https://doi.org/10.26651/allelo.j/2020-50-2-1281 0971-4693/94 Euro 20.00
Allelopathy Journal 50 (2): 153-172 (July, 2020) *International Allelopathy Foundation* 2020
Table: 6, Figs : 8, Photographs : 3

Allelopathic effects of *Parthenium hysterophorus* L. on the growth and yield of *Vigna radiata* L.

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(Received in Revised Form: April 14, 2020)

ABSTRACT

In Laboratory and Field bioassays, we investigated the effects of aqueous extracts from leaves, roots and flowers of parthenium (*Parthenium hysterophorus* L.) on the growth, yield and nodulation of mung bean (*Vigna radiata* L.). The extracts were tested in laboratory bio-assays at 1,3,6 and 9 % concentrations on seed germination and seedling growth of mung bean. Thereafter, they were tested in the field at 3, 6 and 9 % concentrations on germination, growth, yield, nitrogen and phosphorous contents and nodulation of mung bean. The extracts of parthenium were harmful to the physiological and agronomical parameters, and nitrogen and phosphorus content. The leaf extracts caused up to 95 % reduction in different parameters (nodules biomass and nitrogen content) followed by the flower (75 % reduction) and root extract (48 % reduction). The GC-MS analyses identified 33, 35 and 25 compounds in the leaf, flower and root methanolic extracts, respectively. The identified compounds were mainly terpenes, fatty acids, hydrocarbons, phenolics and phytosterols.

Key Words: Allelopathy, aqueous extract, bioassays, flowers, GCMS, growth, leaves, mung bean, nodulation, parthenium, *Parthenium hysterophorus*, seed germination, seedling growth, *Vigna radiata*, yield.

INTRODUCTION

The parthenium (*Parthenium hysterophorus* L.) is common weed on roadsides, wastelands and crop fields. It interferes with the growth of crops, not only due to its fast growth and high seed production but also due to release of allelochemicals in environment (38,43). It releases allelochemicals in the environment by volatilization and leaching from aerial parts, root exudation and decomposition of plant residues (3,21,22,23,31). Parthenium has strong negative impact on the productivity of mung bean (*Vigna radiata*) (29,41). Mung bean is short lifecycle crop (60-90 days) cultivated in India in about 6.0 million ha (16). It has been cultivated by farmers for the last 3500 years (15). It can grow in adverse conditions due to its capability to biologically fix nitrogen. Mung bean contains 20-25 % proteins and 50-60 % carbohydrates. It is often cultivated in rotation with other crops such as wheat, as it increases the available soil nitrogen. In this study, we investigated the effects of aqueous extracts from different parts of parthenium in laboratory and field assays on the growth and yield of mung bean.

MATERIALS AND METHODS

The field experiment was done in Department of Botany, CCS University Meerut (28º 44` and 29º 18` north latitude and 77º 08` and 78º 47` longitude,228 m above sea

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level). Mean summer temperature : 32-42ºC, mean winter temperature : 10-25 ºC, and annual rainfall : 740 mm.

Plant materials

The seeds of mung bean (*V. radiata* variety Pusa-9531) were obtained from Indian Agricultural Research Institute, New Delhi. Parthenium (*Parthenium hysterophorus*) plants (90-95 cm tall) were collected from different sites of our University, Meerut.

Preparation of aqueous extracts of parthenium

The parthenium plants were partitioned into leaves, roots and flowers. Then, they were carefully washed with tap water to remove the dust particles, cut into small pieces (2-3 cms), air-dried for 24 h and finally oven dried at 50 ºC for 48 h.The dried plant parts were separately powdered in a mixer grinder. Each powder (10 g) was soaked into 100 ml distilled water and vigorously stirred for 48 h at room temperature. Then, the extracts were filtered through double-layered muslin cloth followed by Whatman No. 1 filter paper and bacterial filter. The filtrates had concentration of 10 % (w/v) and were diluted with distilled water to obtain concentrations of 1, 3, 6 and 9 % for further studies. Fresh plant material was used for the field experiments and all other processes remained same. In field experiment three concentrations 3, 6 and 9 % of extracts were used.

Laboratory Bioassay

The bioassay was done in Petri dishes (100 x17 mm) lined with filter paper as per (28). The seeds of mung bean (*Vigna radiata* L.) were surface sterilized with a 0.01 % (w/v) $HgCl_2$ solution for 3 min and washed extensively with distilled water. Fifteen healthy seeds of mung bean were placed equidistant on top of filter paper in each Petri dish. The leaf, root and flower extracts of parthenium at 3, 6 and 9 % concentrations were applied at 5 ml/petri dish as per treatment. The experimental treatments were replicated five times. The experiment was done under aseptic conditions in laminar air flow at 25 ^{o}C. Seed germination was recorded every 8 h after sowing. The radical and plumule lengths and the fresh and dry weight of the seedlings were recorded 7 days after sowing.

Field experiment

The field experiments were conducted during March-May, 2018 in microplots (1 x 1 m) in completely randomized design with three replications. One Litre of leaf, root and flower aqueous extracts of parthenium at 3, 6 and 9 % concentrations were applied per plot (1 x 1 m) at mung bean sowing. Double distilled water was used as control. Field soil was sandy loam with pH 7.30-7.37. Fifty healthy seeds of mung bean were sown at 3-4 cm depth in rows spaced 15 cm apart.

Germination (%) (17), germination index (40), mean germination time, 50 % germination time, mean germination rate (36), coefficient of velocity of germination (2) and coefficient of variation of germination time were calculated 20 days after crop sowing as under:

(i). **Germination (%)** = No. of seeds germinated / total no. of seeds x 100

Germination index (GI) = n1 / d1+……+ Nl / Dl

Where, n1: Seeds germinated on the first count, d1: Days to first count, Nl: Seeds germinated on the last count and Dl: Days to last count.

(ii). **Mean germination time(MGT)** =**Σ**Dn/**Σ**n

Where, n : Number of seeds, germinated on day D and D : Number of days counted from the beginning of germination.

(iii). **50 % of germination time** (T50) = ti+ [(n/2) - ni] (tj - ti) /nj-ni

Where, n : Final number of seeds germinated, ni and nj are the cumulative number of seeds germinated by adjacent counts at times ti and tj, when ni <n/2 <nj.

(iv). **Main germination rate** (MGR)= CV/100 = 1/T

Where, T: MGT, CV : Coefficient of velocity.

(v). **Coefficient of velocity of germination** (CVG) = N1+N2+...+Ni/100 x N1T1+...+NiTi

Where, N : Germinated seeds /day, T: Number of days after sowing.

(vi). **Coefficient of variation of germination time** (CVt)={*St*}/*t*X100

Where, *St*: Standard deviation of the germination time, $\bar{t}$: Mean germination time

At 30 days, morphological (plant height, biomass) and nodulation (nodule number, volume and biomass) parameters were examined. Plants were harvested at 75 days after sowing and yield parameters [pods/plant, seeds/pod, pod and seed weight] were recorded. Total nitrogen and phosphorus content were estimated in seeds as per Snell and Snell (44) and Olsen (32) respectively.

