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Allelopathic Potential of *Parthenium hysterophorus*: Is it Growth-stage Dependent?

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Abstract

World's one of the most invasive weeds *Parthenium hysterophorus* L. (Congress grass, family Asteraceae), native to South America, is the most aggressive alien weed in India. The present study aimed to determine the growth stage (early, intermediate and mature) of this weed which was most detrimental to the growth of adjoining crop plants. Impacts of leaf-leachate concentrations (0.5%, 1% and 2%) prepared from leaves at different growth stages of *P. hysterophorus* were investigated on seed germination and growth of crop *Vigna unguiculata*. The germination percentage, shoot length, number of leaves, pods, seeds and total biomass of the crop plants significantly declined with increasing concentration of the *Parthenium* leaf-leachate (maximum at 2%w/v) at all growth stages. However, the leaf-leachate from the leaves of *P. hysterophorus* at the early growth stage showed the highest detrimental impact on the crop *V. unguiculata*, followed by intermediate and mature growth stages. Total phenolic content, antioxidant activity and enzymatic were highest in leaves of *P. hysterophorus* at the early growth stage. In conclusion, the early growth stage of this noxious weed evidently being most detrimental to the growth of adjoining crop plants, suggests *Parthenium's* early growth-centric management implication.

Keywords: Invasive alien weed, Leaf-leachate, Crop biomass, Phenolic content, Enzymatic activity

Introduction:

The control and management of *Parthenium hysterophorus* L. (hereafter referred to as *Parthenium*) for its adverse impact on the growth of adjoining plants in general and crop plants in particular, has been globally an ecological and agronomic concern (Bashar et al., 2021, Boja et al., 2022) ever since it left its native range over two centuries ago, and has presently invaded more than 46 countries and territories (Mao et. al., 2020). Several of its characteristics including its short life cycle of 90-120 days, ability to adapt to various photo-thermal conditions, usually absence of natural enemies, fast growth ability, to spread via waterways and roadways (Gupta and Narayan 2006, 2010; Aggarwal et al., 2012) and its allelopathic traits (Begum et al., 2020) have been attributed to its aggressive expansion in alien areas. *Parthenium*, considered as one of the most noxious weeds of the world (Shabani et al., 2020), was accidentally introduced in India in the 1950's by imported food grains PL480, which has now spread over the entire country (Ramaswami, 1997). Its distribution is likely to expand further globally and locally in the light of future climate changes (Adhikari et. al., 2023). Thus, effective control and management strategy of this aggressive weed is a global concern for countries like India where it has expanded its habitats of dominance at a massive scale. This prolific invasive weed with potent allelopathic qualities for its water-soluble phenolic contents has altered the agricultural scenario of the world, reducing crop production by 40-50% and pasture production by up to 90% (Khan et al., 2022). In the plant invasion process, the allelopathic impact effected by breaking its metabolites and releasing chemicals into the soil is expected to be a crucial essential invasive mechanism because of the incapability of co-evolved resident vegetation to survive the allelochemicals released by the invaders, thus allowing the newly arrived exotic plant species to dominate over the resident plant communities (Hierro & Callaway, 2003).

Parthenium successfully dominated a large variety of habitat conditions e.g., farmsteads, fallow ground, orchards and railway tracts in India and abroad and has transformed the resident species composition (Dogra et al., 2009). It significantly impacted the production and biodiversity of agricultural and natural ecosystems, as it thrived well on all types of soils (Mahadevappa et al., 2001). A single plant of *Parthenium* may release up to 100,000 seeds in one cycle. Sankaran (2008) reported 340 million seeds/ha in surface soil. Adverse impact of *Parthenium* on the growth of several plant species has been reported e.g., *Trifolium repens*, *Imperata, Chrysopogon aciculate, Sporobolium, and Dactyloctenium aegypticum* (Timsina et al., 2011). *Parthenium's* allelopathic effect on germination and growth of various plants viz. *Phaseolus mungo* (Sikha & Jha, 2018), *Zea mays* (Devi et al., 2014), lettuce (Wakjira et al., 2009), three *Brassica* species (Singh et al., 2005). Several studies on the impacts of *Parthenium* on crops have been done in different parts of India. *Parthenium* has been reported to cause losses in maize production to the extent of 3.8 – 7.7 million USD in Africa (Pratt et al., 2017) and sorghum in Ethiopia 40 – 97% (Tamado et al., 2002).

