

Study Of *Parthenium hysterophorus* L. Extracts (First clean-up Fractions) On Seed Germination Behaviour In Search Of Bioactive Fractions for Preparation of Bioherbicide Formulations.

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ABSTRACT: Increase in productivity is directly related with increase pesticide consumption. So there is a strong desire to use “greener” chemistry to produce more toxicologically and environmentally benign pesticides and natural products. In this present study, a comparison was made to evaluate the phytotoxicity potential of first cleanup fractions obtained from sequentially extracted solvent (ethyl-acetate, methanol) extracts of *Parthenium hysterophorus* L. (aerial parts) in vitro through bench-top seed germination assay (*Vigna radiata* L.). One-way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) were done for statistical analysis of the data. The study reveals that germination, growth and vigour was significantly ($P < 0.05$) reduced by ethyl-acetate and methanol fractions. A significant changes in soluble and insoluble sugar content, protein, amino acid content and cellular metabolic activity was also observed by 1F3, 1F4, 1F6, and 1F9 fractions. The present study concluded that phytotoxicity of four fractions (1F3, 1F4, 1F6, and 1F9) from ethylacetate and methanolic crude extracts of *Parthenium hysterophorus* could be exploited as potential bioherbicide for future weed management programme and the development of bioherbicide for commercial use.

1. INTRODUCTION

With the advancement of civilization, mankind has two primary goal in mind – (i) achieving food security and (ii) improving the quality of life. To achieve the earlier point we always ignore our traditional resources and relied on the synthetic agrochemicals for productivity increase and pest management. Today, more than 60% of our population is involved in agriculture sector, which dominates the economic scenario of our country. The increasing pattern of productivity is directly related with increasing trend of pesticide consumption

Due to favourable growth and multiplication criteria offered by a tropical country like India pests (insects, fungal pathogens, nematodes, weeds, rodents, birds, mollusc etc.) assume a serious proportions throughout the year. Year round cropping system also encourage these pest populations. So, losses due to pests are far greater than the temperate countries. Crop reduction due to pests accounts for 25-35%. This has been a reason for intensive use of pesticides.

Natural product especially plant product is used as the one of main sources of biocides as pharmaceuticals, crop protection chemicals and many more since time immemorial. Many highly effective chemicals have so far been isolated from plant sources especially as pest control agents viz., nicotine, pyrethrins, cinerins, rotenone, ryanodine etc. [1]. In recent times number of breakthrough work has been done in respect of biopesticides with particular reference to botanicals, microbial pesticides, pheromones, IGR, plant growth regulators, and new more promising crop protection molecules. A detailed review of naturally occurring bioactive crop protecting chemicals has been discussed by Vyvyan [2] and Dyan [3,4].

However, in today’s perspective, there is a strong desire to use “greener” chemistry to produce more toxicologically and environmentally benign pesticides and natural products. Natural products, especially phytochemicals have been the potential source of many pesticides, either used directly as

crude preparations or as pure compounds. They have been used more often as structural leads for the discovery and development of natural product-based pesticides.

There are several examples of plants used as fungicides, insecticides, molluscicides, algacides and other pesticides than as herbicides [2]. Among the weeds *Parthenium hysterophorus* L., is an aggressive weed native to Southern North America, Central America, the West Indies and Central South America, having allelopathic effect and drastically retards the growth of many species [5]. *Parthenium* is an invasive exotic weed commonly known as Bitter weed, false ragweed, Fever few, Ragweed, Carrot weed, White top, Chatak chandani, Congress grass & Star weed. Its botanical name is *Parthenium hysterophorus* L. and belongs to the Class: Magnoliopsida, Family: Asteraceae (Compositae). It is an annual herb and has a deep taproot and erect stem, which becomes woody with age. Height varies of *Parthenium* weed between 50-150 cm, leaves are deeply lobed. It is pale green in colour and has soft hair. Flower are creamy white in color. It has small (1-2mm long) black seeds with white scales. They are not visible to the naked eye. The countries where it has been reported are South Africa, Mozambique, Madagascar in Africa, China, India, Vietnam, Nepal in Asia, Guatemala, Honduras, Belie etc. in Central America, Mexico, in South America and in United States [cf. 6].