Nitrogen and Phosphorus contents

(i). Nitrogen: The plant material was digested as per Snell and Snell (44). Fifty mg dried plant samples were digested with 5 ml concentrated H_2SO_4 and 2 ml of 30 % H_2O_2. The boiling tubes were kept on a sand bath for 30 min followed by the cooling at room temperature. Three ml of 30 % H_2O_2 was added again to the boiling tubes and kept for further digestion for 60 min. The process was repeated until the digests become transparent. When the samples become clear and transparent the total volume of samples was made 10 ml by adding double-distilled water (DDW). Each sample was divided into three replications by adding 1.0 ml of digests, 3.0 ml of Nessler reagent and 1 ml of DDW. The absorbance of digest was taken at 425 nm.

(ii). Phosphorus: Its content was determined using the method of Olsen (32). 1 g dried plant material was added in the 100 ml conical flask, with one pinch of activated charcoal and 20 ml of 0.5 M $NaHCO_3$. The flask was shaken for 30 min on an electric shaker followed by the filtration through Whatman No.1 filter paper. Five ml of this filtrate was mixed with 5 ml molybdate reagent and 10 ml DDW, followed by stirring the samples. 1 ml of $SnCl_2$ solution was added to the samples and made volume of 25 ml with DDW. After 10 min incubation, the absorbance was recorded at 660 nm.

Final calculations of N and P content were done separately as under:

$$\text{N/P Content (\%)} = \frac{\text{OD} \times \text{Factor} \times \text{Dilution (if any)} \times 1000}{100 \times \text{Total Volume}}$$

GC-MS analysis: Methanolic extracts were prepared from the different plant parts of parthenium following Netsere and Mendesil (29). Twenty g plant material was sequentially extracted with 250 ml methanol in Soxhlet apparatus. The extraction continued till the solvent in the siphon tube of the extractor become colourless. After that, the extract was taken in a beaker and kept on a water bath at 68.5 °C till all the solvent was evaporated and the final concentration will be 2.5 g/ml.
Gas chromatography -Mass spectrometry was performed with a GCMS-QP2010 Plus (Shimadzu, Japan) equipped with an auto-sampler AOC-20iPlus. Each sample was injected in a volume of 2.0 μL. The temperature program started at 50 °C for 3 min and then increased up to 280 °C at a rate of 15°C/min and finally 20 min at 280 °C. Other working conditions were: injection temperature at 260 °C, ion source temperature at 220 °C, split ratio of 1:10. Helium was used as the carrier gas with a flow rate of 1.2 mL/min. Compounds were identified matching their mass spectra with those in the National Institute of Standards and Technology Mass Spectra Library and Wiley library.

Statistical Analysis

The data of germination, plant height, plant biomass, yield attributes and nitrogen and phosphorus content were subjected to ANOVA and the differences among means were evaluated by the tests of Tukey and Duncan (P =0.05). The statistical analyses were done with the SPSS 20.0 software.

RESULTS AND DISCUSSION

LAB BIOASSAY

Seed germination

Various germination parameters [Germination % and germination index] are directly related to seeds germination. The effects of aqueous extracts from different plant parts of parthenium were concentration-dependent. The flower and leaf extracts at 1 and 3 % concentrations and the root extract up to 6 % concentrations enhanced seed germination parameters (Table 4) and germination index (Table 5). As the concentration of different extracts increased, the seeds germination decreased. *Parthenium* low concentrations might have stimulated seed germination due to the presence of many compounds such as parthenin and other sesquiterpenes (8) which might have affected the seed coat of mung bean and thus increased its germination. However, the aqueous extracts of higher concentrations inhibited the seed germination. Various allelochemicals such as terpenes, phytosterols and phenolic acids were detected in the GC-MS analysis (Tables 1,2 and 3) and also reported in literature (6,7,13,19,20). Many of these allelochemicals decreased the seeds germination.

Seedling growth

The aqueous extracts from different parts of parthenium significantly affected the seedlings growth of mung bean. The effects of various extracts of parthenium were concentration-dependent. The flower and leaf extracts at 1 and 3 % concentrations and the root extract up to 6 % concentration stimulated the seedling growth, biomass and seed vigour of mung bean (Photographs 1, 2 and 3). However, these extracts were inhibitory to

Table 1. Chemical compounds detected in the methanolic leaf extract of *Parthenium hysterophorus*

S.N.	Retention Time	Area %	Compound name	Compound nature	Allelopathic activity
1	8.002	0.34	N-O-Dimethylhydroxylamine	Hydroxylamine	+
2	9.945	0.26	1,5-anhydro-6-deoxyhexo-2,3-diulose	Glycoside	-
3	11.444	0.32	1,2,Pentanediol	Pentylene Glycol	+
4	15.792	0.06	Aristol-1(10),8,diene	Sesquiterpenes	+
5	15.883	0.08	Patchoulane	Sesquiterpenes	+
6	15.918	0.13	Tetradecane	Alkane hydrocarbon	+
7	16.406	0.13	Beta-Caryophyllene	Sesquiterpenes	+
8	16.658	0.09	Octyl acetate	Fatty alcohol esters	-
9	16.776	0.23	2-Nepthalenemethanol, 1,2,3,4,4a,8a-hexahydro-.alpha.,. alpha.,4a,8-tetramethyl-,[2R-(2.alp,4a,8a)	Sesquiterpene alcohol	+
10	16.844	0.19	Unknown	Unknown	-
11	18.544	0.65	Neophytadiene	Sesquiterpene	+
12	18.800	0.13	+)-.Beta.-citronellene	Monoterpenoids	-
13	18.991	0.23	1- Octadecyne	Alkene	-
14	19.455	0.38	Hexadecanoic acid	Fatty acid methyl esters	+
15	19.941	7.00	Palmitic Acid	Fatty acid	+
16	20.683	0.40	Unknown	Unknown	-
17	21.085	0.27	Methyl linolelaidate	Fatty acid	+
18	21.143	0.64	Alfa-Methyl linolenate	Fatty acid	+
19	21.257	3.39	Phytol	Diterpene	+
20	21.662	18.79	Linolenic acid	Fatty acid	+
21	21.803	0.38	Stearic acid	Fatty acid	+
22	21.896	0.26	Nonyl salicylaldehyde	Phenol	-
23	22.009	0.64	Abscisic acid	Sesquiterpenes	+
24	22.165	0.41	Farnesol	Sesquiterpene alcohol	+
25	22.416	0.53	(+)- Dolichodial	Iridoids	+
26	22.589	2.20	Unknown	Unknown	-
27	22.785	0.14	Bis(2-(Dimethylamino)ethyl) ether	Ether	-
28	24.560	3.00	Unknown	Unknown	-
29	24.854	0.54	2,11,Dodecanedione	Alkyl-Phenylketones	-
30	25.547	51.06	Unknown	Unknown	-
31	25.667	0.15	Unknown	Unknown	-
32	26.397	3.57	Parthenin	Sesquiterpenes	+
33	27.231	0.18	3-methyl-cis-1,2-epoxycyclohexane	Saturated hydrocarbons	-
34	29.546	0.54	Squalene	Triterpenes	+
35	30.555	0.16	2-Heptanal-(E)	Fatty aldehyde	+
36	33.595	0.80	DL-α-Tocopherol	Acetate esters	-
37	35.792	1.23	Stigmaterol	Triterpenes	+
38	37.074	0.26	Unknown	Unknown	-
39	38.268	0.25	Epoxycaryophyllene oxide	Sesquiterpenes	+