There has been an alarming threat of this weed *Parthenium* to decimate the biodiversity, crop production and cause severe health hazards. While various physical, chemical and cropping system, management practices have been suggested for its control and reduction of its incidence in croplands (Duguma et al., 2019), few attempts have been made to understand whether there existed any variation in the allelopathic potential of this aggressive botanical invader at its varying growth stages. Such a study is likely to have immense implication for its effective control strategy. With this objective, the current study aimed to assess the allelopathic potential of aqueous leaf extract of *Parthenium at* its different growth stages on seed germination and growth of a protein-rich crop *Vigna unguiculata*, a staple pulse crop of India.

Materials and Methods:

Plant material

Parthenium plant individuals were collected from the weed-infested sites in Meerut (28° 59' N lat. And 77° 40' E long.), India, at different growth stages - Early growth stage (Pe), Intermediate growth stage (Pi), and Mature growth stage (Pm). Pe individuals were collected in March, Pi in May, and Pm individuals in September in the year 2022. A total of 150 *Parthenium* plant individuals (50 each at each growth stage) were estimated for their shoot length (up to shoot tip), basal diameter (measured by screw gauge), number of leaves and above-ground biomass according to Gupta and Narayan (2012). Leaves were removed separately from the plants at their respective growth stages and carefully washed with tap water to remove all the dust particles, then dried in the shade and separately powdered by the laboratory blender and kept in an airtight container for further use in this study.

Preparation of aqueous extract of plant leaves

10 g leaf powder of plants at three growth stages of *Parthenium* was soaked in 100 ml of distilled water and vigorously stirred for 48 hrs at room temperature. Then, the extracts were filtered through the muslin cloth, followed by Whatman filter paper no.1. These filters have a concentration of 10% w/v and were diluted for further study with distilled water for desired concentration viz. 0.5%, 1%, and 2% w/v.

Seed germination bioassay

The effect of different concentrations of aqueous leaf extracts of *Parthenium* at different growth stages on germination and early seedling growth of crop *Vigna unguiculata* was studied in a laboratory bioassay. For this, three replicates of 6 seeds of *V. unguiculata* were placed in 8.5 cm Petri dish lined with a Whatman No. 1 filter paper moistened with 6 ml of different concentrations of *Parthenium* leaf aqueous extracts. Three replicates of distilled water treatment were kept similarly that served as control. Petri plates were incubated in a dark chamber in laboratory conditions. The recorded mean temperature during the study period was maximum 25.5 ± 0.6ºC and minimum 23.2 ± 0.7ºC. After seven days, seed germination, seedling root/shoot length, seed vigour index (SVI) and dried biomass were determined following Dahiya & Narwal (2003).

Growth assay

Plastic pots of 13 cm diameter and 15 cm depth were filled with garden soil 1 kg/pot. Fifteen days old, *Vigna* plants were carefully transplanted into these pots. Aqueous extracts of 0.5%,1%, and 2% w/v of leaves of *Parthenium* at its three different growth stages were prepared as described earlier. These extracts were used to irrigate crop plants after every five days of crop transplantation. Plants in the control treatment were irrigated with distilled water. Crop plants were harvested after pods ripened (120 days), and data regarding shoot length, the number of leaves, pods and seeds, and dry biomass were determined according to Javaid et al. (2006).

Quantitative phytochemical screening

Phenolic content and Antioxidant activity: To determine total phenolic content (Bray & Thrope, 1954) and antioxidant activity i.e. DPPH radical scavenging activity (Nile & Park, 2014), FRAP assay (Pulido et al., 2000) and ABTS assay (Re et al., 1999) of leaves of *Parthenium* at its three different growth stages, dried leaf samples were extracted in ethanol by using shaker-assisted extraction procedure (5 g of powdered leaves of each weed sample extracted with 100 ml of absolute ethanol for 48 hrs at 120 rpm). These extracts were filtered and stored at low temperature.

