

## **Allelopathic impact of *Parthenium hysterophorus* L. extracts on germination and growth of weed *Portulaca oleracea* L.**

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### **ABSTRACT**

This study, evaluated the allelopathic effects of *Parthenium hysterophorus* L. aqueous extracts on the germination and seedlings growth of noxious weed *Portulaca oleracea* L. The lower concentrations inhibited the germination and the sub-lethal concentrations decreased the germination and seedlings growth. The Microscopic analysis of the affected seedlings showed necrosis of primordial roots that caused death of seedlings. The phytochemical composition of the extracts was analysed by GC-MS, it revealed the presence with phytotoxic properties. Our study highlighted the herbicidal potential of aqueous extracts of *P. hysterophorus* L. on agricultural weed *P. oleracea* L.

**Keywords:** Allelopathy, aqueous extracts, GCMS, germination, *Parthenium hysterophorus*, phytotoxicity, *Portulaca oleracea*, root necrosis, seedlings growth, weed.

### **INTRODUCTION**

Plant allelopathy is a potential source for use of its novel chemicals with diverse bioactivity for agricultural pests control (17). Bioactive chemicals produced in the plant negatively affects the growth of another plant (reduces germination, or even induce lethality to the plant). The growing environmental and health concerns by use of synthetic herbicides have prompted research into allelopathy as an eco-friendly alternative for weed management. Allelochemicals such as parthenin (from *Parthenium hysterophorus* L.), juglone (from walnut trees), and phenolic acids have herbicidal properties by inhibiting the essential physiological processes like photosynthesis, enzyme activity, and cell division (9,29). Adoption of plant allelopathy for weed control may reduce dependence on synthetic herbicides, contributing to sustainable farming practices. Despite, its advantages, harnessing allelopathy faces continuous challenges which reduce their adaptability. *P. hysterophorus* L. is an aggressive invasive weed (Figure-1A) in tropical and sub-tropical countries that severely reduced crop yields. The superior allelopathic properties of *P. hysterophorus* L. make it an ideal candidate to use its herbicidal properties. The allelochemicals-prompted phytotoxicity of *P. hysterophorus* is well-documented. However, the herbicidal property of parthenium extracts against other agricultural weeds remains elusive. In India, it has infested agriculture farms, forests, roadsides, railway tracks, vacant lands, waste lands, lands adjacent to irrigation canals and industrial areas (70), causing large scale damage to human health, animal husbandry, crop production and biodiversity. Phytochemicals of *P. hysterophorus* L. exhibit negative allelopathy on numerous plants: *Vigna subterranea* L., *Raphanus sativus* L., *Cucurbita maxima* L., *Cucumis sativus* L., *Solanum lycopersicum* L., *Capsicum frutescens* L., *Zea mays* L., *Abelmoschus esculentus* L., *Daucus carota* L., *Digitaria sanguinalis* L. and *Eleusine indica* L. (7).

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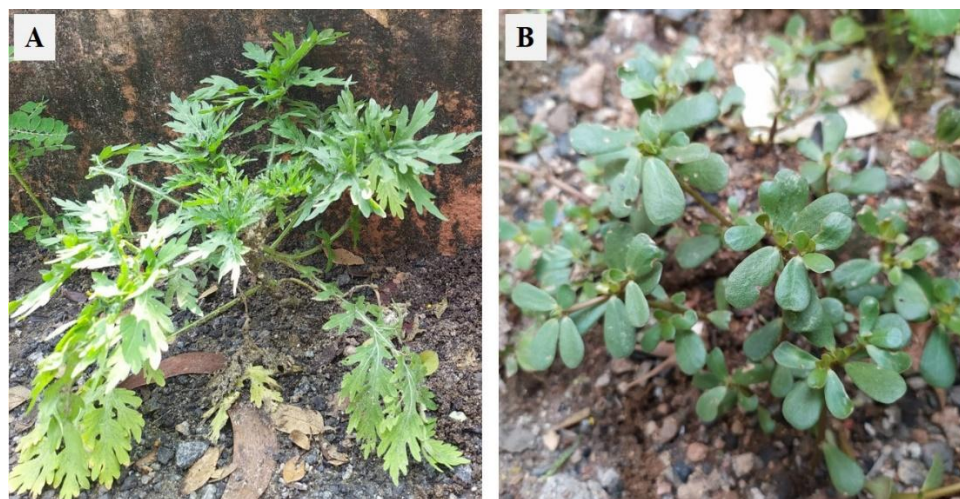


Figure 1A: *Parthenium hysterophorus* L., B: *Portulaca oleracea* L.

Weeds negatively affect the crops growth and cause 20-40 % yield losses through allelopathic activity, due to their secondary metabolites (1,30). *Portulaca oleracea* L. (Figure - 1B) (common purslane, Portulacaceae family) has originated from South or North America (15) and is most noxious weed in 45 crops across 81 countries (16,46). It negatively affected the cultivation of many crops viz., *Triticum aestivum* L., *Saccharum officinarum* L., *Camellia sinensis* L., *Gossypium hirsutum* L., *Capsicum annum* L., *Cucurbita pepo* L. and *Luffa cylindrica* L. etc. (31). In addition, *P. oleracea* weed acts as a vector for various pests like sugar beet nematode, tobacco mosaic virus and Cucumber mosaic virus (14,47), thus affecting the crop production. It has fast growth and reproduction including, metabolism switching ability (C4 to CAM pathway), drought tolerance, salinity tolerance, extensive root system (up to 153 cm), shorter life cycle (2-4 months), palatability to animals and humans, vegetative reproduction (19,47). Weed management strategies also tend to be ineffective against this weed, because of vegetative reproduction from the stem (63). The reliance on chemical methods of weed control for controlling this weed is futile, due to the formation of herbicide resistance biotypes that withstand many herbicides viz., linuron, atrazine, diuron, cyanazine, and prometryn (45).

Plant diversity analysis on grazing lands of Ethiopia revealed that *P. hysterophorus* L. infestation significantly reduced the growth of *P. oleracea* L., due to its negative allelopathy (56). However, the allelopathic nature of *P. hysterophorus* L. in growth reduction of *P. oleracea* L. and the molecular mechanism behind the phytotoxicity was not studied. Hence, we assessed whether allelopathic extracts from congress grass exert herbicidal effects against the germination of *P. oleracea* L. The extract of *P. hysterophorus* L. remarkably inhibited germination and seedlings growth of *P. oleracea* L. Exposure of *P. oleracea* L. to *P. hysterophorus* L. extracts significantly stunted the seedlings growth and deformed the roots formation. Phytochemical analysis of *P. hysterophorus* L. extracts showed the presence of multiple phytotoxic allelochemicals.

## MATERIALS AND METHODS

### Aqueous extract preparation of *P. hysterophorus* L.

Aqueous extracts from the whole plants of *P. hysterophorus* L. were prepared as per methodology (42,50) with minor modifications. Whole parthenium plants were collected from Annamalai University, Chidambaram (11.3918° N, 79.7132° E) for analysis. The collected plants were stored at 4 °C in an ice box and transported to lab for processing. The plant samples were washed thoroughly with tap water to remove adhering soil and impurities and once with distilled water. The washed plant materials were chopped into tiny portions (of 0.5 to 1 cm) in length and dried in shade (indoor conditions) at room temperature for 7-10 days. The plant samples were frequently turned to ensure even drying of samples. After drying, the samples were pulverized using Willey mill and the resultant powder was sieved (2 mm sieve) and stored in an air-tight container at room temperature. Initially, the stock extract of 30 % concentration was prepared by mixing 30 g powder in 100 ml distilled water (W/V). The mixture was left undisturbed for 24 h at 4 °C and strained with double-layered muslin cloth. The filtrate was then filtered using Whatman filter paper (No.1) and the resultant extract (4 %) was stored in a sealed bottle until usage. The working solutions (3 %, 6 %, 9 %, and 12 %) were prepared by appropriately diluting the stock in sterile distilled water. During the experiment, the stock and working solution of the extracts were stored at 4 °C.

