ASSESSMENT OF ALLELOPATHIC STATUS OF *Parthemium hysterophorus* L. BY PHYSIOLOGICAL AND BIOCHEMICAL APPORACHES

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Abstract: An experiment was done to evaluate the allelopathic potential of an obnoxious weed *Parthemium hysterophorus* by using black gram (*Vigna radiata*) seeds as bioassay material. Fresh leaf extracts and dry leaf leachates of young, mature and old leaves were used for the experiment. Different concentrations of aqueous leaf extracts and leaf leachates of the test plant material were used for pretreatment of black gram seeds for 8 hours. Pretreated gram seeds showed reduced germination percentage and speed of seed germination with concomitant enhancement of the time required for 50% germination (T_{50}) of *Vigna* seeds. Biochemical behaviour was measured in terms of leaching of free amino acids and soluble carbohydrate, content of soluble and insoluble carbohydrate, protein, amino acids and nucleic acids in seed kernel was abnormal. The seed pretreating agents enhanced deleterious leaching of soluble carbohydrates and free amino acids from the seeds with concomitant reduction of protein and nucleic acids as well as activities of catalase, dehydrogenase, amylase and protease. The soluble carbohydrate level was higher where as insoluble carbohydrate level found low. The protein and amino acid content in seed kernel was declined and DNA and RNA content were decreased remarkably.

Seeds were also experienced with forced aging treatment and prepared 30 days accelerated aging seeds. The biochemical behaviour measured with the same parameters showed drastic abnormalities.

A conclusion is made that the *Parthemium hysterophorus* possesses allelopathic property.

Key Words: Allelopathy, *Parthemium hysterophorus*, *Vigna radiata*, seed germination behaviour, biochemical behavior, accelerated aging.

I. INTRODUCTION:

Allelopathy is a process of either positive or negative effect of a plant upon another, by the release of allelochemicals from plant parts in both natural and agricultural systems for both crop and weed species. The allelochemicals are mainly water soluble and may persist in soil or leachates out with water, affecting both neighboring plants as well as surrounding plants within the succession. The whole body of the allelopathic plant or different parts of it including flowers, stems, barks, roots, leaves, leaf litter and leaf mulch, soil and soil leachates and the allelochemicals or their derivative compounds can have the allelopathic property. Allelochemicals may involve the interaction of different classes of chemicals like phenolic compounds, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, amino acids etc. The mixture of allelochemicals sometime produces greater effect than an individual compound alone. Allelopathy is also an expression of the ecological phenomenon which is a normal constituent of the environment of the terrestrial plants (Dutta and Sinha-Roy, 1974; Vivanco *et al*, 2004).

In 1996, The International Allelopathy Society (IAS) put forward a definition of 'Allelopathy' in accordance with Rice (1984), but effects of biochemical compounds involved in plant-plant interactions and the effects of allelopathic plants are discussed in a broader perspective than strictly related to plant-plant interactions. The IAS defined the allelopathy as: "Any process involving secondary metabolites produced by plants, micro-organisms, viruses, fungi that influence the growth and development of agricultural and biochemical systems (including animals), including positive and negative effects" (Torres *et al*, 1996). Allelopathy research has been conducted for several decades, but much knowledge knowledge is yet to be achieved in this field (Thorpe *et al*, 2009). An improvement in crop cultivars is the only area that has not been exploited to any great extent as a weed management strategy (Callaway and Vivanco, 2007). The possibility of incorporating allelopathic traits into improved rice cultivars, which would reduce the need for applying herbicides to the crop, is worth exploring. Of course, thus far, no commercial cultivars carrying allelopathic properties have been developed (Duke *et al*., 2002). Application of allelopathic compounds before, along with, or after synthetic herbicide could increase the overall effect of both materials, thereby reducing application rates of synthetic herbicides.

The present study is an attempt to assess the allelopathic potential of a fast growing, exotic tree *Parthemium hysterophorus* L. (Family- Asteraceae). This species has been selected in view of the fact that many exotic displace the local biodiversity through their harmful effects including allelopathy (Bhakat, 2006; Bhattacharjee, 2003; Nayek *et al.*, 2002; Nayek 2012; 2018). Allelopathic efficacy was analysed by using black gram (*Vigna radiata*) seeds as a reliable bioassay material. In fact, allelopathic action of any plant or plant part affects germination behaviour, metabolism as well as growth and development of seeds and seedlings which in turn may discourage a species from thriving, thus influencing the whole community structure (Ghosh and

Dutta, 1989). The potential status of Vigna seeds were evaluated as a result of allelopathic treatment with donor plant under laboratory and field conditions.