Enzymatic Activity: Enzymatic activity of Peroxidase and Catalase was assessed according to Maehly & Chance (1976) and Aebi (1984) respectively. For extraction of the enzyme, 100 mg fresh sample of leaves of *Parthenium* at its different growth stages was homogenized in 10 ml chilled common extraction-cum-buffer, i.e., 0.2M Tris-Malate-NaOH Buffer, pH 7.2 and centrifuged at 1000 rpm at 4°C temperature for 5 minutes. After discarding the pellet, the supernatant was tested for the enzymatic activity assay.

Results:

Plant traits at different growth stages

The plant traits of sampled individuals of *Parthenium* plants at three different growth stages included shoot length, number of leaves, basal diameter and above-ground biomass (ABG) (Table 1). The mean values of these characteristics for plants at early growth stage were - shoot length 8.18 cm, number of leaves 11.97, basal diameter 1.34 cm and AGB 7.76 g in contrast to much higher values at mature stage of growth i.e., shoot length 119.8 cm, number of leaves 34.47, basal diameter 3.85 cm and AGB 45.5 g.

Table 1. *Parthenium hysterophorus* growth traits at its different growth stages (n = 50). Mean values with the same letters for one growth trait do not differ from each other using ANOVA compared to the DMRT test at 0.05 probability level.

Growth Traits	Early growth (Mean ± S.E.)	Intermediate growth (Mean ± S.E.)	Mature growth (Mean ± S.E.)
Shoot length (cm)	8.18 ± 0.31 **a**	64.18 ± 1.38 **b**	119.83 ± 2.38 **c**
No. of leaves	11.97 ± 0.45 **a**	23.27 ± 0.68 **b**	34.47 ± 1.32 **c**
Girth size (cm)	1.34 ± 0.05 **a**	2.55 ± 0.06 **b**	3.85 ± 0.1 **c**
AGB (g)	7.76 ± 0.24 **a**	29.22 ± 0.88 **b**	45.5 ± 0.93 **c**

Leaf-leachate impact on seed germination.

Leaf-leachates of *Parthenium* plants at all three growth stages showed significant inhibitory impact on the seed germination and SVI of *V. unguiculata* (Table 2). The results were concentration-dependent as inhibition increased with increasing leaf-leachate concentration. The early growth-stage leaf-leachate showed maximum inhibition of seed germination (78%), SVI (131) and lowest seeding dry weight (.037g) at the highest leaf-leachate concentration (2%) in the present study.

Table 2. Inhibitory effect of different aqueous leaf-leachate concentrations of *Parthenium hysterophorus* at its different growth stages on the seed germination% and seed vigour index of *Vigna unguiculata*. Mean values with the same letters in the same column do not differ from each other by using ANOVA compared to the DMRT test at 0.05 probability level. Digits affixed to different growth stages of *Parthenium* (P) – Early (e), Intermediate (i)and Mature (m) refer to aqueous leaf-leachate concentration (w/v).

Treatment	Radicle length (cm) (Mean±S.E.)	Plumule length (cm) (Mean±S.E.)	Dry weight (g) (Mean±S.E.)	Germination (%)	Seed Vigour Index
Control	2.76±0.17**a**	4.36±0.1**abc**	0.071±0.002**a**	100 **a**	712**a**
Pe0.5%	2.74±0.1**a**	4.41±0.17**abc**	0.047±0.001**cd**	55.55**cd**	397.2**cd**
Pe1%	2.51±0.34**a**	4.27±0.9**bc**	0.044±0.003**de**	33.33**ef**	226**ef**
Pe2%	2.37±0.18**a**	3.53±0.23**d**	0.037±0.001**e**	22.22**f**	131.1 **f**
Pi0.5%	3.1±0.58**a**	4.76±0.31**ab**	0.052±0.001**bc**	66.67**bc**	524**bc**
Pi1%	2.67±0.41**a**	4.45±0.06**abc**	0.049±0.001**cd**	50**cde**	356**de**
Pi2%	2.4±0.35**a**	4.02±.11**c**	0.044±0.002**de**	33.33**ef**	214**ef**
Pm0.5%	3.26±0.22**a**	4.82±0.15**a**	0.057±0.003**b**	77.78**b**	628.4**b**
Pm1%	2.81±0.1**a**	4.55±0.1**abc**	0.053±0.002**bc**	55.55**cd**	408.8**cd**
Pm2%	2.58±0.25**a**	4.22±0.1**bc**	0.049±0.002**cd**	44.44**de**	302.2**f**

