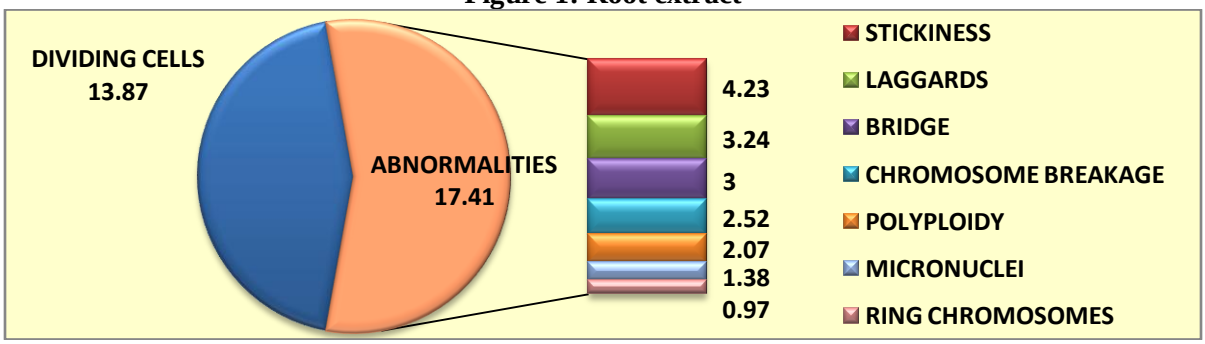

Figure 2: Stem extract

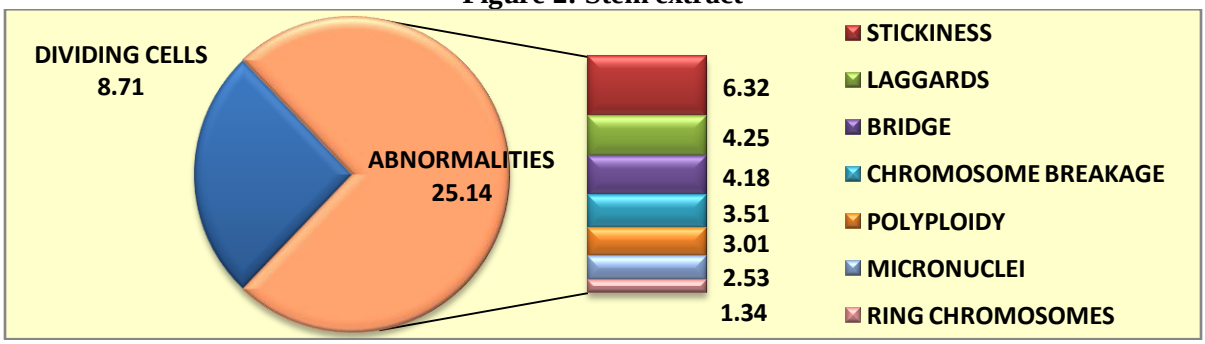

Figure 3: Leaf extract

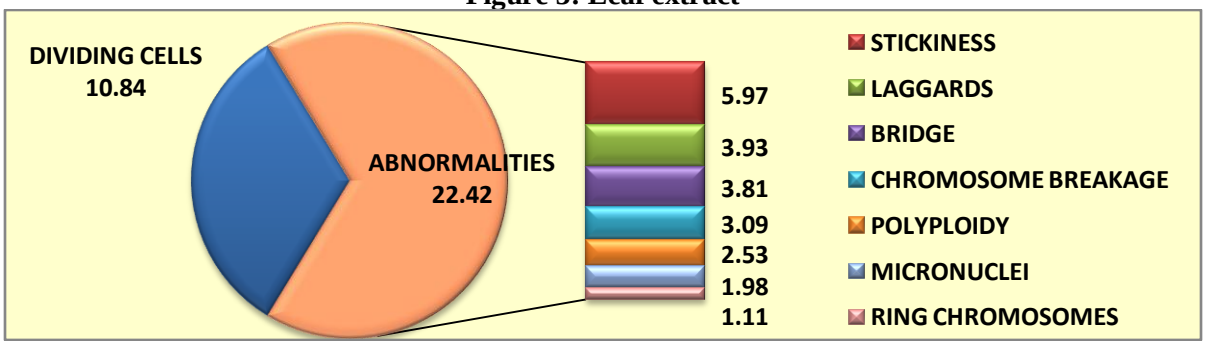

Figure 4: Inflorescence extract

Plate 2: Mitotic divisions, chromosomal abnormalities and their types induced by 25% concentrations of weed extracts in *Oryza sativa* var. L. ASD-16

All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the maximum was recorded at the highest

concentration (Table 2; Plate 1, 2). However, the extracts of leaves and inflorescence caused severe inhibition and greater number of chromosomal abnormalities (25.14 and 22.42% respectively) than the stem and root extracts (17.41% and 15.11% respectively) at 25% concentration, the least being with root extract (Table 2; Plate 1, 2).

