Chapter-1-

Determination of Relative Allelopathic Vigour of Three Weeds of West Bengal by Physio-Biochemical Approach.

Experiments- I to IV

Allelopathic effect of:

Desmostachya bipinnata
Parthenium hysterophorus
Alternanthera sessilis

on

Physiological and Metabolic (biochemical) changes in seeds of

Vigna radiata and
Senna occidentalis

INTRODUCTION

Weeds are redundant, unwanted, unplanted plants that affect the growth of cultivated crops in the field by releasing chemicals called as allelochemicals (Batish *et al.*, 2007). Often they affect growth and dynamicity of crops and physiological functions including mineral nutrition, photosynthesis and respiration through allelopathic mechanism (Benyas *et al.*, 2010). The arrest of germination and reduction of root and shoot growth in early stages affect the establishment of seedling and ultimately yield of the crop. The most common effect among environmental stresses is the oxidative damage to the plant tissue (Smirnoff, 1995). Reactive oxygen species (ROS) are continuously produced as by-products from various metabolic pathways. However under stressful conditions, their formation might be increased which leads to lipid peroxidation (Chen *et al.*, 2018), damage nucleic acid, degrade protein ultimately leading to programmed cell death (Jiang *et al.*, 2007). There are numerous reports that dehydrogenase and catalase enzymes play vital role during the germination (Ghayal *et al.*, 2011; Maiti *et al.*, 2013) which are greatly influenced by some putative allelochemicals present in the leaf extracts and leachates of weed plants.

Dehydrogenase enzyme activity is a good, stable, metabolic marker for estimation of the degree of vigour in seeds (Saxena *et al.*, 1987) and has positive correlation with potential and viability of seeds (Halder and Gupta, 1982; Rudrapal and Basu, 1979).

α-amylase activity is representative of the carbohydrate metabolism in endosperms of seeds and represent a variety of germination vigour as reported in some rice cultivars

due to very low respiratory activity in seeds, which was higher than in storage condition (Sadia Galani, 2011).

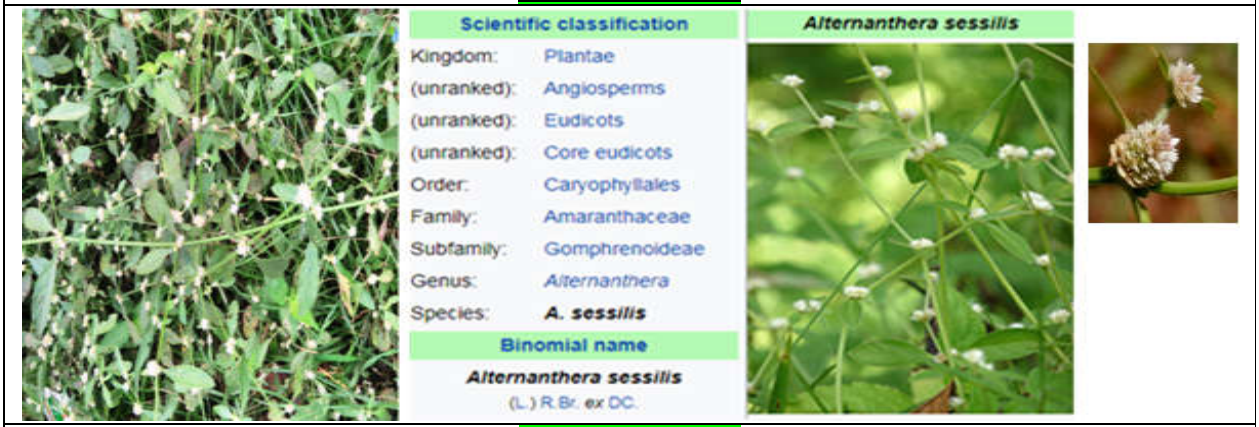
The present allelopathic study aims to throw light on the allelopathic effect of three weeds mof West Bengal *i.e. Desmostachya bipinnata* L. Stapf (Poaceae), *Parthenium hysterophorus* L. (Asteraceae) *and Alternanthera sessilis* L. R. Br. *ex* DC. (Amaranthaceae). They are selected due to their vigorous growing ability, easy availability and sensitive response towards plants used as bioassay. Mungbean /green gram *i.e.Vigna radiata* (L.) R. Wilczek (Fabaceae), is an important vegetable as well as pulse crop in North India and also in West Bengal. Therefore its seeds are chosen as test species or bioassay materials according to the objectives of our study.

On the other hand *Senna occidentalis* L. (Fabaceae) grows rank and wild in nature. Therefore seeds of this weed species are chosen as bioassay material because allelopathy is an ecophysiological phenomenon.Experiments are accordingly so designed as to study the germination responses of seeds of *Vigna* and *Senna* measured in terms of seed germination percentage, T_{50} (time taken for 50% germination of seeds) value and the speed of their germination recorded at 24h interval upto 168h exposed to various concentrations [1:5, 1:10 and 1:20 (w/v)] for each of the leaf extracts and or leachates respectively of the three weeds selected. Physiological parameter like TTC (2,3,5 triphenyl tetrazolium chloride) stainability of the seeds is also tested exposed to the three concentrations of extracts and leachates respectively. Biochemical estimation of metabolic changes of both leaf extract and leaf leachate pretreated seed kernels in terms of soluble and insoluble carbohydrates, amino acids, proteins, nucleic acids like DNA and RNA, enzymes like dehydrogenase, catalase, peroxidase and amylase are done.

MATERIALS AND METHODS

PHOTOPLATE-1 A

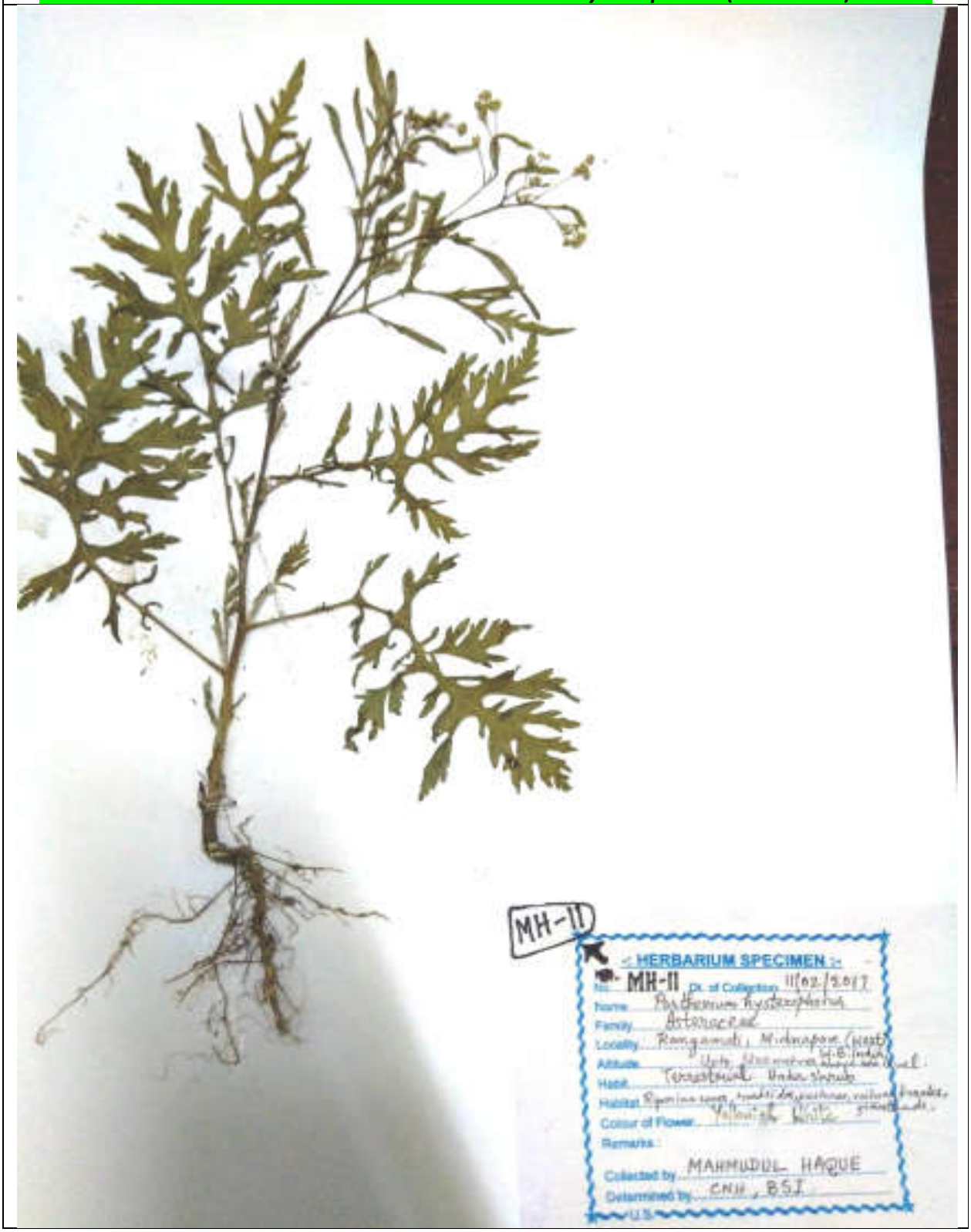
PHOTOPLATE-1 B



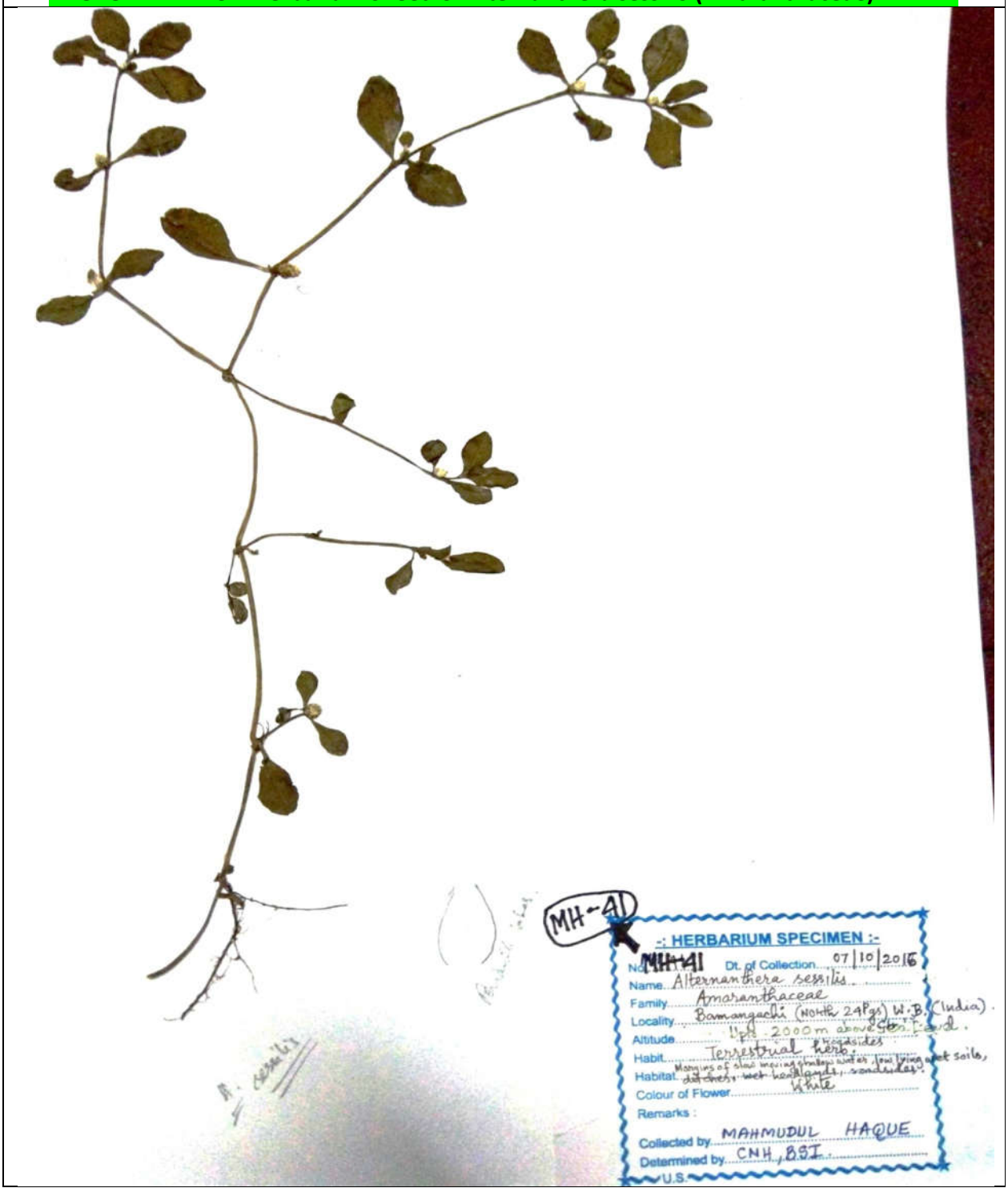
PHOTOPLATE-1 C

PHOTOPLATE-2 A. Herbarium Sheet of *Desmostachya bipinnata* (Poaceae)- MH-51

PHOTOPLATE-2 B. Herbarium Sheet of *Parthenium hysterophorus* (Asteraceae)- MH-11



PHOTOPLATE-2 C Herbarium Sheet of *Alternanthera sessilis* (Amaranthaceae)- MH-41



Authentication of plants at Central National Herbarium, Botanical Survey Of India.

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
फैक्स/ Fax: (033)26686226
दूरभाष/ Phone: (033)26683235/3364
इमेल/ E-mail: calherbarium@yahoo.co.in

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या/No.: CNH/Tech.II/2019/13 दिनांक/Date: 28 -03-2019

To,
Mr. Mahmudul Haque
Department of Botany and Forestry
Vidyasagar Univereity
Midnapore –721102
West Bengal

Sub.: Identification of three plant specimens – reg.

Dear Mr. Haque,

Please refer to your letter dated 26th March 2019 along with three plant specimens for identification. The specimens have been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	MH–11	**Parthenium hysterophorus** L.	Asteraceae
2.	MH–41	**Alternanthera sessilis** (L.) R.Br. ex DC.	Amaranthaceae

Also one specimen is incomplete bearing only vegetative plant parts, without any flower or fruit. The specimen has been tentatively identified as:

3.	MH–51	**Desmostachya bipinnata** (L.) Stapf	Poaceae

A complete specimen in all respects, with vegetative as well as floral parts is necessary for authentication.

The receipt of ₹ 150/- (Rupees one hundred fifty only) Receipt No. TR-5, C-160580 dated 28-03-2019 is enclosed herewith.

Your specimens are returned herewith.

Yours sincerely

28-3-2019

(K. KARTHIGEYAN)
Scientist –'D'

वैज्ञानिक 'डी' Scientist 'D'
केन्द्रीय राष्ट्रीय पादपालय
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावड़ा / Howrah-711103

PHOTOPLATE COLLAGE- 3

Bioassay materials

Senna occidentalis
(Bengali- Kalkasunda)

Vigna radiata (mung bean)

Senna occidentalis seeds and pods.

During collection of ***Senna occidentalis*** pods with seeds.

Vigna radiata seeds

Plant materials:

(a) Three weed species were authenticated vide (Memo number-CNH/TechII/2019/13) at the Central National Herbarium, BSI, Indian Botanic Garden ,Howrah,West Bengal, India and considered for allelopathic studies bearing voucher/tag numbers viz. *Desmostachya bipinnata* (MH-51), *Parthenium hysterophorus* (MH-11) *and Alternanthera sessilis* (MH-41*)* because of their vigorous growing ability, easy availability and good responses towards test plant material. Leaves which are healthy and fresh, of three types viz. young, mature and old were respectively collected from their active growing populations through out their life cycle from sites as mentioned here under.

(b) Materials chosen for bioassay are the pods and seeds of weed species *Senna occidentalis* and the seeds of domesticated species *Vigna radiata* grown as a crop.These were collected from the below mentioned sites.

Sites of collection

Vidyasagar University Campus,West Midnapore, Burdwan University campus ,Burdwan, railway tracks around Bamangachi, North 24 Parganas , Baruipur , South 24 Parganas and Haldia ,East Midnapore ,West Bengal year round at regular time intervals during the investigation periods. The leaf samples were collected from the plants through out their life cycle.

All the experiments and investigations of the present study were carried out with fully healthy and viable seeds of green gram (*Vigna radiata*) procured from the Seed Corporation of India, District Office, Midnapore, West Bengal, India .

The leaves were collected from those three plants and separately washed with distilled water to remove the adherent particles of dust . The same process was followed each time for collection of leaf samples.

Allelochemical extraction:

Leaf Extracts:

Young, mature and old dry leaves of the plants *Desmostachya bipinnata*, *Parthenium hysterophorus and Alternanthera sessilis* which are healthy ,were collected (3 lots , 25g each) from the weed plants respectively and homogenized thoroughly using 100 ml double distilled water.Using a fine cloth the homogenate was strained and stirred manually for five minutes and centrifuged at 5000 *g* for 15 min. It was then filtered with Whatman No.1 filter paper. The filtered solution was then made up to 125 ml, 250 ml and 500 ml volume respectively in three sets using double distilled water. This was considered as stock solution of leaf concentrations of 1:5 w/v, 1:10 w/v and 1:20 w/v proportion respectively. It was used as the test sample for studies on allelopathic potential.

Leaf leachates:

Healthy, old, mature and young leaves which are dry from the plants *Desmostachya bipinnata*, *Parthenium hysterophorus and Alternanthera sessilis* were collected respectively (3 lots of 25g each) from each weed plant. Preparation of Leaf leachates were accomplished by immersing each lot in 125 ml, 250 ml and 500 ml double distilled water in 1000 ml beaker respectively and kept for 72 h at room temperature (28±2°C). Using a fine cloth the leachates were strained, stirred for five minutes manually and filtered

through Whatman No.1 filter paper and decanted in different beakers and the leachate is made upto a total volume of 125 ml, 250 ml and 500 ml using double distilled water and this is taken as 1:5 w/v, 1:10 w/v and 1:20 w/v proportion of stock solution of leaf leachates respectively and was used as the sample to be tested for allelopathic studies.

Pretreatment of the seeds:

Vigna radiata and *Senna occidentalis seeds* which are fully viable are taken in ten lots of 25 g each . These seeds were surface sterilized for 90 seconds with 0.1% Mercuric chloride ($HgCl_{2)}$ solution. Seed lots were then presoaked separately in the three types of leaf extracts & leaf leachates of *Desmostachya, Parthenium and Alternanthera* or in distilled water (control) for 24 h. The seeds were allowed to germinate for testing their germination vigour. The experimental set up was kept in room temperature at around (32±2°C). The seeds were thus considered for biochemical tests.