Leaf-leachate impact on crop growth

All growth parameters estimated in this study viz. shoot length, number of leaves, pods, and seeds, and total biomass of crop plants significantly decreased when treated with different growth-stage leaf-leachates of *Parthenium* (Figure 1). Maximum crop growth inhibitions were recorded at the highest concentration (2%) of leaf-leachate from the leaves of *Parthenium* in its early stage of growth -shoot length 27%, number of leaves 28%, number of pods 50%, number of seeds 55% and total crop biomass 56%.

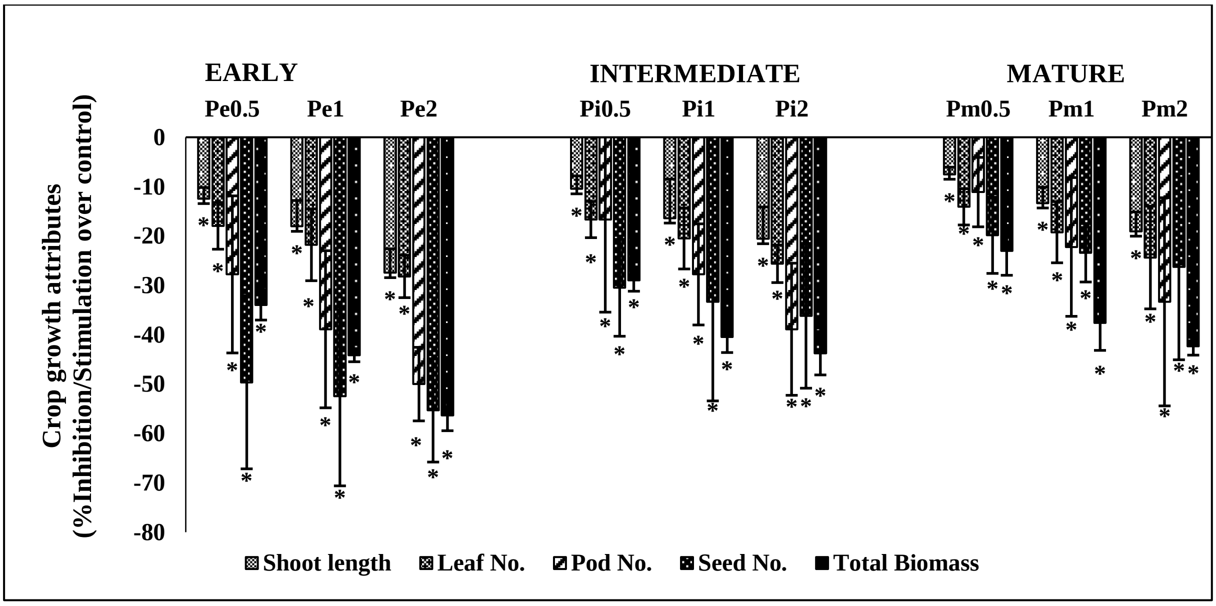

Figure 1. Inhibitory effect of different aqueous leaf-leachate concentrations of *Parthenium hysterophorus* at its different growth stages on the morphological growth attributes of *Vigna unguiculata*. Digits affixed to weed different growth stages of *Parthenium* (P) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v). According to Dunnett's test, * indicates significant inhibition/stimulation over control at $p < 0.01$.

Quantitative phytochemical screening of Parthenium leaves

Total phenolic content and enzymatic activity: Highest total phenolic content was recorded in *Parthenium* leaves at its early stage of plant growth (2.594 mg/g), and their amount decreased with the advancement of its growth (Table 3). The highest activity of both peroxidase and catalase enzymes was also shown by *Parthenium* leaves at the early stage of its growth (0.338 and 1.032, respectively).