The major compounds were : Linolenic acid, Palmitic acid and Phytol.
(+: allelopathic activity present, -: allelopathic activity not known yet)

Table 2. Chemical compounds detected in the methanolic flower extract of *Parthenium hysterophorus*

S.N	Retention Time	Area %	Compound name	Compound nature	Allelopathic activity
1	9.962	0.12	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4one	Flavanoid	+
2	13.811	0.09	β-Elemene	Sesquiterpenes	+
3	14.599	0.19	β-Caryophyllene	Sesquiterpenes	+
4	14.865	0.15	Ylagenol	Sesquiterpenes	+
5	15.662	0.87	Caryophyllene oxide	Sesquiterpenes	+
6	15.885	1.66	Globulol	Sesquiterpenes	+
7	16.127	0.34	7-methyl-3 Octyne	Alkynes	+
8	16.214	0.14	Aromadendrene oxide 2	Sesquiterpenes	+
9	16.725	1.56	4-Methyl-1,4-heptadiene	Unsaturated hydrocarbons	+
10	17.775	0.14	Unknown	Unknown	-
11	18.541	0.20	1-Ethynylcyclopentanol	Alcohol	-
12	18.987	0.17	Dispiro[2.0.2.2]octan-7-ol	Alcohol	-
13	19.050	0.17	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	Sesquiterpenes	+
14	19.455	0.71	Eicosanoic acid, methyl ester	Methyl esters	-
15	19.914	6.62	Palmiticacid	Fatty acid	+
16	21.083	0.65	Methyl linolelaidate	Fatty acid	+
17	21.141	0.67	Linoleoyl Chloride	Fatty acid	-
18	21.251	1.63	Phytol	Diterpene alcohol	+
19	21.375	0.18	Hexadecanoic acid, methyl ester	Fatty acid methyl esters	+
20	21.525	1.24	Linoelaidic acid	Fatty acid	+
21	21.583	1.56	Unknown	Unknown	-
22	21.776	0.96	Octadecanoic acid	Fatty acid	+
23	21.999	0.86	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione	Methyl ester	+
24	22.157	0.50	Farnesol	Sesquiterpene alcohol	+
25	22.403	0.29	Patchoulane	Sesquiterpenes	+
26	22.578	0.13	Sec-Butylcyclohexane	Cycloalkanes	-
27	22.777	0.33	Carbonic acid, 2-dimethy laminoethyl neopentyl ester	Fatty acid ester	-
28	23.322	0.33	7-Octen-2-ol	Secondary alcohol	-
29	24.034	0.50	Unknown	Unknown	-
30	24.383	0.09	Unknown	Unknown	-
31	24.562	2.30	Unknown	Unknown	-
32	25.464	57.90	Unknown	Unknown	-
33	25.648	0.19	3 Methylcyclohexane oxide	Hydrocarbon	+
34	26.365	6.65	Parthenin	Sesquiterpenes	+
35	27.222	0.32	Unknown	Unknown	-
36	27.896	0.74	1-Octadecanol	Fatty alcohol	+
37	29.383	0.82	3-(1-Adamantyl)-2-butanone	Ketone	-
38	29.547	2.71	Squalene	Triterpenes	+
39	30.556	0.31	1-Tetradecanol	Fatty alcohol	+
40	33.592	0.38	α-Tocopherol	Acetate esters	-
41	35.792	3.27	Stigmasterol	Triterpenes	+
42	37.068	0.65	β-Sitosterol	Triterpenes	+

The major compounds were : Parthenin, Palmitic acid, Stigmasterol and Sonalime.
(+: allelopathic activity present, -: allelopathic activity not known)

Table 3. Chemical compounds detected in the methanolic root extract of *Parthenium hysterophorus*.

S.N.	Retention Time	Area %	Compound name	Compound nature	Allelopathic activity
1	14.904	12.04	Guanosine	Purine	-
2	15.145	0.66	Unknown	Unknown	-
3	15.967	0.28	Unknown	Unknown	-
4	16.195	6.62	Unknown	Unknown	-
5	16.377	0.58	Diethyl phthalate	Phthalate esters	+
6	16.560	0.67	(-)-globulol	Sesquiterpenes	+
7	16.925	11.01	15-Copaenol	sesquiterpenes	+
8	17.312	1.49	Unknown	Unknown	-
9	17.376	0.41	Isolongifolene	tricyclic sesquiterpene	-
10	17.511	0.34	Unknown	Unknown	-
11	17.790	0.76	Khusinol	Cyclic sesquiterpene alcohols	+
12	17.939	1.05	Unknown	Unknown	-
13	18.070	0.25	Unknown	Unknown	-
14	18.168	3.04	Oplopanone	Sesquiterpenoids	-
15	18.318	2.80	Aromadendrane-4,10-diol	sesquiterpenoid	+
16	18.421	1.17	Isospathulenol	Sesquiterpenes	+
17	18.585	1.61	Patchoulane	Sesquiterpenes	+
18	18.774	1.17	Drimenol	sesquiterpene alcohol,	+
19	18.996	1.04	Unknown	Unknown	-
20	19.139	1.04	β-Atlantol	Monocyclic sesquiterpene alcohol	-
21	19.275	0.36	4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7	fatty alcohol	+
22	19.526	1.16	Unknown	Unknown	-
23	19.596	2.79	β-Ionone	Monoterpenes	+
24	19.672	1.15	Ylagenol	Sesquiterpenes	+
25	19.821	0.72	Unknown	Unknown	-
26	19.888	6.96	Isoshyobunone	Sesquiterpenes	+
27	20.096	2.71	Unknown	Unknown	-
28	20.224	1.57	Unknown	Unknown	-
29	20.336	2.38	Dibutyl phthalate	Phthalates	+
30	20.560	0.57	Unknown	Unknown	-
31	20.669	0.95	1h-3a,7-methanoazulen-5-ol,octahydro-3,8,8-trimethyl-6-m	Sesquiterpenes	-
32	20.898	3.60	Unknown	Unknown	-
33	21.005	0.68	Ethylene brassylate	Phenylpropanoids	-
34	23.067	0.61	Isoaromadendrene epoxide	Sesquiterpenes	-
35	26.201	0.63	Unknown	Unknown	-
36	30.163	2.19	Neoabi ethyl alcohol, acetate	Alcohol	-
37	31.441	0.49	Zierone	Sesquiterpene ketone	+
38	36.834	1.39	Campestrol	Triterpenoids	+
39	37.427	14.27	Stigmasterol	Phytosterol	+
40	38.910	2.42	Clionasterol	Phytosterol	+

The major compounds were : Stigmasterol, Guanosine, Copalnol and Isohydrobionone.

(+: allelopathic activity present, -: allelopathic activity not known)

Photograph 1. Effects of *Parthenium* root aqueous extracts on seedling growth of *Vigna radiata* L. C: Control, R1: Root extract1 %, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %.

