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Utilization of *Parthenium hysterophorus* Aqueous Extracts as a Bio-Herbicide - An Alternative for Synthetic Herbicides

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ABSTRACT

Invasive weed *Parthenium hysterophorus* has been wreaking havoc all over the terrestrial ecosystems in tropics and sub-tropics. The traits governing successive invasion and colonization of this weed are superficial seed production ability, inedibility to animals and allelopathy. Allelopathy refers to the impact of plants on nearby plants or their associated microflora or macrofauna by the production and release of allelochemicals. The identification and characterization of allelochemicals will be helpful for utilizing in agricultural pest management operations. In this rationale, a preliminary probe has been made to assess the potential of *Parthenium hysterophorus* aqueous extracts as a farmer friendly herbicide. Raddish seeds have been used to test the phytotoxicity

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of the extracts and complete germination inhibition was found with application of 25% extract. Seeds in control has a germination speed of 5.29 seeds germinated/day and produced robust seedlings with 4.81 cm shoot and 2.87 cm, whereas application of 20% aqueous extracts has reduced the germination speed to 0.17 seeds germinated/day with nil seedling formation. On working out phytotoxicity percentage, it is revealed that 10% extract application has produced 100% phytotoxicity. Interestingly, the phytochemical analysis of the *Parthenium hysterophorus* aqueous extracts by GC-MS has revealed the presence of 4 phytotoxins viz., 1-Tetradecene, 2-methyldodecane, Eicosane and 1-Decanol, 2-hexyl-.

Keywords *Parthenium hysterophorus*, Allelopathy, Germination inhibitor, Phytotoxins.

INTRODUCTION

Parthenium hysterophorus is an highly invasive species belonging to Asteraceae, originated from North East Mexico (Patel 2011). It is a rapidly maturing annual weed featuring a deep taproot and an upright stem that hardens over time. This annual herb has spread across the globe (more than 40 countries) with tropical and sub-tropical climate. As a single plant, it has the potential to produce 100,000 seeds over its entire life cycle and its infestation creates a seed bank of 340 million seeds per hectare on the surface soil (Sankaran 2007). Upon germination, the

plant proceeds to a rosette stage, which produces a basal rosette containing bright green leaves which continue to grow even during unfavorable conditions. Apart from weediness, this plant causes serious health hazards which includes dermatitis, and asthma (up on contact) in humans whereas, diarrhea, kidney and liver damage (upon ingestion) in cattles and sheep (Lakshmi & Srinivas 2007). The remarkable invasiveness of P. hysterophorus is attributed to their allelopathic property which makes it easily colonize various terrains by suppressing the natural vegetation. These findings opened a spectrum of phytotoxicity studies of Parthenium hysterophorus on various plant species which includes Oryza sativa, Triticum aestivum, Zea mays, Cicer sp., Raphanus sativus, Brassica campestris, Brassica oleraceae, Ageratina adenophora and Artemisia dubia, Capsicum annuum, Solanum lycopersicum, Solanum melongena and Linum usitatissimum (Masum et al. 2013, Mersie and Singh 1987, Netsere & Mendesil 2011, Motmainna et al. 2021a, Raveena et al. 2022). The methanolic extracts prepared from Parthenium hysterophorus plants has shown germination inhibitory activities on weeds (which includes, Ageratum conyzoides, Euphorbia hirta, Oryza sativa f. spontanea and Echinochloa colona) and crops (Oryza sativa, Zea mays, Abelmoschus esculentus and Amaranthus gangeticus) (Motmainna et al. 2021b). The foliar application of whole plant methanol extracts prepared from P. hysterophorus plants has shown to affect various physiological processes on the tested plant species (Motmainna et al. 2021c). The presence of a huge array of allelochemicals (which includes carbohydrates, fatty acids, proteins, aminoacids, saponins, tannins, carotenoids, flavonoids, alkaloids, glycosides, polyphenols, anthraquinone and steroids) are reported in Parthenium hysterophorus (Kumari & Deepalakshmi 2017). Previous studies on Parthenium hysterophorus revealed the presence of 18 phytocompounds viz., Caffeic acid 4-O-glucoside, 1-Caffeoylquinic acid, 3-Caffeoylquinic acid, p-Anisaldehyde, 3-p-Coumaroylquinic acid, Luteolin 7-O-(2-apiosyl-6-malonyl)-glucoside, Caffeic acid 4-O-glucoside (isomer), Isorhamnetin 4'-O-glucoside, Tetramethylscutellarein, 1,3-Dicaffeoylquinic acid, p-Coumaric acid 4-O-glucoside, Isorhamnetin 3-O-rutinoside, Scutellarein, Luteolin 7-O-glucuronide, 1,3-Dicaffeoylquinic acid, Bisdemethoxycurcumin, Dihydrocaffeic acid 3-sulfate and Rosmanol (Alfaro Jiménez *et al.* 2022). However, preparation of the organic solvent based plant extracts is not financially feasible for the usage by farmers. Hence this investigation has been carried out to test the phytotoxic properties of *Parthenium hysterophorus* in aqueous extracts on test plant *Raphanus sativus*. GC-MS analysis of the aqueous extracts revealed the presence of several phytochemicals which exhibit phytotoxicity.