II. MATERIALS AND METHODS:

2.1 Plant material:

All the experiments of the present study were carried out with the fully viable healthy seeds of black gram (*Vigna radiata*) procured from the Seeds Corporation of India, Midnapore District Office, West Bengal, India. The exotic species was taken into consideration for allelopathic study is *Parthenium hysterophorus* as seeds hardly germinate under its canopy.

Fresh and healthy leaves of three types *viz*. young, mature and old were collected from actively growing populations of *Parthemium hysterophorus* L. (Family- Asteraceae) in Burdwan, West Bengal, during the period of investigation. The leaf samples were collected from the plants throughout its life cycle and washed separately with distilled water to remove the adherent dust particles.

2.2 Extraction of Allelochemicals:

2.2.1 Leaf extracts:

To prepare leaf extracts fresh and healthy young leaves (100g) of *Eucalyptus* were thoroughly homogenized using 250 ml double distilled water. The homogenate was strained using a fine cloth and the filtrate was stirred manually for five min and subsequently centrifuged at 5000 *g* for 15 min. Then the sample was again filtered with the help of Whatman No.1 filter paper. The volume of the filtered solution was then made up to 500 ml using double distilled water and this was considered as 1:5 (W/V) proportion stock solution of the aqueous leaf extract. Thus, the concentration grade of 1:5 w/v of *Eucalyptus* of young and old leaf extract was used as the test sample for allopathic studies.

2.2.2 Leaf Leachates:

To prepare leaf leachates 100g sun-dried young leaf samples of each *Eucalyptus* were kept immersed in 250 ml double distilled water in 1000ml beaker and kept at room temperature (28±2°C) for 48 h. Then it was stirred manually for five min and filtered through Whatman No.1 filter paper to prepare aqueous leachate which was decanted in a separate beaker. The total volume of the leachate was then made up to 500 ml using double distilled water and it was taken as the 1:5 (w/v) proportion stock solution. Thus, the concentration grade of 1:5 w/v of *Eucalyptus* young and old leaf leachate was used as the test samples for allelopathic studies.

2.3 Pretreatment of seeds:

Fully viable *Vigna radiata* seeds in twenty four lots of 25 g each were surface sterilized with 0.1% $HgCl_2$ solution for 90 seconds. The seed lots were then separately presoaked in the three types of leaf leachates and leaf extracts of *Eucalyptus* for 8 h and then allowed the seeds to dry back to original seed moisture level. Thus, the seeds were considered for biochemical tests.

2.4 Accelerated ageing of seeds:

Subsequently the seed sample were taken in separate power cloth begs separately (previously marked samples numbers) and thus stored in a desiccator in which 99.5% relative humidity (RH) was maintained by keeping 500 ml 1.57% H_2S0_4. Thus experimental set up was kept in room temperature (32±2°C) and thus the seeds were allowed to experience forced aging treatment. After 30 days the seeds were taken out from the desiccator and then the seeds were allowed to come back to original seed moisture level and considered as 30 days accelerated aged seeds for experiments.

2.5 Physiological experiments:

2.5.1 Analyses of TTC stainability of both leaf extract and leaf leachate- pretreated seeds:

To analyze TTC stain ability, 100 seeds of each treatment with control were dehusked after pre-soaking with double distilled water and were allowed to imbibe 0.5% TTC (2,3,5-triphenyl tetrazolium chloride) solution (w/v) in petridishes for 24 h in dark condition. The percentage TTC-stained (red coloured) seeds were calculated from the total number of seeds of each treatment. This method was adopted essentially after Halder (1981).

2.5.2 Analysis of speed of germination:

To analyze the speed of germination, the individual seed lots in each groups of 100 seeds of each treatment were transferred separately to Petri dishes (9 cm) containing filter paper moistened with 10 ml double distilled water. Data were recorded at an interval of 24 h in laboratory up to 168 h of seed soaking in distilled water. Germination data were recorded following the rules of International Seed Testing Association (ISTA, 1976).