Photograph 2. Effects of *Parthenium* flowers aqueous extract on seedling growth of *Vigna radiata* L. C: Control, F1: Flower extract 1 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %

Photograph 3. Effects of *Parthenium* flowers aqueous extract on seedling growth of *Vigna radiata* L. C: Control, L1: Leaf extract 1 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

the seedling growth and biomass at higher concentrations (Table 5). The stimulatory effects of aqueous extracts on the seedling growth may be due to low contents of allelochemicals, which might have stimulated the seeds germination and seedlings growth of mung bean. On the other hand, their high amount inhibited the seedlings metabolism and growth. The parthenium releases phenolic acids and sesquiterpenes from its roots, flowers and leaves and from decaying plant residue in the soil (46,47), which may be stimulatory in low amount. This phenomenon is called 'hormesis' in which a stressor (i.e., allelochemicals or herbicides) enhances the stress response capability of other plants. However, it is dose-dependent and age-dependent phenomenon, certain dose can enhance the plant growth and others can inhibit, also they work independently, if one parameter is influenced by the lower amount of allelochemicals another can be inhibited at the same time. The sesquiterpenes present in the parthenium elicits the growth response in neighbouring plants (8,12). Similar results have been reported on *Glycine max*, *Phaseolus vulgaris* and *Allium cepa* (9,29).

Table 4. Effects of aqueous extracts of roots, shoots and leaves of *Parthenium hysterophorus* on the seed germination (%) of mung bean (*Vigna radiata* L.)

Extract conc. (%)	Seed germination (%)							
	8h	16h	24h	32h	40h	48h	56h	64h
Control	0.00	66.00	93.33	93.33	93.33	93.33	93.33	93.33
Root extract								
1	0.00	73.00	91.13	95.55	95.55	97.06	100.00	100.00
3	0.00	66.00	91.13	97.80	97.80	97.00	97.80	97.80
6	0.00	66.00	88.88	95.55	95.55	95.55	97.80	97.80
9	0.00	73.00	73.33	88.88	88.88	87.06	87.06	91.13
Flower extract								
1	0.00	66.00	80.00	88.88	88.88	89.20	93.33	93.33
3	0.00	66.00	84.46	84.46	84.46	88.88	92.00	97.80
6	0.00	66.00	77.80	80.00	80.00	84.06	91.06	91.06
9	0.00	59.00	77.80	86.60	84.46	84.46	80.00	84.46
Leaf extract								
1	0.00	59.00	93.33	93.33	92.00	92.00	93.33	93.33
3	0.00	52.00	75.53	82.20	82.20	82.20	85.20	88.88
6	0.00	66.00	82.20	75.53	85.20	82.20	85.20	86.66
9	0.00	46.00	68.86	68.86	79.60	79.60	82.20	82.20

Table 5. Effects of aqueous extracts of roots, shoots and leaves of *Parthenium hysterophorus* on the Germination index, radical length, plumule length, total seedling length, and seedling dry weight of mung bean (*Vigna radiata* L.)

Extract conc. (%)	Germination index	Radical length(mm)	Plumule length (mm)	Total length (mm)	Seed Vigour Index	Dry wt (g)
Control	4.01	3.74	7.32	15.07	1032.22	0.04
Root extract						
1	4.23	3.78	8.18	16.19	1178.41	0.03
3	4.08	4.92	7.80	16.80	1214.76	0.03
6	4.06	4.84	7.68	16.58	1195.66	0.03
9	3.65	4.08	6.74	14.47	941.98	0.02
Flower extract						
1	3.77	4.92	8.14	16.35	1117.85	0.02
3	3.77	3.76	7.66	15.67	1057.43	0.03
6	3.51	3.90	7.22	14.63	897.71	0.03
9	3.44	2.98	5.96	12.38	731.92	0.03
Leaf extract						
1	4.08	4.38	7.02	15.48	1089.04	0.03
3	3.67	4.22	6.74	14.63	913.29	0.04
6	3.52	3.36	6.74	13.62	867.89	0.03
9	3.37	2.24	5.82	11.43	645.12	0.02

FIELD STUDY

Germination

Germination determines and evaluate the effects of any treatment applied to seeds during the earlier stages of seed germination. Various parameters (Germination percentage, germination index, coefficient of variation of germination time (CV_t), coefficient of velocity to germination (CVG) and mean germination rate, 50 % germination time (T50) and Mean germination time (MGT) were recorded during the field experiments. Maximum germination %, MGR, CV_t, GI, CVG were determined in all treatments and compared with control (Table 6). The 3 %aqueous extract of root, enhanced the seeds germination. While the seeds treated with 9 %aqueous extract of leaf showed minimum germination, followed by 9 % flower extract. Maximum MGT and T50 values were observed in 9 % aqueous extract of leaves, while their minimum values were found in control followed by 3 % root extract. MGT and T50 are inversely proportional to the germination rate, as the values of these parameters increased, germination rate gradually decreased. The 3 % root aqueous extract slightly increased the seedlings length (Table 6), which can be due to the increase in root length, which was stimulated by the release of low amount of sesquiterpenes lactones. Rashid *et al.* (37) also reported similar results at low dose treatment of *Parthenium*. The decreased seeds germination due to increased extract concentrations showed the negative impact of *P. hysterophorus* weed on mung bean germination. It might be due to the various chemicals present in the *P. hysterophorus* extract, which might have interrupted the metabolic activities in embryo (35,45). Our results corroborate the findings of earlier researchers (6,7,13,19,20,38).

Table 6. Effects of aqueous extracts of root, shoot and leaves of *P. hysterophorus* on germination parameters of mung bean (*Vigna radiata*).

Extract conc. (%)	G (%)	MGT (Days)	MGR (%)	CV_t (%)	GI (Days)	CVG (%)	T_{50} (Days)
Control	434.444	11.100	0.090	39.332	14.785	9.010	9.525
Root extract							
3	412.222	11.398	0.088	37.278	13.403	8.774	9.879
6	370.000	11.892	0.084	34.380	11.229	8.409	10.552
9	352.222	12.000	0.083	34.169	10.597	8.333	10.778
Flower extract							
3	430.400	11.214	0.089	39.085	14.667	8.918	9.749
6	384.444	11.364	0.088	38.485	12.743	8.800	9.949
9	342.222	11.533	0.087	38.088	11.222	8.671	10.300
Leaf extract							
3	368.889	11.735	0.085	35.078	11.396	8.522	10.299
6	318.889	11.903	0.084	35.491	9.833	8.401	10.758
9	290.000	12.415	0.081	31.065	8.208	8.055	11.231

G (%) : Germination percentage, MGT: Mean germination time, MGR: Mean germination rate, CV_t: Extract conc. coefficient of variation of germination time GI: Germination index, CVG: Coefficient of velocity of germination time,T_{50}: Time to 50 % germination.

Plant growth

Aqueous extracts of *Parthenium* significantly reduced the plant height and biomass of mung bean. A slight increase was observed in plant height with 3 % aqueous extract of roots followed by control, while the minimum plant height (Figure 1) and biomass (Figures 2 and 3) were observed in aqueous leaf extract at 9 % concentration. These results showed the direct impacts of different concentrations of *P. hysterophorus* plant parts extracts on mung bean in concentration dependent manner (Figure1). These results correlate with the earlier findings (4), they reported that allelochemicals can adversely affect the growth of mung bean by impairing the activity of protease and peroxidase, respiration and protein content. Sesquiterpenes (Tables 1,2 and 3) inhibit the growth of other plants by adversely affecting their physiological activities such as dehydrogenase activity, inhibition in roots, membranes destruction and reduction in the chlorophyll content. Similar results were reported earlier (37) at lower dose of *Parthenium* treatment.