MATERIALS AND METHODS

Sample collection and storage

Sample collection was performed as the protocol described in Motmainna *et al.* (2021b). Healthy whole *Parthenium hysterophorus* plants are collected from premises of Faculty of Agriculture, Annamalai University. The collected plants were washed with tap water to remove the soil and adhering impurities. The washed plants are chopped into tiny pieces of 2 cm in length and shade dried for a week with daily turning for removal of moisture. The dried plant materials are pulverized using wiley mill to fine powder and stored in air tight containers at room temperature for future usage.

Extract preparation

Aqueous extract was prepared from the powdered sample as described by Imad *et al.* (2021) with slight modifications. The powdered plant material was made into aqueous extracts of 5% to 30%, on weight/ volume basis by mixing respective weight (5, 10, 15, 20, 25 and 30 g) of powder into 100 ml of sterile distilled water. The mixture was left undisturbed for 24 hrs at room temperature. After 24 hrs, the extract was collected by filtering the semi solid mass through double layer of muslin cloth two times and once using filter paper. The extract was finally stored at 4° celsius until further usage.

Seed germination assay

Seed germination assay was performed as described by Afridi and Khan (2015) with slight modifications. *Raphanus sativus* seeds of Pusa chetki variety have been used to test the phytotoxicity of the aqueous extracts. Seeds were surface sterilized with 2% sodium hypochlorite and 10 seeds/plate were sown in petri plates lined with 4 layers of tissue paper. The whole setup was sterilized by spraying the top layer with 0.2% of fungicide (carbendazim 12% + Mancozeb 63% wp). The experiment was conducted in a Completely Randomized Design with 7 treatments (control, 5% aqueous extract, 10% aqueous extract, 15% aqueous extract, 20% aqueous extract, 25% aqueous extract, 30% aqueous extract) and 3 replications. 5 ml of respective aqueous extract was applied to every treatment and 5 ml of distilled water was applied to control. Radicle emerged seeds were considered germinated. Germination count was taken daily, shoot and root length, was measured at the end of 7 days. Speed of germination was calculated by the formula given by Gairola et al. (2011). The formula utilized by Sarvadamana (2019) was taken for finding phytotoxicity percentage. The entire experiment was conducted thrice and mean values are used for production of graphical figures.

Germination	_	Number of seeds germinated	— × 100
percent		Total number of seeds	

Speed of germination = n1/d1+n2/d2+n3/d3+...

Where, n = Number of germinated seeds, d = Number of days.

	Seedling length of control-Seedling	
Phytotoxicity	length of treated plant	
percentage	=	× 100

Phytochemical profiling protocol

To identify the phytotoxic properties GC-MS analysis was performed on the prepared aqueous extract. The extract was filtered with Whatman No. 1 filter paper and the filtrate was evaporated at 55°C until removal of water content. The resultant semisolid sludge was analyzed using GC-MS in a Thermo Scientific (Waltham MA), Trace GC Ultra & ISQ Single Quadrupole MS, TG-5 MS fused silica capillary column (30 m \times 0.25 mm \times 0.1 mm film thickness). An electron ionization system with ionization energy of 70 eV was used to detect allelochemicals. Inert gas helium was used as a carrier at a flow rate of 1ml/min. The temperature of the injector and MS transfer line was 280°C. The temperature was program 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.

