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Phytotoxic impact of *Parthenium hysterophorus* L. on *Macrotyloma uniflorum* a pulse crop in a dry tropical environment, Bihar, India

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Abstract
An experiment was conducted to evaluate the effect of aqueous extract of leaf, stem and root of *Parthenium hysterophorus* on the rate of seed germination (%) and seedling growth (cm) in *Macrotyloma uniflorum*. The different concentrations of root, stem and leaf extract used in the experiment were 15, 25, 50, 75 and 100%. The rate of seed germination, length of root and shoot and Seed Vigour Index (SVI) values were recorded. Data collected were analysed for Pearsons Correlation Coefficient, Tukey HSD (Honestly Significant Difference) and Post HOC Tests. It was recorded that SVI value decreased in different concentrations of leaf, stem and root from 23.53 to 100%, 3.33 to 26.21% and 32.44 to 100%, respectively. This indicated that leaf and root extracts were more phytotoxic to *M.uniflorum* than the stem extract of *P.hysterophorus*. The rate of seed germination and length of shoot differed significantly in different concentrations than the length of root, as indicated by Post HOC Test. The effect of plant parts i.e. root, stem and leaf; treatments i.e. different concentrations and interaction of plant parts and treatments were significantly differed at $p < 0.000$. Generally the impacts of different parts were also significantly different at $p < 0.000$. Thus, this study indicated that the leaf and root extracts are more toxic to *M. uniflorum* than the stem extract; and the rate of seed germination and shoot length were more affected than the root length. The seed germination and shoot growth in *M. uniflorum* are affected by leaf and root extracts of *Parthenium*.

Keywords: Phytotoxic effect, *Parthenium hysterophorus*, *Macrotyloma uniflorum*, Seed Vigour Index

INTRODUCTION

In India, *Parthenium* is considered as worst weed due to its allelopathic effects on crops and harmful effects on animals and human beings. It is spread in all states of India. It is called as “Scourge of India”. In 2012 it has covered about 35 million ha of wasteland, cropland and forest lands in India (Kumar 2012). *Parthenium* has spread in all types of cereals, pulses, cash crops, vegetable crops, pastures, forests, plantations etc. Under suitable soil and moisture conditions *Parthenium* becomes dominant species and causes exclusion of beneficial plants. The invasive species having high capability to spread rapidly and high competitiveness are the major threats to the native species and ecosystem (Kathiresan *et al.* 2005). *Parthenium* colonizes wide range of habitats and any type of soil, wastelands, pastures, road sides, agricultural lands etc. The production of crop is affected by *Parthenium.* According to McGinley and Duffy (2011) invasive species rank second as a threat to biodiversity. *P.hysterophorus* had no place in the world’ worst weed till 1977 and during 1987 it became one of the seven most dangerous weed of the world (Kumar 2015). According to Netsere (2015) allelopathy has many effects either positive or negative on many plant species by stimulating or inhibiting the surrounding herbaceous vegetation. *Parthenium* will become more problematic in future when CO_2 levels will increase due to climatic change (Nguyen *et al.* 2017).

The allelopathic effects of different parts of *Parthenium* on seed germination and seedling growth have been evaluated in rice, maize, wheat, *Artemisia dubia*, wall ex. *Ageratina adenophora* and cultivated crucifers, *R.sativus*, *B.compestris* and *B.oleracea*, (Maharajan *et al.* 2007); *Eragrostis tef* (Tafera, 2002), *Brassica* sp. (Singh *et al.* 2005); onion and beans (Demissie *et al.* 2013); three native plants of Himachal Pradesh (Dogra and Sood, 2012); rice, wheat, chickpea, soybean and mustard (Karim and Forzwa 2010; Biswas 2010); *Glycine max* and *Phaseolus vulgaris* (Netsere and Mendesil, 2011); onion (Wakjira, 2009); *Lettuca*