### Preparation of *P. oleracea* L. seed material

Seeds of *P. oleracea* L. exhibit dormancy and therefore exhibit reduced germination. The dormancy of *P. oleracea* L. was broken according to the protocol (6). Whole *P. oleracea* L. plants at the post-flowering (capsule formation) stage (4-5 weeks post-germination) were collected. The whole plants containing intact pods were uprooted entirely along with adhering soil and dried in sunlight for 1 week. The plants were spread on a clean white cloth placed inside plastic trays and exposed to sunlight, in night, the trays were moved to room. Thus, the sample was exposed to both high temperature in day time and low temperature in night time. After a week, the seeds from the dehisced pods were collected and used for experiments. For every experiment, fresh seeds were used to ensure uniform seed germination.

### Seed germination bioassay

Seed germination assay was performed as per (7) with minor modifications. Healthy, uniform seeds were surface-sterilized using 2 % sodium hypochlorite for 3-min and rinsed thrice using sterile distilled water to remove contaminants. Sterile Petri dishes lined with filter papers were moistened with either distilled water (control) or treatment solutions (3 %, 6 %, 9 % and 12 % extracts). Seeds were evenly placed on the filter paper using sterile forceps, ensuring uniform spacing (10 seeds/plate). The Petri dishes were then covered and incubated (25 ± 2 °C) with 12-h photoperiod. Germination (%), root length, shoot lengths and total length were calculated from the 100 seeds (35 seeds per replication). All parameters were recorded for 7 days to assess the treatments effects. The experiment was done thrice and mean values were used for analysis. Based on formulas suggested (24), germination (%) and germination speed were calculated, while seed vigour index and phytotoxicity (%) were calculated as under (69).

- Germination (%) = (Number of seeds germinated/Total number of seeds) X 100
- Speed of Germination =  $n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots$

Where, n : Number of germinated seeds, d : Number of days

- Phytotoxicity (%) = [(Seedling length in control – Seedling length in treated plant)/Seedling length in control] x 100
- Seed vigour index = Germination (%) x Mean seedling length

### Microscopic Analysis

Microscopic analysis of seedlings was done to assess the cellular damage. Thin root/shoot sections of seedlings were made and dipped in 1 % safranin for 60 sec. The samples were then rinsed with distilled water and placed on the glass slide. A drop of glycerol was placed over the sample and coverslip was placed over it. The samples were visualized under ESAW Pathological Binocular compound microscope in 10X and 40X magnification. The images were edited using Image.

### Phytochemicals profiling (GCMS Analysis)

To identify the phytotoxic properties, GC-MS analysis of *P. hysterophorus* extract was done. The extract was filtered with Whatman No-1 filter paper and the filtrate was dried at 55 °C. The resultant semisolid sludge was dissolved in DMSO and again centrifuged. The clear supernatant was analyzed using GC-MS in a Thermo Scientific (Waltham, MA), Trace GC Ultra and ISQ Single Quadrupole MS, TG- 5MS fused silica capillary column (30 m x 0.25 mm x 0.1 mm film thickness). An electron ionization system with ionization energy of 70 eV was used to detect allelochemicals. Inert helium gas was used as carrier at a flow rate of 1 ml/min. The temperature of the injector and MS transfer line was 280 °C. The temperature was programmed as follows: an initial temperature 50 °C at a rate of 2 min, 50-150 °C at a rate of 7 °C/min, 150-270 °C at a rate of 5 °C/min, and a final temperature of 270-310 °C at an increasing rate of 3.5 °C/min.

### Sampling of *P. hysterophorus* L. and *P. oleraceae* L. in fallow lands

The distribution of *P. hysterophorus* L. and *P. oleraceae* L. was surveyed in on our Research Farm (11.3918° N, 79.7132° E), Cuddalore district, Tamil Nadu. Sampling of *P. hysterophorus* L. and *P. oleraceae* L. were done using quadrat method (71). Based on the number of parthenium plants per quadrat, the fields were classified as heavily infested (> 5 plants/quadrat) and lightly infested (< 5 plants/quadrat). Total number of weeds were analyzed in both conditions in random 10 quadrats.

### Statistical Analysis

Each experiment was repeated thrice. Standard deviation and standard error mean values were calculated to analyzed the values within a group. One-way ANOVA was performed among the group using AGRES software package. The graphs were drawn using Graphpad Prism 9 and standard error mean was plotted as error bars.

## RESULTS AND DISCUSSION

### Germination (%)

The effects of aqueous extracts of *P. hysterophorus* L., were evaluated on germination of *P. oleracea* L. with and without extracts. To assess the toxic concentration range, preliminary germination assay was done at wide concentration range 10, 20 and 30 % (data not shown). The growth at 10 % concentration exhibited severe inhibition and 20 and 30 % completely inhibited germination. Therefore, we evaluated the germination inhibition at (3, 6, 9, and 12 %). The extract significantly inhibited the germination of *P. oleracea* L. Our results indicated that the extracts of *P. hysterophorus* significantly inhibited the germination of *P. oleracea* (Table -1, Figure -2). These results are comparable with other results (42,73).

Table 1. Inhibitory effects of *P. hysterophorus* L. extracts on the germination of *P. oleracea* L.

Extract conc (%)	Germination (%)	Phytotoxicity (%)	Speed of germination (seeds germinated/day)
Control	94.44 ± 2.22	0	8.76 ± 0.91
3	85.56 ± 9.88	55.68 ± 5.72	4.31 ± 1.21
6	31.11 ± 16.37	89.99 ± 5.49	1.79 ± 1.11
9	13.33 ± 8.82	98.28 ± 0.91	0.45 ± 0.28
12	1.11 ± 1.11	100 ± 0	0.04 ± 0.03
LSD (p=0.05)	29.87	11.25	2.67

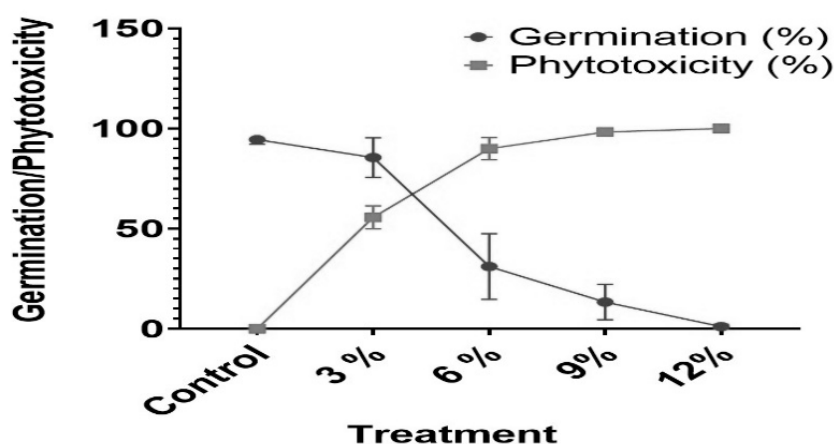


Figure 2. Effects of *P. hysterophorus* L. extract on the seed germination (%) and phytotoxicity (%) *P. oleracea* L.

### Speed of germination

The number of seeds germinated with 6 % extract was drastically reduced to 1.79 seeds germinated/day against control (8.76 seeds germinated/day). Germination speed depicts the seed vigour and play an important role during competition. The *P. hysterophorus*

extracts had remarkable effects on germination speed. Even at lower extract concentrations (3 and 6 %) the speed of germination was reduced (Table -1). Approximately 50 % reduction was observed with 3 % extract and at 6 %, 80 % reduction in speed of germination. The reduction in speed of germination in *P. oleracea* L. certainly give selective advantage, during the later stage of crop growth. Li *et al* (40) studied the allelopathic potential of *Artemisia argyi* water soluble extract on germination speed of *Brassica pekinensis* L., *Lactuca sativa* L., *Oryza sativa* L. and found that the extracts negatively affect the germination speed index.

### Seedling vigour

High vigour of seedlings helps in establishment of seedlings at early stages, offering growing seedlings a competitive edge in withstanding various environmental stresses. After germination, the shoot, root length of seedling were measured to evaluate the inhibitory effects against *P. oleracea*. The germinated seedlings exhibited a stunted growth at lower concentrations (Table -2, Figure -3A, B). The shoot growth was comparably less affected than root. At higher concentrations (12 % extract, the seeds did not produce normal roots and shoots) against control in which healthy seedlings produced 0.73 cm shoot and 1.3 cm root. The absence of shoot growth in 12 % extract was on par with 9 % and 6 % extract, in which severe shoot growth restriction was observed (i.e.) 0.04 cm and 0.19 cm. However, at 3 % extract shoots were formed similar to untreated control (Figure -3A). Development of roots was significantly affected at 6 % and 9 % treatment and exhibited stunted growth than control in which roots grew normally (Figure -3 B).