The invasive nature of the noxious weed *P. hysterophorus* L., is thought to be due to an ability to displace other species by means of allelopathy. The allelochemicals released from *Parthenium* affecting many plant species are sesquiterpene lactones and phenolics. Parthenin is the major sesquiterpene lactone whereas caffeic, vanillic, ferulic, chlorogenic and anisic acids are the major phenolics [5,7]. These two synergistically acting groups of allelochemicals significantly decrease the seed germination and subsequent growth in many crops [7]. Therefore, current study was undertaken to investigate and identify the phytotoxic fractions of the ethylacetate and methanol crude extracts of *Parthenium hysterophorus* L. on germination and early seedling growth of test plant species under laboratory conditions.

2. MATERIAL AND METHODS:

Plant material for extraction: The plant sample (*Parthenium hysterophorus* L) was collected from local vegetation of Baruipur, 24-Paraganas (south) [22°21'29''N Latitude and 88°26'37''E Longitude and altitude of 42 feet], West Bengal (W.B.), India. The samples were cut into small pieces, air dried at room temperature (25±30°C), then oven dried for 2 days at 40°C and powdered by mechanical grinder to store in air tight packets which are now ready for experiment.

Seed material for bioassay: *Vigna radiata* L. Seeds were used in this experiment as target organism for bioassay. The seed materials were procured from the Department of Agronomy, BCKV, Mohanpur, Kalyani, Nadia (W.B.), India.

Extraction: 100g of powdered *Parthenium* was sequentially extracted with solvents (600ml) of increasing polarity [6]. This process were repeated several times till the experiment completed.

Isolation for cleanup: Isolation for clean-up of the crude extracts (Ethyl acetate and Methanol crude extract) was then subjected to liquid column chromatography on silica gel G (Merck 60-100 mesh) as adsorbent following solvent of increasing polarity. The elution was done with solvents wef 100% hexane to 100% methanol as eluent of increasing polarity with two gradients. Elutes, as fractions (200ml each) were collected and marked accordingly (Table-1). The fractions were then concentrated on rotary vacuum evaporator (Buchi type) and were subjected to phytochemical analysis and bioassay.

TABLE-1: Results of the column chromatography of the Ethyl acetate and Methanol extract of *Parthenium hysterophorus* by increasing polarity:

Solvents & mixture	From ethylacetate crude		From methanol crude	
	Residues/fractions on evaporation	Name of the cleanup fractions	Residues/fractions on evaporation	Name of the cleanup fractions
Hexane (100%)	Fraction 1	1F1	Fraction 6	1F6
Hexane: Ethyl acetate (1:1)	Fraction 2	1F2	Fraction 7	1F7
Ethyl acetate (100%)	Fraction 3	1F3	Fraction 8	1F8
Ethyl acetate: methanol (1:1)	Fraction 4	1F4	Fraction 9	1F9
Methanol (100%)	Fraction 5	1F5	Fraction 10	1F10

Phytochemical analysis: The qualitative tests [8] were used for detecting the presence of various phytochemicals to give general idea of chemical constituents present in various fractions as per the Table-2.

TABLE-2: Qualitative tests for phytochemical constituents

Secondary metabolites	Name of the test	Methodology	Observation
Alkaloides	Mayer's test	To 1 ml of extract, 1 ml. of Mayer's reagent was added (Potassium mercuric iodide solution).	Whitish or cream colour precipitated
	Wagner's Test	To 1 ml of extract, 2 ml of Wagner's reagent was added (Iodide in KI)	Reddish brown coloured precipitated
Anthraquinones	Borntrager's Test	2 ml of extract was added with 1ml 10% ferric chloride solution and 1ml of conc. HCl, Cooled and filtered. Shake the filtrate with equal amount of diethyl ether. Further extract the ether extract with strong NH_3 .	Pink or deep red coloration of aqueous layer
Flavonoides	NaOH test	The extract was treated with dil. NaOH, followed by addition of dilute HCl.	A yellow solution with NaOH, turns colorless with dilute HCl
	Lead Acetate Test	Lead acetate solution was added to a small quantity of ethanolic extract of test solution.	Yellow ppt.
Steroids	Liebermann-Burchardt test	To 1ml of methanolic extract, added 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of concentrated H_2SO_4 .	Dark green coloration
	Salkowski test	5 ml extract was added with 2 ml of chloroform and 3 ml of concentrated sulphuric acid H_2SO_4 .	Reddish brown color of interface
Tannins	Braemer's test	10% alcoholic ferric chloride was added to 2-3ml of methanolic extract (1:1)	Dark blue or greenish grey coloration of the solution
	Dilute HNO_3	On addition of dil. HNO_3 solution to the ethanolic extract	Reddish color
6)Terpenoids	Liebermann-Burchardt test	To 1ml of methanolic extract, add 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid.	Pink or red coloration