(Wakjira *et al.*, 2005); maize, sorghum, multipurpose trees; pumpkin and tomato (Tamado *et al.* 2002); *Alysicarpus glumaceus*, *Chloris gayana*, *Zea mays*, barley, wheat, peas, *Helianthus annus*, *Glycine max*, *Phaseolus vulgaris*, sorghum, *Eragrostis tef*, rice, chickpea, soybean, mustard, *Brassica*, green gram, black gram, moth bean, cow pea etc. (Choesin and Boerner 1991; Tafera 2002; Bajwa *et al.* 2003; Singh *et al.* 2005; Maharajan *et al.* 2007; Kumar *et al.* 2008; Rashid *et al.* 2008; Biswas 2010; Netsere and Mendesil 2011; Clarence *et al.* 2013; Purohit and Pandya 2013; Devi *et al.* 2014; Netsere 2015) soybean and hericot bean (Netsere and Mendesil 2011); wheat (Khan *et al.* 2012); *Zea mays* (Devi and Dutta 2012); chili, tomato, brinjal (Jarvis *et al.,* 1985); rice (Oudhia 1998); chickpea, mustard and linseed (Oudhia *et al.* 1997, Oudhia and Tripathi 1998); *Allium cepa* (Karim and Forzwa 2010; Biswas 2010; Demissie *et al.* 2013); *Phaseolus mungo* (Kumar and Kumar 2010); pumpkin and tomato (Guzman 1988); sunflower (Bajwa *et al.* 2004); *Festuca arundinaceae*, *Digitaria sanguinalis* (Peters and Zam 1981); wheat and associated weeds (Amin *et al.* 2007); *Ocimum americanum* (Batish *et al.* 2001); *Vigna radiata* and *Phaseolus vulgaris* (Afjal *et al.* 2000); cultivated and wild herbs;, maize (Pandey 1994); *Brassica* sp. (Rai 2013); sorghum (Rai 2015); chickpea and radish (Rajiv *et al.* 2013); tomato (Rao 1956); barley (Sarita *et al.* 2011); *Arachis hypogea* (Sharma and Bhutani 1988); *P.vulgaris* (Singh and Thapar 2003); onion and bean (Srivastava *et al.* 1985); barley (Kumar 2015); *Oriza sativa* and *Triticum aestivum* (Singh and Sangeeta 1991); soyabean (Bhatt *et al.* 1994); barley, wheat and peas (Srivastava *et al.* 1985); wheat (Patil and Hedge 1988); cabbage (Kohli *et al.* 1985); *Lepidium* (Amin *et al.* 2007); *C.aeritinum*, *P.sativum* and *C.cajan* (Singh *et al.* 2014); and *Phaseolus mungo*, *Cicer aeritinum*, *Pisum sativum*, *Cajanus cajan*, *Zea mays Brassica nigra*, *Triticum aestivum* (Shikha and Jha 2016 a, b, c, d; 2017 a, b; 2018 a, b). Tamado (2000) has reported 90% loss in the yield of sorghum whereas Gnanavel and Natrajan (2013) have reported 40% loss in yield of crops and causing socio-economic impacts. *M.uniflorum* is a pulse crop which have many medicinal uses. *Macrotyloma uniflorum* is an annual plant, densely growing, low-growing of climbing, slender, herbaceous legume reaching 30-60cm in height. The stems, leaves and husks are used as fodder or green manure and seeds are fed to cattle and horses. It is anti-bacterial, antifungal and antihyper glycemic in nature. It is rich in antioxidants. It is useful in preventing constipation, lowering blood pressure, good for weight loss, improves heart health, increases sperm count, lowers cholesterol levels, keeps body warm during winter and useful in treatment of urinary stones (Ranasinghe and Ediriweera 2017).

Thus, the present study was aimed to evaluate the impact of different concentrations of leaf, stem and root aqueous extracts of *P.hysterophorus* on the rate of seed germination and growth of seedlings of *Macrotyloma uniflorum* in laboratory condition.

MATERIALS AND METHODS

Parthenium hysterophorus has invaded the Jai Prakash University campus of about 240 ha area in just ten years. Earlier the whole area was a cropland. The study site is situated between 25^0 36'-26^0 15' N latitude and 84^0 25'-85^0 15' E longitude in the southern part of the newly - created Saran Division of North Bihar. Total area of the Saran district is 2641 sq. km.