2.5.3 Analysis of T_{50} values:

The time required for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al.* (1984).

2.5.4 Analysis of Percentage germination:

Percentage germination was recorded by analyzing speed of germination of individual seed lots of each treatment after 7 days of seed soaking following the values of International Seed Testing Association (ISTA, 1976).

2.6 Biochemical experiments:

2.6.1 Analysis of Free amino acid:

Free amino acid levels from the seed leachates of each treatment were analyzed after immersing 10 seed sample of black gram in 10 ml distilled water for 24 h. The leachates were carefully separated from the seeds in separate test tubes. From the leachate stock, free amino acid level was quantified following the method of Moore and Stein (1948) modified by Bhattacharjee (1984).

2.6.2 Analysis of Soluble carbohydrate:

Soluble carbohydrate from seed leachate, sampling procedure was the same as done in case of leachable free amino acids, and from the same leachate stock. Soluble carbohydrate level was determined following the method of Mc Cready *et al* (1950) after simple modifications.

2.6.3 Analysis of Soluble carbohydrate (From seed kernel):

Extraction of soluble carbohydrate was same as that of amino acid from seed kernels. One ml sample was taken the stock solution and quantitative analysis was done as the method mentioned earlier using 0.2% anthrone reagent.

2.6.4 Analysis of Insoluble carbohydrate (from seed kernels):

For the analysis of insoluble carbohydrate from seed kernel 100 mg seed kernel of lack sample was thoroughly however in mortar with pastle using 5 ml 80% boiling ethanol. After centrifugation at 6000 *g* for 10 min, the residue was digested with 5 ml 25% H_2SO_4 (v/v) at 80 °C in a water bath for 30 min. The extracted material was taken as the source of insoluble carbohydrate. For quantitative measurement 1 ml. of extracted sample was taken in test tubes after necessary dilution (preferably 10 times) and insoluble carbohydrate level was determined with 0.2% anthrone reagent (Following the method of Mc Cready *et al*, 1950) as described in soluble carbohydrate.

2.6.5 Protein (from seed kernel):

Protein extraction and estimation was done with the treated seeds kernels. Hundred mg seed kernel were homogenized in a mortar pastle with 80% ethanol and centrifuged at 6000 *g* for 10 min. Phenol free pellet was done by thoroughly washing successively with 10% cold trichloroacetic acid (w/v, twice), ethanol (once), ethyl alcohol: chloroform (3:1, v/v once) and finally with solvent ether following the method of Kar and Mishra (1976). Then the pellet was evaporated to dryness to remove the ether. The protein was then solubilized by treating with 0.5 N NaOH at 80 °C for 1 h. A volume of 4 ml was made with the extraction medium i.e. 80% ethanol. Protein content was then estimated by reacting protein solution with Folin phenol reagent and measuring the OD value at 650 nm according to the method of Lawry *et al* (1951). Quantitative determination was made by comparing the OD value with a standard curve previously prepared using bovine serum albumin (BSA Fractin-v, Sigma Chemical Co., USA).

2.6.6 Amino acid (from seed kernels):

To analyze amino acid levels from seed kernel 100 mg seed kernel of each sample was thoroughly homogenized in a mortar with pastle using 5 ml 80% boiling ethanol. After centrifugation at 6000 *g* for 10 min, the filtrate was taken as the source of free amino acids. One ml sample was taken from the stock and quantitative analysis was done following the method of Moore and Stein (1948) modified by Bhattacharjee (1984), as mentioned earlier.

2.6.7 Analysis of Nucleic acids (from seed kernels):

Extraction of nucleic acids (DNA & RNA) were done from seed kernel following the method described by Biswas and Chowdhuri (1978) and estimated as per the method of Cherry (1962), modified by Choudhuri and Chatterjee (1970).

2.6.7.1 Estimation of DNA:

One ml of the nucleic acid extra was taken in a test table and 5 ml freshly prepared diphenyl amine reagent (100 ml glacial acetic acid (BDH, AR) + 2.7 ml conc. H_2SO_4 + 1 g AR grade diphenyl amine) was mixed. Then the mixture was boiled in a water bath for 30 min with glass marble at the top at the test tube. After cooling in running tap water, shining ball colour appears. The intensity of blue colour was measured spectrophotometrically at 610 mm. DNA content was quantified from the OD values of standard curse prepared with Harring sperm DNA.