Table 2. Inhibitory effects of *P. hysterothorus* extracts on seedling growth of *P. oleracea*

Extract conc (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)
Control	0.73 ± 0.05	1.3 ± 0.05	2.03 ± 0.08
3	0.73 ± 0.06	0.16 ± 0.06	0.89 ± 0.12
6	0.19 ± 0.11	0.01 ± 0.00	0.21 ± 0.11
9	0.04 ± 0.02	0 ± 0.00	0.04 ± 0.02
LSD (p=0.05)	0.19	0.11	0.26

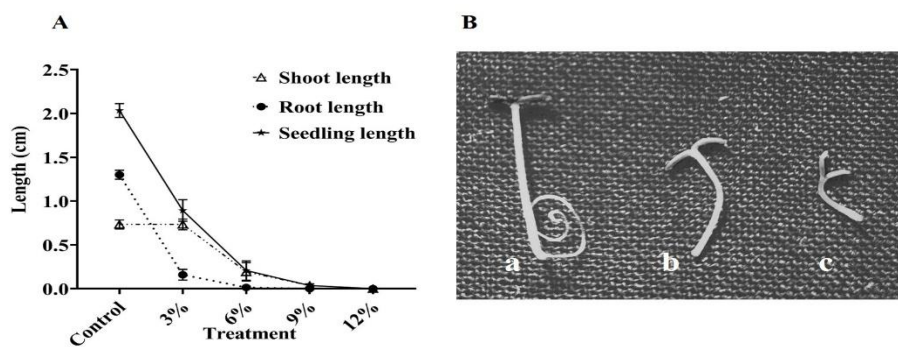


Figure 3A. Inhibitory effects of *P. hysterothorus* L. extract on seedling growth of *P. oleracea* L.

Figure 3B. Inhibitory effects of *P. hysterothorus* L. extract on the development of *P. oleracea* L. seedlings (a: Control, b: 3 % extract and b: 6 % extract)

The toxicity was analyzed by assessing the total length of the seedling after the treatment (7 days). The length of the seedling was greatly reduced in treatments. The application of 9 % and 6 % extract suppressed the seedling growth (0.04 cm and 0.21 cm). Finally, phytotoxicity (%) produced by *P. hysterophorus* L. extract was worked out, 100 % phytotoxicity was achieved in 12 % extract and was on par with 9 % and 6 % extract, in which 98.28 % and 89.99 % phytotoxicity was observed, respectively. These results are in comparison with other results (73,75). In addition, Borghetti and his coworkers (13) suggested that in the practice of aqueous extracts reflects natural conditions more closely than other methodology, which signifies the importance of this methodology. These results highlight the excellent allelotoxic effects of *P. hysterophorus* L. aqueous extracts against the germination and early development of *P. oleracea* L. seedlings.

#### Microscopic analysis revealed severe tissue damage in roots

The application of *P. hysterophorus* L. extracts significantly damaged the roots of treated plants. To ascertain the nature of damage and assess the effects on plant growth, a detailed microscopic analysis was done on roots exposed to extracts and compared with control. The roots of treated seedlings exhibited stunted growth with distortion of root tissues, turned black and died (Figure-4 A, B, C).

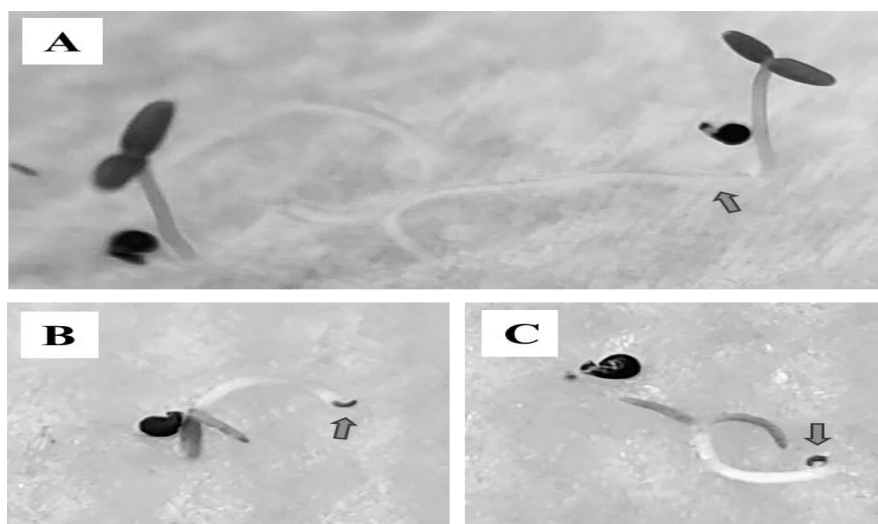


Figure 4. Root necrosis of *P. oleracea* L. seedlings caused by *P. hysterophorus* L. extract (A-control and B, C- 5 % extract)

⇨ - arrows indicate the root portion of *P. oleracea*

Microscopic examination revealed the damaged epidermal tissue with impact on root hairs. Affected roots produce fewer root hairs than control, in which a tuft of root hairs was noticed (Figure-5 A, B). The distorted root tissues with loss of root hairs could be attributed to the necrosis of exposed seedlings. Necrosis is a multifaceted process of cell death characterized by tissue discolouration, darkening (76) and the emergence of a brittle

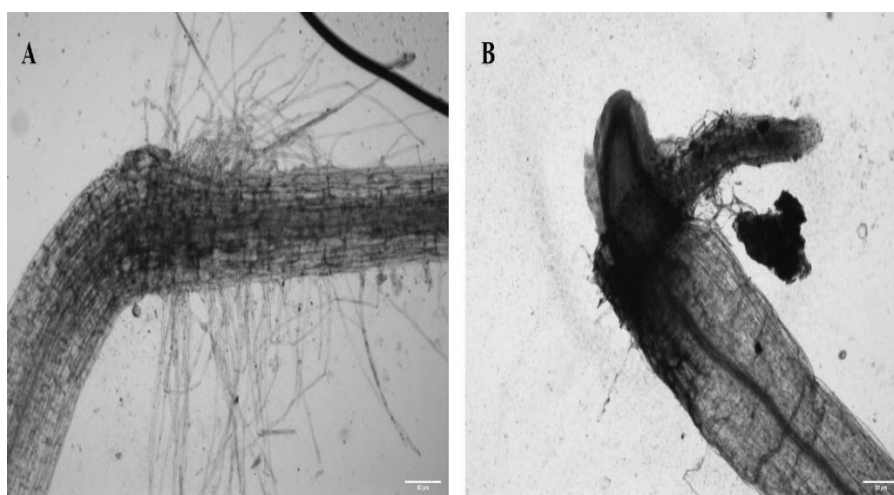


Figure 5. Microscopic observation of *P. oleracea* L. roots damaged by *P. hysterophorus* L. extract (A: control, B: 5 % extract)

texture. It can affect any part of plant including stem, root, leaves, flowers and fruits. It is a complex process mediated by a combination of internal and external factors. Various factors like pathogens, stress conditions and physical damage disrupt the cellular homeostasis and acts as a triggering factor for necrosis (59,79). Once triggered, this process is irreversible and leads to the generation of reactive oxygen species and elicitation of oxidative stress. Organelle dysfunction result in rupturing of vacuoles which releases hydrolytic enzymes and digest the cellular contents. Loss of osmotic imbalance and membrane integrity result in the cellular rupture and produce the characteristic symptoms of necrosis (28). The behaviour of seedlings of *P. oleracea* L. exhibited the similar pattern up on exposure to the extracts of *P. hysterophorus* L. Soon after the treatment, the roots begins to lose its texture and becomes brittle, which significantly stunted the growth of seedlings This is followed by discolouration and darkening of root tips and subsequent death of the seedlings (Figure 4 and 5). Other studies on *P. hysterophorus* L. extracts exhibited a similar pattern (i.e severe root damage and necrosis of root tips) (12,48,49,61) The inhibitory effects of aqueous extract of *P. hysterophorus* L. stems from the water soluble allelochemicals present in the extracts, which correlates with other studies (2, 22, 54). Datta and his associates (21) observed a necrosis of root region in *Cassia tora* L. and revealed the effect is due to the allelochemical parthenin. Exposure of seeds to purified parthenin inhibited germination and produced severe root necrosis on lower concentrations which corroborate with our results. Similar results were observed on other weed species viz., *Amaranthus viridis* L., *Cassia occidentalis* L., *Echinochloa crusgalli* L. and *Phalaris minor* Retz. (10). The affected roots swelled, black root tips, shrivelling, damage to the epidermal tissue and non-formation of root hairs.