RESULTS

Aqueous extract of *Parthenium hysterophorus* exhibit remarkable toxicity on the seeds of *Raphanus sativus*

Incubation of radish seeds in presence of aqueous extract of P. hysterophorus severely hampered the rate of germination. The observed inhibition of germination was directly proportional to the concentration of the extract. Though the inhibitory activity was observed at lowest concentration tested (5%), complete inhibition (100%) (Fig.1A) of germination was observed in 25% extract concentration whereas the control seeds germinated normally. Speed of germination which was observed over the experimental period revealed that the application of Parthenium hysterophorus aqueous extracts has slackened the germination process in which the control shows 5.29 seeds germinated/day against 0.17 seeds germinated/day on the usage of 20% extract concentration (Fig.1B). Perhaps, the seeds germinated in control developed into healthy seedlings with 4.81 cm shoot, 2.87 cm root and seedling size of 7.68 cm. The usage of 5% aqueous extract has highly hindered the seedling growth which is evident with development of smaller seedlings with 0.64 cm shoot, 0.24 cm root and 0.87 cm seedlings (Fig. 2 A). Although seeds germinated in 10% - 20% aqueous extracts, it fails to develop into seedlings with a defined root and shoot architecture as clearly observed in control and 5% aqueous extracts. The usage of 5% aqueous extracts on Raphanus sativus has provided 91.1% of phytotoxicity which hampers seedling growth, while 100% phytotoxicity was attained at 10% aqueous extracts (Fig.1A). These results highlight the phytotoxic effect of the aqueous extract of P. hysterophorus on germination and growth of R. sativus (Fig. 2B).

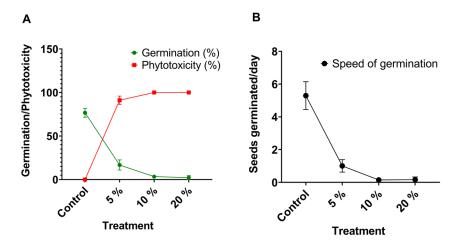


Fig. 1A. Effect of *Parthenium hysterophorus* extracts on germination % and phytotoxicity % of *Raphanus sativus*. Fig. 1B. Effect of *Parthenium hysterophorus* extracts on speed of germination.

Phytochemical profiling of the aqueous extract reveals the presence of several bioacive compounds

In order to ascertain the bioactive phytochemicals present in the aqueous extract, we performed GC-MS analysis. A total of 46 peaks have been detected in the analysis which starting from retention time 5.217 and ending at 36.971 minutes (Fig. 3). Interestingly 24 compounds have been detected from the aqueous extracts of *P. hysterophrous* (Table 1). Various phytochemicals have been detected multiple times in the analysis viz., Octadecane, 5-methyl- have been

detected 11 times with occupied area of 21.01%, Carbonic acid, decyl nonyl ester have been detected 7 times with occupied area of 18.21%, Eicosane have been detected 3 times with occupied area of 5.85%, 2-methyldodecane have been detected 2 times with occupied area of 1.33%, 1-Decanol, 2-hexyl- have been detected 2 times with occupied area of 3.66%, Eicosane, 2,4-dimethyl- have been detected 2 times with occupied area of 3.15% and Dichloroacetic acid, undecyl ester have been detected 2 times with occupied area of 5.29%. Other than this, Phenol, 2,4-bis (1,1-dimethylethyl)-, phosphite (3:1) which

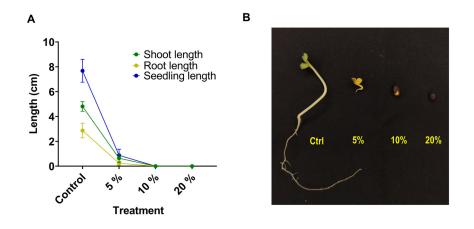


Fig. 2A. Effect of *Parthenium hysterophorus* extracts on early growth of *Raphanus sativus*. Fig. 2B. Growth inhibition caused by *Parthenium hysterophorus* aqueous extracts.

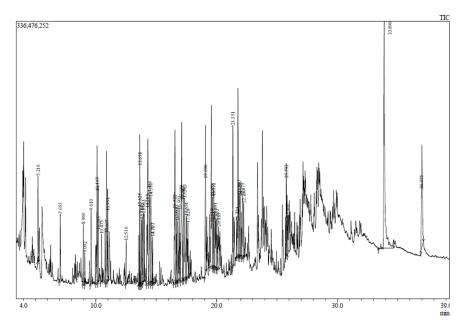


Fig. 3. Chromatogram of allelochemicals present in Parthenium hysterophorus aqueous extracts.

was detected single time, occupies 11.40% of the total area.