Common Details of the experiments I- IV:

i) Analysis of germination behavior of both leaf extract and leaf leachate of *Desmostachya, Parthenium and Alternanthera* pretreated seeds:

Under this category data recorded include:

A) Experiments on seed Germination behaviour:

a) Percentage germination of seeds.

b) Values in terms of T_{50}

c) Speed of germination.

a) Percentage germination of seeds of bioassay plants :

To analyse the germination percentage of seeds, the individual seed lots in four groups of 100 seeds of each treatment were transferred to different petri dishes with filter paper moistened with 10 ml distilled water. The data of germination were recorded after 7 days of seed soaking following ISTA (1976) rules.

b) **T_{50} values**:

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

c) Speed of germination of seeds :

To analyze the speed of germination, the individual seed lots 100 seeds in each treatment were transferred to separate petridishes (9 cm) containing filter paper moistened with 10 ml double distilled water . Recording of data was done at an interval of 24 h. in laboratory up to 168 h. of soaking of seeds .Germination data were recorded following the rules of International Seed Testing Association (ISTA, 1996).

B) Physiological Experiments:

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

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

C) **Experiments on Biochemical parameters :**

Estimation of metabolic changes of pretreated seeds of both extract- andleachate of leaves from three weeds -:

These include: Free amino acids and Soluble carbohydrates. .

Other important biochemical parameters were recorded from the seed kernels of treated green gram *Vigna radiata* and *Senna occidentalis* .These are:

a) Soluble and insoluble carbohydrates

b) Proteins and amino acids

c) Nucleic acids (DNA & RNA)

d) Enzymes: Dehydrogenase , Catalase, Peroxidase and Amylase .

Free amino acids (from seed leachate):

From the seed leachates of each treatment free amino acid levels were analysed by immersing 10 g seed sample of treated *Vigna radiata* or *Senna occidentalis* which ever may be the case in 100 ml distilled water for 24 h. From the stock solution of leachate, the level of free amino acid was quantitavely analysed adopting the method of Moore and Stein (1948) with required modifications by Bhattacharjee (1984). 1 ml seed leachate and 3 ml of 0.1% ninhydrin solution (in 80% ethanol)were taken in test tubes with glass marbles at the top. These were kept for 15 min in a water bath. The reaction mixture soon turned violet in colour. The test tubes were taken out, cooled and up to 4 ml volume was made up with 80% ethanol. With a UV-VIS spectrophotometer the absorbance of the solution was measured at 580 nm. The quantitative estimation was carried out by comparing the O.D. values from a standard curve of glycine.

Soluble carbohydrates (from leachate of seed):

Sampling procedure was the same as done in case of leachable free amino acids, and from the same leachate stock, the quantitative measurement of soluble carbohydrate was determined following the method of Mc Cready *et al.* (1950) with simple modifications.

From each treatment 1 ml of the leachate from seed was taken in a test tube after needed dilution (almost 20 times) . To it 3 ml, precooled, fleshly prepared 0.2% anthrone reagent (200 mg anothrone powder dissolved in 10 ml concentrated H_2SO_4). After 30 minutes, the intensity of green colour in terms of optical density (OD) was measured at 610 nm by a UV-VIS spectrophotometer. The actual quantity was estimated from a formerly prepared standard curve with glucose.

Amino acid (from seed kernels):

100 mg seed kernel of each sample was thoroughly homogenized in a mortar with pestle using 5 ml 80% boiling ethanol. For 10 min after centrifugation at 6000 *g*, the filtrate was taken as the source of free amino acids. 1 ml sample was taken from this stock solution and quantitative analysis was done following the method of Moore and Stein (1948) modified by Bhattacharjee (1984), as mentioned earlier.

Soluble carbohydrate (From seed kernel):

The same method was used for the extraction of soluble carbohydrate as that of amino acid from seed kernels. 1ml sample was then taken from the stock solution and quantitative analysis was done as the method mentioned earlier according to Mc Cready *et al.* (1950) with simple upgradations using 0.2% anthrone reagent.

Insoluble carbohydrates (from seed kernels): The same residue after centrifugation of the sample (vide seed kernel extracted soluble carbohydrate) was digested at 80°C in a water bath for 30 min with 5 ml 25% H_2SO_4 (v/v). The extracted material served as a source of insoluble carbohydrates. For quantitative measurement, 1 ml of the extracted sample was taken in test tubes after necessary dilution (preferably 10 times) and insoluble carbohydrates level was determined in the same way as followed for soluble carbohydrates.

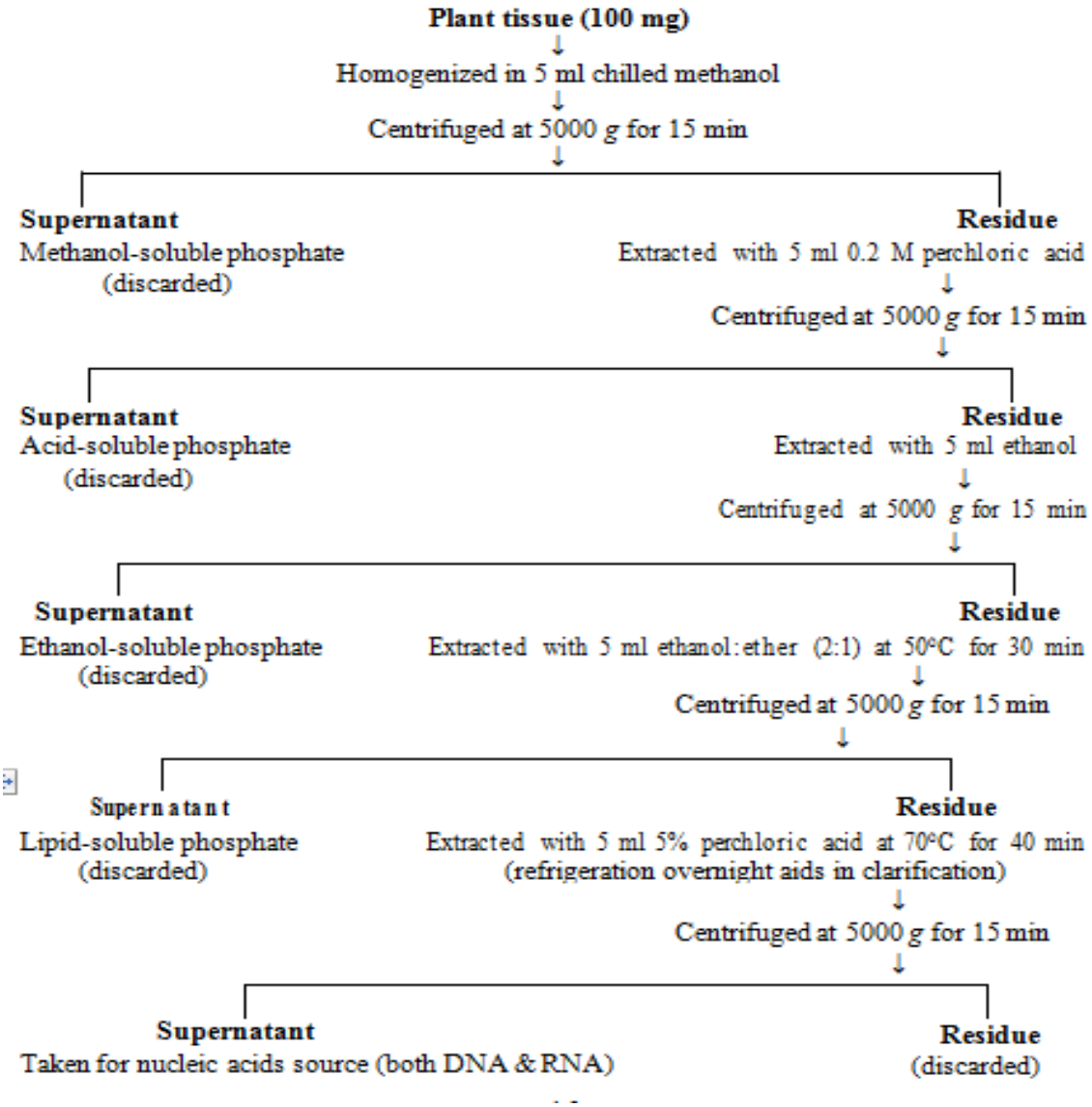
Protein (from seed kernel):

Protein extraction and estimation was done with the treated seeds kernels. 100 mg seed kernel were homogenized in a mortar with pestle in 80% ethanol and centrifuged for 10 min at 6000 *g*. Phenol free pellet was achieved by successively washing thoroughly with 10% w/v cold trichloroacetic acid w/v(twice), ethanol (once) and ethyl alcohol: chloroform 3:1 v/v (once) and finally with solvent ether following the method of Kar and Mishra (1976). To remove the ether the pellet was then evaporated to dryness. Then by treating with 0.5 N NaOH at 80 °C for 1 h the protein was solubilized. 4 ml volume was made with the extraction medium using 80% ethanol. Protein content was then estimated by reacting protein solution with Folin phenol reagent and the OD value was measured at 650 nm after the method of **Lowry *et al* (1951).** Quantitative analysis was done by comparing the OD value with a standard curve already prepared using bovine serum albumin (BSA, Fractin-V, Sigma Chemical CO., USA).

Nucleic acids (from seeds kernels):

Extractions of nucleic acids (DNA& RNA) were done from 100mg seeds kernel adopting the method of Cherry (1962). Nucleic acid estimation was done from a common stock and the sample was finally extracted with 5% perchloric acid.

The outline of extraction procedure of nucleic acid (DNA& RNA) is as follows :.



Estimation of DNA:

In a test tube an admixture of 1ml of the nucleic acid extract and freshly prepared 5 ml diphenyl amine reagent in 100 ml glacial acetic acid (BDH, AR) +2.7 ml conc. H_2SO_4 + 1 g diphenyl amine AR grade). in a water bath the mixture was boiled for 30 min with glass marble at the top of the test tube. It is cooled in running tap water. The intensity of blue colour was measured with a UV-VIS spectrophotometer at 610 nm. The content of DNA was then quantified from the O.D. values of a standard curve prepared with Herring sperm DNA.

Estimation of RNA:

Nucleic acid extract (3ml in 5% perchloric acid) was taken and treated with freshly prepared single volume of orcinol reagent (1g AR grade orcinol dissolved in 100 ml conc. HCl containing 0.1% $FeCl_3.6\ H_2O$).The mixture is then boiled in a water bath with glass marble at the top of the test tubes for 20 min. After cooling the blue green colour was measured spectrophotometrically at 700 nm following the method of Markham (1955) modified by Choudhuri and Chatterjee (1970). A mixture of 3 ml distilled water and 3 ml orcinol reagent , treated in an identical manner is used as blank. The amount of RNA was calculated with the help of a standard curve prepared with yeast RNA.

12) Dehydrogenase activity is analyzed adopting the methods of Rudra Pal and Basu (1979), with slight modifications. Procedure:-

1 g seeds which are dehusked ,of each treatment were immersed in 0.5% solution of TTC within test tubes and incubated in the dark for 12 h. The hydrogen atoms released by the total dehydrogenase enzymes release hydrogen atoms which are involved in the respiratiort activity of

living tissues reduce Tetrazolium chloride to red coloured Formazan (Moore, 1973). Then for 24h the TTC stained seeds are extracted with 5 ml of 2- methoxyethanol and OD values were recorded at 520 nm.

13) Catalase enzyme is extracted and estimated following the method of Snell and Snell (1971), with slight modifications by Biswas and Chowdhury (1978). Methodology:-

500 mg seed kernel of individual treatments were homogenized with 8 ml chilled 0.1 M phosphate buffer (NaH_2PO_4) (pH value - 6.5). Centrifugation of the homogenate was done in cold condition at 1000 *g* for 15 min followed by 10,000 *g* for 20 min. Using the same buffer the supernatant volume was made up to 10 ml, which was used as the crude source of enzyme. The reaction mixture for catalase enzyme consisted of 1 ml of the above extract along with 2 ml 0.05 M H_2O_2, incubated together for 2 min at 37^0C. The reaction was halted by adding 2 ml 0.1% titanium sulphate in 25% H_2SO_4 (v/v), with centrifugation of the mixture at 6000 *g* for 15 min. A golden yellow colour appears whose intensity was measured at 420 nm. A blank was prepared by inactivating (heat killed) catalase with the addition of titanium sulphate prior to the addition of H_2O_2 .

14) The **activity of peroxidase enzyme** was assayed following the method of Kar and Mishra (1976) with little modifications. Methodology:-

200 mg of treated seed kernel were homogenized with cold 0.05 M sodium phosphate buffer about 10 ml whose pH is 6.5 . The homogenate was centrifuged for 15 min at 10,000 *g*. The filtrate was taken as the enzyme source.

5 ml of the assay mixture containing 1 ml of the crude enzyme extract , 2 ml 50µM H_2O_2, 1 ml 300 mM of sodium phosphate buffer (pH 6.8) and 1 ml 50µM catechol and were incubated for 30 min. With the addition of 1 ml of 10% H_2SO_4 the reaction was stopped for 5 min after incubation at 25°C. Golden yellow coloured purpurogallin was formed which was read at 430 nm with the help of a UV-VIS spectrophotometer.

15) The amylase enzyme activity was estimated adopting the procedure described by Khan and Faust (1967) with needed modifications.

Method:-

Seed kernel (500 mg) from each sample was homogenized using 0.1 M phosphate buffer (10 ml; pH value 6.5). Homogenate was then centrifuged for 15 min at 5000 *g*. The supernatant served as the crude source of amylase . 1 ml amylase solution was mixed with same volume of 0.1% solution of starch in 0.1 N Sodium acetate buffer whose pH is 5.0 and incubated for 10 min at 37^0C. The reaction was stopped with 3 ml of iodine-HCl solution (60 mg I_2 ,600 mg KI and in 100 ml - 0.05 N HCl). After inactivating the enzyme with 3 ml iodine-HCl solution a blank solution was prepared prior to addition of starch. A blue colour formed the intensity of which was measured at 620 nm.

During each enzyme estimation and assay, the value at zero time was by default the blank. The activity of each enzyme was calculated using the formula according to (Fick and Qualset, 1975) :

[(∆A x Tv) / (t x v) x g fr wt. of tissue].

Where:

[∆A = OD value of (blank minus sample);

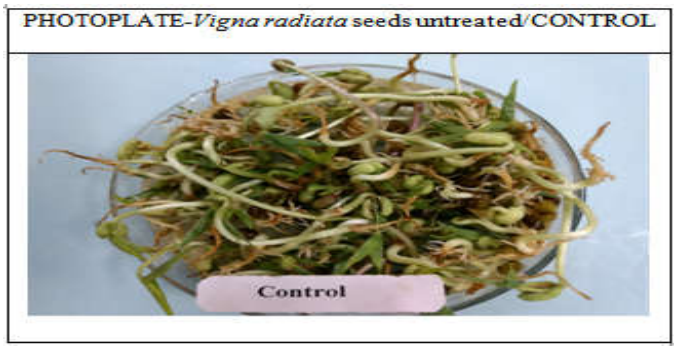
Tv = total volume of the filtrate;

t = time (min) of incubation with the substrate ;

v = volume of filtrate taken for incubation].

Least Significant Difference **(LSD)** was calculated at **95%** confidence limits according to (Panse and Sukhatme, 1967).

PHOTOPLATES



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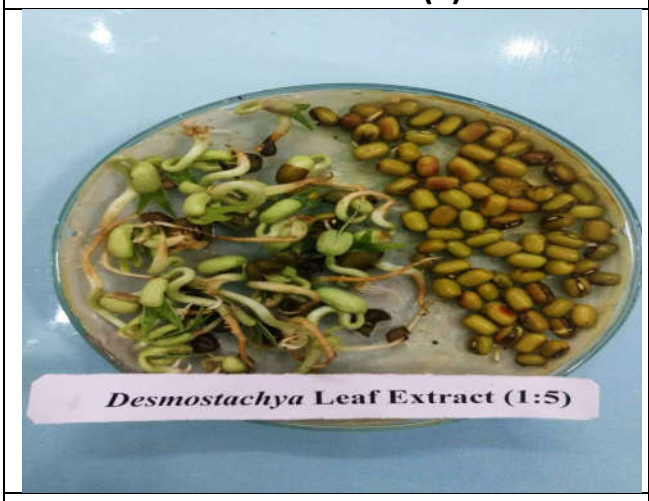	
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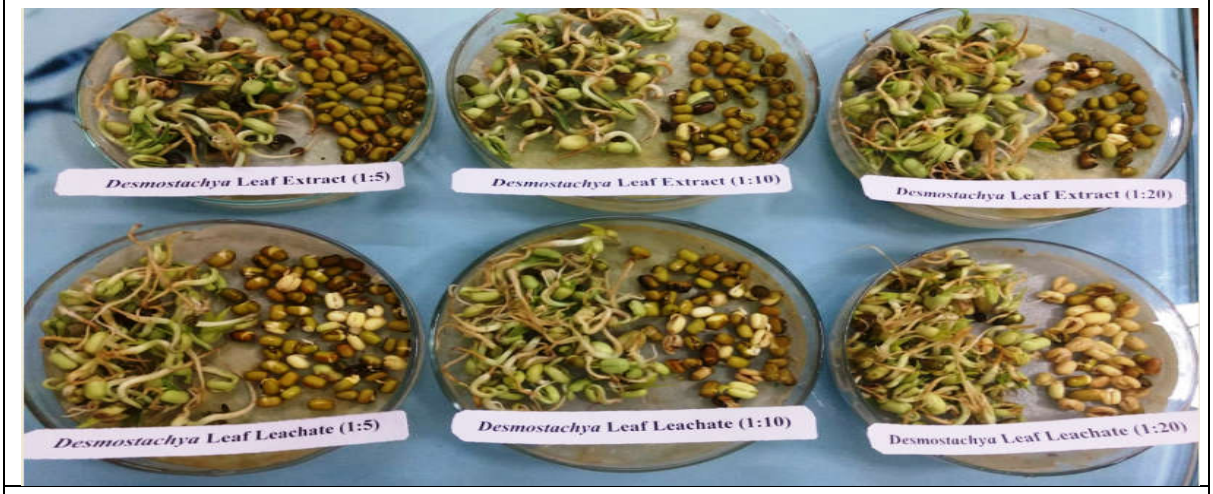
PHOTOPLATE-6(a)	**PHOTOPLATE-6(b)**
	

PHOTOPLATE-7(a)	PHOTOPLATE-7(b)
	
PHOTOPLATE-8(a)	PHOTOPLATE-8(b)
	
PHOTOPLATE-9(a)	PHOTOPLATE-9(b)
	

PHOTOPLATE-10(a)	PHOTOPLATE-10(b)
	