### Phytochemical profiling

The secondary metabolites potential of *P. hysterophorus* L. aqueous extracts were evaluated by GC-MS analysis. Phytochemical profiling of the extracts exhibited 57 peaks from 4.088 min to 32.91 min. A total of 54 compounds were found in the *P. hysterophorus* L. extracts (Table 3). The compounds identified were: 14-Deoxyandrographolide with 2.91 % of occupied area; Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester which occupied 3.04 % of area and Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl- with 2.35 % occupied area. Fewer compounds like 2-Cyclohexen-1-one, 2-methyl-; 2-Dodecen-1-yl (-) succinic anhydride; Sclareolide and Epoxylathyrol occupied > 5 % area (Figure-6, Table-3).

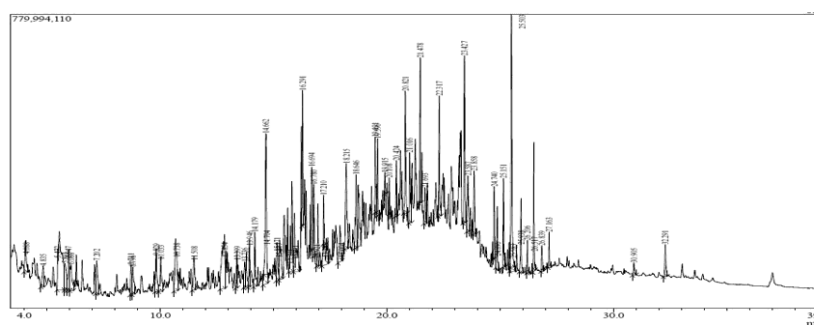


Figure 6. Chromatogram of allelochemicals present in *P. hysterophorus* L. extract

Table 3. List of chemicals identified in the aqueous extracts of *P. hysterophorus* L.

Peak	RT	Area (%)	Compound
1	4.088	1.02	3-Methyl-3-butenic acid
2	4.835	0.78	2,2-Dimethyl-3-hydroxypropionaldehyde
3	5.472	5.17	2-Cyclohexen-1-one, 2-methyl-
4	5.801	1.15	trans-1,4-Cyclohexanediol, bis(trifluoroacetate)
5	5.947	1.18	cis-2-Hexen-1-ol, trifluoroacetate
6	6.068	1.18	4-Hexen-1-ol, trifluoroacetate
7	7.202	1.29	N-Aminopyrrolidine
8	8.711	0.80	Carbamic acid, phenyl ester
9	8.796	1.00	Hexanoic acid, 2,2-dimethylpropyl ester
10	9.829	1.27	cis-2,3-Epoxyoctane
11	10.033	1.10	Pentanoic acid, pentyl ester
12	10.738	2.25	trans-2,3-Epoxy-nonane
13	11.508	1.13	Bicyclo[2.2.1]heptan-2-ol, 2,3,3-trimethyl-
14	12.854	3.56	Cyclopropane, 1-bromo-2,2,3,3-tetramethyl-1-prop-1-ynyl-
15	13.399	0.78	1,5,5-Trimethyl-6-methylene-cyclohexene
16	13.736	0.80	N-(1-Cyanopropenyl)formamide
17	13.946	1.07	Bicyclo[3.2.0]hepta-3,6-diene-1-carbonitrile
18	14.179	1.20	1-Cyclohexene-1-methanol, .alpha.,2,6,6-tetramethyl-
19	14.662	4.18	2,6S-Diethyl-3,5S-dimethyl-3,4-dihydro-2H-pyran
20	14.704	5.10	2-Dodecen-1-yl(-)succinic anhydride

21	15.171	0.98	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-
22	15.247	1.60	2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl-
23	15.701	2.95	Bicyclo[4.3.0]nonan-2-one, 8-isopropylidene-
24	15.971	2.35	2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl
25	16.291	2.11	(3S,3aR,6R,8aS)-3,7,7-Trimethyl-8-methylenehexahydro-1H-3a,6-methanoazulen-2(3H)-one
26	16.694	1.61	2,4,6-Trinitro-N-methyl-aniline
27	16.780	1.61	4-Cycloocten-1-one, 8-(4-octen-4-yl)-
28	16.921	1.05	Fumagillol
29	17.210	0.19	3-Isopropyltricyclo[4.3.1.1(2,5)]undec-3-en-10-ol
30	17.283	2.40	1,2-Dihydropyridine, 1-(1-oxobutyl)-
31	18.004	1.19	14-Deoxyandrographolide
32	18.215	2.54	1-Cyclohexene-1-butanal, .alpha.,2,6,6-tetramethyl-
33	18.646	1.72	14-Deoxyandrographolide
34	19.491	1.35	Bicyclo[4.1.0]heptane, 1-(3-oxo-4-phenylthiobutyl)-2,2,6-trimethyl-
35	19.596	1.08	Ppropionic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-
36	20.108	0.37	1-Cyclohexene-1-butanal, .alpha.,2,6,6-tetramethyl-
37	20.424	0.83	Eudesma-4(15),7-dien-1.beta. -ol
38	20.821	2.62	2-(4,8,12-Trimethylcyclotetradeca-3,7,11-trien-1-yl) propan-2-ol
39	21.016	1.90	1-(2-Hydroxypropan-2-yl)-3a-methyl-6,10-dimethylidene-2,3,4,5,7,8,9,11,12,12a--decahydro-1H-cyclopenta[11]annulene-5,9-diol
40	21.478	2.60	1-Methyl-1-n-dodecyloxy-1-silacyclobutane
41	21.693	0.98	Pent-4-enoylamide, 2-methyl-N-allyl-N-pentyl-
42	22.317	2.13	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
43	23.427	3.38	i-Propyl nonadecanoate
44	23.587	1.51	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester
45	23.858	1.53	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester
46	24.740	1.18	(-)-Globulol
47	24.896	1.16	6-Phenylhexanoic acid, TMS derivative
48	25.151	1.58	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
49	25.503	5.62	Sclareolide
50	25.583	6.06	Epoxyathyrol
51	25.938	1.38	1-Heptatriacotanol
52	26.206	0.48	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-
53	26.517	1.87	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-
54	26.839	0.46	Docosanoic acid, methyl ester
55	27.163	0.57	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-, [1R-[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]-
56	30.905	0.30	Cholesterol
57	32.291	0.84	Stigmasterol

The presence of several metabolites allies with the toxic effect of the extracts. Allelochemical parthenin is a sesquiterpene lactone biosynthesized during the entire life cycle of the *P. hysterophorus* L. plant and this compound exhibits broad spectrum phytotoxicity (11). In addition, this compound is one of the major phytochemical produced by *P. hysterophorus* L. plants (8,20,23,27,51,57) etc. However, parthenin was not detected in phytochemical analysis. This could be due to the presence of parthenin at very low levels below the detection range. Further, in the observed compounds at least nine compounds (Table 4 and Figure 7) exhibit phytotoxicity with other bioactive molecules of antibacterial, antifungal and larvicidal properties.

Table 4. Phytotoxic chemicals in aqueous extracts of *P. hysterophorus* and their Bio-activity.