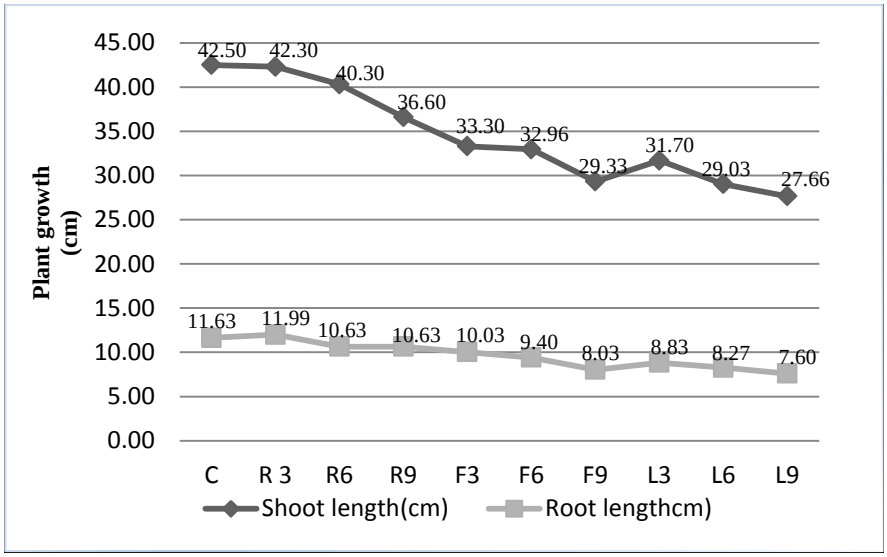

Figure 1. Effects of aqueous extracts from different parts of *P. hysterophorus* on plant length of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

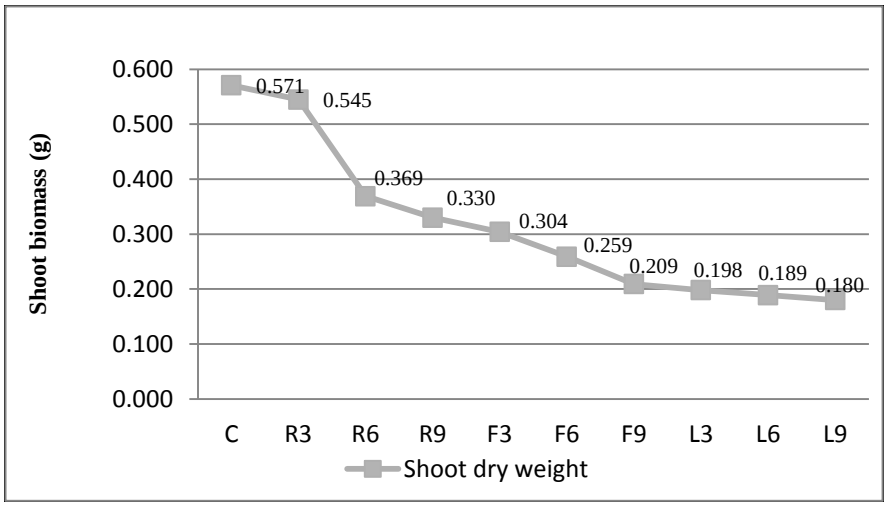

Figure 2. Effects of aqueous extracts from different parts of *P. hysterophorus* on shoot biomass of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

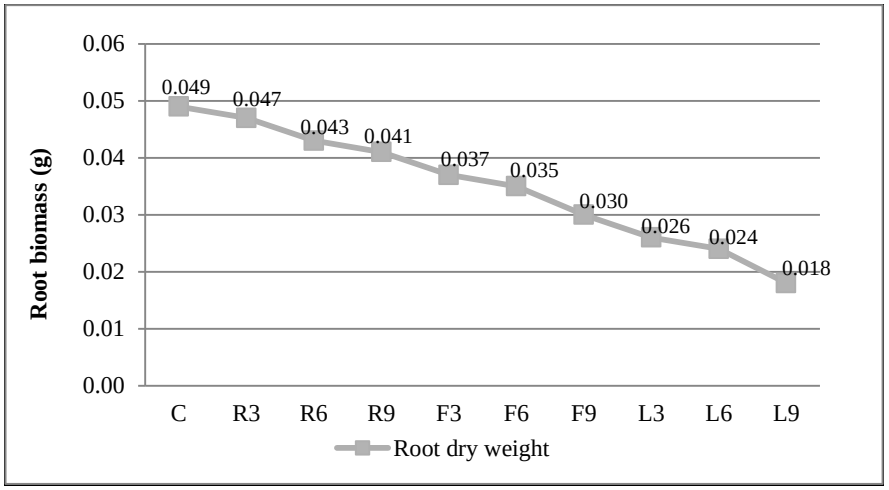

Figure 3. Effects of aqueous extracts from different parts of *P. hysterophorus* on root biomass of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

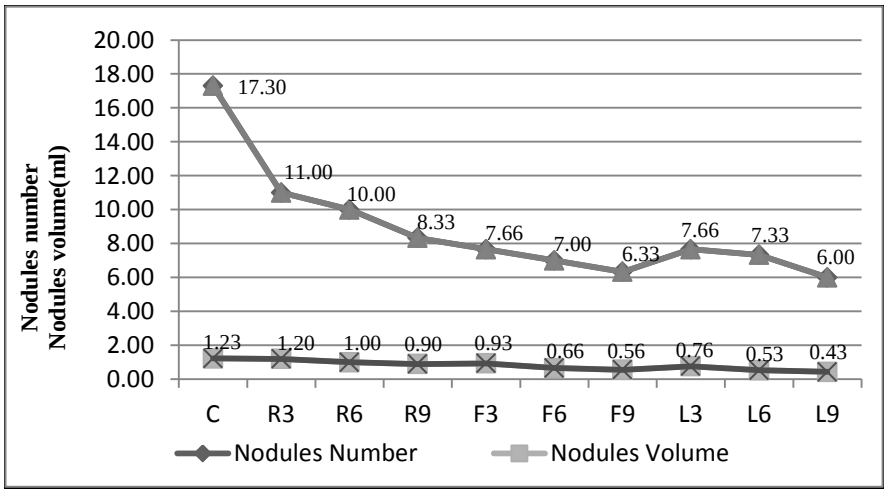

Figure 4. Effects of aqueous extracts from different parts of *P. hysterophorus* on Nodules numbers and volume of mung bean (*Vigna radiata*).

C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

Nodulation

Nodulation parameters [nodule number, nodule volume and nodule weight] were recorded during the field study. There was slight variation in the number of nodules

(Figure 4) in different treatments but their volume (Figure 4) and weight (Figure 5) varied significantly. Maximum numbers of nodules were found in untreated plants and minimum numbers were observed for the leaf and flower aqueous extract at 9 % concentration. These allelochemicals can impair the legume- *Rhizobium* symbiosis and can decrease the nitrogenase activity; however, fewer data are available on these studies. Kohli and Batish (20) reported that high amounts of sesquiterpenes and phenolics were present in the rhizosphere of *P. hysterophorus* infested areas. The variable inhibitory influence of different plant parts and concentrations on root growth might be due to the differences in the amount of secondary metabolites found in different plant parts. These results agree with earlier findings (41), they reported the effects of *Parthenium* extract on growth and yield of soybean plants.