Bioassay: The first cleanup fractions 1F1, 1F2, 1F3, 1F4, 1F5, 1F6, 1F7, 1F8, 1F9, and 1F10 from ethyl acetate and methanol crude were used for bioassay with distilled water as control set. Physiological and biochemical tests were then performed with the pre-treated seeds.

3. ANALYSIS OF PHYSIOLOGICAL PARAMETERS

Germination behavior: Germination data were recorded every 12 h. intervals up to 120 h. of seed soaking following the International Rules for seed Testing [9]. Seven germination indices, germination percentage (GP), germination index (GI), germination energy (GE), speed of emergence (SE), mean germination time (MGT), seedling vigour index (SVI), and coefficient of the rate/velocity of germination (CRG) were obtained & then calculated from the same data by using the equations described below (Table-3).

TABLE-3: Equations used to calculate Germination indices and other physiological parameters.

<i>Germination parameters</i>	<i>Equation</i>	<i>Reference</i>
Germination percent (<i>GP</i>)	$GP = [(\text{number of germinated seeds at final count}) / \text{total number of seeds sets for bioassay}] \times 100$	Global method
Germination index (<i>GI</i>)	$GI = \sum G_T / T_t$	AOSA [10]
Mean germination time (<i>MGT</i>)	$MGT = [\sum (T_i \times N_i) / \sum N_i]$ $N_i = \text{number of newly germinated seeds at time } T_i.$	Mavi et al. [11]
Seedling vigour index (<i>SVI</i>)	$SVI = (\text{seedling length} \times \text{germination percent}) / 100$	Islam et al. [12]
Speed of emergence (<i>SE</i>)	$SE = (\text{number of germinated seeds at the starting day of germination} / \text{number of germinated seeds at the final day of measurements}) \times 100$	Modified from Islam et al. [12]
Germination energy (<i>GE</i>)	$GE = (\text{percent of germinated seeds at the day of germination} / \text{total number of seeds set for bioassay}) \times 100$	Modified from Ruan et al. [13]
Coefficient of the rate of germination (<i>CRG</i>)	$CRG = [(N_1 + N_2 + \dots + N_n) / (N_1 * T_1) + (N_2 * T_2) + \dots + (N_n * T_n)] * 100$ where, N_1 =number of germinated seeds at time T_1 , N_2 =number of germinated seeds at time T_2 , N_n =number of germinated seeds at time T_n .	Bewely and Black [14], Chiapusio et al. [15]
Phytotoxicity (<i>P</i>)	$(\text{Root length in control} - \text{Root length in treatment}) / \text{Root length in control}$	Chou and Muller, [16]
Inhibition (<i>I</i>)	$100 - (E_2 \times 100 / E_1)$, Where I =%Inhibition, E_1 =Response in control, E_2 =Response in treatment plant.	Rho & Kil, [17].
Relative root Elongation	$R = (L / L_r) \times 100$, where R is the relative root elongation, L is the mean root length in treatments, L_r is mean root length the of control.	Rho & Kil, [17].

Growth Study: Morphological parameters like root length, shoot length and seedling height were measured in fresh samples after 120 hrs. TTC (2, 3, 5-Triphenyl Tetrazolium Chloride) stain ability was done according to the method of Halder [18]. TTC stain ability, were analysed by differential staining pattern, 3 separate categories of seeds (viz., fully stained, partly stained, and not stained) were visually made.