Compound	Bioactivity	Ref
Carbamic acid, phenyl ester	Germination inhibition, shoot and root growth inhibition on radish	78
Pentanoic acid, pentyl ester	Inhibition of Germination and radical growth in <i>Raphanus sativus</i> , <i>Sinapis arvensis</i> and <i>Lolium multiflorum</i>	37
14-Deoxyandrographolide	Suppressing germination, shoot growth and root growth on <i>Eleusine indica</i> , <i>Ageratum conyzoides</i> , <i>Cyperus distans</i> and <i>Oryza sativa</i>	74
Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	Increases the phenol content, flavonoid content, ROS production and antioxidant production, reduction of protein and nucleic acid content in <i>Portulaca oleracea</i> leaves.	24
Globulol	Produces inhibition of germination, shoot growth and root growth in <i>Solanum elaeagnifolium</i>	80
	Germination inhibition and suppression of radical growth on <i>Raphanus sativus</i> , <i>Lolium multiflorum</i> and <i>Sinapis arvensis</i>	34
	Germination inhibition on <i>Raphanus sativus</i>	44
	Radical growth suppressive effect on <i>Raphanus sativus</i>	43
	Growth reduction in <i>Agrostis stolonifera</i>	35
	Restricts germination, shoot growth and root growth on lettuce, watermelon, cucumber and tomato	68
	Arrests growth of <i>Lactuca sativa</i> and <i>Agrostis stolonifera</i> seedlings	36
	Restricted germination, shoot growth and root growth on <i>Sinapis arvensis</i> , <i>Lolium rigidum</i> and <i>Trifolium campestre</i>	33
	Restricts growth of <i>Lemna minor</i>	60
Sclareolide	Growth inhibition of <i>Lemna minor</i> plants	18
1-Heptatriacotanol	Restricted germination, shoot growth and root growth on <i>Sinapis arvensis</i> , <i>Lolium rigidum</i> and <i>Trifolium campestre</i>	33
	Root growth inhibition on seedlings of <i>Lactuca sativa</i>	72
	Suppress germination, plant growth and yield, reduces photosynthetic pigments and protein content, increases production of phenols and proline on <i>Lens culinaris</i>	41

	Inhibition of germination on <i>Eragrostis teff</i> under water stressed conditions	26
Docosanoic acid (or) Behenic acid, methyl ester (or) Methyl behenate	Inhibition of germination on <i>Eragrostis teff</i> under water stressed conditions	26
	Produces growth regulatory effects and death of <i>Lemna minor</i> fronds	58
	Growth inhibition of <i>Lemna minor</i>	4
	Growth inhibition and death of <i>Lemna minor</i>	64
	Shoot and root growth inhibition on <i>Cynodon dactylon</i> seedlings	5
Stigmasterol	Growth inhibition and death of <i>Lemna minor</i>	64
	Radicle growth inhibition on <i>Lens esculentum</i>	77
	Inhibits the germination, shoot growth and radicle growth of <i>Bidens pilosa</i>	65
	Restricts growth of <i>Lemna minor</i>	60
	Reduced germination, shoot growth and fresh weight on <i>Vigna unguiculata</i>	38
	Growth inhibition of <i>Lemna minor</i>	52
	Reduction in root/shoot length and dry weight of <i>Cassia tora</i> seedlings. Ruptures and shrink leaf epidermal cells; damaged margins and necrosis on <i>Cassia tora</i> leaf	53
	Inhibition of growth in <i>Lemna minor</i>	32

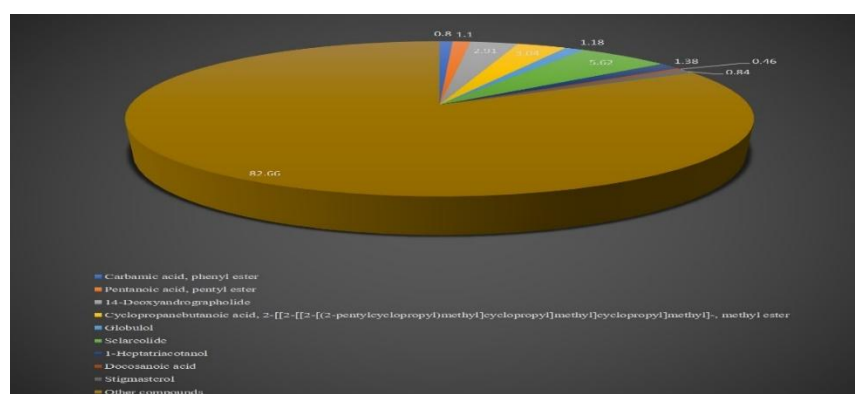


Figure-7 Quantity of phytotoxic compounds in *P. hysterophorus* L. extract

Several studies proved their herbicidal properties in various plant species. For example: phytochemicals like Carbamic acid, Pentanoic acid and phenyl ester showed inhibition of germination and restricting shoot along with root growth in radish seeds (24,37,78). Herbicidal potential of *Rumex dentatus* L. is due the presence of Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl]methyl] cyclopropyl] methyl]-, methyl ester has been confirmed (24). Other observed compounds like globulol (34,80), sclareolite (18), 1-Heptatriacotanol (41,72), Docosanoic acid methyl ester (26,58) exhibits poor germination, restricted shoot and root growth, in various

seedlings. A list of phytochemicals of *P. hysterophorus* L. identified in the aqueous extract and their phytotoxic activities are given in the table 4. From these results, it is clear that, aqueous extract of *P. hysterophorus* L. contains multiple phytotoxins that might contribute to the observed toxicity on the weed plant *P. oleracea* L. The challenges of using organic solvents to prepare plant extracts in field conditions led us to use its aqueous extracts.

#### Distribution of *P. hysterophorus* L. and *P. oleracea* L. in experimental fields

Allelopathic suppression of weeds is a natural mechanism found in many ecosystems, where weeds influence the growth and survival of neighboring plants (66). By producing the of phytochemicals and phytotoxic exudates, they exert allelopathic effects and thereby shape the plant community, biodiversity and influence species composition (3). In order to assess the allelopathic activity of *P. hysterophorus* over the growth of *P. oleracea* L., the distribution of *P. oleracea* L. was assessed in fields heavily and lightly infested with *P. hysterophorus* L. We found an antagonistic interaction between the two species (Figure- 8 A) in field conditions. The number of *P. oleracea* L. is significantly reduced in the fields with heavy parthenium infestation, whereas, the fields with light parthenium infestation have relatively higher number of *P. oleracea* L. In addition, we found that in presence of *P. hysterophorus* L., *P. oleracea* L. exhibited stunted growth with greatly reduced plant size (Figure- 8 B and C).

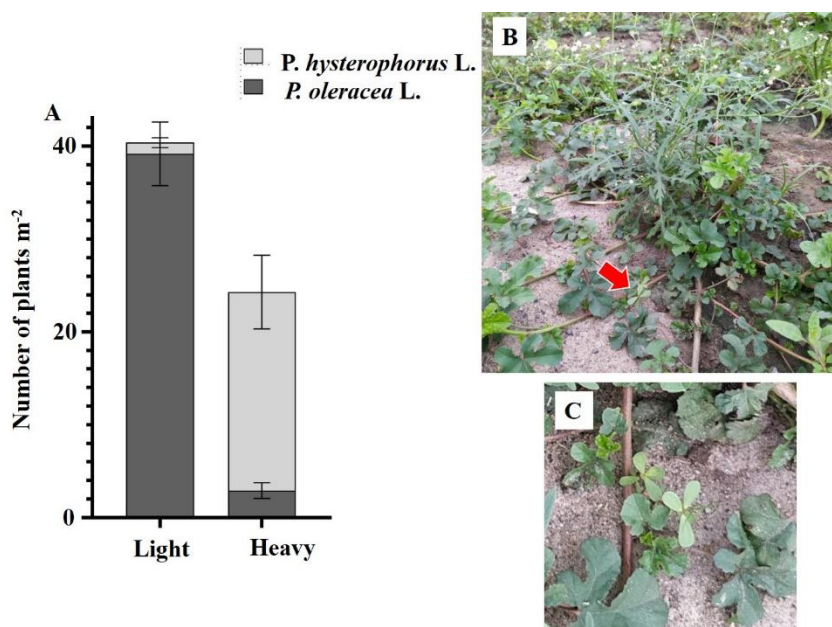


Figure 8. Bar graph showing the allelopathic effect of *P. hysterophorus* L. on distribution *P. oleracea* L. **A**: Number of *P. oleracea* L. (black shade) and the number of *P. hysterophorus* L. (grey shade) is inversely proportional. Light : Light parthenium infested fields; Heavy - Heavy infested fields. **B**: *P. oleracea* L. (exhibited stunted growth when seen with *P. hysterophorus* L. Red arrows showing the under developed *P. oleracea* L. **C**: enlarged image