2.6.7.2 Estimation of RNA:

Three ml of the nucleic acid extract (in 5% perchloric acid) was taken and treated with ingle volume of freshly prepared orcinol reagent (1g AR grade orcinol dissolved in 100 ml conc. HCl containing 0.1% $FeCl_3$,6 H_2O).The treated mixture then boiled in a water bath for 20 min with glass marble at the top of the test tubes. The mixture was then allowed to cool and blue green colour was measured at 700 nm in a spectrophotometer. The procedure was followed by the method developed by Markham (1955) modified by Choudhuri and Chatterjee (1970).The black used contained a mixture of 3 ml distilled water and 3 ml orcinol reagent which was treated in an similar manner. RNA level was calculated from the OD Values of standard curve prepared with Yeast RNA.

2.6.8 Analysis of Dehydrogenase activity (from seed kernels):

One g dehusked seeds of each treatment in the control were immersed in 0.5% TTC solution in test tubes and incubated for 12 hr dark. The hydrogen atoms released by the total dehydrogenase enzymes which are involved in the respiration process of living tissues, reduce tetrazolium to red coloured formazan (Moore, 1973).The TTC stained 1 g embygnal ares of the seeds of each treatment was extracted with 5 ml of 2-methorythanol for 24 h and OD values of the solutions were recorded at 520 nm. The activity of total dehydrogenase of infact seeds was analyzed by the reception of tetrazolium chloride according to the method of Rudrapal & Basu (1979).

2.6.9 Analysis of Catalase activity (from seed kernels):

To analyses catalase activity 500 mg seed kernel of each treatment was homogenized with 8 ml of chilled 0.1 M phosphate (Na_2HPO_4 / NaH_2PO_4) buffer (pH 6.5). The homogenate was centrifuged at 3000 *g* for 15 min followed by 10000 *g* for 20 min in cold condition then the supernatant taken and the volume made up to 10 ml with the same buffer, and this was used as crude enzyme source. The enzyme activity was determined followed the method of smell and Smell (1971) modified by Biswas & Choudhuri (1978) To estimate to catalase activity 2 ml 0.05 M H_2O_2 was mixed with 1 ml of the above extract and incubated the reaction mixture 37 °C for 2 min. the reaction was stopped by adding 2 ml 0.1% titanium sulphate in 25% H_2SO_4 (v/v).The the mixture was centrifuged at 4000 *g* for 20 min. Supernatant became goes yellow colour and the intensity was measured at 420 nm. The black was prepared by inactivating (heat killed) enzyme with the addition of titanium sulphate prior to H_2O_2 addition.

2.6.10 Analysis of Protease activity (from seed kernels):

The method was estimated following the method described by F. D. Snell and C. T. Snell (1971) after necessary modifications. Five hundred milligram (500 mg) of seed of each sample were taken and homogenized with 5 ml. chilled phosphate buffer (pH 6.5) in cold condition. The homogenate was then centrifuged at 5000 *g* for 10 min. the supernatant was taken and the volume was made up to 10 ml with phosphate buffer and used as crude enzyme source. One ml of enzyme solution was mixed with 0.1 ml of 0.1 M Mg SO_4 and 1 ml of 50 ppm Bovin Serum Albumin and kept the set up in incubation at 37 °C for 1 h. Then the reaction was stopped with 50% TCA. After the reaction mixture was centrifuged at high speed (10000 – 15000 *g*). Supernatant was rejected and pellet was taken. The pellet was dissolved in 1 ml of 1 M NaOH solution and 1 ml of distilled water was added to it. Then the mixture was incubated at 80 °C for 15 min. After incubation the mixture was diluted 10 times. One ml Cu-tartrate and NaOH - Na_2CO_3 (1:10) was added with 1 ml of diluted solution and wait for 10 min. ten ml of Folin reagent was added to it and blue colour appeared. The intensity of blue colour was measured at 650 nm.