4. ANALYSIS OF BIOCHEMICAL PARAMETERS

In the phytotoxicity experiment soluble, insoluble carbohydrate, total free amino acid, protein and cellular respiratory activity were recorded. Soluble and insoluble carbohydrate from seed Kernels was determined following method described by McCready et al, [19]. Quantification of total free amino acid in seed kernel was followed as per the method of Moore and Stein [20] modified by Bhattacharjee [21]. Protein in seed kernels was estimated following the method by Bradford [22] using BSA as standard. The respiratory activity of intact seeds was analysed by the reaction of tetrazolium chloride (TTC) according to the method of Rudrapal and Basu [23].

Statistical Analysis: One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) were done for statistical analysis of the data at 5% level using IBM SPSS v.20.

5. RESULTS

Phytochemical characterization of P. hysterophorus extracts (first cleanup fractions) obtained from ethylacetate and methanolic crude extracts.

The first cleanup fractions (1F1 to 1F10) were tested for qualitative chemical tests for various phytochemical constituents and they confirmed the presence of triterpenes, steroids, alkaloids and flavonoids (Table-4).

TABLE-4: Qualitative phytochemical analysis of chromatographed fractions

Sl. No.	Elutes designation number	First fraction elutes.	Alkaloids -Mayer's test	Alkaloids - Wagner's test	Flavonoid(NaOH/HCl test)	Flavonoid (Lead acetate test)	Triterpines	Tannins (FeCl ₃ 5%)	Tannins (Lead acetate)	Tannins (dil. HNO ₃)	Steroids (Salkowski test)	Steroids (liberman's test)	Anthraquinones (Borntrager's test)
1.	1F1	Hex elute	-	-	-	-	-	-	-	-	+	-	-
2.	1F2	Hex:EA elute	-	-	-	-	-	-	-	-	-	-	-
3.	1F3	EA elute	-	-	-	-	++	-	-	-	+	-	-
4.	1F4	EA:M elute	+	-	-	-	+	-	-	-	+	-	-
5.	1F5	M elute	-	-	+	-	-	-	-	-	-	-	-
6.	1F6	Hex elute	-	++	-	+	++	-	-	-	-	-	-
7.	1F7	Hex:EA elute	-	-	-	-	-	-	-	-	+	-	-
8.	1F8	EA elute	-	-	-	-	+	-	-	-	-	-	-
9.	1F9	EA:M elute	-	+	-	+	+	-	-	-	-	-	-
10.	1F10	M elute	-	-	-	+	-	-	+	-	-	-	-

Positive tests for terpenoides were indicated for the fractions 1F3, 1F4, 1F6, 1F8 and 1F9 whereas positive for steroids for 1F1, 1F3, 1F4, 1F7 were found. Fractions 1F4, 1F6, 1F9 yield positive for alkaloids and 1F5, 1F6, 1F9, 1F10 for flavonoids. Fraction On the basis of the above results all the fractions were selected for phytotoxicity bioassay.

Effect of P. hysterophorus extracts (first cleanup fractions) on germination and growth of V. radiata

There is a significant change in germination parameters of ethyl acetate and methanol fractions (Figure-1). In methanolic fractions 1F6, 1F7, 1F8, 1F9, 1F10 the kinetics are much lower in comparison to control.

The ethylacetate fractions (Table-5) have significant ($P < 0.05$) effect on all calculated germination indices. The GP, GI, CRG, SE, GE, SVI showed a reduced trend in 1F3, 1F4, followed by 1F2 and 1F1 except MGT.