These results clearly signify the phytotoxic effect exerted by *P. hysterophorus* L. over the germination and growth of *P. oleracea* L. Allelopathic effect of weed species like *P. hysterophorus* L., species were well documented on the growth of other weed species which correlates with the observed effect (55). Altogether, this study unravels the bioherbicidal potential of *P. hysterophorus* against *P. oleracea* L. The diverse array of phytochemicals with allelopathic properties in the extract not only underscores its potential as a bioherbicide but also paves the way for its application in field conditions using aqueous preparations

### CONCLUSIONS

We proposed an effective way to utilize *P. hysterophorus* L. as bioherbicide against weed *P. oleracea* L. The allelochemicals present in *P. hysterophorus* L., aqueous extract significantly inhibited the germination and growth of *P. oleracea* L. The extracts caused the root necrosis showing the inhibitory activity of toxic allelochemicals including parthenin in weed management. GC-MS identified 9-phytotoxic allelochemicals (Carbamic acid phenyl ester, Pentanoic acid pentyl ester, 14-Deoxyandrographolide, Cyclopropane butanoic acid, Globulol, Sclareolide, 1-Heptatriacotanol; Docosanoic acid and Stigmasterol) and validated the efficacy of aqueous extract. This study showed the allelotoxic potential of *Parthenium hysterophorus* L. weed against another weed *Portulaca oleracea* L.

### AUTHORS CONTRIBUTION

**TS** : Conceptualization, methodology, investigation, data analysis, visualization, Writing: original draft and revision; **RR**: Supervision, Writing – original draft and revision; **SG** - Data analysis, Investigation, **AA**: investigation, **SS** - Data analysis, Investigation.

### DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

### ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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## REFERENCES

1. Abbas, M.N., Rana, S.A., Mahmood-Ul-Hassan, M., Rana, N. and Iqbal, M. (2013). Phytochemical constituents of weeds: Baseline study in mixed crop zone agroecosystem. *Pakistan Journal of Weed science Research* **19**(2):231-238.
2. Afridi, R.A. and Khan, M.A. (2015). Comparative effects of water extract of *Parthenium hysterophorus*, *Datura alba*, *Phragmites australis* and *Oryza sativa* on weeds and wheat. *Sains Malaysiana* **44**(5): 693-699.
3. Al Musalami, A.A., Al Marshoudi, M.S., Farooq, S.A. and Al-Reasi, H.A. (2023). Allelopathic effects of the invasive species (*Prosopis juliflora*) on seedlings of two common arid plants: Does free proline play roles?. *Journal of Arid Environments* **211**: 104931.
4. Alam, M., Uddin, G., Siddiqui, B.S., Sadat, A., Khan, A.A. and Rauf, A. (2014). Gas chromatography-mass spectrometry (GC-MS) analysis, antimicrobial, phytotoxic, insecticidal and leishmanicidal activities of fixed oil from *Viburnum grandiflorum*. *African Journal of Pharmacy and Pharmacology* **8**(46): 1173.
5. Alsaadawi, I.S. and Rice, E.L. (1982). Allelopathic effects of *Polygonum aviculare* L. II. Isolation, characterization, and biological activities of phytotoxins. *Journal of Chemical Ecology* **8**: 1011-1023.
6. Aroonrungsikul, C., Sukprakarn, S., Nawata, E. and Sakuratani, T. (2001). Effects of physical treatments on breaking of seed dormancy in Thai cucumber. *Japanese Journal of Tropical Agriculture* **45**(4): 251-258.
7. Bashar, H.K., Juraimi, A.S., Ahmad-Hamdani, M.S., Uddin, M.K., Asib, N., Anwar, M.P., Rahaman, F., Haque, M.A. and Hossain, A. (2023). Evaluation of allelopathic effects of *Parthenium hysterophorus* L. methanolic extracts on some selected plants and weeds. *Plos one* **18**(1): e0280159.
8. Bashar, H.K., Juraimi, A.S., Ahmad-Hamdani, M.S., Uddin, M.K., Asib, N., Anwar, M.P., Rahaman, F., Karim, S.R., Haque, M.A., Berahim, Z., Mustapha, N.A.N. and Hossain, A. (2022). Determination and quantification of phytochemicals from the leaf extract of *Parthenium hysterophorus* L. and their physio-biochemical responses to several crop and weed species. *Plants* **11**(23): 3209.
9. Bashar, H.K., Juraimi, A.S., Ahmad-Hamdani, M.S., Uddin, M.K., Asib, N., Anwar, M.P., Karim, S.R., Rahaman, F., Haque, M.A. and Hossain, A. (2022). Documentation of phytotoxic compounds in *Parthenium hysterophorus* L. leaf and their phytotoxicity on *Eleusine indica* (L.) Gaertn. and *Digitaria sanguinalis* (L.) Scop. *Toxins* **14**(8): 561.
10. Batish, D.R., Pal Singh, H., Kohli, R.K., Kaur, S., Saxena, D.B. and Yadav, S. (2007). Assessment of phytotoxicity of parthenin. *Zeitschrift für Naturforschung C* **62**(5-6): 367-372.
11. Belz, R.G. (2008). Stimulation versus inhibition-Bioactivity of parthenin, a phytochemical from *Parthenium hysterophorus* L. *Dose-Response* **6**(1): 80-96.
12. Bhadoria, P.B.S. (2010). Allelopathy: A natural way towards weed management. *American Journal of Experimental Agriculture* **1**(1): 7-20.
13. Borghetti, F., Lima, E.C.D. and Silva, L.D.C.R. (2013). A simple procedure for the purification of active fractions in aqueous extracts of plants with allelopathic properties. *Acta Botanica Brasiliica* **27**: 50-53.
14. Bottenberg, H., Masiunas, J., Eastman, C. and Eastburn, D. (1997). Weed management effects on insects and diseases of cabbage and snapbean. *HortTechnology* **7**(4): 400-403.
15. Bristone, B., Md Zain, N., Muhamad, S., Ywih, C.N.H. and Naher, L. (2022). Herbicidal activity of isolated fractions and identified compounds from the ethyl acetate extract of *Parthenium hysterophorus* L. leaves on *Echinochloa colona* (L.) Link and *Hedyotis verticillata* (L.) Lam. *Annals of Agri-bio Research* **27**(2):158-167.
16. Chauhan, B.S. and Johnson, D.E. (2009). Seed germination ecology of *Portulaca oleracea* L.: An important weed of rice and upland crops. *Annals of Applied Biology* **155**(1): 61-69.
17. Cheng, F. and Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science* **6**: 1020.
18. Choudhary, M.I., Musharraf, S.G. and Sami, A. (2004). Microbial Transformation of Sesquiterpenes, (-) - Ambrox® and (+) - Sclareolide. *Helvetica Chimica Acta* **87**(10): 2685-2694.
19. Coleman, M., Kristiansen, P., Sindel, B. and Fyfe, C. (2018). Pigweed (*Portulaca oleracea*): Weed management guide for Australian vegetable production. School of Environmental and Rural Science, University of New England, Armidale.
20. Das, B. and Das, R. (1995). Chemical investigation in *Parthenium hysterophorus* L. an allelopathic plant. *Allelopathy Journal* **2**: 99-104.
21. Datta, S. and Saxena, D.B. (2001). Pesticidal properties of parthenin (from *Parthenium hysterophorus*) and related compounds. *Pest Management Science* **57**(1): 95-101.