2.6.11 Analysis of Amylase activity (from seed kernels):

Five hundred mg seed kernel of each sample was homogenized with 10 ml to 0.1M phosphate buffer (pH 6.5). The homogenate was centrifuged at 5000 *g* for 15 min. The supernatant was taken and used as the crude source of the enzyme. To estimate amylase activity, the method described by khan & Fast (1976) was followed. One ml of the enzyme solution was mixed with an equal volume of 0.1% starch solution in 0.1 N sodium acetate buffer (pH 5.0) and incubated at 37 °C for 10 min. The reaction was stopped with 3 ml Iodine HCl solution (600 mg KI & blue colour appears & the intensity was measured at 20 min with spectrophotometer 60 mg1 in 100 ml of 0.05N HCl) The blank was prepared after inactivating the enzyme with 3 ml Iodine HCl solution period to addition of starch. The amylase enzyme activity was estimated following the method described by Khan and Faust (1967) with necessary modifications.

III. CODES USED FOR THE EXPERIMENT:

CON/Control = Seeds treated with double distilled water.
P1 = Seeds treated with Parthenium Young Leaf Leachate 1:5 (w/v).
P2 = Seeds treated with Parthenium Mature Leaf Leachate 1:5 (w/v).
P3 = Seeds treated with Parthenium Old Leaf Leachate 1:5 (w/v).
P4 = Seeds treated with Parthenium Young Leaf Extract 1:5 (w/v).
P5 = Seeds treated with Parthenium Mature Leaf Extract 1:5 (w/v).
P6 = Seeds treated with Parthenium Old Leaf Extract 1:5 (w/v).

IV. RESULT AND DISCUSSION:

4.1 Result:

Table 1: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of percentage germination (%), time (h) for 50% germination (T_{50}) and TTC stainability (%) of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing					
	0 day			30 days		
	Germination	T_{50}	TTC stainablity	Germination	T_{50}	TTC stainablity
Control	99.77	15.8	100	12.1	NA	18.3
P1	45.6	NA	71.3	6.5	NA	9.8
P2	43.2	NA	66.6	5.4	NA	7.6
P3	44.5	NA	70.5	5.7	NA	8.1
P4	45.1	NA	70.8	6.2	NA	8.5
P5	41.4	NA	65.3	3.2	NA	6.2

P6	42.0	NA	68.1	4.1	NA	7.1
LSD	1.826844	NA	2.627266	1.434188	NA	1.83738

Table 2: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of speed of germination of 0 day accelerated ageing black gram seeds.

Treatments	Germination at 12 h intervals									
	12	24	36	48	60	72	84	96	108	120
CON	26.8	57.8	68.1	74.9	84.1	89.8	93.7	95.7	97.4	99.5
P1	7.5	10.3	14.3	27.5	32.6	36.5	39.4	43.2	44.2	45.6
P2	7.1	10.5	15.2	28.1	32.5	37.3	38.5	42.8	43.2	43.2
P3	7.0	10.2	15.1	29.0	33.2	37.4	38.6	42.5	43.3	44.5
P4	7.5	11.1	15.6	28.22	33.4	36.4	39.8	43.4	44.1	45.1
P5	6.3	10.1	15.0	27.1	31.5	34.3	37.5	39.4	40.1	41.4
P6	6.6	10.0	14.2	26.0	32.6	35.1	38.6	40.8	42.0	42.0
LSD	1.240348		1.18085		1.422686		1.581182		1.843259	

Table 3: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of speed of germination of 30 days accelerated ageing black gram seeds.

Treatments	Germination at 12 h intervals									
	12	24	36	48	60	72	84	96	108	120
Control	1.0	3.2	3.5	4.5	4.9	6.8	8.4	9.3	10.2	12.1
P1	0.0	0.0	0.0	1.3	2.7	3.5	4.6	5.6	6.5	7.1
P2	0.0	0.0	0.0	0.0	1.4	2.6	3.4	4.2	5.1	5.4
P3	0.0	0.0	0.0	1.2	22.8	3.7	4.2	5.2	5.7	5.7
P4	0.0	0.0	0.0	1.4	2.7	3.5	4.8	5.3	6.0	6.2
P5	0.0	0.0	0.0	0.0	1.1	1.8	2.1	2.7	3.0	3.2
P6	0.0	0.0	0.0	0.0	1.2	1.8	2.7	3.2	4.1	4.1
LSD	0.148824		0.749885		0.89122		1.071607		1.510857	