After establishment of the University campus and abandonment of cropping *P.hysterophorus* invaded the whole area in just ten years. It is classified as below:

Division: Eukaryota
Kingdom: Plantae
Phyllum: Spermetophyta
Sub – Phyllum: Angiospermae
Class: Dicotyledonae
Order: Asterales
Family: Asteraceae
Genus: *Parthenium*
Species: *hysterophorus*

Parthenium grows luxuriantly in wastelands, public lawns, orchards, forestlands, flood plains, agricultural areas, urban areas, overgrazed pastures, industrial areas, playgrounds, roadsides, railway tracks and residential plots. Drought and subsequent reduced pasture cover creates the ideal situation for the *Parthenium* weed to establish itself. Although it is capable of growing in moist soil types, it is most dominant in alkaline, clay loam soils (Kaur *et al.* 2014; Tafera 2002). Plant samples were collected from the University campus from vegetative phase of *P.hysterophorus* during the period 2017. Root, stem and leaves were separated and air dried in shade and crushed with the help of laboratory blender. Stems and roots were cut into small pieces and dried samples were grounded using laboratory blender. The leaves were dried in shade and then crushed with the help of laboratory blender. 35gm. of leaf, stem and root powder was soaked with 100ml sterilized water for 24 h at room temperature. After soaking solutions were filtered through whatman's filter paper and final volume was adjusted for further use. The extract was considered as stock solution and a series of solutions with different strengths (15%, 25%, 50%, 75% and 100%) were prepared by dilution with distilled water. Dried samples were powdered A separate control condition was set up by using only distilled water. Experiments were set up in petri dishes covered with

whatman's filter paper. For each treatment ten replicates were maintained and in each petridish ten seeds of *Macrotyloma uniflorum* was placed. Distilled water was added when needed in petridishes. The rate of seed germination, length of root and shoot were determined after seven days of setting up of the experiment. Seed Vigour Index (SVI) was calculated by using the following formula:

SVI = (Length of root + Length of shoot) × Seed germination %

Data collected were statistically analysed by using the SPSS (Statistical Package for the Social Sciences) programme through Pearson Correlation Coefficient and Tukey HSD, Post Hoc Tests.

RESULTS AND DISCUSSION

Seed germination rate (%): The rate of seed germination in control condition varied from 93% to 100% whereas in aqueous extracts of leaf, stem and root of different concentrations it varied from 0% to 96%; 87% to 98%; and 0% to 93%, respectively (Table 1). The rate of seed germination decreased from 4 to 100% in aqueous extract of leaf; 1.08% to 6.45% in stem extract; and 19.35 to 100% in root extract (Table 2). Marwat *et al.* (2015) have collected data on chemicals isolated from *Parthenium* by various authors and about 123 compounds have been reported. All parts of *Parthenium* (leaves, stems, leaf hairs, flowers, pollen grains etc.) contain toxic and inhibitory constituents such as terpenoids, sesquiterpene lactones , volatile oils, flavanoides (Barnes *et al.*, 2007; Pareek *et al.*, 2011); phenolic derivatives (Parsons and Cuthbertson 2001); Parthenin (Zhou *et al.*, 2011); caffic, vanllic , ferfulic, chlorogenic and anisic acids (Parsons and Cuthbertson, 2001). Parthenin is one of the important alkaloids which is responsible for phytotoxic and allelopathic effect of *Parthenium*. The visible effects of allelochemicals on other plants include inhibition or retardation of germination rate; darkening and swelling of seeds; reduction of root and shoot length; swelling or necrosis of root tips; curling of the root axis; decoloration, lack of root hairs; increased number of seminal roots; reduced dry weight accumulations; and lowered reproductive capacity (Bhadoria 2011). Khalaj *et al.* (2013) reported that secondary metabolites are released through volatilization, leaching, root exudation and decomposition of plant residues in the soil. Bhowmik *et al.* (2007) have reported that parthenin is the active principal component among all the chemicals secreted by *P.hysterophorus* which have the strong allelopathic

Table 1. Seed germination rate, length of root, shoot and Seed Vigour Index in *M.uniflorum* in different concentrations of leaf, stem and root extract of *P.hysterophorus*.

Extracts	Growth parameters	Control	Concentration				
			15%	25%	50%	75%	100%
Leaf Extract	Seed Germination (%)	100	96	86	73	33	0
	Root Length (cm)	5.19	3.15	3.03	1.89	0.49	0
	Shoot Length (cm)	6.04	4.82	2.64	2.27	0.46	0
	SVI	1123	765	488	304	31	0
Stem Extract	Seed Germination (%)	93	98	92	91	92	87
	Root Length (cm)	6.8	6.62	6.35	5.3	5.59	9.85
	Shoot Length (cm)	10.16	10.01	9.47	8.68	7.36	6.84
	SVI	1577	1630	1455	1272	1164	1452
Root Extract	Seed Germination (%)	93	75	78	56	10	0
	Root Length (cm)	4.78	3.47	3.03	1.13	0.06	0
	Shoot Length (cm)	8.69	5.63	5.98	2.51	0.17	0
	SVI	1253	683	703	204	2.3	0

Table 2. Per cent increase or decrease in seed germination rate and growth parameters in *M.uniflorum* in different concentrations of leaf, stem and root extract of *P.hysterophorus*.