22. Devi, O.I., Dutta, B.K. and Zea, L. (2012). Allelopathic effects of the aqueous extract of *Parthenium hysterophorus* and *Chromolaena odorata* on the seed germination and seedling vigour of *Zea mays* L. *Academic Journal of Plant Sciences* **5(4)**: 110-113.
23. Dukpa, R., Tiwari, A. and Kapoor, D. (2020). Biological management of allelopathic plant *Parthenium* sp. *Open Agriculture* **5(1)**: 252-261.
24. El-Shora, H.M., Alharbi, M.M., Darwish, D.B. and Gad, D. (2022). Allelopathic potential of aqueous leaf extract of *Rumex dentatus* L. on metabolites and enzyme activities of common purslane leaves. *Journal of Plant Interactions* **17(1)**: 267-276.
25. Gairola, K.C., Nautiyal, A.R. and Dwivedi, A.K. (2011). Effects of temperatures and germination media on seed germination of *Jatropha curcas* L. *Advances in Bioresarch* **2(2)**: 66-71.
26. Galt, N. (2018). *The Role of Phytotoxic and Antimicrobial Compounds of Euphorbia Gummifera in the Cause and Maintenance of the Fairy Circles of Namibia*. M.Sc. Thesis. Department of Plant and Soil Sciences, Faculty of Natural and Agricultural sciences, University of Pretoria, South Africa.
27. Hernandez, Y.S., Sanchez, L.B., Bedia, M.M.G., Gomez, L.T., Rodríguez, E.J., San Miguel, H.M.G., Mosquera, D.G., García, L.P., Dhooche, L., Theunis, M., Pieters, L. and Apers, S. (2011). Determination of parthenin in *Parthenium hysterophorus* L. by HPLC-UV: Method development and validation. *Phytochemistry Letters* **4(2)**: 134-137.
28. Hryvusevich, P.V., Samokhina, V.V. and Demidchik, V.V. (2022). Stress-induced electrolyte leakage from root cells of higher plants: Background, mechanism and physiological role. *Experimental Biology and Biotechnology* **2**: 4-18.
29. Islam, A.M. and Widhalm, J.R. (2020). Agricultural uses of juglone: Opportunities and challenges. *Agronomy* **10(10)**: 1500.
30. Kamala, B., Ashwini, R.N. and Geetha, K.N. (2022). Allelopathy in weed management: A review. *Mysore Journal of Agricultural Science* **56(3)**:1-15.
31. Kaur, H., Kaur, N. and Bhullar, M.S. (2021). Germination ecology and management of *Portulaca oleracea* L.- a weed of summer vegetable crops in Punjab. *Agricultural Research Journal* **58(1)**:51-59.
32. Khaliq-uz-Zaman, S.M., Simin Shameel, S.S., Mustafa Shameel, M.S., Leghari, S.M. and Ahmad, V.U. (1998). Bioactive compounds in *Chara corallina* var. wallichii (A. BR.) RD Wood (Charophyta). *Pakistan Journal of Botany* **30(1)**:19-31.
33. Khedhri, S., Polito, F., Caputo, L., Khamassi, M., Hamrouni, L., Nazzaro, F., Fratianni, F., Scognamiglio, M.R., De Feo, V. and Amri, I. (2024). Chemical composition, phytotoxic and antibiofilm activity of *Pinus canariensis*, *P. jeffreyi* and *P. taeda* essential oils. *Journal of Essential Oil Bearing Plants* **27(2)**: 482-497.
34. Khedhri, S., Polito, F., Caputo, L., Manna, F., Khamassi, M., Hamrouni, L., Amri, I., Nazzaro, F., De Feo, V. and Fratianni, F. (2022). Chemical composition, phytotoxic and antibiofilm activity of 7-*Eucalyptus* species from Tunisia. *Molecules* **27(23)**: 8227.
35. Kobaisy, M., Tellez, M.R., Dayan, F.E. and Duke, S.O. (2002). Phytotoxicity and volatile constituents from leaves of *Callicarpa japonica* Thunb. *Phytochemistry* **61(1)**: 37-40.
36. Kobaisy, M., Tellez, M.R., Webber, C.L., Dayan, F.E., Schrader, K.K. and Wedge, D.E. (2001). Phytotoxic and fungitoxic activities of the essential oil of kenaf (*Hibiscus cannabinus* L.) leaves and its composition. *Journal of Agricultural and Food Chemistry* **49(8)**: 3768-3771.
37. Kouki, H., Polito, F., De Martino, L., Mabrouk, Y., Hamrouni, L., Amri, I., Fratianni, F., De Feo, V. and Nazzaro, F. (2022). Chemistry and bioactivities of six Tunisian *Eucalyptus* species. *Pharmaceuticals* **15(10)**: 1265.
38. Kpoviessi, D.S.S., Gbaguidi, F.A., Gbenou, J.D., Accrombessi, G.C., Moudachirou, M. and Quetin-Leclercq, J. (2006). Allelopathic effects on cowpea (*Vigna unguiculata* (L.) Walp) plant and cytotoxic activities of sterols and triterpenes isolated from *Justicia anselliana* (NEES). *Electronic Journal of Natural Sciences* **1**: 12.
39. Kumar, S. and Varshney, J.G. (2010). Parthenium infestation and its estimated cost management in India. *Indian Journal of Weed Science* **42(1,2)**: 73-77.
40. Li, J., Chen, L., Chen, Q., Miao, Y., Peng, Z., Huang, B., Guo, L., Liu, D. and Du, H. (2021). Allelopathic effects of *Artemisia argyi* on the germination and growth of various weeds. *Scientific reports* **11(1)**: 4303.
41. Lomas, M.K., Anjali, A., Agrawal, S. and Narayan, R. (2024). *Applied Powdered Leaf-Biomass of Alien Weed Hyptis suaveolens (L.) Poit. In: Soil Adversely Impacts Germination, Growth and Yield of Crop Lens culinaris Medik. despite Enhancing Soil Fertility.*; doi:10.21203/rs.3.rs-3864136/v1
42. Mahajan, S., Shrestha, B.B. and Jha, P.K. (2007). Allelopathic effects of aqueous extract of leaves of *Parthenium hysterophorus* L. on seed germination and seedling growth of some cultivated and wild herbaceous species. *Scientific world* **5(5)**: 33-39.