PHOTOPLATE-11(a)	**PHOTOPLATE-11(b)**
	
PHOTOPLATE-12(a)	**PHOTOPLATE-12(b)**
	

PHOTOPLATE-13



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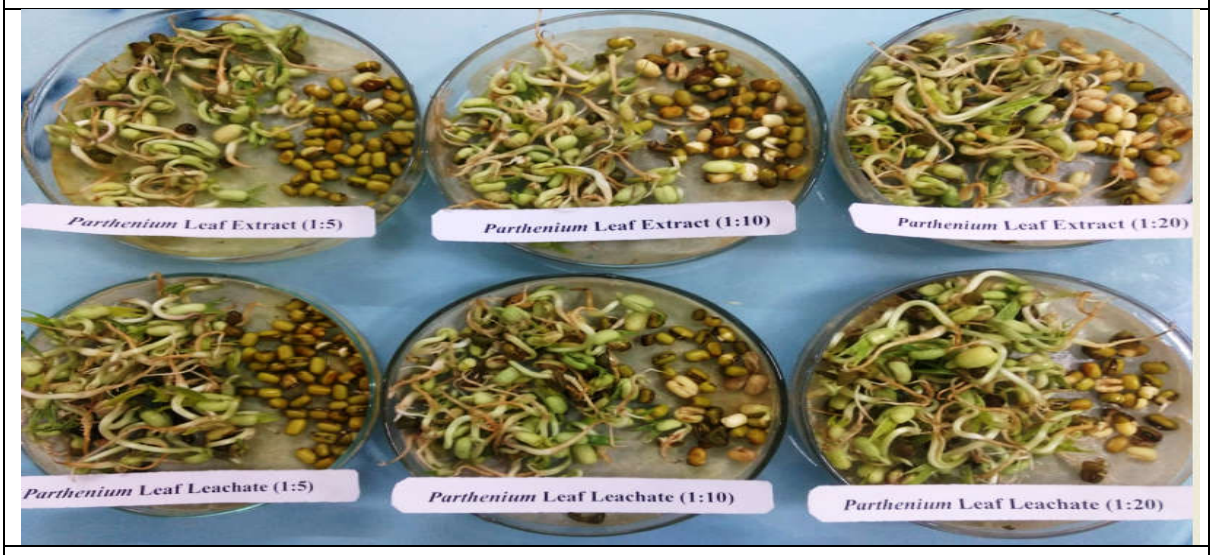

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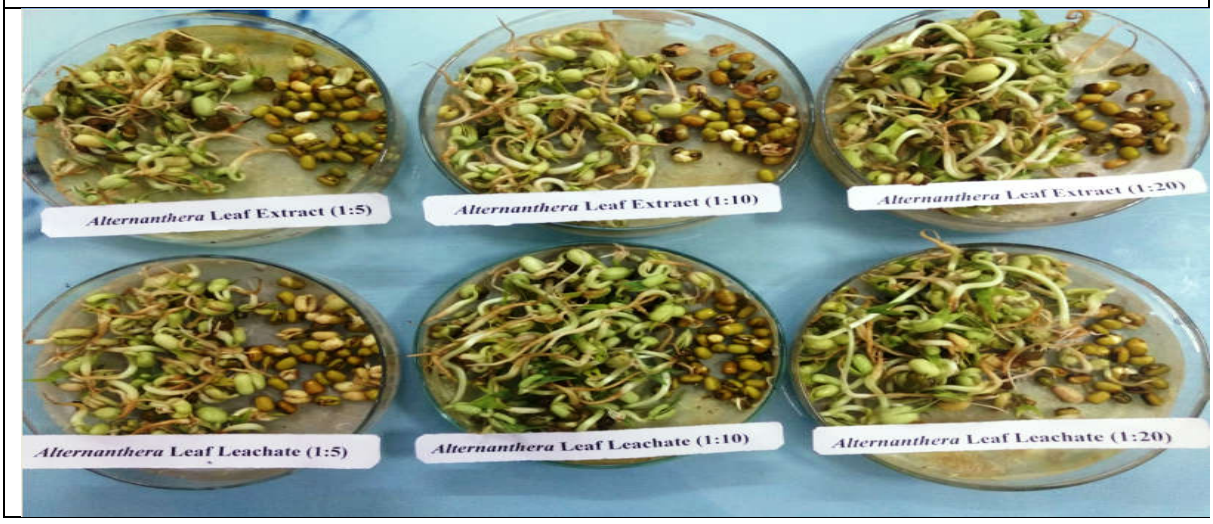

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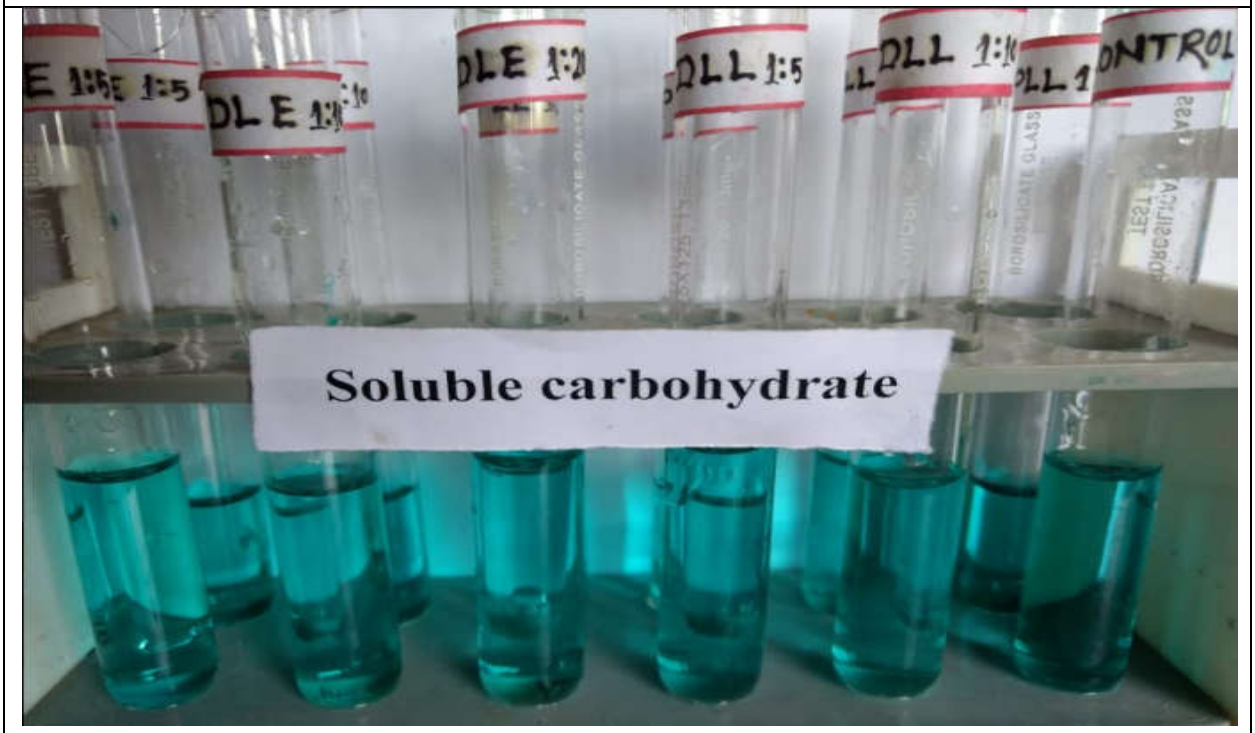

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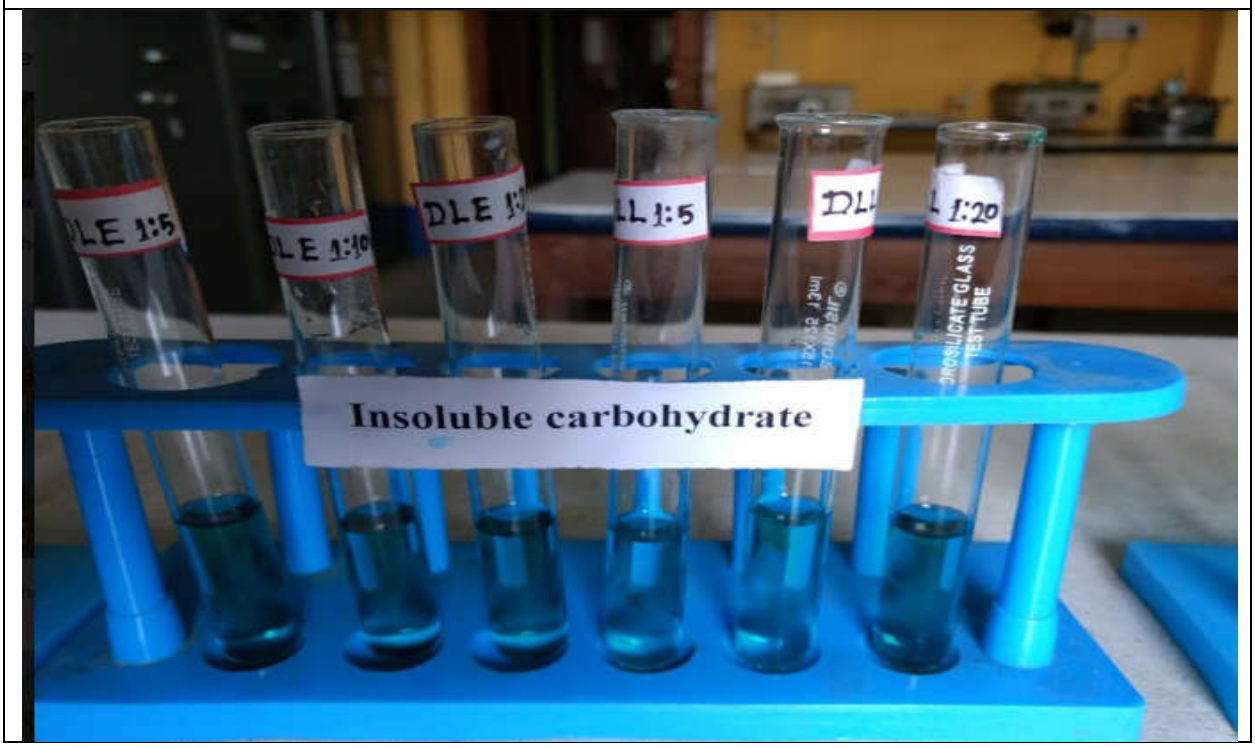

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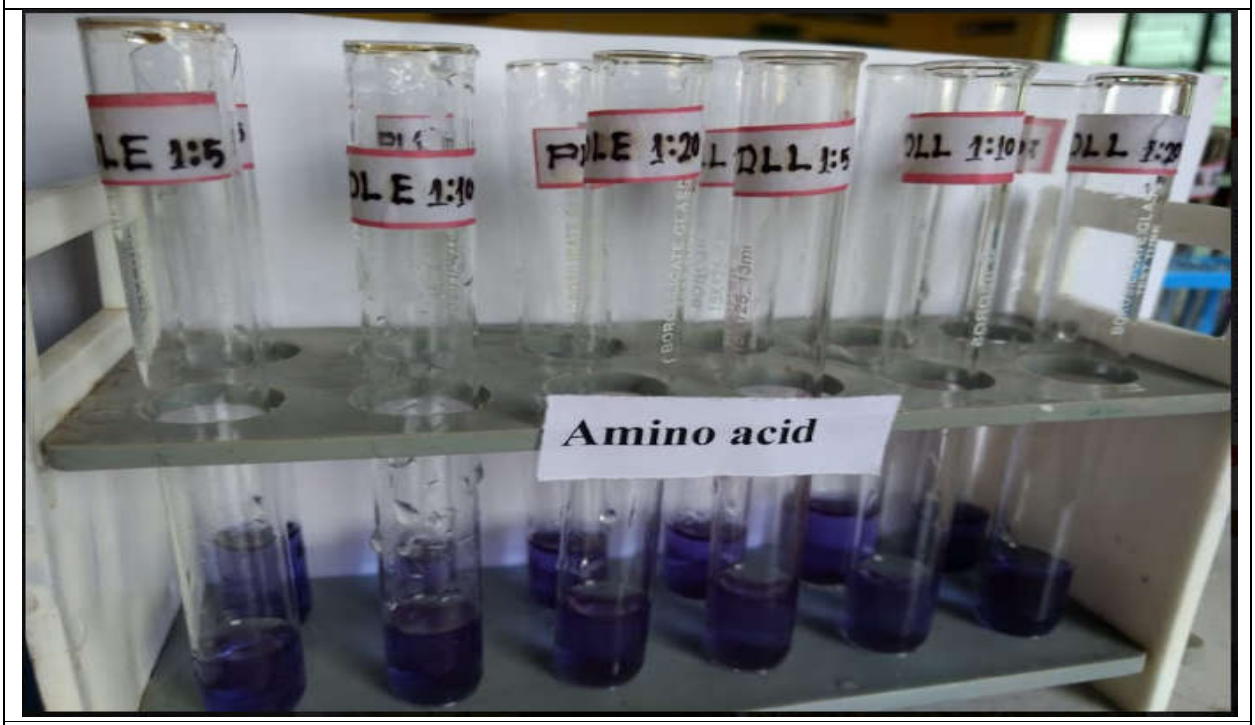
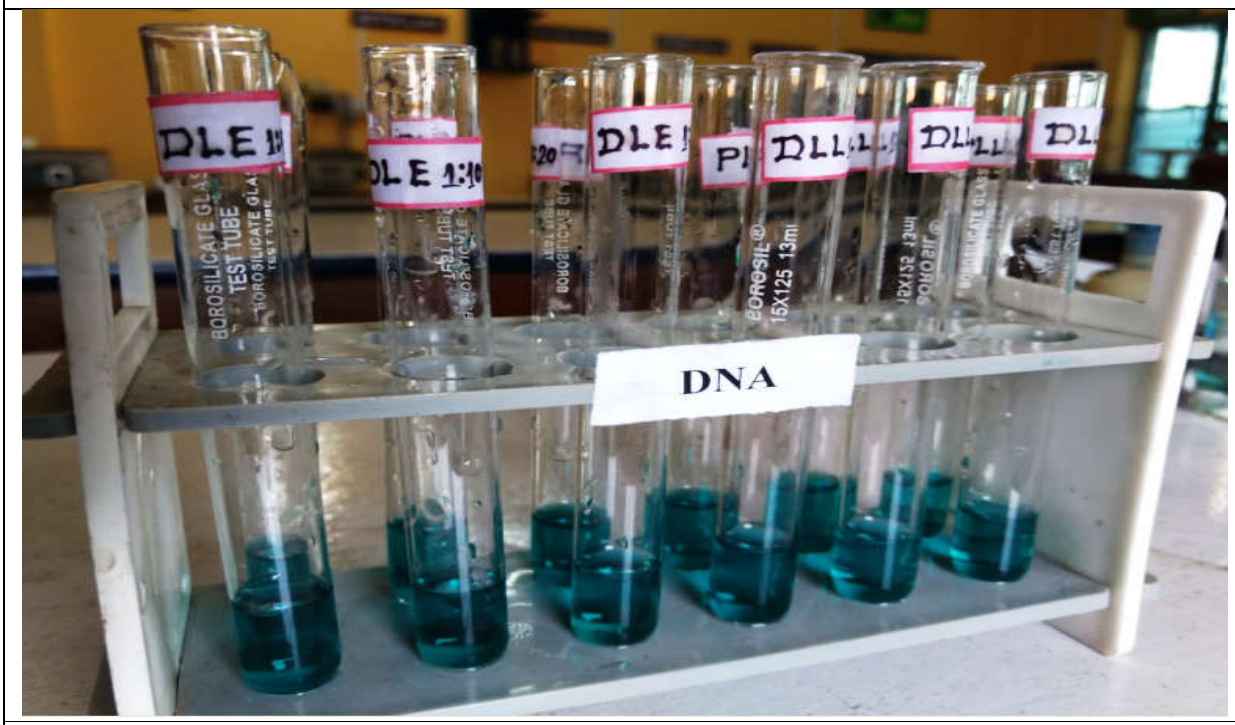


PHOTOPLATE-19

PHOTOPLATE-20



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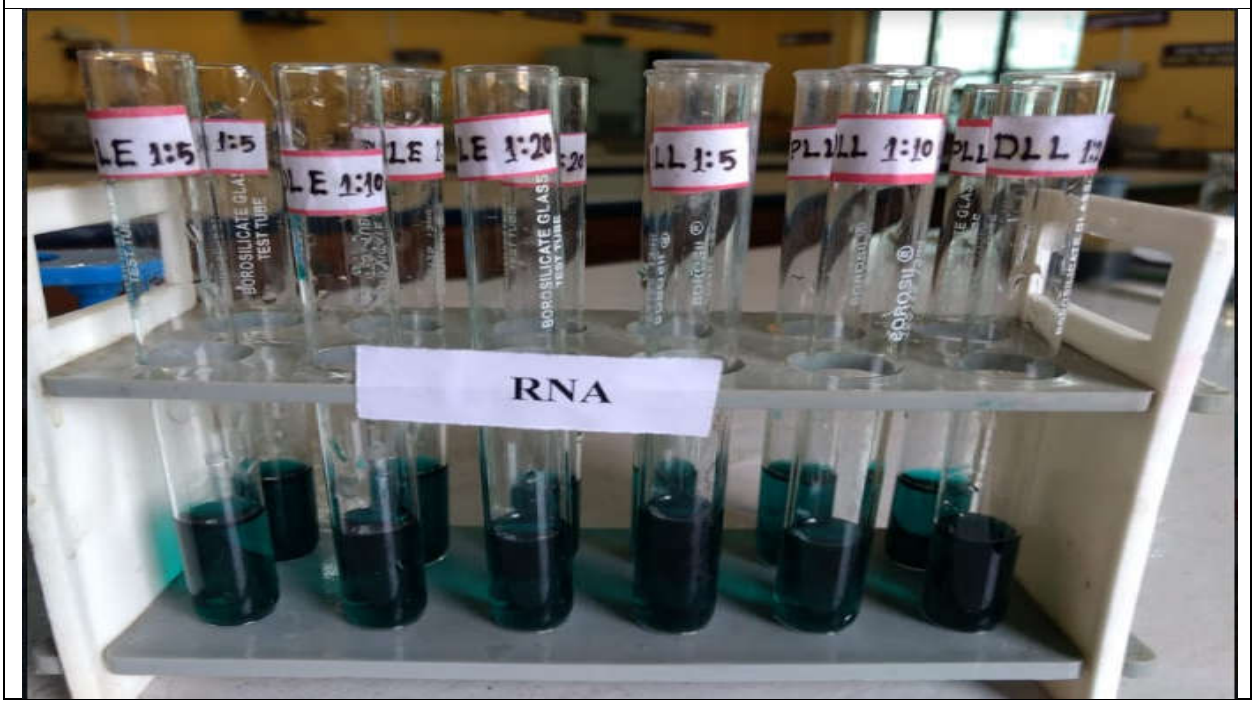

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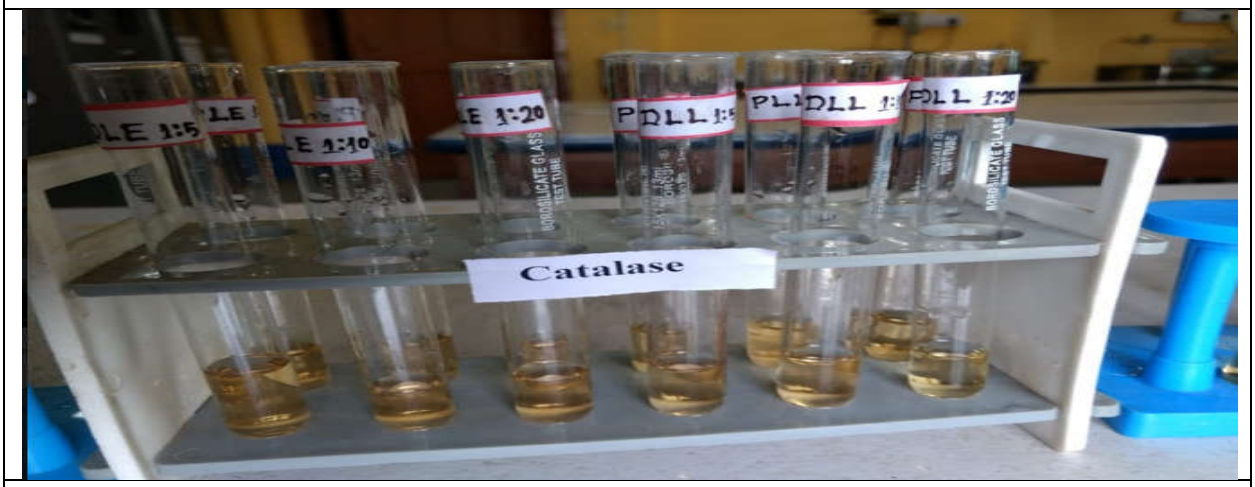
PHOTOPLATE-23



PHOTOPLATE-24

RESULTS

Experiment No.I:

Table-1. Effect of seed pretreatment with dry leaf extracts of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on percentage germination, time (h) for 50% germination (T_{50}) and TTC stainability of *Vigna radiata* seeds.