Grain yield

Reduction in yield parameters [pods per plant, seeds per pod, weight of pods and seeds], increased with increase in the concentration of aqueous extract. Various yield parameters were maximum in control plants and minimum in plants treated with 9 % leaf aqueous extract (Figures 6 and 7). Allelochemicals in the donor plant can alter the availability of macro and micronutrients in soil for plants (18). Parthenin, abscisic acid, β-caryophyllene, β-Sitosterol, squalane, etc. (Tables 1, 2 and 3) were allelopathic to plant growth and these allelochemicals may be responsible for the yield loss in mung bean. The effects of various concentration of leaf, root and flower aqueous extracts are dose-dependent, as the maximum reduction in yield parameters were observed with the highest concentration of extracts. Maximum yield reduction was observed in leaf extract treated plants. Shehzad (41) also reported similar results in soybean.

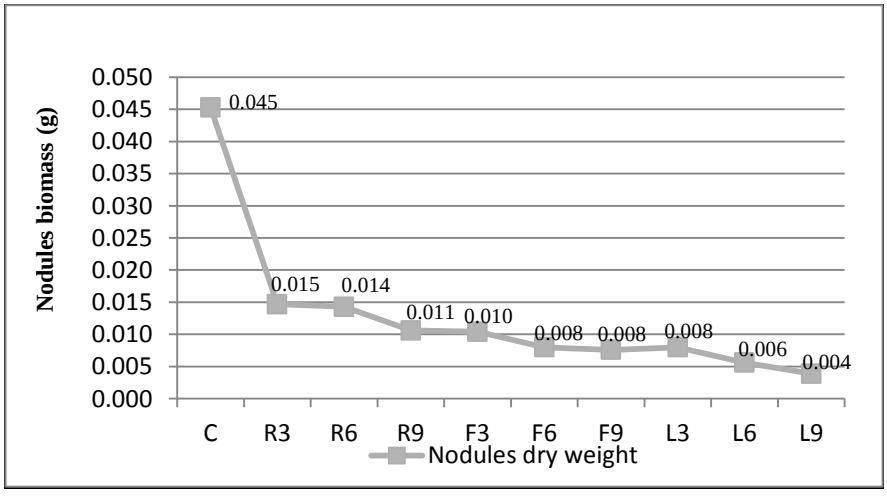

Figure 5**.** Effects of aqueous extracts from different parts of *P. hysterophorus* on nodules biomass of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

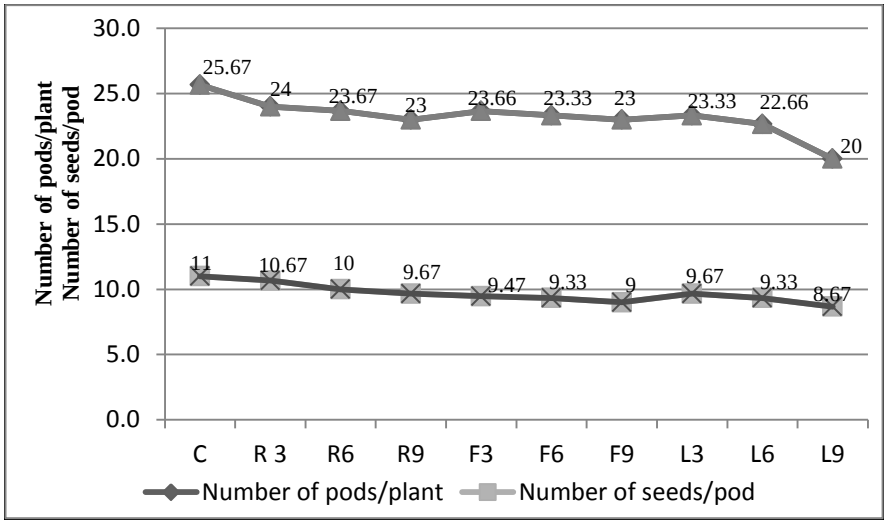

Figure 6**.** Effects of aqueous extracts from different parts of *P. hysterophorus* on yield attributes of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

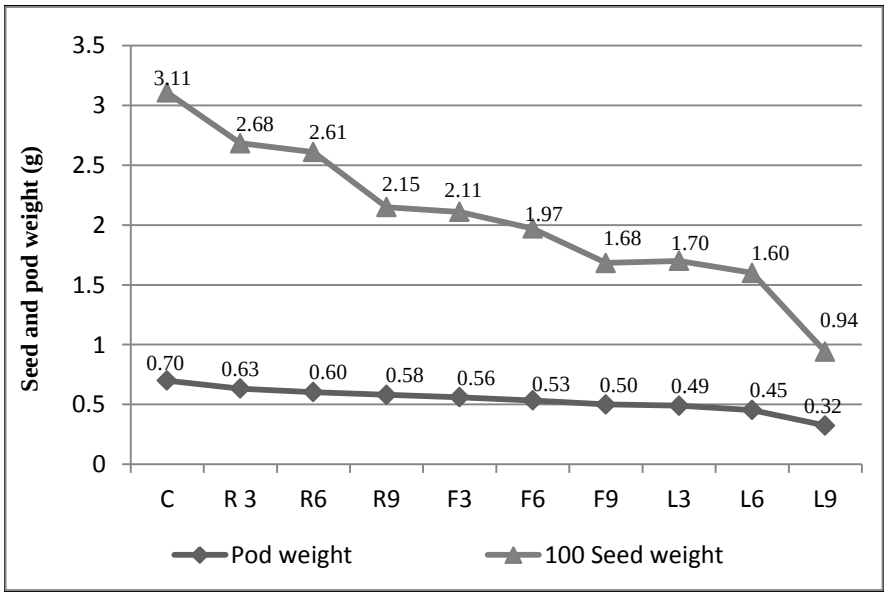

Figure 7**.** Impact of aqueous solution extracted from different parts of *P. hysterophorus* on pods and seed weight of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

Nitrogen and Phosphorus content

Minimum N and P contents were observed in plants treated with 9 % leaf aqueous extract (Figures 8 and 9). Maximum nitrogen content was found in untreated plants followed by 3 % aqueous extract of root (Figure 8). Reduction in the nitrogen content may be due to the inhibition of biological nitrogen fixation efficiency of legumes by allelochemicals. The allelochemicals adversely affected the legume-*Rhizobium* symbiosis and decreased the nitrogenase enzymes activity and nodulation parameters. *P. hysterophorus* extract suppresses the beneficial microflora (including the nitrogen fixing bacteria and phosphate solubilising bacteria), thereby, less soluble Phosphorus and Nitrogen are available for the plant uptake. Sesquiterpenes associated with the parthenium weed reduces the nutrients contents of neighboring plants (18), due to the reduction in nutrients content of *P. hysterophorus* infested soil (16,41). The allelochemicals present in various plants parts influences the availability of macro and micronutrients to plants (4,14,18). The different inhibitory influences of various plant parts extracts and their concentrations on plant growth and yield might be due to the variability in the amount of allelochemicals present in various plant parts.