Table 4: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of leaching of soluble carbohydrate and free amino acid levels of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing			
	0 day		30 days	
	Soluble carbohydrate (mg/g/10ml)	Amino acid (mg/g/10ml)	Soluble carbohydrate (mg/g/10ml)	Amino acid (mg/g/10ml)
Control	11.69	1.73	18.30	8.20
P1	40.35	5.10	38.60	11.90
P2	44.60	7.65	42.25	13.85
P3	43.16	6.77	41.60	13.44
P4	42.00	5.85	40.42	12.80
P5	48.52	8.90	44.85	15.23
P6	46.75	8.13	43.50	14.66
LSD	4.711496	2.180672	1.332209	1.227580

Table 5: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of soluble carbohydrate and insoluble carbohydrate levels in seed kernels of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing			
	0 day		30 days	
	Soluble carbohydrate (mg/g fr. wt.)	Insoluble carbohydrate (mg/g fr. wt.)	Soluble carbohydrate (mg/g fr. wt.)	Insoluble carbohydrate (mg/g fr. wt.)
Control	06.99	30.89	16.35	18.45
P1	15.88	20.44	26.81	10.18
P2	17.32	16.35	29.78	6.95
P3	16.90	18.21	28.38	8.50

P4	16.49	18.98	27.21	9.33
P5	19.27	14.11	31.95	5.20
P6	18.59	15.73	30.50	6.12
LSD	1.378857	1.738151	2.201560	2.072079

Table 6: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of protein content and amino acid levels in seed kernels of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing			
	0 day		30 days	
	Protein (mg/g fr. wt.)	Amino acid (mg/g fr. wt.)	Protein (mg/g fr. wt.)	Amino acid (mg/g fr. wt.)
Control	41.72	1.58	20.90	3.34
P1	28.66	5.76	12.42	8.18
P2	23.75	7.84	8.14	9.75
P3	24.24	7.12	9.73	9.25
P4	26.50	6.61	11.01	8.76
P5	20.16	8.90	6.47	10.78
P6	21.60	8.10	7.26	10.25
LSD	2.888484	2.104858	1.203782	1.009349

Table 7: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of DNA and RNA contents in seed kernels of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing			
	0 day		30 days	
	DNA (µg/g fr. wt.)	RNA (µg/g fr. wt.)	DNA (µg/g fr. wt.)	RNA (µg/g fr. wt.)
Control	75.42	868.46	37.29	495.35
P1	28.53	459.23	12.73	306.44
P2	24.89	379.13	9.19	255.29
P3	26.18	402.88	10.45	271.76
P4	27.33	431.70	11.52	288.23
P5	21.44	333.65	7.11	226.17
P6	23.48	358.11	8.26	241.66
LSD	2.341234	2.279743	49.720630	30.809370

Table 8: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of dehydrogenase and catalase activities in seed kernels of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing			
	0 day		30 days	
	Dehydrogenase (ΔOD/g/10ml)	Catalase (Unit/h/g fr. wt.)	Dehydrogenase (ΔOD/g/10ml)	Catalase (Unit/h/g fr. wt.)
Control	0.46	128.70	0.23	59.50
P1	0.19	73.77	0.07	16.26
P2	0.12	64.11	0.04	13.50
P3	0.15	67.40	0.04	12.80
P4	0.17	71.63	0.05	16.10
P5	0.07	51.27	0.02	8.89
P6	0.10	57.55	0.03	10.11
LSD	2.311234	0.022984	8.852401	3.327267

Table 9: Effect of seed pretreatment with leaf extracts and leaf leachates of *Parthenium* on changes of protease and amylase activities in seed kernels of 0 day and 30 days accelerated ageing black gram seeds.

Treatments	Accelerated ageing			
	0 day		30 days	
	Protease (Unit/h /g fr. wt.)	Amylase (Unit/h /g fr. wt.)	Protease (Unit/h /g fr. wt.)	Amylase (Unit/h /g fr. wt.)
Control	24.51	29.32	43.66	54.43
P1	40.55	65.62	67.89	89.97

P2	46.41	70.30	73.93	96.11
P3	45.11	69.12	72.13	93.34
P4	43.62	67.44	70.75	91.66
P5	51.13	77.51	78.10	99.81
P6	48.26	74.19	75.68	97.33
LSD	3.915129	4.230324	4.635436	3.671480

4.2 Findings:

The leaf extracts and leaf leachates of *Parthenium* strongly retarded percentage germination of black gram seeds.