Treatments	Concentration	Germination (%)	T_{50} (h)	TTC stainability (%)
Control		**100.00**	**45.00**	**100.00**
Desmostachya	**1:5**	23.76	NA	39.62
	1:10	38.62	NA	47.21
	1:20	57.31	120.25	69.55
Parthenium	**1:5**	30.35	NA	42.65
	1:10	43.79	NA	62.42
	1:20	59.50	112.66	71.31
Alternanthera	**1:5**	37.56	NA	45.14
	1:10	48.21	NA	69.22
	1:20	63.51	94.66	84.41
LSD($p \leq 0.05$)		**2.40**	**4.26**	**3.69**

NA: Non-attainment of 50% germination.

Statistical analysis: (Table 1)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Germination (%)	Between Groups	2894.940	3	964.980	4.349	.060
	Within Groups	1331.271	6	221.879		
	Total	4226.211	9			
T50 (h)	Between Groups	181.777	3	60.592	.015	.997
	Within Groups	24075.236	6	4012.539		
	Total	24257.013	9			
TTC stainability (%)	Between Groups	1808.360	3	602.787	2.129	.198
	Within Groups	1698.828	6	283.138		
	Total	3507.188	9			

Table-2. Effect of seed pretreatment with dry **leaf extracts** of of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on speed of germination of *Vigna radiata* seeds.

Treatments	Speed of germination at 24h intervals							
	Concentration	24	48	72	96	120	144	168
Control		**32.15**	**56.60**	**88.71**	**100.00**	**100.00**	**100.00**	**100.00**
Desmostachya	**1:5**	0.00	0.00	8.25	18.57	21.00	23.76	23.76
	1:10	0.00	3.65	13.11	19.31	31.22	37.36	38.62
	1:20	0.00	14.96	26.82	38.21	50.35	55.77	57.31
Parthenium	**1:5**	0.00	0.00	10.25	13.18	23.66	30.35	30.35
	1:10	0.00	10.93	18.45	28.19	37.28	42.65	43.79
	1:20	0.00	16.35	28.14	39.95	57.21	58.66	59.50
Alternanthera	**1:5**	0.00	8.71	19.71	27.66	30.66	36.55	37.56
	1:10	0.00	16.92	22.86	28.11	39.75	47.28	48.21
	1:20	0.00	30.45	42.10	52.65	58.52	62.88	63.51
LSD(p≤0.05)		**NC**	**0.41**	**0.78**	**1.28**	**1.30**	**2.01**	**2.26**

NC= Not Calculated.

Statistical analysis: (Table 2)

Two way Analysis of Variance

Groups: 1= Control, 2= *Desmostachya,, 3= Parthenium, 4= Alternanthera*
Hours: 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs

Tests of Between-Subjects Effects					
Dependent Variable: Speed of germination					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	40752.272[a]	27	1509.343	10.264	.000
Intercept	93775.000	1	93775.000	637.705	.000
Group	20262.374	3	6754.125	45.931	.000
Hours	17902.189	6	2983.698	20.290	.000
Group * Hours	1153.684	18	64.094	.436	.970
Error	6176.129	42	147.051		
Total	121352.249	70			
Corrected Total	46928.402	69			
a. R Squared = .868 (Adjusted R Squared = .784)					

Post Hoc analysis: LSD (Independent variable= Group)

Multiple Comparisons						
Dependent Variable: Speed of germination LSD						
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Desmostachya	59.540476*	5.2924149	.000	48.859950	70.221002
	Parthenium	56.356667*	5.2924149	.000	45.676141	67.037192
	Alternanthera	50.394762*	5.2924149	.000	39.714236	61.075288
Desmostachya	Control	-59.540476*	5.2924149	.000	-70.221002	-48.859950
	Parthenium	-3.183810	3.7423025	.400	-10.736082	4.368463
	Alternanthera	-9.145714*	3.7423025	.019	-16.697986	-1.593442
Parthenium	Control	-56.356667*	5.2924149	.000	-67.037192	-45.676141
	Desmostachya	3.183810	3.7423025	.400	-4.368463	10.736082
	Alternanthera	-5.961905	3.7423025	.119	-13.514177	1.590367
Alternanthera	Control	-50.394762*	5.2924149	.000	-61.075288	-39.714236
	Desmostachya	9.145714*	3.7423025	.019	1.593442	16.697986
	Parthenium	5.961905	3.7423025	.119	-1.590367	13.514177
Based on observed means. The error term is Mean Square(Error) = 147.051.						
*. The mean difference is significant at the 0.05 level.						

Fig1. Graphical presentation of coefficient of determination (R^2) at 1:5 leaf extract of *Desmostachya, Parthenium* and *Alternanthera on Vigna radiata seeds* using MS Excel

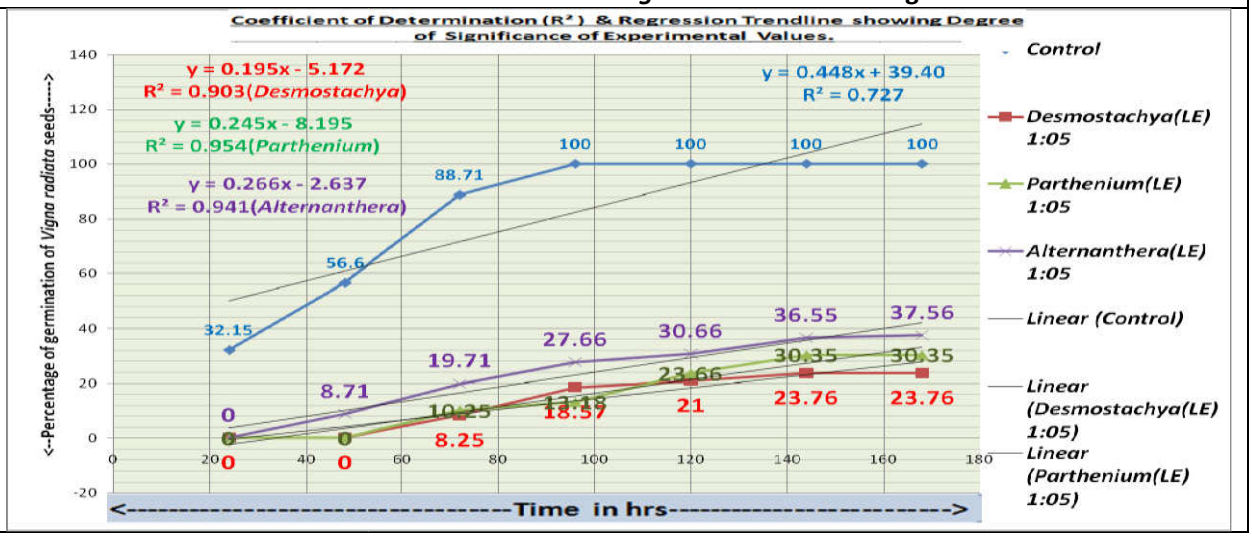


Fig2. Graphical presentation of coefficient of determination (R^2) at 1:10 leaf extract of *Desmostachya, Parthenium* and *Alternanthera on Vigna radiata seeds* using MS Excel

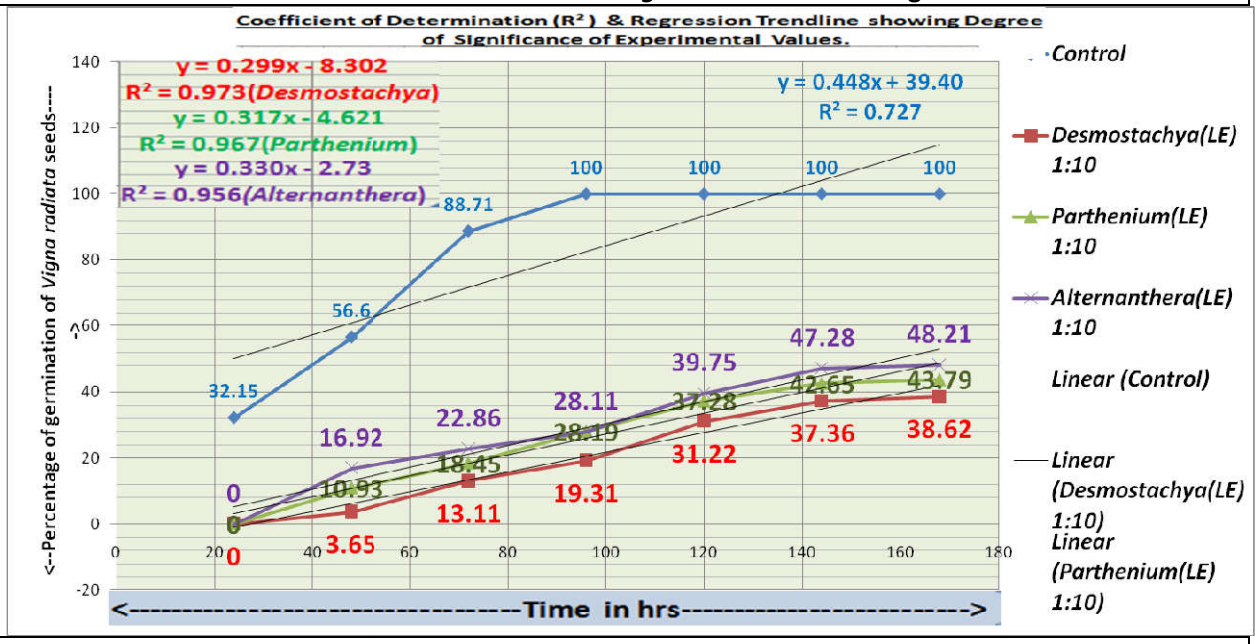


Fig3. Graphical presentation of coefficient of determination (R^2) at 1:20 leaf extract of *Desmostachya, Parthenium* and *Alternanthera on Vigna radiata seeds* using MS Excel

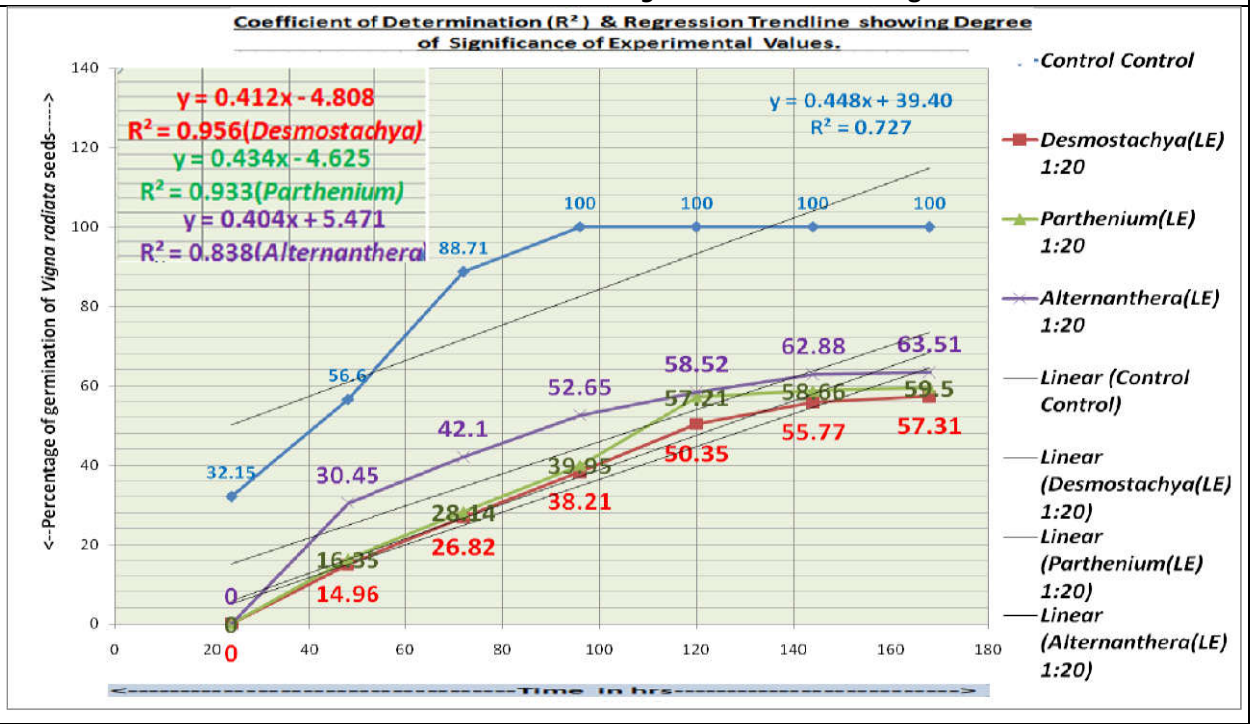


Table-3. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of amino acids and soluble carbohydrates level in *Vigna radiata* seeds.

Treatments	Concentration	Amino acids (mg/g /10ml.)	Soluble carbohydrates (mg/g /10ml.)
Control		**1.92**	**14.66**
Desmostachya	**1:5**	7.79	48.97
	1:10	7.10	43.55
	1:20	6.28	38.21
Parthenium	**1:5**	6.22	38.05
	1:10	5.63	37.22
	1:20	5.05	34.76
Alternanthera	**1:5**	5.21	30.09
	1:10	4.48	28.29
	1:20	4.02	26.86
LSD($p \leq 0.05$)		**0.21**	**1.52**

Statistical analysis: (Table 3)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	22.565	3	7.522	17.715	.002
	Within Groups	2.548	6	.425		
	Total	25.113	9			
Soluble carbohydrates	Between Groups	764.256	3	254.752	22.157	.001
	Within Groups	68.984	6	11.497		
	Total	833.240	9			

Pearson's Correlation:

Correlations			
		Amino acids	Soluble carbohydrates
Amino acids	Pearson Correlation	1	.986**
	Sig. (2-tailed)		.000
	N	10	10
Soluble carbohydrates	Pearson Correlation	.986**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-4. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble carbohydrates and insoluble carbohydrates level in kernels of *Vigna radiata* seeds.

Treatments	Concentration	Soluble carbohydrates (mg/g fr wt.)	Insoluble carbohydrates (mg/g fr wt.)
Control		**13.90**	**132.81**
Desmostachya	**1:5**	21.67	116.37
	1:10	21.23	122.55
	1:20	19.34	123.43
Parthenium	**1:5**	20.72	122.90
	1:10	19.68	123.33
	1:20	17.66	123.66
Alternanthera	**1:5**	19.36	124.21
	1:10	18.55	126.28
	1:20	17.40	126.38
LSD($p \leq 0.05$)		**1.26**	**3.36**

Statistical analysis: (Table 4)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Soluble carbohydrates	Between Groups	36.465	3	12.155	7.406	.019
	Within Groups	9.847	6	1.641		
	Total	46.312	9			
Insoluble carbohydrates	Between Groups	117.678	3	39.226	7.155	.021
	Within Groups	32.895	6	5.483		
	Total	150.573	9			

Pearson's Correlation:

Correlations			
		Soluble carbohydrates	Insoluble carbohydrates
Soluble carbohydrates	Pearson Correlation	1	-.902**
	Sig. (2-tailed)		.000
	N	10	10
Insoluble carbohydrates	Pearson Correlation	-.902**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-5. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata* *Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble amino acids and protein level in kernels of *Vigna radiata* seeds.

Treatments	Concentration	Amino acids (mg/g fr wt.)	Protein (mg/g fr wt.)
Control		**2.03**	**160.32**
Desmostachya	**1:5**	6.02	122.38
	1:10	5.78	122.77
	1:20	5.20	123.64
Parthenium	**1:5**	5.66	126.23
	1:10	5.12	128.56
	1:20	4.30	132.46
Alternanthera	**1:5**	4.55	127.71
	1:10	4.32	128.65
	1:20	3.80	136.66
LSD(p≤0.05)		**0.19**	**9.21**

Statistical analysis: (Table 5)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	10.767	3	3.589	6.460	.026
	Within Groups	3.333	6	.556		
	Total	14.100	9			
Protein	Between Groups	1045.333	3	348.444	30.447	.001
	Within Groups	68.667	6	11.444		
	Total	1114.000	9			

Pearson's Correlation:

Correlations			
		Amino acids	Protein
Amino acids	Pearson Correlation	1	-.910**
	Sig. (2-tailed)		.000
	N	10	10
Protein	Pearson Correlation	-.910**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-6. . Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata Parthenium hysterophorus* and *Alternanthera sessilis* on changes of DNA and RNA contents in kernels of *Vigna radiata* seeds.