Table3. Total phenolic content and enzymatic activity of aqueous leaf-leachate of *Parthenium hysterophorus* at its different growth stages. Mean values with the same letters for one biochemical analytic test do not differ from each other using ANOVA compared to the DMRT test at 0.05 probability level. Digits affixed to weed different growth stages of *Parthenium* (P) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v).

S.No.	Leaves	Phenolics	Peroxidase	Catalase
1.	Pe	2.59 ± 0.07 **a**	0.34 ± 0.003 **a**	1.03 ± 0.008 **a**
2.	Pi	2.38 ± 0.11 **b**	0.28 ± 0.005 **b**	0.68 ± 0.018 **b**
3.	Pm	1.99 ± 0.38 **c**	0.21 ± 0.006 **c**	0.62 ± 0.005 **c**

Antioxidant activity: In addition to phenolic estimation in the present study, antioxidant capacity was also performed by the DPPH (2,2-diphenyl -1- picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (ferric reducing/antioxidant power) in leaf extract of *Parthenium* at its different growth stages (Table 4). The DPPH assay determines the scavenging of stable radical species DPPH by antioxidant compounds present in the extracts. The highest activity was recorded in the leaf extract of leaves at *Parthenium's* early stage of growth (79.36%). The ABTS scavenging assay was used as an index reflecting the antioxidant activity in the present study. Radical-scavenging activities of leaves at all three growths tages of *Parthenium* were recorded. The leaf-extract of plant at early growth stage was found to be most effective in scavenging radicals and showed maximum ABTS (96.91%). FRAP assay represented a direct correlation between high reducing power and high content of phytochemicals in leaves of *Parthenium* plants at the early growth stage (8.346 µg/g).

Table 4. Antioxidant activity of aqueous leaf-leachate of *Parthenium hysterophorus* at its different growth stages. Mean values with the same letters in the same column do not differ from each other using ANOVA compared to the DMRT test at 0.05 probability level. Digits affixed to weed different growth stages of *Parthenium* (P) – Early (e), Intermediate (i) and Mature (m) refer to aqueous leaf-leachate concentration (w/v).

S.No.	Leaves	DPPH (%) (Mean ± S.E.)	ABTS (%) (Mean ± S.E.)	FRAP (µg/g) (Mean ± S.E.)
1.	Pe	79.36±0.1 **a**	96.91±0.1 **a**	8.35±0.19 **a**
2.	Pi	70.56±0.41 **b**	94.13±0.36 **b**	7.29±0.1 **b**
3.	Pm	63.79±0.24 **c**	92.74±0.26 **c**	1.58±0.03 **c**

Discussion:

The present study revealed in clear terms that the leaf-leachate of this exotic invasive weed had significant adverse impact on seed germination, seedling growth and seed vigour index of the staple protein-rich pulse crop *Vigna unguiculata* and this adverse impact increased with increasing concentration of leaf-leachate (Table 2). Comparable phytotoxic impacts of this aggressive invader in India have been reported by Hassan et al. (2018) on the seed germination of *Triticum aestivum, Cicer arietinum, Brassica campestris, Avena fatua, Asphodelus tenuifolius,* and *Lolium rigidum*. The phytotoxic impact of this weed in the present study also varied with the stage of its growth: maximum at early stage and minimum at mature stage. Kohli and Batish, 1994 suggested that the allelopathic influence of *Parthenium* was due to some allelochemicals produced by its plant parts, especially roots and leaves. Of the different plant organs, leaves have mainly been suggested to influence the allelopathic potential of a weed (Gupta and Narayan, 2012). These allelochemicals in the leaf extract are reported to prevent embryo growth or thus cause the death of seeds *via* various chromosomal mutations in dividing cells. Such a phenomenon significantly depended on the concentration and duration of exposure (Rajendiran, 2005). Several workers have detected and reported various allelochemicals such as terpenes, phytosterol, and phenolics in *Parthenium* (Batish et al., 2002 and Kil et al., 2006).