Extracts	Growth parameters	Concentration				
		15%	25%	50%	75%	100%
Leaf Extract	Seed Germination (%)	-4	-14	-27	-67	-100
	Root Length (cm)	-39.31	-41.62	-63.58	-90.56	-100
	Shoot Length (cm)	-19.7	-56.29	-62.42	-92.38	-100
	SVI	-23.53	-62.23	-72.49	-97.48	-100
Stem Extract	Seed Germination (%)	5.38	-1.08	-2.15	-1.08	-6.45
	Root Length (cm)	-2.65	-6.62	-22.06	-17.79	44.85
	Shoot Length (cm)	-1.48	-6.79	-14.57	-27.6	-32.68
	SVI	3.33	-7.72	-19.34	-26.21	-7.94
Root Extract	Seed Germination (%)	-19.35	-16.13	-39.78	-89.25	-100
	Root Length (cm)	-27.41	-36.61	-76.36	-98.74	-100
	Shoot Length (cm)	-35.21	-31.19	-71.12	-98.04	-100
	SVI	-32.44	-33.11	-72.98	-98.29	-100

Table 3. Tukey HSD and significance levels after statistically analysing the data collected by Post HOC tests.

Sl. No.		Seed Germination Rate (%)	Root Length (cm)	Shoot length (cm)
1	Plant Parts	0.000	0.000	0.000
2	Treatments	0.000	0.003	0.000
3	Plant Parts × Treatments	0.000	0.039	0.000
4	Leaves × Stem	0.000	0.000	0.000
5	Leaves × Root	0.000	0.945	0.000
6	Stem × Root	0.000	0.000	0.000
7	Control / 15%	0.432	0.801	0.000
8	Control / 25%	0.027	0.622	0.000
9	Control / 50%	0.000	0.033	0.000
10	Control / 75%	0.000	0.003	0.000
11	Control / 100%	0.000	0.114	0.000
12	15% /25%	0.817	1.000	0.153
13	15% /50%	0.000	0.489	0.000
14	15% /75%	0.000	0.115	0.000
15	15% /100%	0.000	0.790	0.000
16	25% /50%	0.004	0.683	0.000
17	25% / 75%	0.000	0.218	0.000
18	25% / 100%	0.000	0.920	0.000
19	50% / 75%	0.000	0.970	0.000
20	50% / 100%	0.000	0.997	0.000
21	75% / 100%	0.000	0.804	0.848

and allergic effect. Different concentrations of parthenin have been reported in different plant parts on dry weight basis such as leaf (3.40%), stem (0.12%), flower (1.08%) and trichomes (1.20%). Under laboratory conditions, parthenin released by aquous extraction of freash leaf material of *P.hysterophorus* proved to have 16-100% relative role (Belz *et al.* 2007). Dogra and Sood (2012) analysed the phytotoxicity of *Parthenium* on three native plants of Himachal Pradesh under in – vivo condition and found that soil mixed with residues of *Parthenium* adversely affect the per cent of seed germination and seedling growth. Maharajan *et al.* (2007) have indicated that increase in concentration of extract was invariably associated with decrease in germination and seedling characteristics of the crops. Veena and Maurya (2012) have reported that due to inhibitory role of mainly parthenin present in *P.hysterphorus* inhibits the germination and growth of plants such as pasture grasses, cereals, vegetables and other plant species. Rajendiran (2005) and Sorecha *et al.* (2017) have reported that allelochems present in the *Parthenium* plant parts could prevent the embryonic development and embryo growth and caused death. The present study revealed that increase in the concentration of *Parthenium* plant extracts inhibited the germination of *M.uniflorum*. Similar findings have been reported by Sorecha *et al.* (2017) in case of peanut and soybean in Ethiopia. The impact of inhibition in the rate of seed germination in *M.uniflorum* by extracts of leaf, stem and root it was leaf extract that highly reduced the germination followed by root than stem. It may be because leaf of *Parthenium* contains more parthenin than root and stem. Complete failure in seed germination in *M.uniflorum* was observed in 100% concentrations of leaf and root extracts of *Parthenium* (Table 1). Tafera (2002) has also reported that aqueous extracts of *Parthenium* leaf and flower inhibited completely seed germination in *Eragrostis teff*. The higher the concentration of *Parthenium* plant parts extracts, the higher the influences on the germination of *M.uniflorum* was observed.