Treatments	Concentration	DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
Control		**108.56**	**857.98**
Desmostachya	**1:5**	38.67	530.22
	1:10	40.21	571.75
	1:20	43.66	586.71
Parthenium	**1:5**	44.61	610.41
	1:10	52.12	628.32
	1:20	52.98	635.78
Alternanthera	**1:5**	60.87	711.70
	1:10	63.55	740.11
	1:20	70.11	760.00
LSD(p≤0.05)		**3.89**	**38.60**

Statistical analysis: (Table 6)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
DNA((µg/g fr. wt.)	Between Groups	3774.014	3	1258.005	74.989	.000
	Within Groups	100.655	6	16.776		
	Total	3874.669	9			
RNA((µg/g fr. wt.)	Between Groups	88997.765	3	29665.922	55.077	.000
	Within Groups	3231.783	6	538.631		
	Total	92229.548	9			

Pearson's Correlation:

Correlations			
		DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
DNA((µg/g fr. wt.)	Pearson Correlation	1	.949**
	Sig. (2-tailed)		.000
	N	10	10
RNA((µg/g fr. wt.)	Pearson Correlation	.949**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-7. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes on Dehydrogenase, Catalase, Peroxidase, Amylase activities in kernels of *Vigna radiata* seeds.

Treatments	Concentration	Dehydrogenase (ΔOD/g/10ml)	Catalase (Unit/h/g fr wt.)	Peroxidase (Unit/h/g fr wt.)	Amylase (Unit/h/g fr wt.)
Control		**0.42**	**121.33**	**68.66**	**44.87**
Desmostachya	**1:5**	0.19	82.20	31.21	64.37
	1:10	0.22	86.77	38.71	64.00
	1:20	0.23	89.03	40.22	62.33
Parthenium	**1:5**	0.26	88.35	48.14	60.29
	1:10	0.27	89.00	55.41	60.00
	1:20	0.32	93.31	57.22	58.11
Alternanthera	**1:5**	0.30	98.22	55.00	58.98
	1:10	0.31	102.71	55.80	57.33
	1:20	0.33	109.30	58.43	52.11
LSD(p≤0.05)		**0.02**	**6.88**	**3.02**	**4.30**

Statistical analysis: (Table 7)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Dehydrogenase	Between Groups	.081	3	.027	47.888	.000
	Within Groups	.003	6	.001		
	Total	.085	9			
Catalase	Between Groups	1189.948	3	396.649	23.595	.001
	Within Groups	100.865	6	16.811		
	Total	1290.814	9			
Peroxidase	Between Groups	1044.181	3	348.060	21.052	.001
	Within Groups	99.202	6	16.534		
	Total	1143.384	9			
Amylase	Between Groups	278.160	3	92.720	18.011	.002
	Within Groups	30.888	6	5.148		
	Total	309.048	9			

Pearson's Correlation

Correlations					
		Dehydrogenase	Catalase	Peroxidase	Amylase
Dehydrogenase	Pearson Correlation	1	-.366	-.117	.435
	Sig. (2-tailed)		.298	.747	.209
	N	10	10	10	10
Catalase	Pearson Correlation	-.366	1	.844**	-.967**
	Sig. (2-tailed)	.298		.002	.000
	N	10	10	10	10
Peroxidase	Pearson Correlation	-.117	.844**	1	-.875**
	Sig. (2-tailed)	.747	.002		.001
	N	10	10	10	10
Amylase	Pearson Correlation	.435	-.967**	-.875**	1
	Sig. (2-tailed)	.209	.000	.001	
	N	10	10	10	10
**. Correlation is significant at the 0.01 level (2-tailed).					

Experiment II

Table-8. Effect of seed pretreatment with dry **leaf leachates** of of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on percentage germination, time (h) for 50% germination (T_{50}) and TTC stainability of *Vigna radiata* seeds.

Treatments	Concentration	Germination (%)	T_{50} (h)	TTC stainability (%)
Control		**100.00**	**45.00**	**100.00**
Desmostachya	**1:5**	32.71	NA	49.39
	1:10	43.51	NA	61.89
	1:20	61.35	110.00	76.77
Parthenium	**1:5**	37.11	NA	55.43
	1:10	46.25	NA	68.71
	1:20	63.16	96.00	82.66
Alternanthera	**1:5**	42.26	NA	55.16
	1:10	56.39	94.00	67.35
	1:20	66.29	72.00	89.53
LSD($p \leq 0.05$)		**2.97**	**4.35**	**4.76**

Statistical analysis: (Table 8)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Germination (%)	Between Groups	2389.546	3	796.515	4.511	.056
	Within Groups	1059.451	6	176.575		
	Total	3448.997	9			
T50 (h)	Between Groups	4857.433	3	1619.144	.885	.500
	Within Groups	10978.667	6	1829.778		
	Total	15836.100	9			
TTC stainability (%)	Between Groups	1060.654	3	353.551	1.567	.292
	Within Groups	1353.869	6	225.645		
	Total	2414.523	9			

Table 9. Effect of seed pretreatment with dry leaf leachates of of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on speed of germination of *Vigna radiata* seeds.

Treatments	**Speed of germination at 24h intervals**							
	Concentration	**24**	**48**	**72**	**96**	**120**	**144**	**168**
Control		**32.15**	**56.60**	**88.71**	**100.00**	**100.00**	**100.00**	**100.00**
Desmostachya	**1:5**	0.00	8.11	15.96	20.15	26.55	30.22	32.11
	1:10	0.00	14.81	19.45	28.22	36.75	42.89	43.51
	1:20	6.81	23.75	38.41	43.44	56.48	59.99	61.35
Parthenium	**1:5**	0.00	16.33	19.37	21.21	29.95	36.51	37.11
	1:10	0.00	18.25	24.24	30.75	39.88	46.25	63.16
	1:20	7.25	30.12	42.11	50.01	56.85	62.00	37.56
Alternanthera	**1:5**	5.50	15.88	28.77	34.66	38.00	39.99	42.66
	1:10	6.25	29.56	39.92	50.05	53.11	56.00	56.39
	1:20	8.66	32.11	50.00	56.11	59.18	65.67	66.29
LSD($p \leq 0.05$)		**0.61**	**0.79**	**1.76**	**2.21**	**2.73**	**3.03**	**3.18**

Statistical analysis: (Table 9)

Two way Analysis of Variance

Groups: 1= Control, 2= *Desmostachya,, 3= Parthenium, 4= Alternanthera*

Hours: 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs

Tests of Between-Subjects Effects					
Dependent Variable: Speed of germination					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35832.848[a]	27	1327.143	10.020	.000
Intercept	117334.142	1	117334.142	885.876	.000
Groups	16404.858	3	5468.286	41.286	.000
Hours	17389.339	6	2898.223	21.882	.000
Groups * Hours	1024.361	18	56.909	.430	.972
Error	5562.896	42	132.450		
Total	144775.519	70			
Corrected Total	41395.744	69			
a. R Squared = .866 (Adjusted R Squared = .779)					

Post Hoc test: LSD (Independent Variable: Group)

Multiple Comparisons						
Dependent Variable: Speed of germination LSD						
(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Desmostachya	53.496190*	5.0228034	.000	43.359763	63.632618
	Parthenium	50.641429*	5.0228034	.000	40.505001	60.777856
	Alternanthera	42.743810*	5.0228034	.000	32.607382	52.880237
Desmostachya	Control	-53.496190*	5.0228034	.000	-63.632618	-43.359763
	Parthenium	-2.854762	3.5516583	.426	-10.022299	4.312775
	Alternanthera	-10.752381*	3.5516583	.004	-17.919918	-3.584844
Parthenium	Control	-50.641429*	5.0228034	.000	-60.777856	-40.505001
	Desmostachya	2.854762	3.5516583	.426	-4.312775	10.022299
	Alternanthera	-7.897619*	3.5516583	.032	-15.065156	-.730082
Alternanthera	Control	-42.743810*	5.0228034	.000	-52.880237	-32.607382
	Desmostachya	10.752381*	3.5516583	.004	3.584844	17.919918
	Parthenium	7.897619*	3.5516583	.032	.730082	15.065156
Based on observed means. The error term is Mean Square(Error) = 132.450.						
*. The mean difference is significant at the 0.05 level.						

Table 10. Effect of seed pretreatment with dry **leaf leachates** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of amino acids and soluble carbohydrates level in *Vigna radiata* seeds.

Treatments	Concentration	Amino acids (mg/g /10ml.)	Soluble carbohydrates (mg/g /10ml.)
Control		**1.92**	**14.66**
Desmostachya	**1:5**	5.99	41.29
	1:10	5.20	38.71
	1:20	4.39	36.83
Parthenium	**1:5**	4.78	31.33
	1:10	4.10	28.00
	1:20	3.36	27.27
Alternanthera	**1:5**	3.86	27.97
	1:10	3.22	22.08
	1:20	3.09	21.28
LSD($p \leq 0.05$)		**0.20**	**1.52**

Statistical analysis: (Table 10)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	9.733	3	3.244	7.405	.019
	Within Groups	2.629	6	.438		
	Total	12.362	9			
Soluble carbohydrates	Between Groups	584.115	3	194.705	25.346	.001
	Within Groups	46.092	6	7.682		
	Total	630.207	9			

Pearson's Correlation:

Correlations			
		Amino acids	Soluble carbohydrates
Amino acids	Pearson Correlation	1	.956**
	Sig. (2-tailed)		.000
	N	10	10
Soluble carbohydrates	Pearson Correlation	.956**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-11. Effect of seed pretreatment with dry **leaf leachates** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble carbohydrates and insoluble carbohydrates level in kernels of *Vigna radiata* seeds.

Treatments	Concentration	Soluble carbohydrates (mg/g fr wt.)	Insoluble carbohydrates (mg/g fr wt.)
Control		**13.90**	**132.81**
Desmostachya	**1:5**	20.44	124.42
	1:10	20.24	126.37
	1:20	18.12	127.36
Parthenium	**1:5**	20.56	124.18
	1:10	19.42	124.23
	1:20	18.28	125.18
Alternanthera	**1:5**	18.31	127.40
	1:10	15.29	127.82
	1:20	14.08	128.40
LSD($p \leq 0.05$)		**1.44**	**8.66**

Statistical analysis: (Table 11)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Soluble carbohydrates	Between Groups	43.668	3	14.556	5.672	.035
	Within Groups	15.397	6	2.566		
	Total	59.066	9			
Insoluble carbohydrates	Between Groups	56.720	3	18.907	20.204	.002
	Within Groups	5.615	6	.936		
	Total	62.334	9			

Pearson's Correlation:

Correlations			
		Soluble carbohydrates	Insoluble carbohydrates
Soluble carbohydrates	Pearson Correlation	1	-.840**
	Sig. (2-tailed)		.002
	N	10	10
Insoluble carbohydrates	Pearson Correlation	-.840**	1
	Sig. (2-tailed)	.002	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-12 . Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata* *Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble amino acids and protein level in kernels of *Vigna radiata* seeds.

Treatments	Concentration	Amino acids (mg/g fr wt.)	Protein (mg/g fr wt.)
Control		**2.03**	**160.32**
Desmostachya	**1:5**	5.53	120.59
	1:10	5.02	121.56
	1:20	4.62	126.44
Parthenium	**1:5**	4.66	127.38
	1:10	4.41	132.32
	1:20	3.87	138.30
Alternanthera	**1:5**	4.41	138.33
	1:10	4.10	140.39
	1:20	3.67	144.42
LSD($p \leq 0.05$)		**0.19**	**9.81**

Statistical analysis: (Table 12)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	7.131	3	2.377	14.004	.004
	Within Groups	1.018	6	.170		
	Total	8.149	9			
Protein	Between Groups	1192.909	3	397.636	24.184	.001
	Within Groups	98.654	6	16.442		
	Total	1291.562	9			

Pearson's Correlation:

Correlations			
		Amino acids	Protein
Amino acids	Pearson Correlation	1	-.963**
	Sig. (2-tailed)		.000
	N	10	10
Protein	Pearson Correlation	-.963**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-13. . Effect of seed pretreatment with dry **leaf leachates** of *Desmostachya bipinnata* *Parthenium hysterophorous* and *Alternanthera sessilis* changes of DNA and RNA contents in kernels of *Vigna radiata* seeds.

Treatments	Concentration	DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
Control		**108.56**	**857.98**
Desmostachya	**1:5**	40.55	550.44
	1:10	43.61	565.78
	1:20	45.43	589.00
Parthenium	**1:5**	55.78	680.00
	1:10	56.10	693.45
	1:20	63.77	721.66
Alternanthera	**1:5**	70.28	762.03
	1:10	82.19	779.23
	1:20	88.71	807.11
LSD(p≤0.05)		**3.83**	**34.71**

Statistical analysis: (Table 13)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
DNA((µg/g fr. wt.)	Between Groups	4156.821	3	1385.607	36.501	.000
	Within Groups	227.762	6	37.960		
	Total	4384.583	9			
RNA((µg/g fr. wt.)	Between Groups	97474.157	3	32491.386	72.391	.000
	Within Groups	2692.987	6	448.831		
	Total	100167.144	9			

Pearson's Correlation:

Correlations			
		DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
DNA((µg/g fr. wt.)	Pearson Correlation	1	.955**
	Sig. (2-tailed)		.000
	N	10	10
RNA((µg/g fr. wt.)	Pearson Correlation	.955**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-14. Effect of seed pretreatment with dry **leaf leachates** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes on Dehydrogenase, Catalase, Peroxidase, Amylase activities in kernels of *Vigna radiata* seeds.

Treatments	Concentration	Dehydrogenase (ΔOD/g/10ml)	Catalase (Unit/h/g fr wt.)	Peroxidase (Unit/h/g fr wt.)	Amylase (Unit/h/g fr wt.)
Control		**0.42**	**121.33**	**68.66**	**44.87**
Desmostachya	**1:5**	0.22	87.25	42.35	65.65
	1:10	0.25	89.36	44.72	61.22
	1:20	0.29	94.37	50.80	58.43
Parthenium	**1:5**	0.28	98.77	56.11	57.36
	1:10	0.29	100.14	60.89	54.71
	1:20	0.33	103.71	60.22	53.55
Alternanthera	**1:5**	0.33	102.77	60.33	53.61
	1:10	0.35	109.73	62.90	49.33
	1:20	0.36	110.38	62.11	48.82
LSD($p \leq 0.05$)		**0.02**	**7.32**	**4.30**	**4.56**

Statistical analysis: (Table 14)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Dehydrogenase	Between Groups	.094	3	.031	43.415	.000
	Within Groups	.004	6	.001		
	Total	.098	9			
Catalase	Between Groups	867.951	3	289.317	23.038	.001
	Within Groups	75.349	6	12.558		
	Total	943.301	9			
Peroxidase	Between Groups	591.274	3	197.091	21.557	.001
	Within Groups	54.858	6	9.143		
	Total	646.132	9			
Amylase	Between Groups	295.386	3	98.462	12.313	.006
	Within Groups	47.981	6	7.997		
	Total	343.367	9			

Pearson's Correlation:

Correlations					
		Dehydrogenase	Catalase	Peroxidase	Amylase
Dehydrogenase	Pearson Correlation	1	-.295	-.124	.163
	Sig. (2-tailed)		.408	.732	.652
	N	10	10	10	10
Catalase	Pearson Correlation	-.295	1	.946**	-.982**
	Sig. (2-tailed)	.408		.000	.000
	N	10	10	10	10
Peroxidase	Pearson Correlation	-.124	.946**	1	-.958**
	Sig. (2-tailed)	.732	.000		.000
	N	10	10	10	10
Amylase	Pearson Correlation	.163	-.982**	-.958**	1
	Sig. (2-tailed)	.652	.000	.000	
	N	10	10	10	10
**. Correlation is significant at the 0.01 level (2-tailed).					

Experiment No.III:

Table15. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on percentage germination, time (h) for 50% germination (T_{50}) and TTC stainability of *Senna occidentalis* seeds.

Treatments	Concentration	Germination (%)	T_{50} (h)	TTC stainability (%)
Control		**100.00**	**43.00**	**100.00**
Desmostachya	**1:5**	27.94	NA	48.33
	1:10	42.33	NA	59.26
	1:20	60.24	120.00	73.88
Parthenium	**1:5**	41.05	NA	57.61
	1:10	48.66	NA	62.76
	1:20	65.92	98.00	88.10
Alternanthera	**1:5**	49.78	NA	72.46
	1:10	61.24	96.25	78.10
	1:20	77.55	92.00	92.36
LSD($p \leq 0.05$)		**2.67**	**4.25**	**4.81**

Statistical analysis: (Table 15)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Germination (%)	Between Groups	2574.909	3	858.303	4.160	.065
	Within Groups	1237.996	6	206.333		
	Total	3812.905	9			
T50 (h)	Between Groups	1480.465	3	493.488	.135	.936
	Within Groups	21918.042	6	3653.007		
	Total	23398.506	9			
TTC stainability (%)	Between Groups	1425.365	3	475.122	2.660	.142
	Within Groups	1071.819	6	178.637		
	Total	2497.184	9			

Table 16. Effect of seed pretreatment with dry **leaf extracts** of of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on speed of germination of *Senna occidentalis* seeds.

Treatments	**Speed of germination at 24h intervals**							
	Concentration	**24**	**48**	**72**	**96**	**120**	**144**	**168**
Control		**24.11**	**52.45**	**80.00**	**96.75**	**100.00**	**100.00**	**100.00**
Desmostachya	**1:5**	0.00	4.66	10.44	14.88	20.13	27.94	27.94
	1:10	0.00	6.75	16.21	28.11	34.56	39.85	42.33
	1:20	0.00	19.44	38.66	45.52	53.78	58.26	60.24
Parthenium	**1:5**	0.00	7.08	13.99	26.76	32.71	40.00	41.05
	1:10	0.00	10.56	19.21	30.44	40.69	48.50	48.66
	1:20	0.00	16.30	36.71	50.90	58.25	64.13	65.92
Alternanthera	**1:5**	0.00	13.55	30.66	38.11	42.65	48.05	49.78
	1:10	0.00	28.11	42.16	50.05	56.77	60.48	61.24
	1:20	0.00	39.68	46.72	52.66	66.11	75.99	77.55
LSD(p≤0.05)		**NC**	**0.50**	**1.02**	**1.51**	**2.00**	**2.17**	**2.73**

Statistical analysis: (Table 16)
Post Hoc Test: LSD (Independent variable: Group)

Multiple Comparisons						
Dependent Variable: Speed of germination LSD						
(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Desmostachya	52.868095*	5.1712394	.000	42.432112	63.304079
	Parthenium	48.003333*	5.1712394	.000	37.567350	58.439317
	Alternanthera	37.124286*	5.1712394	.000	26.688302	47.560269
Desmostachya	Control	-52.868095*	5.1712394	.000	-63.304079	-42.432112
	Parthenium	-4.864762	3.6566185	.191	-12.244117	2.514593
	Alternanthera	-15.743810*	3.6566185	.000	-23.123164	-8.364455
Parthenium	Control	-48.003333*	5.1712394	.000	-58.439317	-37.567350
	Desmostachya	4.864762	3.6566185	.191	-2.514593	12.244117
	Alternanthera	-10.879048*	3.6566185	.005	-18.258402	-3.499693
Alternanthera	Control	-37.124286*	5.1712394	.000	-47.560269	-26.688302
	Desmostachya	15.743810*	3.6566185	.000	8.364455	23.123164
	Parthenium	10.879048*	3.6566185	.005	3.499693	18.258402
Based on observed means. The error term is Mean Square(Error) = 140.394.						
*. The mean difference is significant at the 0.05 level.						