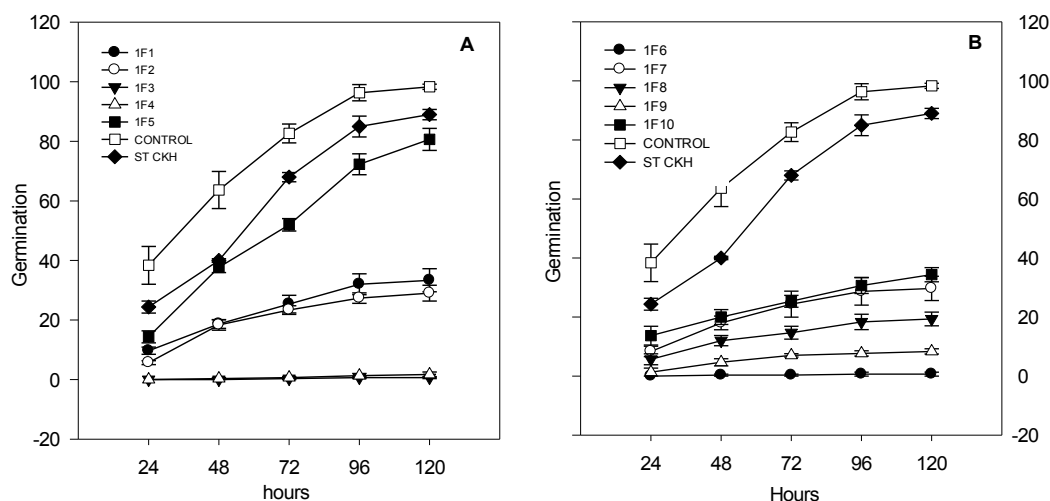


FIGURE-1: Germination kinetics of *Vigna* seeds treated with first clean-up fractions (A- ethylacetate fractions; B- methanol fractions)

The methanol fractions (Table-6) also have significant ($P < 0.05$) effect on all calculated germination indices. The GP, GI, CRG, SE, GE, SVI decreased in 1F6, 1F9, followed by 1F8, 1F7, and 1F10 except MGT, which is increased.

TABLE-5: Effect of ethylacetate crude cleanup fractions of *Parthenium* on germination:

Treatments	GP	GI	MGT	CRG	SE	GE	SVI
1F1	33.33 ^d	18.32 ^d	2.40 ^a	42.08 ^a	29.81 ^{ab}	33.33 ^d	5.44 ^c
1F2	29.00 ^d	15.00 ^d	2.41 ^a	41.58 ^a	19.90 ^{bc}	29.00 ^d	5.89 ^c
1F3	0.67 ^e	0.19 ^e	2.33 ^a	19.44 ^b	0.00 ^d	0.67 ^e	0.05 ^c
1F4	1.67 ^e	0.51 ^e	2.39 ^a	18.61 ^b	0.00 ^d	1.67 ^e	0.20 ^c
1F5	80.67 ^c	37.53 ^c	2.82 ^a	35.54 ^{ab}	17.66 ^c	80.67 ^c	9.05 ^c
CONTROL	98.33 ^a	61.15 ^a	2.14 ^a	47.32 ^a	39.02 ^a	98.33 ^a	69.30 ^a
ST CHK	89.00 ^b	46.55 ^b	2.56 ^a	39.12 ^a	27.32 ^{bc}	89.00 ^b	43.74 ^b

Data are mean of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

TABLE-6: Effect of methanol crude cleanup fractions of parthenium on germination:

Treatments	GP	GI	MGT	CRG	SE	GE	SVI
1F6	0.67 ^f	0.25 ^f	3.00 ^a	11.11 ^b	0.00 ^b	0.67 ^f	0.21 ^d
1F7	29.67 ^c	16.56 ^{cd}	2.35 ^b	42.73 ^a	27.74 ^a	29.67 ^c	2.43 ^d
1F8	19.33 ^d	10.84 ^{de}	2.36 ^b	42.45 ^a	31.79 ^a	19.33 ^d	6.96 ^d
1F9	8.33 ^e	4.08 ^{ef}	2.55 ^b	39.55 ^a	13.33 ^b	8.33 ^e	1.52 ^d
1F10	34.33 ^c	20.68 ^c	2.40 ^b	41.97 ^a	39.37 ^a	34.33 ^c	20.06 ^c
CONTROL	98.33 ^a	61.15 ^a	2.14 ^b	47.32 ^a	39.02 ^a	98.33 ^a	73.25 ^a
ST CHK	89.00 ^b	46.55 ^b	2.56 ^b	39.12 ^a	27.32 ^a	89.00 ^b	43.74 ^b

Data are mean of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

Ethylacetate fractions, 1F3, 1F4 and 1F5 shows maximum inhibitory effect on the root and shoot growth of the test species followed by 1F1 and 1F2 (Figure-2A). The maximum root, shoot and seedling growth was inhibited by 1F3 fraction; while the minimum inhibition was noted for 1F2 fraction. Methanol fractions 1F7 shows maximum inhibitory effect on root and shoot growth of germinating *Vigna* seeds (Figure-2B).