Root length: The root length values in *M.uniflorum* in different concentrations of leaf, stem and root of *Parthenium* ranged from 0.0 to 3.15cm; 5.3 to 9.85 cm; and 0.0 to 3.47 cm, respectively. The decrease in root length varied from 39.31% to 100%; 2.65 to 44.85%; and 27.41 to 100%, respectively. The leaf extract was recorded more inhibitory for root length in *M.uniflorum* followed by root extract than stem extract of *Parthenium*.

Rashid *et al.* (2008) have reported root extract of *Parthenium* reduces the germination and growth of barley and maize. Picman and Picman (1984), and Mersie and Singh (1988) have suggested that water soluble allelochemicals parthenin is the cause of high degree of phytotoxicity of *Parthenium* residues.

Shoot length: The shoot length values in *M. uniflorum* in different concentrations of leaf extract of *Parthenium* varied from 0.0 to 4.82 cm; in stem extract from 6.84 to 10.01 cm and in root extract

from 0.0 to 5.98 cm (Table 1). The per cent decrease in shoot length in *M.uniflorum* varied from 19.7% to 100%; 1.48 to 32.68% and 31.19 to 100%, respectively, in leaf, stem and root extracts of *Parthenium.* The germination and growth of agricultural crops like rice, wheat, maize, pigeonpea, blackgram, sorghum etc. are inhibited by the allelopathic effect of *P.hysterophorus*. Nodulation in legumes due to inhibition of activity of nitrogen fixing and nitrifying bacteria such as *Rhizobium*, Actinomycetes, *Azitobacter* and *Azospirillum* is affected by *P.hysterophorus*. Wakjira *et al.* (2009) reported that the adverse effect of residues on seed germination and plant growth could be the result of immobilization of large amounts of nutrients by micro-organisms involved in decomposition, by allelochemicals or both. Upadhyay *et al.* (2013) have evaluated the ecological impacts of *P.hysterophorus* invasion in saline soil in India. Anwar *et al.* (2016) have reported phytotoxic effects of leaf powder and aquous extract of *P.hysterophorus* on seed germination and seedling growth in *Avena fatua*, *Rumex dentatus*, *Helianthus annus*, *Zea mays* and *Triticum estivum* in Pakistan. Singh *et al.* (2005) have shown the strong positive correlation between extract concentration of residue of *Parthenium* and reduction in seedling length of *Brassica species*.

Seed vigour index (SVI): The seed vigour index values of *M.uniflorum* in leaf, stem and root extracts of *Parthenium* varied from 0.0 to 765; 1164 to 1630 and 0.0 to 683 in different treatments (Table 1). The seed vigour index values decreased from 23.53% to 100%; 7.72 to 26.21% and 32.44 to 100%, respectively, in leaf, stem and root extracts of *Parthenium* compared to control treatment.

Parthenium inhibited the growth of water hyacinth due to loss of dehydrogenase activity in roots, damage of cellular membrane and loss of chlorophyll in leaves (Pandey 1996). Similarly Batish *et al.* (2002) have reported that parthenin inhibited mung bean growth by affecting protease and peroxidase enzyme activities, respiration and protein content. In the present study, thus, the parthenin and other phenolic compounds including ferulic, caffeic, chlorogenic, vanillic and anisic acid reported by several researchers in *Parthenium* may be probably growth retardants in *M. uniflorum*.

Conclusion

The phytotoxic effects of different concentrations of aqueous extracts of leaf, stem and root of *P.hysterophorus* were recorded on seed germination and growth of *M.uniflorum*. Leaf and root extracts were more phytotoxic than the stem extract in the present study. Thus prevention and control of *P.hysterophorus* is needed. All control strategies of invasive species such as physical, chemical and biological should be integrated. A holistic approach having integrated long-term management programme should be carried out to control the weed. Coordination among social people, scientists, governments and NGO'S is needed including people awareness programme. To overcome the economic loss it is essential to use *Parthenium* alternatively in beneficial purposes such as biofertilizer, green manure, anticancer, pesticidal, antimicrobial, lervicidal, ovicidal, herbicidal etc. There is need to do clinical researches and investigations to establish *Parthenium* as a standard medicinal plant.

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