DISCUSSION

Seed production in annual and biennial weed plants constitutes an important part of their life cycle, in which their survivability is entirely depended (Rao 2000). Agriculture soil contains deposition of weed seeds and their reproductive propagules at various depths (Hossain & Begum 2016). Soil is also known as weed seed bank because of the huge deposition of weed seeds which will germinate and infest the crop plants and produces considerable yield loss. As herbicides are highly preferred for weed control, formation of herbicide resistance in weeds, environmental damage and presence of toxic residues in food are all problems allied with usage of herbicides for weed control (Bhullar et al. 2017). Identification of novel herbicidal molecules and eco-friendly weed management strategies are of paramount importance in maintaining the quality and quantity of agricultural production. Allelopathy refers to the impact of plants on nearby plants or their associated microflora or macrofauna by the production and release of allelochemicals, which impedes with plant growth or stimulation (Anonymous 2024). Plant derived allelochemicals can be used as herbicides in farm with minuscule damages to the agro-ecosystem (Masum *et al.* 2013).

P. hysterophorus is well known for their allelopathic capability which produces various growth impeding allelochemicals that suppresses other plant growth (Khamare et al. 2022). The phytotoxicity of aqueous extracts on the germination of R. sativus clearly indicated the presence of water soluble allelochemicals and these results are in line with the findings of Maharjan et al. (2007). This effect was previously documented by Wardle et al. (1991), in which the speed of germination of rye grass was declined by the allelopathic effect of seeds of Carduus nutans. Higher degree of phytotoxicity percentage was seen with usage of P. hysterophorus extracts might be due to the reduced shoot length and root length which are highly affected by the treatments was already documented by Bashar et al. (2023) and our results are in agreement with it.

Among 24 phytochemicals which are detected on the GC-MS analysis, 4 compounds viz.,1-Tetra-