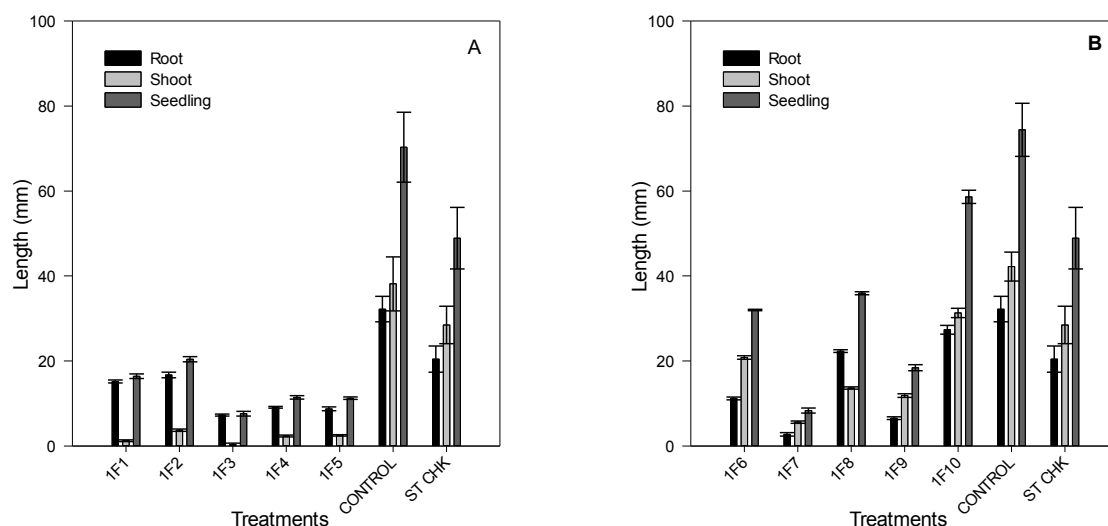


FIGURE-2: Effect of first clean-up fractions on growth of *Vigna radiata* (A-ethylacetate fractions; B- methanol fractions)

The toxicity potential of ethylacetate and methanol extracts was also supported by phytotoxicity, inhibition and relative root elongation (Figure-3A,B). The cleanup fractions 1F3, 1F4, 1F6, 1F9 and 1F7 shows significant ($P < 0.05$) toxicity on growth of test species. The strong phytotoxicity, inhibitory activity and least root elongation was noted for 1F3, 1F4, 1F6, 1F9 fraction; whereas minimal toxicity potential were observed in 1F5, 1F8, 1F10. TTC staining pattern and TTC stainability (Table-7-8) reveals a significant effect of the fractions on *Vigna* seeds germination. Fraction 1F4, 1F2 and 1F6 shows minimum TTC stainability of 81, 82 and 85% respectively. Overall TTC stainability was reduced in all treatments in comparison to control.