Two way Analysis of Variance

Groups: 1= Control, 2= *Desmostachya,, 3= Parthenium, 4= Alternanthera*

Hours: 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs

Tests of Between-Subjects Effects					
Dependent Variable: Speed of germination					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42574.409[a]	27	1576.830	11.231	.000
Intercept	111120.175	1	111120.175	791.488	.000
Groups	16059.182	3	5353.061	38.129	.000
Hours	23140.554	6	3856.759	27.471	.000
Groups * Hours	1342.502	18	74.583	.531	.926
Error	5896.549	42	140.394		
Total	147674.191	70			
Corrected Total	48470.958	69			
a. R Squared = .878 (Adjusted R Squared = .800)					

Fig4. Graphical presentation of coefficient of determination (R^2) at 1:10 leaf extract of *Desmostachya, Parthenium* and *Alternanthera on Senna occidentalis seeds* using MS Excel

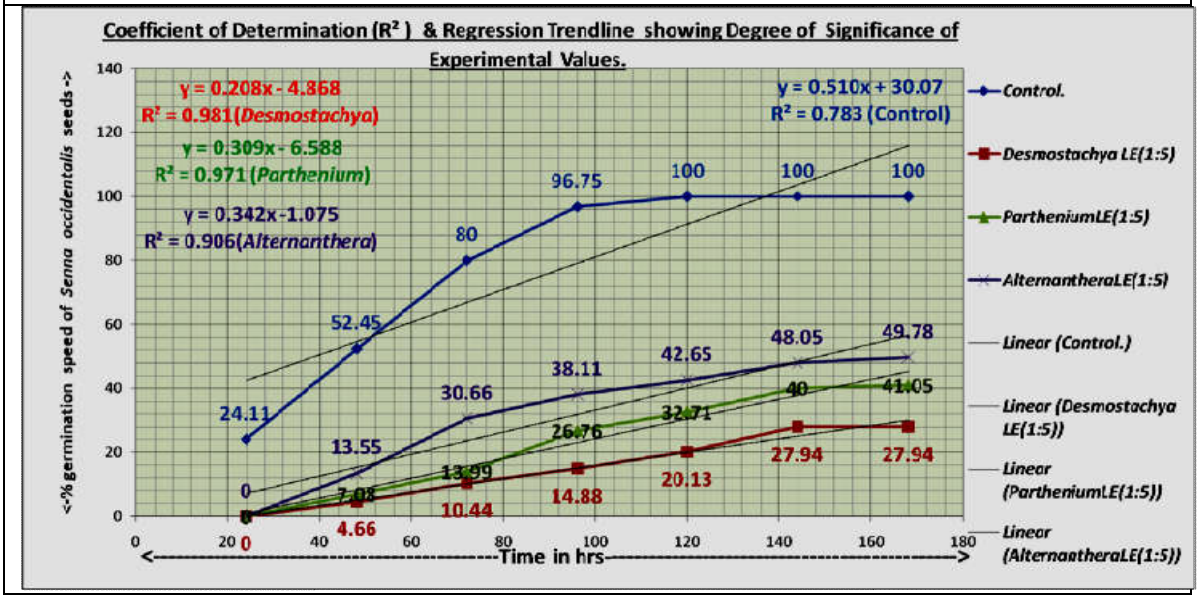


Fig5. Graphical presentation of coefficient of determination (R^2) at 1:10 leaf extract of *Desmostachya, Parthenium* and *Alternanthera on Senna occidentalis seeds* using MS Excel

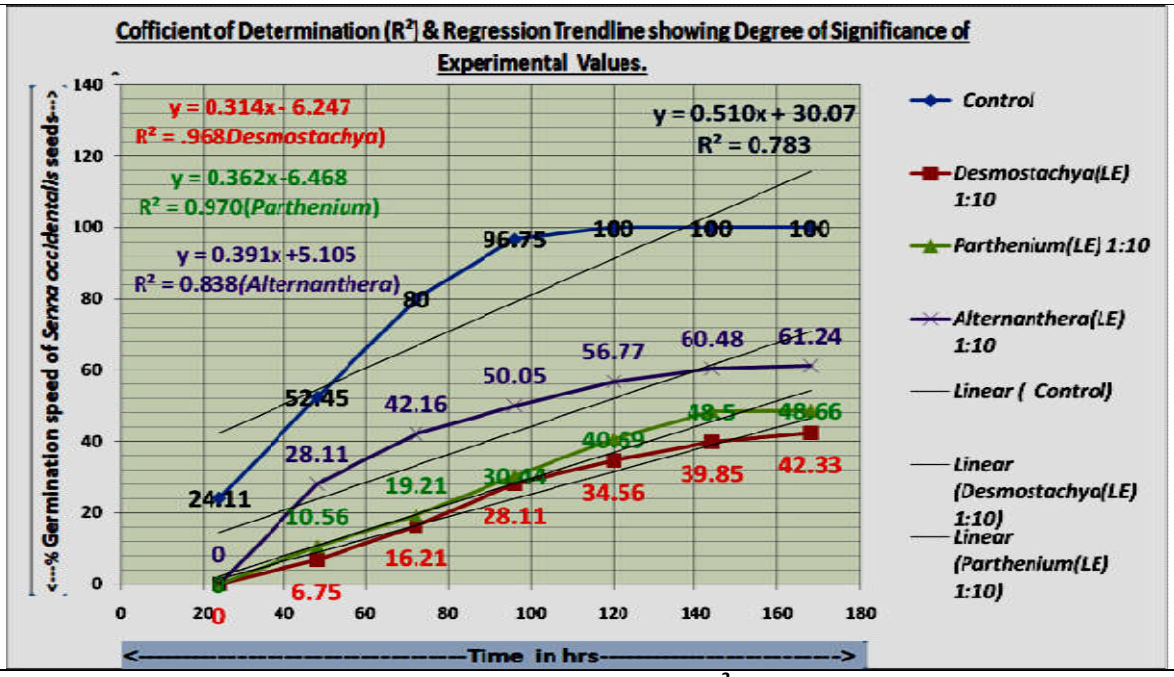


Fig6. Graphical presentation of coefficient of determination (R^2) at 1:20 leaf extract of *Desmostachya, Parthenium* and *Alternanthera on Senna occidentalis seeds* using MS Excel

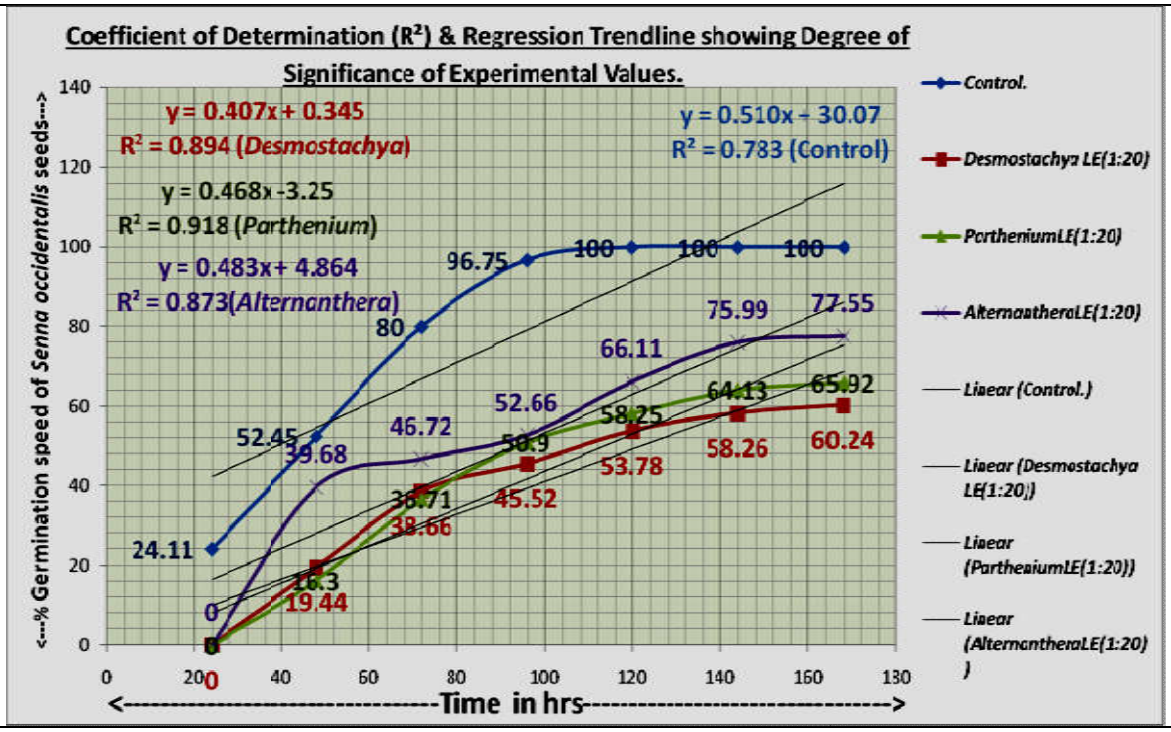


Table-17. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata* ,*Parthenium hysterophorus* and *Alternanthera sessilis* on changes of amino acids and soluble carbohydrates level in *Senna occidentalis* seeds.

Treatments	Concentration	Amino acids (mg/g /10ml.)	Soluble carbohydrates (mg/g /10ml.)
Control		**1.30**	**12.05**
Desmostachya	**1:5**	6.33	42.23
	1:10	6.43	41.37
	1:20	5.39	37.49
Parthenium	**1:5**	5.73	37.89
	1:10	5.35	36.76
	1:20	4.89	32.21
Alternanthera	**1:5**	5.21	30.00
	1:10	4.25	27.35
	1:20	3.89	25.49
LSD($p \leq 0.05$)		**0.12**	**1.18**

Statistical analysis: (Table 17)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	18.067	3	6.022	18.593	.002
	Within Groups	1.943	6	.324		
	Total	20.011	9			
Soluble carbohydrates	Between Groups	704.074	3	234.691	34.254	.000
	Within Groups	41.109	6	6.851		
	Total	745.182	9			

Pearson's Correlation:

Correlations			
		Amino acids	Soluble carbohydrates
Amino acids	Pearson Correlation	1	.979**
	Sig. (2-tailed)		.000
	N	10	10
Soluble carbohydrates	Pearson Correlation	.979**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-18. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble carbohydrates and insoluble carbohydrates level in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	Soluble carbohydrates (mg/g fr wt.)	Insoluble carbohydrates (mg/g fr wt.)
Control		**13.00**	**127.33**
Desmostachya	**1:5**	19.44	110.68
	1:10	19.42	118.26
	1:20	18.32	119.36
Parthenium	**1:5**	18.53	116.39
	1:10	17.60	120.27
	1:20	16.64	122.22
Alternanthera	**1:5**	16.58	117.36
	1:10	16.46	120.28
	1:20	15.35	122.81
LSD($p \leq 0.05$)		**1.28**	**8.61**

Statistical analysis: (Table 18)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Soluble carbohydrates	Between Groups	31.866	3	10.622	18.067	.002
	Within Groups	3.528	6	.588		
	Total	35.394	9			
Insoluble carbohydrates	Between Groups	97.304	3	32.435	2.522	.154
	Within Groups	77.161	6	12.860		
	Total	174.466	9			

Pearson's Correlation:

Correlations			
		Soluble carbohydrates	Insoluble carbohydrates
Soluble carbohydrates	Pearson Correlation	1	-.843**
	Sig. (2-tailed)		.002
	N	10	10
Insoluble carbohydrates	Pearson Correlation	-.843**	1
	Sig. (2-tailed)	.002	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-19 . Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata* *Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble amino acids and protein level in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	Amino acids (mg/g fr wt.)	Protein (mg/g fr wt.)
Control		**1.88**	**148.75**
Desmostachya	**1:5**	5.66	120.89
	1:10	5.32	122.39
	1:20	4.58	125.44
Parthenium	**1:5**	5.38	124.83
	1:10	4.79	126.76
	1:20	4.32	127.66
Alternanthera	**1:5**	4.68	125.63
	1:10	3.52	128.60
	1:20	3.45	130.66
LSD(p≤0.05)		**0.18**	**9.70**

Statistical analysis: (Table 19)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	9.545	3	3.182	8.968	.012
	Within Groups	2.129	6	.355		
	Total	11.673	9			
Protein	Between Groups	515.914	3	171.971	37.221	.000
	Within Groups	27.721	6	4.620		
	Total	543.636	9			

Pearson's Correlation:

Correlations			
		Amino acids	Protein
Amino acids	Pearson Correlation	1	-.930**
	Sig. (2-tailed)		.000
	N	10	10
Protein	Pearson Correlation	-.930**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-20 . Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata* *Parthenium hysterophorus* and *Alternanthera sessilis* on changes of DNA and RNA contents in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
Control		**101.33**	**830.00**
Desmostachya	**1:5**	42.36	512.21
	1:10	46.22	537.33
	1:20	46.89	550.48
Parthenium	**1:5**	55.30	630.17
	1:10	58.59	685.39
	1:20	64.31	730.56
Alternanthera	**1:5**	72.11	702.72
	1:10	72.36	730.66
	1:20	74.16	733.22
LSD(p≤0.05)		**4.18**	**37.99**

Statistical analysis: (Table 20)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
DNA((µg/g fr. wt.)	Between Groups	2754.550	3	918.183	98.320	.000
	Within Groups	56.032	6	9.339		
	Total	2810.583	9			
RNA((µg/g fr. wt.)	Between Groups	89909.401	3	29969.800	28.165	.001
	Within Groups	6384.569	6	1064.095		
	Total	96293.970	9			

Pearson's Correlation:

Correlations			
		DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
DNA((µg/g fr. wt.)	Pearson Correlation	1	.939**
	Sig. (2-tailed)		.000
	N	10	10
RNA((µg/g fr. wt.)	Pearson Correlation	.939**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-21. Effect of seed pretreatment with dry **leaf extracts** of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes on Dehydrogenase, Catalase, Peroxidase, Amylase activities in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	Dehydrogenase (ΔOD/g/10ml)	Catalase (Unit/h/g fr wt.)	Peroxidase (Unit/h/g fr wt.)	Amylase (Unit/h/g fr wt.)
Control		**0.41**	**118.75**	**64.25**	**45.61**
Desmostachya	**1:5**	0.19	70.98	33.26	67.35
	1:10	0.23	72.33	36.35	63.22
	1:20	0.24	72.98	39.66	60.98
Parthenium	**1:5**	0.29	75.33	44.66	58.71
	1:10	0.30	76.71	47.34	55.14
	1:20	0.33	83.66	48.41	55.00
Alternanthera	**1:5**	0.33	76.76	48.42	50.81
	1:10	0.35	82.42	50.35	50.04
	1:20	0.36	89.35	55.69	49.39
LSD(p≤0.05)		**0.03**	**6.98**	**3.28**	**4.31**

Statistical analysis: (Table 21)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Dehydrogenase	Between Groups	.038	3	.013	27.715	.001
	Within Groups	.003	6	.000		
	Total	.041	9			
Catalase	Between Groups	1682.234	3	560.745	27.698	.001
	Within Groups	121.470	6	20.245		
	Total	1803.704	9			
Peroxidase	Between Groups	693.407	3	231.136	24.626	.001
	Within Groups	56.316	6	9.386		
	Total	749.723	9			
Amylase	Between Groups	396.793	3	132.264	25.818	.001
	Within Groups	30.737	6	5.123		
	Total	427.531	9			

Pearson's Correlation:

Correlations					
		Dehydrogenase	Catalase	Peroxidase	Amylase
Dehydrogenase	Pearson Correlation	1	.806**	.978**	-.982**
	Sig. (2-tailed)		.005	.000	.000
	N	10	10	10	10
Catalase	Pearson Correlation	.806**	1	.884**	-.753*
	Sig. (2-tailed)	.005		.001	.012
	N	10	10	10	10
Peroxidase	Pearson Correlation	.978**	.884**	1	-.957**
	Sig. (2-tailed)	.000	.001		.000
	N	10	10	10	10
Amylase	Pearson Correlation	-.982**	-.753*	-.957**	1
	Sig. (2-tailed)	.000	.012	.000	
	N	10	10	10	10
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Experiment No.IV:

Table-22. Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on percentage germination, time (h) for 50% germination (T_{50}) and TTC stainability of *Senna occidentalis* seeds.

Treatments	Concentration	Germination (%)	T_{50} (h)	TTC stainability (%)
Control		100	43.00	100
Desmostachya	1:5	37.92	NA	53.28
	1:10	48.71	NA	63.71
	1:20	64.66	108.25	82.14
Parthenium	1:5	49.22	NA	70.05
	1:10	57.81	122.10	79.76
	1:20	76.21	96.20	89.45
Alternanthera	1:5	52.33	118.00	74.18
	1:10	63.89	98.50	80.03
	1:20	80.37	70.50	91.77
LSD($p \leq 0.05$)		3.88	4.12	4.79

NA: Non-attainment of 50% germination.

Statistical analysis: (Table 22)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Germination (%)	Between Groups	1873.151	3	624.384	3.288	.100
	Within Groups	1139.376	6	189.896		
	Total	3012.527	9			
T50 (h)	Between Groups	5993.485	3	1997.828	.696	.588
	Within Groups	17222.880	6	2870.480		
	Total	23216.365	9			
TTC stainability (%)	Between Groups	944.459	3	314.820	2.435	.163
	Within Groups	775.783	6	129.297		
	Total	1720.241	9			

Table 23. Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on speed of germination of *Senna occidentalis* seeds.

Treatments	**Speed of germination at 24h intervals**							
	Concentration	**24**	**48**	**72**	**96**	**120**	**144**	**168**
Control		**24.11**	**52.45**	**80.00**	**96.75**	**100.00**	**100.00**	**100.00**
Desmostachya	**1:5**	0.00	6.76	14.18	24.48	34.65	37.92	37.92
	1:10	0.00	15.61	29.28	37.55	42.68	47.99	48.71
	1:20	8.98	20.35	33.33	43.99	54.60	63.56	64.66
Parthenium	**1:5**	0.00	10.23	28.56	33.86	42.66	49.00	49.20
	1:10	6.22	18.41	29.32	41.71	50.89	56.75	57.81
	1:20	10.14	38.82	44.39	53.22	69.26	73.36	76.21
Alternanthera	**1:5**	11.15	23.66	37.19	42.15	50.92	51.29	52.33
	1:10	12.66	28.56	39.33	53.66	58.17	63.00	63.89
	1:20	13.00	36.11	52.26	66.50	72.86	79.59	80.37
LSD(p≤0.05)		**0.57**	**0.70**	**1.38**	**2.37**	**3.50**	**3.82**	**3.88**

Statistical analysis: (Table 23)

Post Hoc Test: LSD (Independent variable: Group)

Multiple Comparisons						
Dependent Variable: Speed of germination LSD						
(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Desmostachya	47.272857*	4.7167974	.000	37.753975	56.791740
	Parthenium	39.043333*	4.7167974	.000	29.524451	48.562216
	Alternanthera	31.965714*	4.7167974	.000	22.446832	41.484597
Desmostachya	Control	-47.272857*	4.7167974	.000	-56.791740	-37.753975
	Parthenium	-8.229524*	3.3352794	.018	-14.960390	-1.498657
	Alternanthera	-15.307143*	3.3352794	.000	-22.038009	-8.576276
Parthenium	Control	-39.043333*	4.7167974	.000	-48.562216	-29.524451
	Desmostachya	8.229524*	3.3352794	.018	1.498657	14.960390
	Alternanthera	-7.077619*	3.3352794	.040	-13.808485	-.346753
Alternanthera	Control	-31.965714*	4.7167974	.000	-41.484597	-22.446832
	Desmostachya	15.307143*	3.3352794	.000	8.576276	22.038009
	Parthenium	7.077619*	3.3352794	.040	.346753	13.808485
Based on observed means. The error term is Mean Square(Error) = 116.803.						
*. The mean difference is significant at the 0.05 level.						