Application of extracts of the weed changed the normal cycle of events of mitosis in *Oryza sativa* L. var. ASD-16 root tip cells producing chromosome fragments (Plate 1, Figure 2), stickiness of chromosome ends (Plate 1, Figure 3), chromosome bridges during anaphase (Plate 1, Figure 4), micronuclei (Plate 1, Figure 5), laggard formation (Plate 1, Figure 6), precocious movement of chromosomes (Plate 1, Figure 7) and polyploidy (Plate 1, Figure 8).

Table 1: Lethality of the leaf, stem, root and inflorescence extracts of *Parthenium hysterophorus* L. on the 7 day old seedlings of *Oryza sativa* L. var. ASD-16 after 3 days of treatment

Expt. Set No.	Extract Concentration	Root (%)	Stem (%)	Leaf (%)	Inflorescence (%)
1	25 %	41.66	42.33	46.33	45.66
	50 %	100	100	100	100
	75 %	100	100	100	100
	100 %	100	100	100	100
2	25 %	41.66	42.33	46.33	45.66
	30 %	56	57.7	68.7	64.3
	35 %	96.7	96.7	100	100
	40 %	100	100	100	100
	45 %	100	100	100	100
	50 %	100	100	100	100
3	25 %	41.66	42.33	46.33	45.66
	26 %	43.3	46.3	50	50
	27 %	50	50	53.3	51.7
	28 %	53.3	54.7	58.7	59.3
	29 %	54.3	56.6	63.3	61.7
	30 %	56	57.7	68.7	64.3

Table 2: Mitosis and chromosomal aberrations induced by *Parthenium hysterophorus* L. extracts in *Oryza sativa* L. var. ASD-16 root tip cells

Extract	Conc. (%)	Dividing cells (%)	Abnormal cells (%)	Stickiness (%)	Laggards (%)	Bridge (%)	Chromosome breakage (%)	Polyploidy (%)	Micronuclei (%)	Ring chromosomes (%)
Control		34.44	-	-	-	-	-	-	-	-
	5	21.02	2.87	1.33	1.54	-	-	-	-	-
	10	19.82	5.46	2.48	1.16	0.95	0.87	-	-	-
Root	15	17.48	8.84	2.22	1.73	1.57	1.30	0.67	-	-
	20	16.77	10.12	2.97	2.25	2.08	1.70	1.12	-	-
	25	15.23	15.11	3.75	2.77	2.53	2.16	1.77	1.16	0.97
	5	19.69	2.90	1.44	0.73	0.73	-	-	-	-
	10	18.78	6.57	2.58	1.33	1.17	1.00	0.38	-	-
Stem	15	16.74	9.14	2.44	1.88	1.69	1.38	0.78	0.39	0.60
	20	14.44	11.69	3.03	2.64	2.19	1.78	1.38	0.67	-
	25	13.87	17.41	4.23	3.24	3.00	2.52	2.07	1.38	0.97
	5	15.79	6.73	1.94	1.53	0.38	1.11	-	0.77	-
	10	14.72	9.34	2.79	2.01	1.93	1.55	0.97	-	-
Leaf	15	10.60	14.11	3.61	2.67	2.43	2.03	1.78	0.93	0.66
	20	9.69	19.22	4.48	3.48	3.22	2.87	2.23	1.74	1.20
	25	8.71	25.14	6.32	4.25	4.18	3.51	3.01	2.53	1.34
	5	17.23	4.79	1.79	1.11	0.89	0.91	-	-	-
	10	15.59	7.42	2.28	1.69	1.36	1.23	0.86	-	-
Inflorescence	15	13.18	11.24	3.18	2.19	1.91	1.79	1.32	0.38	0.38
	20	11.57	16.47	4.08	3.00	3.09	2.36	1.97	0.90	1.07
	25	10.84	22.42	5.97	3.93	3.81	3.09	2.53	1.98	1.11

The leaves and inflorescence of the weed showed severe inhibitory effects and were extremely clastogenic and spindle poisoning when compared with the extracts of the stem and root. This result correlated with 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 present study with *Oryza sativa* L. var. ASD-16 revealed that the leaves and inflorescence of *Parthenium hysterophorus* L. were more potent mitodepressive agents and they played a vital role in maintaining the dominance of the weed by suppressing the growth of surrounding plant species. Thus, it is concluded that prompt action to check the population of this weed is

urgent as the aqueous extracts of *Parthenium hysterophorus* L. have proved to be highly effective in wearing away the genotype of the food crops.

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