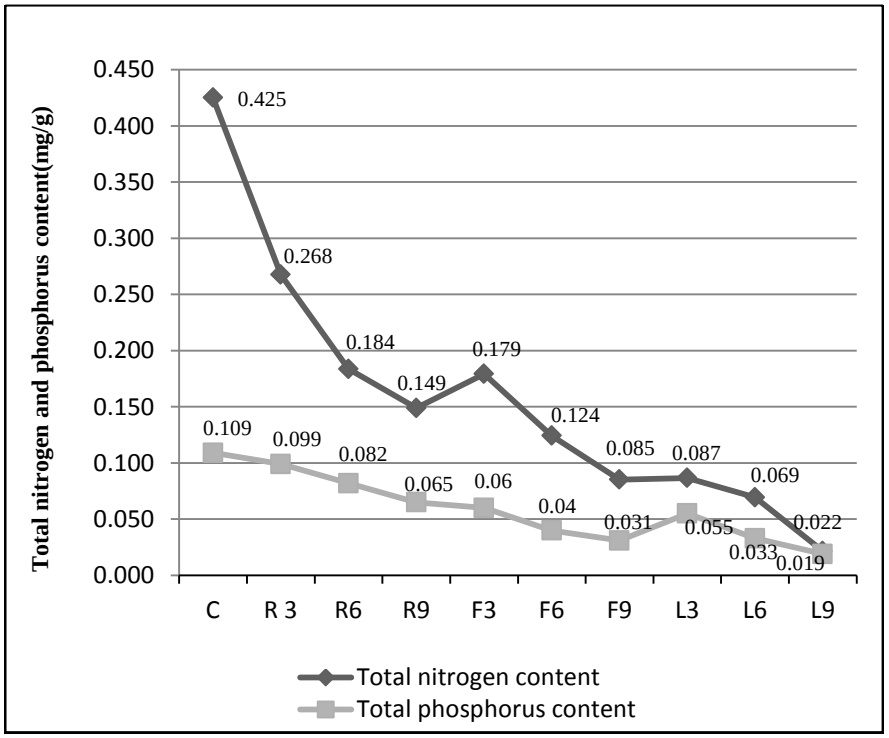

Figure 8. Impact of aqueous solution extracted from different parts of *P. hysterophorus* on nitrogen and phosphorous content of mung bean (*Vigna radiata*).
C: Control, R3: Root extract 3 %, R6: Root extract 6 %, R9: Root extract 9 %, F3: Flower extract 3 %, F6: Flower extract 6 %, F9: Flower extract 9 %, L3: Leaf extract 3 %, L6: Leaf extract 6 %, L9: Leaf extract 9 %.

GC-MS analyses of methanolic extracts

The GC-MS analyses identified 33, 35 and 25 compounds in the Parthenium leaf, flower and root methanolic extracts, respectively (Tables 1,2 and 3). The identified compounds were mainly terpenes, however, fatty acids, hydrocarbons, phenolics and phytosterols were also detected. More than 45 % of detected volatile compounds in leaf and flower extracts had inhibitory allelopathic activities followed by the root extract (40 %). Several of these compounds [N-O-Dimethylhydroxylamine neophytadiene, linolenic acid, hexadecanoic acid, patchoulane, stigmasterol, oplopanone, drimenol, isoshyobunone, parthenin, abscisic acid, β- caryophyllene, β-Sitosterol, squalane, etc.)] have allelopathic activity (1,5,10,11,18,19,24,26,27,33). However, some unknown compounds were also present in the GC-MS data, these could not be identified and indicated as unknown in the tables (1,2,3).

CONCLUSIONS

The results of this study showed that the extracts from all parts of parthenium inhibited the growth and agronomic parameters of mung bean under field conditions. The extracts at low concentrations in laboratory experiments showed hormetic effects which were not evident in field assays. The highest allelopathic inhibitory activity was observed in leaf extract. To understand the impact of parthenium weed on yield of mung bean, further research is needed to know, if under natural field conditions the plant parts of parthenium are able to release high quantity of phytotoxic compound and for How long time they persist in soil?

ACKNOWLEDGMENTS

I would express my deep gratitude to Dr. Ashok Kumar, my research supervisor, for his enlightening guidance. I am highly obliged to CSIR for funding and AIRF, JNU for providing GC-MS facility.

REFERENCES

1. Adams, R.P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Allured Publishing. Carol Stream IL.
2. Al-Mudaris, M. (1998). Notes on various parameters recording the speed of seed germination. *Der Tropenland wirt* **99**: 147-54.
3. Anaya, A.L., Calera, M.R., Mata, R. and Miranda, P.R. (1990). Allelopathic potential of compounds isolated from *Ipomoea tricolor*Cav. (Convolvulaceae). *Journal of Chemical Ecology* **16**: 2145-2152.
4. Appel, H.M. (1993). Phenolics in ecological interactions: The importance of oxidation. *Journal of Chemical Ecology***19**: 1521-1552.
5. Baratelli, T.D.G., Gomes, A.C.C., Wessjohann, L.A., Kuster, R.M., and Simas, N.K. (2012). Phytochemical and allelopathic studies of *Terminalia catappa* L. (Combretaceae). *Biochemical Systematics and Ecology* **41**: 119-125.
6. Batish, D.R., Singh, H.P., Kohli, R.K., Saxena, D.B. and Kaur S. (2002). Allelopathic effects of parthenin against two weedy species, *Avena fatua* and *Bidens pilosa*. *Environmental and Experimental Botany* **47**: 149-155.
7. Batish, D.R., Singh, H.P., Pandher, J.K. and Kohli, R.K. (2005). Phytotoxic effects of *Parthenium hysterophorus* residues on three *Brassica* sp. *Weed Biology Management* **5**: 105-109.
8. Belz, R.G. and Cedergreen, N. (2010). Parthenin hormesis in plants depends on growth conditions. *Environmental and Experimental Botany* **6:** 293-301.