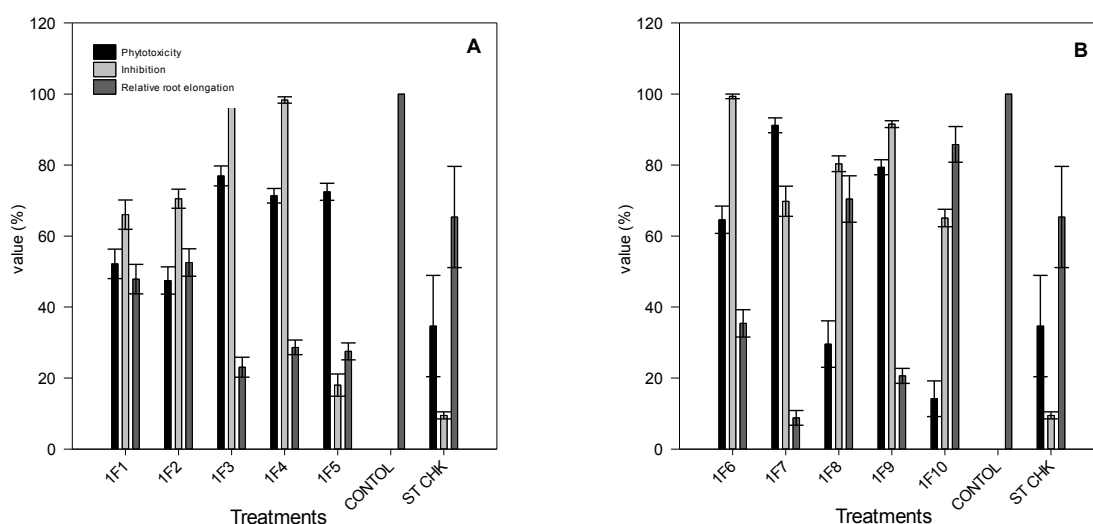


FIGURE-3: Effect of first clean-up fractions on Phytotoxicity, inhibition and relative root elongation of *Vigna* (A-ethylacetate fractions; B- methanol fractions)

TABLE-7: Effect of ethylacetate crude cleanup fractions of on seed health:

Treatments	Full stained	Partly stained	Not stained	TTC stained (%)
1F1	90.33 ^a	1.67 ^c	8.00 ^{bc}	92.00 ^{ab}
1F2	62.00 ^{bc}	20.33 ^{ab}	17.67 ^{ab}	82.33 ^{bc}
1F3	70.00 ^b	24.33 ^a	5.67 ^c	94.33 ^a
1F4	55.00 ^c	26.33 ^a	18.67 ^a	81.33 ^c
1F5	87.67 ^a	10.33 ^{bc}	2.00 ^c	98.00 ^a
CONTROL	93.33 ^a	5.00 ^c	1.67 ^c	98.33 ^a
ST CHK	62.00 ^{bc}	27.00 ^a	11.00 ^{abc}	89.00 ^{abc}

Data are mean of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

TABLE-8: Effect of methanol crude cleanup fractions of on seed health:

Treatments	Full stained	Partly stained	Not stained	TTC stained (%)
1F6	61.33 ^b	23.67 ^a	15.00 ^a	85.00 ^c
1F7	84.33 ^a	7.67 ^b	8.00 ^{abc}	92.00 ^{abc}
1F8	85.00 ^a	9.67 ^b	5.33 ^{bc}	94.67 ^{ab}
1F9	87.33 ^a	5.67 ^b	7.00 ^{bc}	93.00 ^{ab}
1F10	93.33 ^a	3.67 ^b	3.00 ^c	97.00 ^a
CONTROL	93.33 ^a	5.00 ^b	1.67 ^c	98.33 ^a
ST CHK	62.00 ^b	27.00 ^a	11.00 ^{ab}	89.00 ^{bc}

Data are mean of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

Effect of ethyl acetate and methanolic fractions on biochemical parameters of germinating Vigna seeds:

The Biochemical component (Table-9-10) shows high soluble carbohydrate in 1F3 and 1F6 treatments (11.46 mg g⁻¹ and 9.76 mg g⁻¹ fresh weight respectively) followed by 1F2, 1F9. On the contrary, insoluble carbohydrate at highest quantity was noted in 1F4, 1F3 and 1F6 treatments (260.14, 245.77 and 248 mg g⁻¹ fresh weight respectively). Total free aminoacids follows the same pattern as soluble carbohydrate. Cellular respiration measured through formazan percent were drastically reduced in 1F3, 1F6, 1F9, and 1F4 treatments.

TABLE-9: Effect of ethyl acetate fractions on biochemical parameters of mungbeans:

Treatments	Soluble Carbohydrate (mg g ⁻¹ fresh weight)	Insoluble Carbohydrate (mg g ⁻¹ fresh weight)	Total Free Amino Acids (mg g ⁻¹ fresh weight)	Cellular Respiration (%)	Total protein (mg g ⁻¹ fresh weight)
1F1	6.61 ^b	172.73 ^b	3.57 ^{ab}	79.92 ^{ab}	125.70 ^a
1F2	7.92 ^b	89.36 ^{cd}	0.79 ^d	82.93 ^{ab}	90.30 ^{bc}
1F3	11.46 ^b	245.77 ^a	4.06 ^a	36.87 ^c	74.00 ^{bcd}
1F4	7.22 ^b	260.14 ^a	1.20 ^d	48.25 ^c	98.97 ^{ab}
1F5	7.34 ^b	86.09 ^c	1.73 ^{cd}	76.19 ^b	59.88 ^d
CONTROL	7.48 ^b	31.24 ^d	0.83 ^d	100.00 ^a	48.42 ^d
ST CHK	6.92 ^b	53.46 ^{cd}	2.56 ^{bc}	99.89 ^a	66.85 ^{cd}