43. Mancini, E., Apostolides, N., De Feo, V., Formisano, C., Rigano, D., Piozzi, F. and Senatore, F. (2009). Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta nuda* L. subsp. *albiflora* growing wild in Lebanon. *Journal of Plant Interactions* **4**(4): 253-259.
44. Martino, L.D., Formisano, C., Mancini, E., Feo, V.D., Piozzi, F., Rigano, D. and Senatore, F. (2010). Chemical composition and phytotoxic effects of essential oils from four *Teucrium* species. *Natural Product Communications* **5**(12): 1934578X1000501230.
45. Masabni, J.G. and Zandstra, B.H. (1999). Discovery of a common purslane (*Portulaca oleracea*) biotype resistant to linuron. *Weed Technology* **13**(3): 599-605.
46. Matthews, J.F., Ketron, D.W. and Zane, S.F. (1993). The biology and taxonomy of the *Portulaca oleracea* L. (Portulacaceae) complex in North America. *Rhodora* **95**(882): 166-183.
47. Miyanasishi, K. and Cavers, P.B. (1990). The Biology of Canadian Weeds. 40. *Portulaca oleracea* L. *Canadian Journal of Plant Science* **60**(3):953-963.
48. Mohanan, H. and Rajendiran, K. (2013). Allelopathy and cytotoxicity of aqueous extracts of *Parthenium hysterophorus* L. on *Oryza sativa* L. Var. Asd-16. *International Journal of Food, Agriculture and Veterinary Sciences* **3**(3):55-61.
49. Mohanan, H. and Rajendiran, K. (2014). Allelopathic and mitodepressive effects of *Parthenium hysterophorus* L. leachates on ornamental sunflower. *Global Journal of Bio-science and Biotechnology*, 3 (1): 1-5.
50. Motmainna, M., Juraimi, A.S., Uddin, M.K., Asib, N.B., Islam, A.M., Ahmad-Hamdani, M.S., Berahim, Z. and Hasan, M. (2021). Physiological and biochemical responses of *Ageratum conyzoides*, *Oryza sativa* f. *spontanea* (weedy rice) and *Cyperus iria* to *Parthenium hysterophorus* methanol extract. *Plants* **10**(6): 1205.
51. Motmainna, M., Juraimi, A.S., Uddin, M.K., Asib, N.B., Islam, A.M., Ahmad-Hamdani, M.S. and Hasan, M. (2021). Phytochemical constituents and allelopathic potential of *Parthenium hysterophorus* L. in comparison to commercial herbicides to control weeds. *Plants* **10**(7): 1445.
52. Muhammad, S. and Simin, K. (2001). Antimicrobial activity and phytotoxicity of sterols from *Chara wallichii* A. Br. (Charophyta). *Biological Sciences-PJSIR* **44**(5): 301-304.
53. Mushtaq, W., Ain, Q., Siddiqui, M.B. and Hakeem, K.R. (2019). Cytotoxic allelochemicals induce ultrastructural modifications in *Cassia tora* L. and mitotic changes in *Allium cepa* L.: A weed versus weed allelopathy approach. *Protoplasma* **256**: 857-871.
54. Netsere, A. (2015). Allelopathic effects of aqueous extracts of an invasive alien weed *Parthenium hysterophorus* L. on maize and sorghum seed germination and seedling growth. *Journal of Biology, Agriculture and Healthcare* **5**(1): 120-124.
55. Nguyen, T., Bajwa, A.A., Belgeri, A., Navie, S., O'Donnell, C. and Adkins, S. (2017). Impact of an invasive weed, *Parthenium hysterophorus*, on a pasture community in south east Queensland, Australia. *Environmental Science and Pollution Research* **24**: 27188-27200.
56. Nigatu, L., Hassen, A., Sharma, J. and Adkins, S.W. (2010). Impact of *Parthenium hysterophorus* on grazing land communities in north-eastern Ethiopia. *Weed Biology and Management* **10**(3): 143-152.
57. Niranjana, A., Mishra, S., Lehri, A., Amla, D.V., Upadhyay, R.S. and Nautiyal, C.S. (2013). Identification and quantification of heterologous compounds parthenin and organic acids in *Parthenium hysterophorus* L. using HPLC-PDA-MS-MS. *Analytical Letters* **46**(1): 48-59.
58. Nisar, M., Khan, S.A. and Ali, I. (2013). GC-MS analysis and pharmacological potential of oil of *Eluphia dabia*. *Middle-East Journal of Scientific Research* **14**(3): 375-380.
59. Ogburn, A.A., Finnie, J.F. and Van Staden, J. (2020). The role of endophytes in secondary metabolites accumulation in medicinal plants under abiotic stress. *South African Journal of Botany* **134**: 126-134.
60. Orumwensodia, K.O., Uadia, P.O. and Choudhary, M.I. (2021). Phytotoxicity, cytotoxicity and chemical composition of *Spondias mombin* L. stem bark. *Clinical Phytoscience* **7**: 1-9.
61. Pandey, D.K. (2015). Allelochemicals from *Parthenium* for water hyacinth control. *Indian Journal of Weed Science* **47**(3): 321-328.
62. *Portulaca oleracea*. (2024, June 27). In Wikipedia. [https://en.wikipedia.org/wiki/Portulaca\\_oleracea](https://en.wikipedia.org/wiki/Portulaca_oleracea).
63. Proctor, C.A., Gaussoin, R.E. and Reicher, Z.J. (2011). Vegetative reproduction potential of common purslane (*Portulaca oleracea*). *Weed technology* **25**(4): 694-697.
64. Razaullah, S.S.K., Nawaz, M.S., Shmad, V.U. and Wang, Y. (2024). Phytotoxicity, cytotoxicity and chemical composition of *Phlomidioschema parviflorum*. *Pakistan Journal of Botany* **56**(3): 1143-1149.
65. Ricardo, L.L., Bernardi, D.I., Mantovanelli, G.C., Moreno, B.P., Mito, M.S., Silva, A.A., De Oliveira Jr, R.S., Ishii-Iwamoto, E.L., Sarragiotto, M.H. and Baldoqui, D.C. (2018). Phytochemical investigation and phytotoxic activity of aerial parts of oilseed radish (*Raphanus sativus* var. *oleifer* Stokes). *Biochemical Systematics and Ecology* **78**: 52-58.

66. Saha, B., Devi, C., Khwairakpam, M. and Kalamdhad, A.S. (2018). Vermicomposting and anaerobic digestion–viable alternative options for terrestrial weed management–A review. *Biotechnology Reports* **17**: 70-76.
67. Saini, A., Aggarwal, N.K., Sharma, A., Kaur, M. and Yadav, A. (2014). Utility potential of *Parthenium hysterophorus* for its strategic management. *Advances in Agriculture* **2014(1)**: 381859.
68. Sarić-Krsmanović, M., Umiljendić, J.G., Radivojević, L., Rajković, M., Šantrić, L. and Đurović-Pejčev, R. (2020). Chemical composition of *Ambrosia trifida* essential oil and phytotoxic effects on other plants. *Chemistry and Biodiversity* **17(1)**: e1900508.
69. Sarvadamana, A.K. (2019). *Assessment of Allelopathic Potential of Sorghum and Sunflower on Little seed canary grass (Phalaris minor Retz.) and Wheat (Triticum aestivum L.)*. M.Sc. Agriculture (Agronomy) Thesis. Department of Agronomy, G.B. Pant University of Agriculture, Pantnagar, Uttarkhand, India.
70. Singh, P.K., Pawar, D., Gharde, Y., Kumar, S. and Dhagat, S. (2023). *Integrated Management of Parthenium*. Extension Bulletin: DWR/68/2023. ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh.
71. Sinha, M.K. (2017). Studies on weed diversity and its associated phytosociology under direct dry seeded rice systems in Korla District (CG) India. *Advances in Plants and Agriculture Research* **7(2)**: 246-52.
72. Sotero, V., Suarez, P., Vela, J.E., de Sotero, D.G. and Fujii, Y. (2016). Allelochemicals of three Amazon plants identified by GC-MS. *International Journal of Engineering and Applied Sciences* **3(2)**: 257731.
73. Srinithan, T. and Raman, R. (2024). Utilization of *Parthenium hysterophorus* aqueous extracts as a bio-herbicide-an alternative for synthetic herbicides. *Environment and Ecology* **42(4)**: 1606-1614.
74. Taupik, S.A.M., Aani, S.N.A., Chia, P.W. and Chuah, T.S. (2023). Phytotoxic compounds of cassava leaf extracts for weed inhibition in aerobic rice. *South African Journal of Botany* **159**: 563-570.
75. Tefera, T. (2002). Allelopathic effects of *Parthenium hysterophorus* extracts on seed germination and seedling growth of *Eragrostis tef*. *Journal of Agronomy and Crop Science* **188(5)**: 306-310.
76. Teixeira da Silva, J.A., Nezami-Alanagh, E., Barreal, M.E., Kher, M.M., Wicaksono, A., Gulyás, A., Hidvégi, N., Magyar-Tábori, K., Mandler-Drienyovszki, N., Márton, L., Landín, M., Gallego, P.P., Driver, J.A. and Dobránszki, J. (2020). Shoot tip necrosis of *in-vitro* plant cultures: A reappraisal of possible causes and solutions. *Planta* **252**: 1-35.
77. Velasco-Azorsa, R., Cruz-Santiago, H., Cid del Prado-Vera, I., Ramirez-Mares, M.V., Gutiérrez-Ortiz, M.D. R., Santos-Sánchez, N.F., Salas-Coronado, R., Villanueva-Cañongo, C., Lira-de León, K.I. and Hernández-Carlos, B. (2021). Chemical characterization of plant extracts and evaluation of their nematocidal and phytotoxic potential. *Molecules* **26(8)**: 2216.
78. Xuan, T.D., Roni, Y., Andriana, Y., Khanh, T.D., Anh, T.T.T., Kakar, K. and Haqani, M.I. (2018). Chemical profile, antioxidant activities and allelopathic potential of liquid waste from germinated brown rice. *Allelopathy Journal* **45**: 1-12.
79. Zechmann, B. (2020). Subcellular roles of glutathione in mediating plant defense during biotic stress. *Plants* **9(9)**: 1067.
80. Zhang, J., An, M., Wu, H., Liu, D. L. and Stanton, R. (2014). Phytotoxic activity and chemical composition of aqueous volatile fractions from *Eucalyptus* species. *PLoS One* **9(3)**: e93189.