4.2.1 Effect on changes of percentage germination, T_{50} value and TTC stainability (Table 1):

Results further showed that the leaf extracts and leaf leachates significantly decreased the rate of germination of the pretreated seed samples as compared to control ones. The lowest germination percentage was recorded in P5 treatment in acceleratedly aged seed samples for 0 and 30 days. *Parthenium* treated seeds failed to attain 50% germination even after 120 h of seed soaking, whereas, control seed samples achieved 50% germination within a very short period as here T_{50} value was recorded to be 16.5 h. *Vigna* seeds treated with leaf extracts and leaf leachates of *Parthenium* showed reduced TTC stainability but such reduction was found to be differential. The reduction was much higher in and P5 respectively in both 0 day and 30 days accelerated ageing condition.

4.2.2 Effect on changes of speed of germination of 0 day accelerated ageing seeds (Table 2):

Result showed that in control sample the speed of germination of black gram seeds started increasing with the advancement of the germination period as recorded from 12 to 120 h after soaking. Leaf extracts and leaf leachates of both *Parthenium* rendered inhibition of germination during the observation period. Speed of germination was significantly slowed down at all the treatments. The inhibitory effect was found to be drastic in comparison to control.

4.2.3 Effect on changes of speed of germination of 30 days accelerated ageing seeds (Table 3):

The result shows that in control sample percentage germination of black gram seeds increased with the advancement of the germination period as recorded from 12 to 120 h after soaking. Leaf extracts and leaf leachates of both *Parthenium* rendered inhibition of germination during the observation period. Speed of germination was significantly slowed down at all the treatments. In *Parthenium* treated samples, germination starts after 48 hrs except P1 which starts after 36 hrs. So, the inhibitory effect was found to be drastic in comparison to control.

4.2.4 Effect on changes of leaching of soluble carbohydrate and free amino acid levels of 0 day and 30 days accelerated ageing black gram seeds (Table 4):

Data showed that the leaching of free amino acid and soluble carbohydrate was higher than control when black gram seeds underwent treatment with leaf extracts and leachates of *Parthenium* in 0 days accelerated ageing treatment. In 30 days accelerated treatment the magnitude of leaching of free amino acid was highest in the samples treated with *Parthenium*. But in all the treated samples leaching were higher than the control sample.

4.2.5 Effect on changes of soluble carbohydrate and insoluble carbohydrate levels in seed kernels of 0 day and 30 days accelerated ageing black gram seeds (Table 5):

The data revealed that in all the treated samples the soluble carbohydrate levels increased irrespective of accelerated ageing treatments than the control sample. On the other hand the insoluble carbohydrate levels decreased drastically in all the treated samples than the control sample irrespective of treatments. The decrease of insoluble carbohydrate and increment of soluble carbohydrate was more significant in this regard. The soluble carbohydrate levels increased when accelerated ageing treatment increased and insoluble carbohydrate levels decreased with the increment of accelerated ageing in *Parthenium* leaf extracts and leaf leachate treated samples including the control sample.

4.2.6 Effect on changes of protein content and amino acid levels in seed kernels of 0 day and 30 days accelerated ageing black gram seeds (Table 6):

The data obtained from the experiment it was clear that the total protein content in the seed kernels of treated samples were declined than that of control sample in 0 day accelerated ageing treatments. But the amino acid level increased in all treated samples than the control sample in 0 day accelerated ageing treatments, which was much more significant. The declining of protein levels continued its trends in 30 days accelerated ageing treatments in all the treated samples than the control sample. Concomitantly, the rapid rise of amino acid levels in treated samples was also found in 30 days accelerated treatments. In both the accelerated ageing treatments P5 sample showed the lowest protein content and highest amino acid content among the *Parthenium* treatments.

4.2.7 Effect of on changes of DNA and RNA contents in seed kernels of 0 day and 30 days accelerated ageing black gram seeds (Table 7):

The DNA and RNA content in seed kernels both of the *Parthenium* treated seed samples was decreased than that of control sample in 0 day accelerated ageing treatment. The drastic decrease of DNA and RNA content were accelerated ageing treatment dependent. In accelerated treatment P5 sample showed the lowest content respectively.