Two way Analysis of Variance

Groups: 1= Control, 2= *Desmostachya,, 3= Parthenium, 4= Alternanthera*

Hours: 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs

Tests of Between-Subjects Effects					
Dependent Variable: Speed of germination					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38647.615[a]	27	1431.393	12.255	.000
Intercept	137068.838	1	137068.838	1173.505	.000
Groups	12258.311	3	4086.104	34.983	.000
Hours	23361.295	6	3893.549	33.334	.000
Groups * Hours	904.297	18	50.239	.430	.972
Error	4905.723	42	116.803		
Total	176374.748	70			
Corrected Total	43553.338	69			
a. R Squared = .887 (Adjusted R Squared = .815)					

Fig7. Graphical presentation of coefficient of determination (R^2) at 1:5 leaf leachate of *Desmostachya, Parthenium* and *Alternanthera on Senna occidentalis seeds* using MS Excel

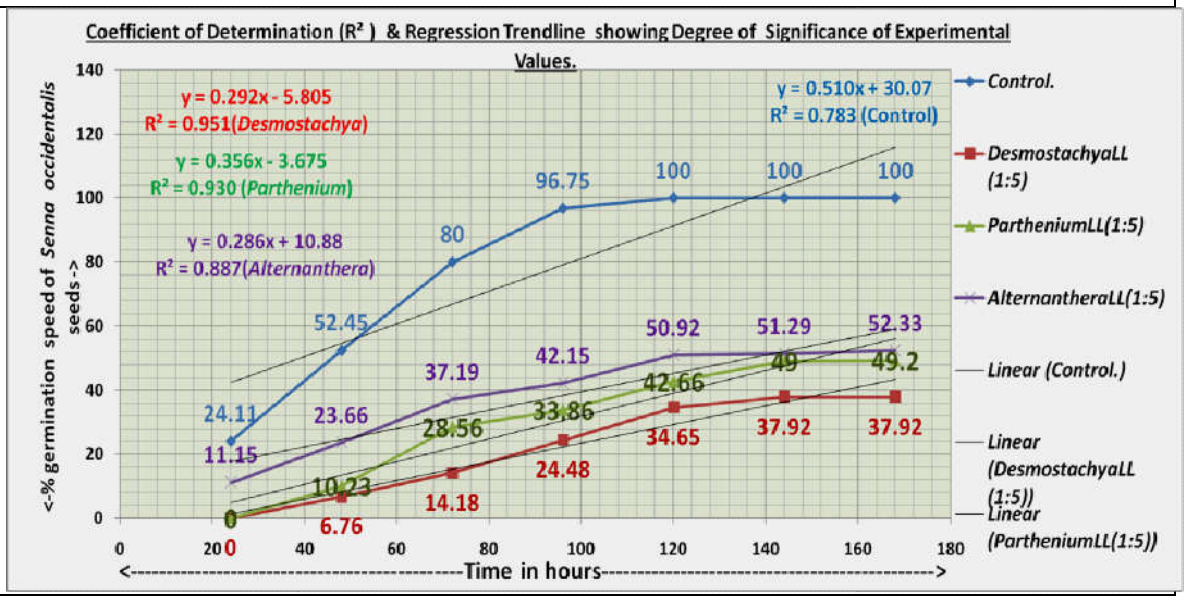


Fig8. Graphical presentation of coefficient of determination (R^2) at 1:10 leaf leachate of *Desmostachya, Parthenium* and *Alternanthera on Senna occidentalis seeds* using MS Excel

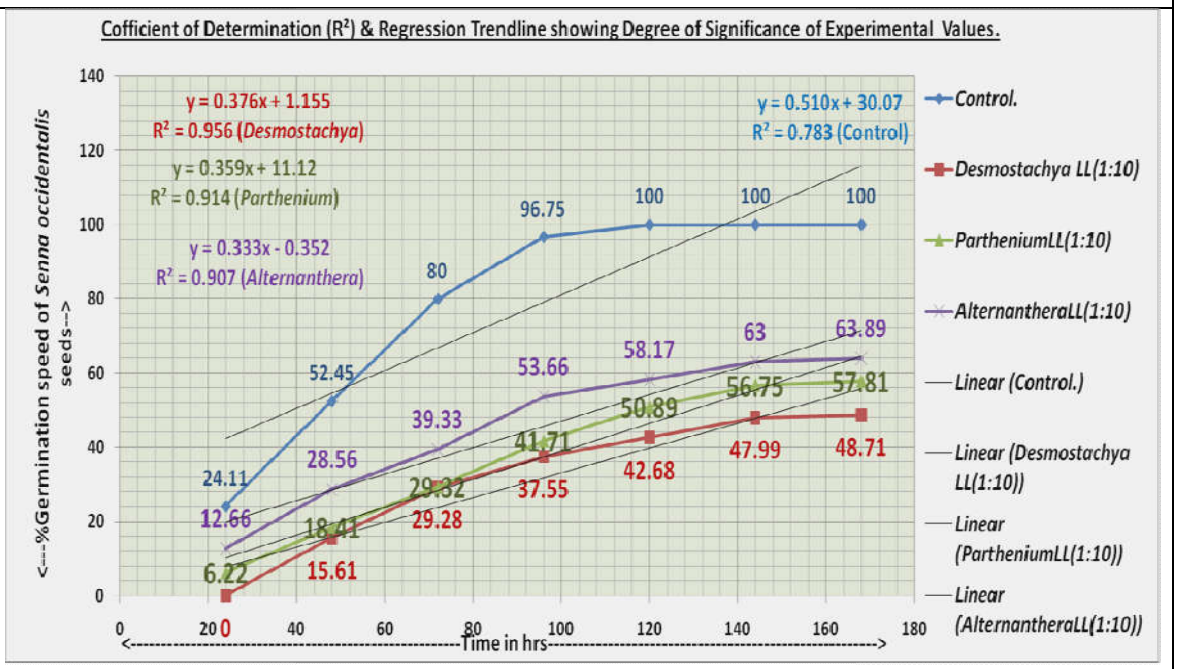


Fig9. Graphical presentation of coefficient of determination (R^2) at 1:20 leaf leachate of *Desmostachya, Parthenium* and *Alternanthera on Senna occidentalis seeds* using MS Excel

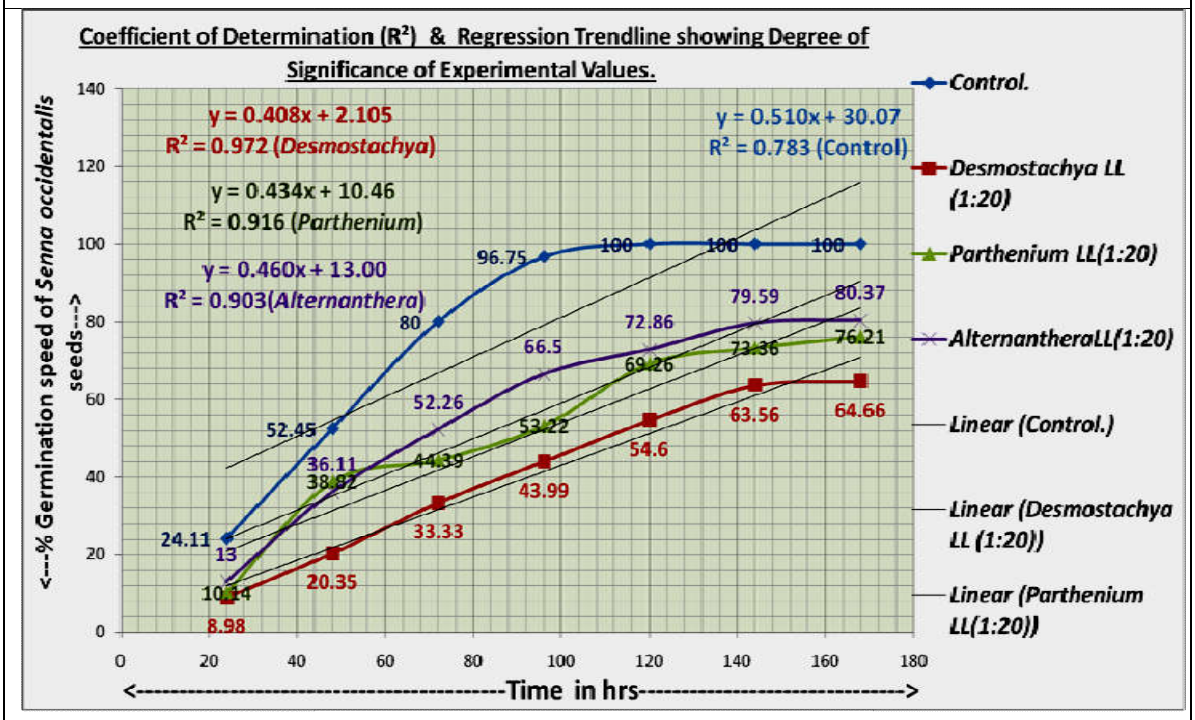


Table-24.. Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorous* and *Alternanthera sessilis* on changes of amino acids and soluble carbohydrates level in *Senna occidentalis* seeds.

	Concentration	**Amino acids (mg/g /10ml.)**	**Soluble carbohydrates (mg/g /10ml.)**
Control		**1.30**	**12.05**
Desmostachya	**1:5**	5.28	40.66
	1:10	4.31	41.53
	1:20	4.09	35.71
Parthenium	**1:5**	4.66	35.96
	1:10	4.56	34.33
	1:20	3.33	30.48
Alternanthera	**1:5**	3.34	28.65
	1:10	3.27	23.66
	1:20	2.53	20.89
LSD(p≤0.05)		**0.13**	**1.18**

Statistical analysis: (Table 24)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	9.949	3	3.316	8.644	.013
	Within Groups	2.302	6	.384		
	Total	12.251	9			
Soluble carbohydrates	Between Groups	712.880	3	237.627	21.447	.001
	Within Groups	66.477	6	11.080		
	Total	779.358	9			

Pearson's Correlation:

Correlations			
		Amino acids	Soluble carbohydrates
Amino acids	Pearson Correlation	1	.946**
	Sig. (2-tailed)		.000
	N	10	10
Soluble carbohydrates	Pearson Correlation	.946**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-25. Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble carbohydrates and insoluble carbohydrates level in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	Soluble carbohydrates (mg/g fr wt.)	Insoluble carbohydrates (mg/g fr wt.)
Control		**13.00**	**127.33**
Desmostachya	**1:5**	18.30	120.46
	1:10	17.26	120.98
	1:20	15.14	121.09
Parthenium	**1:5**	17.59	120.20
	1:10	17.56	123.19
	1:20	14.39	123.26
Alternanthera	**1:5**	14.32	121.37
	1:10	13.79	123.31
	1:20	13.29	124.28
LSD($p \leq 0.05$)		**1.32**	**9.01**

Statistical analysis: (Table 25)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Soluble carbohydrates	Between Groups	23.868	3	7.956	3.825	.076
	Within Groups	12.481	6	2.080		
	Total	36.349	9			
Insoluble carbohydrates	Between Groups	32.492	3	10.831	6.062	.030
	Within Groups	10.720	6	1.787		
	Total	43.212	9			

Pearson's Correlation:

Correlations			
		Soluble carbohydrates	Insoluble carbohydrates
Soluble carbohydrates	Pearson Correlation	1	-.710*
	Sig. (2-tailed)		.021
	N	10	10
Insoluble carbohydrates	Pearson Correlation	-.710*	1
	Sig. (2-tailed)	.021	
	N	10	10
*. Correlation is significant at the 0.05 level (2-tailed).			

Table-26. . Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata* *Parthenium hysterophorus* and *Alternanthera sessilis* on changes of soluble amino acids and protein level in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	Amino acids (mg/g fr wt.)	Protein (mg/g fr wt.)
Control		**1.88**	**148.75**
Desmostachya	**1:5**	5.39	126.38
	1:10	5.37	128.25
	1:20	4.27	128.56
Parthenium	**1:5**	5.44	128.62
	1:10	4.46	130.31
	1:20	3.36	131.43
Alternanthera	**1:5**	4.39	130.33
	1:10	3.38	130.41
	1:20	3.21	132.39
LSD($p \leq 0.05$)		**0.14**	**8.82**

Statistical analysis: (Table 26)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Amino acids	Between Groups	8.298	3	2.766	4.366	.059
	Within Groups	3.801	6	.634		
	Total	12.099	9			
Protein	Between Groups	346.522	3	115.507	72.895	.000
	Within Groups	9.507	6	1.585		
	Total	356.029	9			

Pearson's Correlation:

Correlations			
		Amino acids	Protein
Amino acids	Pearson Correlation	1	-.825**
	Sig. (2-tailed)		.003
	N	10	10
Protein	Pearson Correlation	-.825**	1
	Sig. (2-tailed)	.003	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-27. . Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata* *Parthenium hysterophorus* and *Alternanthera sessilis* on changes of DNA and RNA contents in kernels of *Senna occidentalis* seeds..

Treatments	Concentration	DNA((µg/g fr. wt.)	RNA((µg/g fr. wt.)
Control		**101.33**	**830.00**
Desmostachya	**1:5**	50.55	548.37
	1:10	56.78	562.80
	1:20	59.41	604.44
Parthenium	**1:5**	68.11	732.22
	1:10	72.30	741.79
	1:20	73.15	750.00
Alternanthera	**1:5**	80.25	765.39
	1:10	84.33	782.14
	1:20	85.97	795.33
LSD(p≤0.05)		**4.95**	**42.30**

Statistical analysis: (Table 27)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
DNA	Between Groups	2054.148	3	684.716	56.031	.000
	Within Groups	73.321	6	12.220		
	Total	2127.470	9			
RNA	Between Groups	89673.202	3	29891.067	77.841	.000
	Within Groups	2304.006	6	384.001		
	Total	91977.209	9			

Pearson's Correlation:

Correlations			
		DNA	RNA
DNA	Pearson Correlation	1	.933**
	Sig. (2-tailed)		.000
	N	10	10
RNA	Pearson Correlation	.933**	1
	Sig. (2-tailed)	.000	
	N	10	10
**. Correlation is significant at the 0.01 level (2-tailed).			

Table-28. Effect of seed pretreatment with dry leaf leachates of *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* on changes of Dehydrogenase, Catalase, Peroxidase, Amylase activities in kernels of *Senna occidentalis* seeds.

Treatments	Concentration	Dehydrogenase (ΔOD/g/10ml)	Catalase (Unit/h/g fr wt.)	Peroxidase (Unit/h/g fr wt.)	Amylase (Unit/h/g fr wt.)
Control		**0.41**	**118.75**	**64.25**	**45.61**
esmostachya	**1:5**	0.20	76.33	42.68	63.71
	1:10	0.24	80.15	45.93	62.11
	1:20	0.25	89.64	50.38	60.44
Parthenium	**1:5**	0.31	89.75	50.32	52.25
	1:10	0.33	91.37	50.81	52.01
	1:20	0.34	95.32	54.66	50.78
Alternanthera	**1:5**	0.33	97.00	56.00	54.31
	1:10	0.35	100.2	56.11	50.88
	1:20	0.37	109.81	58.67	48.10
LSD(p≤0.05)		**0.02**	**7.52**	**4.09**	**4.48**

Statistical analysis: (Table 28)

Analysis of Variance

Groups: Control, *Desmostachya,, Parthenium, Alternanthera*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Dehydrogenase	Between Groups	.035	3	.012	26.057	.001
	Within Groups	.003	6	.000		
	Total	.037	9			
Catalase	Between Groups	1253.570	3	417.857	12.583	.005
	Within Groups	199.250	6	33.208		
	Total	1452.819	9			
Peroxidase	Between Groups	309.716	3	103.239	13.540	.004
	Within Groups	45.749	6	7.625		
	Total	355.466	9			
Amylase	Between Groups	308.006	3	102.669	23.744	.001
	Within Groups	25.944	6	4.324		
	Total	333.949	9			

Pearson's Correlation:

Correlations					
		Dehydrogenase	Catalase	Peroxidase	Amylase
Dehydrogenase	Pearson Correlation	1	.932**	.944**	-.982**
	Sig. (2-tailed)		.000	.000	.000
	N	10	10	10	10
Catalase	Pearson Correlation	.932**	1	.985**	-.895**
	Sig. (2-tailed)	.000		.000	.000
	N	10	10	10	10
Peroxidase	Pearson Correlation	.944**	.985**	1	-.892**
	Sig. (2-tailed)	.000	.000		.001
	N	10	10	10	10
Amylase	Pearson Correlation	-.982**	-.895**	-.892**	1
	Sig. (2-tailed)	.000	.000	.001	
	N	10	10	10	10
**. Correlation is significant at the 0.01 level (2-tailed).					

Chapter-1

DISCUSSION

&

CONCLUSION

A) <u>Analysis and discussion of tables -1,8,15 and 22.</u>

As clearly evident from the data in results section, percentage germination of both *Vigna* and *Senna* seeds were found to be greatest among the controls (100%) in each of the cases, followed in a descending order by *Alternanthera sessilis, Parthenium hysterophorus* and *Desmostachya bipinnata*-dry leaf extract or leachate treated *V.radiata* or *S. occidentalis* seeds as the case may be. Thus *Desmostachya* is more inhibitory to germination of bioassay seeds than *Parthenium* and *Alternanthera.*

Germination percentage of test seeds were reduced with increasing concentration of leaf extract or leachate of each of the test weeds indicating that - a more concentrated one was found to be more inhibitory to germination of *V,radiata* and or *S.occidentalis* seeds than that of diluted one.

Among the seeds of either *V. radiata* or *S. occidentalis* treated with *Desmostachya* LE or LL at 1:5 and 1:10 concentrations there was non attainment of T_{50} value (time required for 50% germination of total seeds) and among seeds treated with *Parthenium LE,* and LL at 1:5 concentration only. Thus the time required for 50% germination increased with increase in concentration of leaf extracts or leachates (1:5>1:10>1:20) maintaining the order (ALE/ALL< PLE/PLL< DLE/DLL).