Data are mean of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

TABLE-10: Effect of methanol fractions on biochemical parameters of mungbeans:

Treatments	Soluble Carbohydrate (mg g ⁻¹ fresh weight)	Insoluble Carbohydrate (mg g ⁻¹ fresh weight)	Total Free Amino Acids (mg g ⁻¹ fresh weight)	Cellular Respiration (%)	Total protein (mg g ⁻¹ fresh weight)
1F6	9.76 ^a	248.80 ^a	4.91 ^a	26.93 ^c	58.61 ^{dc}
1F7	5.80 ^{bc}	148.49 ^{bc}	3.18 ^b	64.27 ^b	106.67 ^b
1F8	7.26 ^{bc}	97.83 ^{cd}	2.99 ^b	56.03 ^b	122.48 ^a
1F9	7.59 ^b	167.75 ^b	4.53 ^a	31.79 ^c	84.42 ^c
1F10	5.50 ^c	67.05 ^{de}	1.78 ^c	66.95 ^b	84.42 ^c
CONTROL	7.48 ^b	31.24 ^c	0.83 ^d	100.00 ^a	48.42 ^e
ST CHK	6.92 ^{bc}	53.46 ^{de}	2.56 ^{bc}	99.89 ^a	66.85 ^d

Data are mean of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

6. DISCUSSION

Presence of terpenoides, steroides, flavonoids, tannins and alkaloids in the ethylacetate and methanol elutes are in agreement with the findings of [24-35].

Gross allelopathic potential of *P. hysterophorus*, revealed from the seed germination bioassay. Among the ethylacetate and methanol extracts, 1F3, 1F4 elutes from ethylacetate and 1F6, 1F9 elutes from methanol fraction indicates a significant inhibitory response on seed germination as also explained from the germination indices. Overall the extract (1F3, 1F4, 1F6, 1F9) reduces germinability and slows down germination kinetics which is considered to be the important visible and reliable indices for the evaluation of allelopathic Effect. Germination percent (GP) index indicated the total germination percent of a seed lot after certain period of time (120 h.) when germination became constant. As it is measured by total germination relative to total number of seeds set for germination, GP alone cannot explain the delayed germination. In contrast, GI is a measure of both percentage and speed of germination and assigns maximum arithmetic weight to seeds that germinate during first count and less weight to those that germinate later. The higher the GI, GE, SE, SVI, and CRG values compared to control, the lower the inhibition, and vice versa, except for MGT [36]. On the other hand, mean germination time (MGT) was calculated as the weighted mean of germination time. The number of seeds germinated in the interval of time is used as weight. Here the use of weighted mean is extremely important as it considers that a different number of seeds germinate in the interval of time [c.f. 37]. Hence, MGT is proportional to reduced and poor germinability. The delay or inhibition of germination caused by phytotoxic plant extracts or substances was also reported by Anjum et al [38] and Hussain et al. [39] as also corroborated by Tefera [5], Regina et al. [40], Rashid et al, [41], Dhole and Dhole [42]. Similar trends of germination retardation were also reported by Marwat et al [43] in *Avena fatua* and *Lepidium pinnatifidum*. The data thus indicating a strong allelopathic effect.

Respiratory ability were also reduced in these treatments over other treatments. These results are in strong agreement with Batish et. al. [44]. Abraham [45] reported that interference of monoterpenes with respiratory ability can lead to germination and growth retardation. Cellular metabolic activity in terms of sugar, total protein, total free amino acids were also impaired in the four treatments (1F3, 1F4, 1F6, and 1F9). Similar type of observation were also recorded by Kiran et al. [46], Padhy et al. [47].

7. CONCLUSION

The present study concluded that phytotoxicity of four fractions (1F3, 1F4, 1F6, and 1F9) from ethylacetate and methanolic crude extracts of *Parthenium hysterophorus* could be exploited as potential bioherbicide for future weed management programme and the development of bioherbicide for commercial use.

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