Peak	Retention time	Area (%)	Compound name	Chemical formula
1	5.216	1.29	Disiloxane, 1, 3-diethoxy-1,1,3,3-tetramethyl-	C ₈ H ₂₂ O ₃ Si ₂
2	7.055	1.11	Oxime-, methoxy-phenyl	C ₈ H ₉ NO ₂
3	8.960	0.85	Cyclohexane, 1,2,4-tris (methylene)-	C ₉ H ₁₂
4	9.092	0.46	Dodecane	$C_{12}H_{26}$
5	9.610	1.10	1-Undecene	$C_{11}^{12}H_{22}^{20}$
6	10.117	2.88	1-Hexanol, 5-methyl-2-(1-methylethyl)-	$C_{10}^{11}H_{22}^{12}O$
7	10.215	1.35	Octadecane, 5-methyl-	$C_{19}H_{40}^{22}$
8	10.475	0.63	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$
9	10.907	2.82	Octadecane, 5-methyl-	$C_{19}H_{40}$
10	10.991	1.50	Octadecane, 5-methyl-	$C_{19}^{19}H_{40}^{40}$
11	12.516	0.74	Dodecane, 2,6,11-trimethyl-	C15H32
12	13.625	1.32	.beta Alanine, N-(3-methylbenzoyl)-, ethyl ester	C ₁₃ H ₁₇ NO ₃
13	13.658	2.78	1-Tetradecene	$C_{14}H_{28}$
14	13.772	1.26	Octadecane, 5-methyl-	$C_{19}^{14}H_{40}^{28}$
15	13.857	1.13	Octadecane, 5-methyl-	$C_{19}^{19}H_{40}^{40}$
16	13.963	1.33	Octadecane, 5-methyl-	$C_{19}^{19}H_{40}^{40}$
17	14.288	4.21	Octadecane, 5-methyl-	$C_{19}^{19}H_{40}^{40}$
18	14.451	2.39	Octadecane, 5-methyl-	$C_{19}^{19}H_{40}^{40}$
19	14.570	2.24	Octadecane, 5-methyl-	$C_{19}^{19}H_{40}^{40}$
20	14.707	0.63	2-methyldodecane	$C_{13}^{19}H_{28}^{40}$
21	14.707	0.70	2-methyldodecane	$C_{13}^{13}H_{28}^{28}$
22	16.522	3.73	Carbonic acid, decyl nonyl ester	$C_{20}^{13}H_{40}^{28}O_{3}$
23	16.670	1.17	Octadecane, 5-methyl-	$C_{19}^{20}H_{40}^{40}$
24	16.772	1.54	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}^{19}H_{22}^{40}O$
25	16.901	1.67	1-Decanol, 2-hexyl-	$C_{16}^{14}H_{34}^{22}O$
26	17.079	5.07	Carbonic acid, decyl nonyl ester	$C_{20}^{16}H_{40}^{34}O_{3}$
27	17.249	1.77	Carbonic acid, decyl nonyl ester	$C_{20}^{20}H_{40}^{40}O_{3}^{3}$
28	17.363	2.41	Carbonic acid, decyl nonyl ester	$C_{20}^{20}H_{40}^{40}O_{3}^{3}$
29	17.504	1.61	Octadecane, 5-methyl-	$C_{19}^{20}H_{40}^{40}$
30	17.628	1.57	Eicosane, 2, 4-dimethyl-	$C_{22}^{19}H_{46}^{40}$
31	19.096	3.17	Dichloroacetic acid, undecyl ester	$C_{13}^{22}H_{24}^{46}Cl_2O_2$
32	19.619	4.80	Heneicosane, 5-methylester	$C_{22}^{13}H_{46}^{24}$
33	19.678	1.68	Carbonic acid, decylester nonyl ester	$C_{20}^{22^{-46}}H_{40}^{-22^{-46}}O_{3}^{-22^{-46}}$
34	19.791	2.41	Carbonic acid, decyl nonyl ester	$C_{20}^{20}H_{40}^{40}O_{3}^{3}$
35	19.891	1.58	Eicosane, 2,4-dimethyl-	$C_{22}H_{46}$
36	20.033	1.05	Eicosane	$C_{20}^{22}H_{42}^{46}$
37	20.183	1.55	Eicosane	$C_{20}H_{42}$ $C_{20}H_{42}$
38	21.351	2.12	Dichloroacetic acid, undecyl ester	$C_{13}H_{24}Cl_2O_2$
39	21.724	3.25	Eicosane	$C_{13} + C_{20} + C$
40	21.867	1.69	Octadecane, 1-isocyanato-	$C_{19}^{20}H_{42}^{42}$ $C_{19}H_{37}^{42}$ NO
41	21.966	1.30	Carbonic acid, decyl dodecyl ester	$C_{19}^{19}H_{37}^{11}C_{10}$ $C_{20}^{19}H_{40}O_{3}$
42	22.171	1.99	1-Decanol, 2-hexyl-	$C_{16} H_{34} O$
43	22.250	1.14	Carbonic acid, decyl nonyl ester	$C_{10}^{16}H_{34}^{10}O_{3}$
44	25.792	1.64	2,2-Dimethyl-piperazine, N, N-diacetyl-	$C_{10}H_{18}N_2O_2$
45	25.900	1.54	Carbonic acid, decyl tetradecyl ester	$C_{10}H_{18}C_{20}C_{25}H_{50}O_{3}$
46	33.890	11.40	Phenol, 2,4-bis (1,1-dimethylethyl)-, phosphite (3:1	

Table 1. GC-MS analysis of the aqueous extracts of Parthenium hysterophorus aqueous extracts.

decene, 2-methyldodecane, Eicosane and 1-Decanol, 2-hexyl- have shown to produce phytotoxic activity based on the earlier reports (Table 2). Faria *et al.* (2016), reported the root growth inhibition properties of *Ruta graveolens* essential oils on *Solanum tuberosum* hairy roots cultures and *Solanum tuberosum* hairy roots with *Meloidogyne chitwoodi* co-cultures. When GC-MS analysis was performed after addition of *Ruta graveolens* essential oils on the *Solanum tuberosum* hairy roots cultures and *Solanum tuberosum* hairy roots with *Meloidogyne chitwoodi* co-cultures, it shows the presence of 1-Tetradecene. Yadav &