Besides the inhibitory impact of *Parthenium* leaf-leachate on seed germination and seedling growth, its growth retardatory impact on the target crop *V. unguiculata* was evinced in this study. Various growth characteristics of the crop viz., shoot length, number of leaves, pods, seeds and plant biomass declined with increasing concentrations of leaf-leachate. In the present study, the plant parts varied in the scale of their growth inhibition by the weed leaf-leachate (shoot length 27%, no. of leaves 8%, no. of pods 50% and no. of seeds 55%). A comparable 40-97% reduction in the yield of *Sorghum* was reported by Tamado et al. (2002) and 18.8-86.4% reduction in common beans under the impact of *Parthenium* (Mitiku, 2011). This may presumably be due to the release of inhibitory compounds that remained active and stable for a considerable duration (Shaukat & Siddiqui, 2001). Such growth retardatory impacts of *Parthenium* on *Vigna* growth could be attributed to the presence of various phytotoxic compounds in the aqueous extract of *Parthenium viz.* water-soluble phenolics including caffeic, ferulic, vanillic, anisic, and fumaric acids, and sesquiterpene lactones including parthenin and coronopilin as suggested by Meirse & Singh (1987). Parthenin, the major allelochemical causing the bioactivity, is considered ecologically significant and a possible cause of its detrimental impact and invasiveness. Reinhardt et al**.** **(**2006) and Belz **(**2016) opined that this parthenin can be released by leaching from living plant parts or by decomposition of plant residues. Dropping of parthenin-containing leachates on the leaves of target plants growing under the canopy of *Parthenium* may be one mechanism of this weedy plant's allelopathic capacity. Several research reports from other plant species demonstrated that such natural leachates could exhibit allelopathic inhibitory effects, *e.g.*, *Acacia dealbata* **(**Msafiri et al., 2013).

The present study's findings indicated that the leaves of the same *Parthenium* plant differed in their allelopathic impact at different stages of its growth. The early stage of its growth in this study exhibited a much higher growth retardatory effect towards the target crop plant compared to that at mature growth stage. This could be on account of difference in the biochemical status, as evinced in the present investigation on phytochemical screening of *Parthenium* leaves at different growth stages. At its early-growth stage, the leaves showed significantly higher phenolic content compared to those at the mature growth stage (Table 3). The crude extract of its floral part has been reported to contain phenolic compounds such as gallic acid, chlorogenic acid, ellagic acid and P-coumaric acid (Iqbal et al., 2022). The phenolic acids isolated from *Parthenium* plant parts, in general, are also reported to include caffeic acid, p-coumaric acid, p-anisic acid, ferulic acid, fumaric acid, p-hydroxy benzoic acid, chlorogenic acid, neochlorogenic acid, protocatechuic acid, ferulic acid, and vanillic acid (Das & Das, 1995). These primary phenolic acids are suggested to play a crucial role in the allelopathic activity of *Parthenium* (Richa et al., 2015). The phenolic compounds extracted from plants possessed multiple biological properties, viz. antioxidant, antimicrobial, and anti-inflammatory properties (Shui & Leong, 2002).

Assessment of antioxidant activity of leaves at all growth stages of *Parthenium*, done by using DPPH, FRAP and ABTS assay, also exhibited maximum activity in leaves of *Parthenium* at its early-growth stage. This is probably due to the presence of a diverse variety of terpenes, fatty acids, sterols, and their derivatives in different parts of *Parthenium*, mainly terpene and terpenoids as major antioxidants combating oxidative stress by donating hydrogen to free radicals, as suggested by Ahmad et al. (2018). The reducing potential of the antioxidant components is closely associated with their total phenolic content. Thus, plant extracts with higher amounts of total phenolics also have more reducing ability. (Cheng et al., 2006; Siddhuraju & Becker, 2003).

Enzymatic activity of peroxidase and catalase was also estimated to be highest in *Parthenium* leaves at its early growth stage. Reactive oxygen species (ROS), such as OH, O_2^-, and H_2O_2, are harmful to cells as they cause oxidative stress. Peroxidase and catalase are reportedly responsible for reducing or reversing these adverse effects (Kumaraswamy et al., 2019).

Conclusion:

In conclusion, the study revealed significant dose-dependent inhibitory impact of *Parthenium* leaf-leachate on seed germination and growth of crop *Vigna unguiculata*. This inhibitory impact differed with growth stages in the order Early > Intermediate > Mature stages, corresponding to phenolic content, antioxidant activity and enzymatic activity in recording the same order.

Conflict of Interest:

Nil

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