9. Demissie, A.G., Ashenafi, A., Arega, A., Etenash, U., Kebede, A. and Tigist, A. (2013). Effect of *Parthenium hysterophorus* on germination and elongation of onion (*Allium cepa*) and bean (*Phaseolus vulgaris*). *Research Journal of Chemical and Environmental Sciences* **1**(2): 17-21.
10. Desai, N. and Gaikwad, D.K. (2015). Allelopathic effects of leaf litter leachates of mangrove *Excoe caria* agal alcohol on rice seedlings. *Allelopathy Journal* **36**: 293-302
11. Duke, S.O., Oliva, A. (2004). Mode of action of Phytotoxic terpenoids: *In : Allelopathy, Chemistry and Mode of Action of Allelochemicals*. *CRC Press*, Boca Raton, pp: 201-216.
12. Duke, S.O., Cedergreen, N., Velini, E.D. and Belz, R.G. (2006). Hormesis: Is it an important factor in herbicide use and allelopathy? *Outlooks Pest Management* **17**: 29-33.
13. Einhellig, F.A. (1996). Interactions involving allelopathy in cropping systems. *Agronomy Journal* **88**: 886-893.
14. Facelli, J.M. and Pickett, S.T.A. (1991). Plant litter: Its dynamics and effects on plant community structure. *Botanical Reviews* **57**: 1-32.
15. Ganesan, K. and Xu, B.A. (2017). Critical review on phytochemical profile and health promoting effects of mung bean (*Vigna radiata*). *Food Science and Human Wellness* **7**: 11-13.
16. Hou, D., Yousuf, L., Xue, Y., Hu, J., Wu, J., Hu, X., Feng, N. and Shen, Q. (2019). Mung bean (*Vigna radiata* L.): Bioactive polyphenols, polysaccharides, peptides and health benefits. *Nutrients* **11**(6): 1-28.
17. ISTA. (1993). *Hand Book for Seedling Evaluation*. International Seed Testing Association, Zurich, Switzerland.
18. Kanchan, S.D. and Jayaehandra (1980). Allelopathic effects of *Parthenium hysterophorus* L. and Identification of inhibitors. *Plant and Soil* **55**: 67-75.
19. Kil, J.H. and Shim, K.C. (2006). Allelopathic effects of *Tagetes minuta* L. and *Eupatorium rugosa* Houtt. aqueous extracts on seedling growth of some plants. *Allelopathy Journal* **18**: 315-322.
20. Kohli, R.K. and Batish, R. (1994). Exhibition of Allelopathy by *Parthenium hysterophorus* in Agroecosystem. *Journal of Tropical Ecology* **35**: 295-307.
21. Kumar, A. (2014). *Parthenium hysterophorus* L. and its impact on living world. *Indian Journal of Scientific Resources* **4** (1): 08-14.
22. Lalita, and Kumar, A. (2018). Review on a weed *Parthenium hysterophorus* (L.). *International Journal of Current Research and Review* **10** (17): 23-32.
23. Lalitha, P., Sripathi, S.K. and Jayanthi, P. (2012). Secondary metabolites of *Eichhornia crassipes* (Water hyacinth): a review (1949 to 2011). *Natural Product Communication* **7**(9): 1249-56.
24. Lin W. X., Kim K. U., and Shin D. H. (2000). Rice allelopathic potential and its modes of action on *Barnyard grass* (*Echinochloa crus-galli*). *Allelopathy Journal* **7**: 215-224.
25. Makoi, J.H. and Ndakidemi, P.A. (2007). Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. *African Journal of Biotechnology* **6**: 1358-1368.
26. Malarkodi, E. and Manoharan, A. (2015). Qualitative and GC-MS analysis of phytochemical constituents of Congress grass (*Parthenium hysterophorus* L.) an allelopathic plant. *International Journal of Current Trends in Research* **4**(1): 98-108.
27. Mediterranean, S., Kirici, M. Inan, M. and Giray,E.S.(2011).To study the essential oil and agricultural properties of vetiver (*Vetiveria zizanioides*) in the southeastern of florin. *Advances in Environmental Biology* **5**(2): 447-451.
28. Narwal, S.S., Sampietro, D.A. and Catalán, C.A.N. (2009). Laboratory bioassays in allelopathy. In: *Plant Bioassays*. Studium Press, Houston, Texas, USA: pp. 1-20.
29. Netsere, A. and Mendesil E. (2011). Allelopathic effects of *Parthenium hysterophorus* L. aqueous extracts on soybean (*Glycine max* L.) and haricot bean (*Phaseolus vulgaris* L.) seed germination shoot and root growth and dry matter production. *Journal of Applied Botany and Food Quality* **84**: 219-222.
30. Nganthoi, D.Y., Dutta, B.K., Sagolshemcha, R. and Singh, N.I. (2014). Allelopathic effect of *Parthenium hysterophorus* L. on growth and productivity of *Zea mays* L. and its phytochemical screening. *International Journal of Current Microbiology and Applied Sciences* **3**(7): 837-846.
31. Nikki, L.P. and Scott, J.M. (2010). Relative allelopathic potential of invasive plant species in young disturbed woodland. *The Journal of the Torrey Botanical Society* **137**(1): 81-87.
32. Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. USDA, Washington.
33. Prasad, D.R. (2018). Pharmacology and clinical research chemical composition, antioxidant and antifungal activity of essential oils of *Pogostemon amaranthoides* from Raya-Bajeta Valley, Pithoragarh, Uttarakhand Himalayas, India. *Journal of Pharmacological and Clinical Research* **6**: 555-691.

34. Purchase, H.F. and Nutman, P.S. (1957). Studies on the physiology of nodule formation: Influence of bacterial number in the rhizosphere of nodule inhibition. *Annals of Botany* (London) **21**: 439.
35. Rajendaran, K. (2005). Mito-depressive effects of aqueous extracts of *Parthenium hysterophorus* L. on *Vigna radiata* (L) Wilczek. *Geobios* **32**(4): 237-240.
36. Ranal, M.A., Santana, D.G., Ferreira, W.R. and Mendes-Rodrigues, C. (2009). Calculating germination measurements and organizing spreadsheets. *Brazilian Journal of Botany* **32**: 849-855.
37. Rashid, H., Khan, M.A., Amin, A., Nawab, K., Hussain, N. and Bhowmik, P.K. (2008). Effects of *Parthenium hysterophorus* L., root extracts on seed germination and growth of maize and barley. *The American Journal of Plant Science and Biotechnology* **2**: 51-55.
38. Reinhardt, C., Vanderlaan, M., Belz, R.G., Hurle, K. and Foxcroft, L. (2006). Production of the allelochemicals parthenin in the leaves of *Parthenium hysterophorus* L. *Journal of Plant Diseases and Protection* **20**: 427-433.
39. Rice E.L. (1984). *Allelopathy*. (2nd ed). Academic Press, Inc, Florida (USA). pp. 422.
40. Scott, S., Jones, R. and Williams, W., (1984). Review of data analysis methods for seed germination. *Crop Science* **24**: 1192-1199.
41. Shehzad, M. Hussain, S., Siddiqui, M.H. and Awan, S.I. (2016). Allelopathic effects of *Parthenium hysterophrous* L. mulch on growth and yield of soybean (*Glycine max*). *Planta Daninha* **34**: 631-638.
42. Shikha, R. and Jha, A.K. (2016). Allelopathic effect of leaf extract of *Parthenium hysterophorus* L. on seed germination and growth of *Cicer aeritinum* L. *International Journal of Scientific Research* **5**(3): 652-655.
43. Siddiqui, M.H., Khalid, S., Shehzad, M., Shah, Z.A., and Ahmad, A. (2018). *Parthenium hysterophorus* herbage mulching: A potential source of weeds control in soybean (*Glycine max*). *Planta Daninha* **36**: 5-25.
44. Snell, F.D. and Snell, C.T. (1967). *Colorimetric Method of Analysis Including Photometric Methods*. Van Nostrand, Inc; Princerton, New Jersey **4**: 217.
45. Sorecha, E.M. and Bayissa, B. (2017). Allelopathic effect of *Parthenium hysterophorus* L. on germination and growth of peanut and soybean in Ethiopia. *Advances in Crop Science and Technology* **5**(3): 1-4.
46. Swaminathan, C., Rai, R.S.V. and Sureshi, K.K. (1990). Allelopathic effects of *Parthenium hysterophoru* L. on germination and seedling growth of few multipurpose trees and arable crops. *The International Tree Crops Journal* **6**: 143-150.
47. Valliappan, K. and Towers, G.H.N. (1988). Allelopathic effect of root exudates from obnoxious weed *Parthenium hysterophorus*. *Indian Journal of Weed Science* **20**(1): 18-22.
48. Verma, P. and Majee, M. (2013). Seed germination and viability test in tetrazolium (TZ) assay. *Bio-Protocol* **3**(17): 1-3.