Sl. No.	Compound	Phytotoxic activity	References
1	1-Tetradecene	Root growth inhibition on <i>S. tuberosum</i>	Faria <i>et al.</i> (2016)
		Germination inhibition on <i>Phaseolus mungo</i> and <i>Triticum aestivum</i>	Yadav and Chandra (2018)
		Germination and root growth inhibition on Latuca sativa	El Ayeb-Zakhama <i>et al.</i> (2016)
2	2-Methyldodecane	Germination and radicle growth inhibition on <i>Raphanus sativus</i>	Marandino et al. (2011)
		Germination and seedling growth inhibition on	
		Seteria verticillata	Jaballah et al. (2019)
3	Eicosane	Germination inhibition in Cenchrus echinatus	Hagaggi & Abdul-Raouf (2023)
		Germination inhibition in Ergrostis teff	Galt (2018)
4	1-Decanol, 2-hexyl-	Inhibits radicle elongation on Oryza sativa	Wang et al. (2021)
		Phytotoxic activity on Lemna minor plants	Ahmad et al. (2012)

 Table 2. Phytotoxic compounds present in P. hysterophorus.

Chandra 2018 reported the germination inhibition properties of pulp and paper mill effluent-contaminated sediment on *Phaseolus mungo* and *Triticum aestivum* and furthermore the GC-MS analysis of extracts has shown the presence of 1-Tetradecene. El Ayeb-Zakhama *et al.* (2016) found that the essentials oils produced from leaf of *Citharexylum spinosum* has germination and root growth inhibition properties on *Latuca sativa*. The results of GC-MS analysis have detected the presence of 1-Tetradecene.

The essential oil produced from *Hypericum perforatum* has inhibited the germination and radicle elongation process in *Raphanus sativus* seeds. The performance of GC-MS has detected 2-Methyldodecane in its phytochemical composition (Marandino *et al.* 2011). Jaballah *et al.* (2019) discovered that the *Cicer arietinum* aerial part extracts were producing germination and seedling growth inhibition on *Setaria verticillate* weed, and on GC-MS analysis it was revealed that phytochemical 2-Methyldodecane was present in it.

The culture filtrates produced from endophytic bacteria viz., *Bacillus inaquosorum* NL1 and *Bacillus safensis* NL2 of *Nerium oleander* plant has shown to produce 100 % germination inhibition on the invasive weed *Cenchrus echinatus* (Hagaggi & Abdul-Raouf 2023). On studying the chemical composition of the phytotoxic culture filtrates of *Bacillus inaquosorum* NL1 and *Bacillus safensis* NL2 through GC-MS analysis, eicosane has been detected. Galt (2018) reported that the methanol extracts prepared from *Euphorbia* gummifera has shown to inhibit germination on *Eragrostis teff* under water stressed conditions. Furthermore, GC-MS analysis of the extract has shown the presence of eicosane in it.

Wang *et al.* (2021) reported that pathogen *Rhizoctonia oryzae-sativae* has inhibited radicle elongation in rice seeds by production of toxins. The GC-MS analysis of crude extracts of *Rhizoctonia oryzae-sativae* shows the presence of 1-Decanol, 2-hexyl-compound. The essential oil produced from aerial parts of *Acacia modesta* has shown to produce phytotoxic activity on aquatic weed *Lemna minor* and the GC-MS analysis of essential oil has detected the presence of 1-Decanol, 2-hexyl- in it (Ahmad *et al.* 2012).

Furthermore, it is also suspected that the remnant compounds detected in the GC-MS analysis may have the potential to exhibit phytotoxicity based on their concentration, test species and given environmental conditions. Pre-emergence herbicides are chemicals which are applied in the field prior to crop or weed emergence, in which they are taken up by weed seeds before germination and by roots, hypocotyls, cotyledons (dicots), coleoptiles (grasses) or leaves before emergence (Krähmer *et al.* 2021). Marwat *et al.* (2008) reported that the application of *Parthenium hysterophorus* leaf aqueous extracts in maize field, significantly reduced the weed density by inhibiting weed seed germination. Our results also agree with the findings of Marwat *et al.* (2008), that the *Parthenium hysterophorus* aqueous extracts exhibit greater inhibition on seed germination, speed of germination and its growth, it would be of greater usage in development of a pre-emergence herbicide for weed management of agricultural crops which will assure weed free environment for the crops during early crop growth stages.

CONCLUSION

This study clearly demonstrated the phytotoxic effect of aqueous extract of *P. hysterophorus* in germination and growth of *Raphanus sativus* seedlings at early stages. GC-MS anlaysis revealed the presence of multiple allelochemicals which has phytotoxic properties. These allelochemical faction which when further studied, will pave way for development of novel herbicide from the noxious weed *P. hysterophorus*.

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