4.2.8 Effect on changes of dehydrogenase and catalase activities in seed kernels of 0 day and 30 days accelerated ageing black gram seeds (Table 8):

The data revealed that the dehydrogenase and catalase enzyme activity were reduced significantly in the seed kernels undergone pretreatments with *Parthenium*. The activities were remarkably lower than the control sample in both the accelerated ageing treatments. And the results showed that higher the accelerated ageing lower were the enzyme activity. The rates of the decreasing enzyme activities were found rapid.

4.2.9 Effect on changes of protease and amylase activities in seed kernels of 0 day and 30 days accelerated ageing black gram seeds (Table 9):

The results showed that the protease and and amylase activities were increased in the seeds pretreated with leaf extracts and leachates of *Parthenium.* The activities of those two enzymes were increased parallelly with the increment of accelerated ageing including the control sample. The amylase enzyme activity was higher than that of protease enzyme activity. The activity was higher in P5 sample within *Parthenium* treated seeds.

4.3 Discussion:

The physiological parameters found in all the treatments reduced percentage seed germination and days of accelerated ageing (0 day and 30 days). Effect on seed germination was found inhibitory in association with reduction of TTC stainability and deleterious enhancement or non attainment of 50% of germination (T_{50}) (Table 1). Concomitantly the speed of germination was slowed down both at 0 day (Table 2) and 30 days (Table 3) after treatment. Leaching of soluble carbohydrates and free amino acids (Table 4) were significantly enhanced irrespective of seed samples and accelerated ageing days (0 and 30).

The physiological variables were associated with the treatment-induced changes of a number of bio chemical parameters analysed in seed kernels. Treatments with leaf extracts and leaf leachates the levels of amino acids, soluble carbohydrates, along with activities of amylase and protease were enhanced significantly. Concomitantly reduction of the levels of proteins, nucleic acids (DNA and RNA), insoluble carbohydrates, as well as that of the activities of dehydrogenase and catalase were recorded.

These reliable allelopathy-indicating parameters (Maiti, 2007)) of experiment no. 1 may add convincing evidences to determine the allelopathic potentiality in the experimental plant. Reports from various literatures reveals that plants having allelopathic potential can reduce seed germinability, speed of germination and TTC stainability along with deranging of seed membranes causing leaky membrane structure (Nayek, 2000, 2018; Maiti, 2008). Further, some biochemical parameters are reported to be adversely affected as a result of seed pretreatment with plant extracts / leachates having allelopathic effect (Chou, 1999; Vyvyan, 2002; Nayek, 2002, 2018; Inderjit, 2003, Yadav, 2010; Maiti, 2010). My results are thus, confirming the reported observations done by some of previous workers. In fact, damage of seed membrane by putative allelochemicals present in leaf extracts and leaf leachates rendered the seed membranes leaky / porous which consequently increase membranes permeability and therebyenhancing the levels of soluble substances in seed leachates. Concomitant reduction of macromolecules like protein, nucleic acids, and insoluble carbohydrates in the kernels of *Vigna* might be due to the reduced biosynthesis of these substances and/ or due to the augmented activities of the some catabolic enzymes like protease, DNAase, RNAase etc. by the active inference of the allelochemicals. Again, enhancement of soluble carbohydrates in seed kernels might possibly be due to enhanced amylase activity. Subdued activities of dehydrogenase and catalase might be attributed to the detrimental effect of the allelochemicals present in the extracts and leachates of the test samples. So, the treatment-induced alteration of a number of biochemical variables recorded in this investigation corroborated the changes of physiological parameters, and hence it might be taken for granted that the treatments do possess allelopathic property.

The biochemical parameters recorded from leaves of 0 day and 30 days accelerated ageing seed kernels also clearly showed subdued levels of protein, insoluble carbohydrate, nucleic acids (DNA and RNA), as well as activity of catalase enzyme.

Increment of the soluble carbohydrate contents might be due to the enhanced activities of amylase which break down the insoluble carbohydrate level and thus, enriching the soluble fraction of carbohydrate. Likewise, enhanced protease activity might be the causal factor for reduction of protein level. DNA and RNA levels are usually reduced due to the activities of corresponding enzymes like DNAase and RNAase. There are reports that the reduction of scavenger enzymes is due to activities of some inhibitory/ hazardous chemicals (Kamp, U., 2007). In the present study, the deleterious changes of biochemicals variables might be attributed to some putative allelochemicals present in the pretreating agents i.e. leaf extracts and leaf leachates of *Parthenium.*

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VII. REFERENCES:

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