At 1:20 concentration of leaf extracts or leachates from *Desmostachya*, *Parthenium* and *Alternanthera*. the T_{50} values with DLE was120.0 h and DLL was 108.25 h , 98.00h with PLE and 96.2 h with PLL while 92.0h with ALE and ALL value 70.5 h respectively indicating that leachates are comparatively less effective and inhibitory to seed germination

than extracts. Similar results as evident from tables 1 and 8 are obtained with *Vigna* seeds as well ,i.e. extract treated seeds take greater T_{50} values compared to leachate treated.

Control group showed gross TTC stainability of 100% which was reduced by the treatment of *Vigna/Senna* seeds with all the three i.e.- D/P/A- leaf extracts/ leachates concentrations (1:5,1:10,1:20) depicting reduced respiratory activitiesat higher concentrations. The TTC stainability was found to be lowest among Vigna /*Senna* seeds treated with DLE/DLL followed by PLE/PLL and ALE/ALL . As evident from the values in the tables1,8,15and 22 a more concentrated leaf leachate of each of the three invasive weeds respectively was found to be more inhibitory to the percentage of seed staining than that of diluted leachate i.e.(1:5>1:10>1:20.

DLE/DLL followed by PLE/PLL and then *by ALE/ALL* are more inhibitory to TTC staining in the order as mentioned of *Vigna* or *Senna* seeds indicating reduced respiratory activities giving testimony to the comparative allelopathic potentiality of the weeds.

B)Analysis and discussion of tables -2,9,16 and 23.

Effect on speed of germination of Vigna radiata seeds pretreated with D/P/A Extracts and Leachates.

Tables 2 and 9 depict a decrease in speed of germination of *Vigna* seeds treated with dry leaf extracts and leachates of *Desmostachya, Parthenium* and *Alternanthera.* Also the speed of germination increased with the increase in duration from 24 hours to 168 hours. Among control group 100% speed of germination was attained at 96 hours. A nil speed of germination was attained by *Vigna* seeds after 24 hours treated with 1:5 ,1:10 and 1:20 leaf extracts of *D,P,A* similar results were obtained for the leachate treated groups (DLL and PLL) except 1:20 LL of *Parthenium* (7.25). *Alternanthera* however showed enhanced speed of germination with dreceasing concentrations. The speed of germination decreased with increase in concentration of all three dry leaf extracts. The strongest inhibition of speed of germination was attained with *Desmostachya* leaf extract followed by *Parthenium* and *Alternanthera* leaf extract..Leachates also yield somewhat similar trend of results. The maximum speed of germination attained at 168 hours and 1:20 concentration among the three leaf extract groups - *Desmostachya, Parthenium* and *Alternanthera* at were 57.31, 59.5 and 63.51 respectively while it was 61.35,67.56 and 66.29 respectivelywith their corresponding leachates. The difference in speed of germination among different groups was found to be significant among the leaf extract/leachate and control groups when time interval was not taken into consideration were in case of table 2-($F=45.931$, $p=0.000$) and table 9-($F=41.286$, $p=0.000$). Also the difference was found to be statistically significant

among different time intervals when leaf extract and leachate groups were not taken into consideration respectively.Table2- (F=20.29, p=0.000) and table 9- (F=21.882, p=0.000).

Post hoc analysis revealed a significant difference in speed of germination of Control group with all the three dry leaf extracts ($p<0.05$). Also a significant difference (p=0.019) and (p=0.004) was observed among the dry leaf extracts and leachates of *Desmostachya* and *Alternanthera* respectively while with *Parthenium* leachates it was (p=0.032).

Post hoc analysis comparing speed of germination among different time intervals revealed statistically significant differences among almost all time intervals. However, speed of germination at 96 hours did not show significant difference with speed of germination at 72 hours (p=0.114) and 120 hours (p=0.13). Also, speed of germination at 144 hours did not show significant difference with speed of germination at 120 hours (p=0.411) and 168 hours (p=0.99).

Effect on speed of germination of Senna occidentalis seeds pretreated with D/P/A Extracts and Leachates.

Table-16:-

Data showed that in control samples of *Senna occidentalis* seeds all seeds have germinated in 120 h (i.e. 100% germination achieved). Whereas for all other concentrations of the three weed treated seeds, germination almost ceases after 144 h.

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), in case of control is R^2=0.783, not very near the +1.0

value as evident from Figures 2,3 and 4 respectively indicate a comparatively low but acceptable level of goodness of fit.

With **1:5** leaf extracts germination speed fails to cross 50% even after 168 h in case of all *Desmostachya, Parthenium and Alternanthera* treated seed samples. as clearly evident from the tabulated data. At 144 h and 168 h *Desmostachya* treated seeds cease germination with a constant value of 27.94% in both the cases. Whereas *Parthenium* treated seeds germinate an extra 1.05% with very less speed of 40.00% to 41.05% only, similarly *Alternanthera* treated seeds germinate an extra 1.73% with very less speed of 48.05% to 49.78% only.

Notably during the first 24 h *Senna occidentalis* seeds have totally failed to germinate when treated with *Desmostachya*, *Parthenium and Alternanthera* leaf extracts proving that the three species render high allelopathic action on the seeds of *Senna occidentalis*. After 48 h only 4.66% germination occurs in *Desmostachya* treated *Senna* seeds .while *Parthenium* and *Alternanthera* treated seeds show germination of 7.08% and 13.55% respectively. After 72 h only 5.78% increase in germination occurs in *Desmostachya* treated *Senna* seeds from its value at 48 h., While *Parthenium* and *Alternanthera* treated seeds show 6.91% and 17.11% extra increase in germination respectively from their values at 48 hours. Clearly their relative allelopathic potential in an ascending order is established from the data. Similar results are obtained after 96 h and 120 h for each of the cases as evident from the table.

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.981 and R^2=0.971 very near the +1.0 value) in

case of *Desmostachya and Parthenium* treated *Senna* seeds respectively indicates a very high level of goodness of fit of the regression line representing the relationship between the time elapsed and response in terms of speed of germination of *Senna occidentalis* seeds, for a fixed concentration of 1:5. R^2= 0.906 in case of *Alternanthera* treated *Senna* seeds also indicate a high level of goodness of fit, indicating that for all the three cases, the experimental data is highly significant.

With **1:10** leaf extracts germination speed fails to cross 50% even after 168 h in case of *Desmostachya and Parthenium* treated seed samples as clearly evident from the tabulated data. At 144 h and 168 h *Desmostachya* treated seeds show least germination with values of 39.85% and 42.33% respectively. *Parthenium* treated seeds also germinate with very less speed of 48.50% to 48.66% whereas *Alternanthera* treated seeds germinate from. 60.48% to 61.24% only indicating germination speed of *Senna occidentalis* seeds will become negligible or stop after 168 h in all the three cases.

Also it is notable that during the first 24 h s *Senna* seeds have totally failed to germinate when treated with *Desmostachya* , *Parthenium and Alternanthera* treated leaf extracts proving that the three species render high allelopathic action on the seeds of *Senna occidentalis*.

After 48 h *Desmostachya* treated *Senna occidentalis* seeds show a meagre 6.75% germination. *Parthenium* shows 10.56% while *Alternanthera* leaf extract treated shows 28.11%. After 72 h only 9.46% increase in germination occurs in *Desmostachya* treated *Senna* seeds from its value at 48 h., While *Parthenium* and *Alternanthera* treated seeds show

8.65% and 14.05% extra increase in germination respectively from their values at 48 hours. Clearly their relative allelopathic potential in an ascending order is established from the data. Similar results are obtained after 96 h and 120 h for each of the cases as evident from the table, giving testimony to the relative allelopathic potential of the three weeds in a descending order as *Desmostachya* > *Parthenium* > *Alternanthera*..

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.970 very near the +1.0 value) in case of *Parthenium* treated seeds indicates a very high level of goodness of fit of the regression line representing the relationship between the time elapsed and response in terms of speed of germination of *Senna occidentalis* seeds, for a fixed concentration of 1:10. R^2 =0.968 in case of *Desmostachya* treated *Senna* seeds also indicate a very high level of goodness of fit, indicating that in both cases, the experimental data is highly significant ,except in case of *Alternanthera* treated *Senna* seeds where value of R^2 =0.838 with acomparatively low but definitely acceptable level of goodness of fit indicating that the experimental data is significant.

With **1:20** leaf extract germination speed at 96 h has only crossed 50%, i.e. 50.90% in case of *Parthenium* and 52.66% in *Alternanthera* treated seed samples whereas for *Desmostachya* treated seed samples germination percentage has failed to cross 50%, i.e. 45.52% as clearly evident from the tabulated data, indicating once again their allelopathic potential in an ascending order. At 120 h *Desmostachya* treated seed samples just barely cross the 50% mark i.e. 53.78%. *Parthenium* and *Alternanthera* treated seed samples comparatively show greater germination speed than *Desmostachya* i.e 58.25% and 66.11%

as evident from table 2. At 144 h and 168 h *Desmostachya* treated seeds show least speed of germination with values of 58.26% and 60.24% respectively with 1.98% increment only. *Parthenium* treated seeds also germinate with very less speed of 1.79% (64.13% to 65.92). *Alternanthera* treated seeds germinate an extra 1.56% (75.99% to 77.55%) only, lower than expected may be due to the fact that its speed has reached its germination threshold level crossing the 75% mark indicating that there will be cessation of seed germination after this.

Also it is notable that during the first 24 h *Senna occidentalis* seeds have totally failed to germinate when treated with *Desmostachya* , *Parthenium and Alternanthera* treated leaf extracts proving that the three species render high allelopathic action on the seeds of *Senna occidentalis*. After 48 h *Desmostachya* treated *Senna* seeds show a 19.44% germination. *Parthenium* shows 16.30% while *Alternanthera* leaf extract treated shows 39.68%. After 72 h *Desmostachya* treated *Senna* seeds show a 38.66% germination. *Parthenium* shows 36.71% while *Alternanthera* leaf extract treated shows 46.72%. Evidently there is a hike of 19.22% in the speed of germination in case of *Desmostachya* treated *Senna* seeds and 20.41% in case of *Parthenium* treated *Senna* seeds respectively from 48 hours to 72 hours giving testimony to the relative allelopathic potential of the three weeds in a descending order as *Desmostachya* > *Parthenium* > *Alternanthera.*

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.918 very near the +1.0 value) in case of *Parthenium* treated seeds indicates a very high level of goodness of fit of the regression line representing the relationship between the time elapsed and response in terms of speed of germination of *Senna occidentalis* seeds, for a fixed concentration of 1:20. R^2 for the other

two viz, 0.873 in case of *Alternanthera* and 0.894 in case of *Desmostachya* treated *Senna* seeds also indicate a high level of goodness of fit, indicating that for all the three cases, the experimental data is highly significant.

Data showed that in control samples of *Senna* seeds all seeds have germinated in 120 h (i.e.100% germination achieved). Whereas for all other concentrations of the three weed treated seeds, germination almost ceases after 120 h.

The difference in speed of germination among different was found to be significant among the leaf extract and control groups when time interval was not taken into consideration ($F=38.129$, $p=0.000$). Also the difference was found to be statistically significant among different time intervals when leaf extract groups was not taken into consideration ($F=27.471$, $p=0.000$)

Post hoc analysis revealed a significant difference in speed of germination of Control group with all the three dry leaf extracts ($p<0.05$). Also a significant difference was observed among the dry leaf extracts of *Alternanthera* with *Desmostachya* ($p=0.000$) and *Parthenium* ($p=0.005$). However, speed of germination at 96 hours did not show significant difference with speed of germination at 72 hours ($p=0.068$) and 120 hours ($p=0.185$).

Table-23:

With **1:5** leaf leachate concentrations germination speed at 120 h has only crossed 50%, i.e.50.92% in case of *Alternanthera* treated seed samples whereas for other two weed plants i.e. *Parthenium* and *Desmostachya* treated seed samples germination percentage has failed to cross 50%, i.e. 42.66% and 34.65% respectively as clearly evident from the tabulated data indicating their allelopathic potential in an ascending order. At 144 h and 168 h *Desmostachya* treated seeds cease germination with a constant value of 37.92% in both the cases. *Parthenium* treated seeds germinate with a negligible speed of 0.2% (49.0% to 49.20%) whereas *Alternanthera* treated seeds germinate an extra 1.04% (51.29% to 52.33%) only.

Notably during the first 24 h *Senna* seeds have totally failed to germinate when treated with *Desmostachya* and *Parthenium* leaf leachates except for *Alternanthera* treated which shows 11.15% indicating that they are least affected than the other two.

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. R^2=0.951 very near the +1.0 value in case of *Desmostachya* treated seeds indicates a very high level of goodness of fit of the regression line representing the relationship between the time elapsed and response, for a fixed concentration of 1:5, as compared to the other two. R^2 for the other two viz, 0.930 in case of *Parthenium* and 0.887 in case of *Alternanthera* treated *Senna* seeds also indicate a very high level of goodness of fit, indicating that for all the three cases, the experimental data is highly significant.

With **1:10** leaf leachate concentrations germination speed at 96 h has only crossed 50%, i.e. 53.66% in case of *Alternanthera* treated seed samples whereas for other two weed plants i.e. *Parthenium* and *Desmostachya* treated seed samples germination percentage has failed to cross 50%, i.e. 41.71% and 37.55% respectively as clearly evident from the tabulated data, indicating their allelopathic potential in an ascending order. At 144 h and 168 h *Desmostachya* treated seeds show least germination with values of 47.99% and 48.71% respectively. *Parthenium* treated seeds also germinate with very less speed of 56.75% to 57.81% whereas *Alternanthera* treated seeds germinate an extra 0.89% i.e. 63.0% to 63.89% only. Indicating germination speed of *Senna* seeds will become negligible or stop after 168 h in all the three cases.

Also it is notable that during the first 24 h *Senna* seeds have totally failed to germinate when treated with *Desmostachya*. *Parthenium* leaf leachates show a meagre 6.22% germination except for *Alternanthera* leaf leachate treated, which shows 12.66% indicating that they are least affected as compared to the other two

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.956 very near the +1.0 value) in case of *Parthenium* treated seeds indicates a very high level of goodness of fit of the regression line representing the relationship between the time elapsed and response in terms of speed of germination of *Senna* seeds, for a fixed concentration of 1:10. R^2 for the other two viz, 0.914 in case of *Alternanthera* and 0.907 in case of *Desmostachya* treated *Senna* seeds also indicate a very high level of goodness of fit, indicating that for all the three cases, the experimental data is highly significant.

With **1:20** leaf leachate concentrations germination speed at 72 h has only crossed 50%, i.e. 52.26% in case of *Alternanthera* treated seed samples whereas for other two weed plants i.e. *Parthenium* and *Desmostachya* treated seed samples germination percentage has failed to cross 50%, i.e. 44.39% and 33.33% respectively as clearly evident from the tabulated data and indicating once again their allelopathic potential in an ascending order. At 144 h and 168 h *Desmostachya* treated seeds show least germination with values of 63.56% and 64.66% respectively with 1.10% increment only. *Parthenium* treated seeds also germinate with very less speed of 2.85% (73.36% to 76.21%) but greater than *Desmostachya* treated seeds whereas *Alternanthera* treated seeds germinate an extra 0.78% (79.59% to 80.37%) only lower than expected may be due to the fact that its speed has reached its germination threshold level crossing the 80% mark indicating that there will be cessation of seed germination after this.

Also it is notable that during the first 24 h *Senna* seeds have only germinated very meagerly 8.98 % when treated with *Desmostachya* leaf leachates. *Parthenium* leaf leachates treated seeds show a comparatively less meagre 10.14% germination except for *Alternanthera* leaf leachate treated which shows 13.00% indicating that they are least affected as compared to the other two.

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.972 very near the +1.0 value) in case of *Desmostachya* treated seeds indicates a very high level of goodness of fit of the regression line representing the relationship between the time elapsed and response, for a fixed concentration of 1:20, as compared to the other two. R^2 for the other two viz, 0.916 in case

of *Parthenium* 0.903 in case of *Alternanthera* treated *Senna* seeds also indicate a very high level of goodness of fit, indicating that for all the three cases, the experimental data is highly significant.

However, the difference was found to be significant among the leaf leachate and control groups when time interval was not taken into consideration ($F=34.983$, $p=0.000$). Also the difference was found to be statistically significant among different time intervals when leaf leachate groups was not taken into consideration ($F=33.334$, $p=0.000$)

Post hoc analysis revealed a significant difference in speed of germination of Control group with all the three dry leaf leachates ($p<0.05$). Intragroup comparison showed a significant difference in speed of germination among all the three dry leaf leachates ($p<0.05$).

Post hoc analysis comparing speed of germination among different time intervals revealed statistically significant differences among almost all time intervals. However, speed of germination at 96 hours did not show significant difference with speed of germination at 120 hours ($p=0.094$).

Tables 3, 10, 17 and 24, revealed a significantly greater amino acids ($F=17.715$, $p=0.002$) , ($F=7.405$, $p=0.019$) , ($F=18.593$, $p=0.002$) , ($F=8.644$, $p=0.013$) and soluble carbohydrate ($F=22.157$, $p=0.001$), ($F=25.346$, $p=0.001$), ($F=34.254$, $p=0.000$), ($F=21.447$, $p=0.001$) levels respectively among the three leaf extract/ leachate groups as compared to control group. Among the three LE or LL groups, amino acids and soluble carbohydrates were

found to be highest among seeds treated with *Desmostachya* LE/LL followed by *Parthenium* and *Alternanthera* (LE/LL.) Moreover, there was an increase in level of amino acids and soluble carbohydrates with increase in concentrations of all the three dry leaf extracts/leachates from 1:20 to 1:5. When correlation test was applied, amino acids and soluble carbohydrate levels showed a strong positive correlation (r=0.986, p=0.000),T-3;(r=0.956, p=0.000),T-10; (r=0.979, p=0.000),T-17 and (r=0.946, p=0.000),T-24; with each other.

In fact, damage of seed membrane by putative allelochemicals present in leaf extracts and leaf leachetes rendered the seed membranes leaky / porous which consequently enhances membranes permeability and thereby, increasing the levels of soluble substances in seed leachates. Increase of the soluble carbohydrate contents might be due to the enhanced activities of amylase which break down the insoluble carbohydrate level within the seeds thus enriching the soluble fraction of carbohydrate.

showed subdued levels of protein, insoluble carbohydrate, nucleic acids (DNA and RNA), chlorophyll as well as activity of catalase enzyme. On the other hand, the treatments significantly increase the levels of soluble carbohydrates, along with activities of amylase and protease in leaves

Tables 4, 11, 18 and 25, depicted significantly lower soluble carbohydrates (F=7.406, p=0.019), (F=5.672, p=0.035) , (F=18.067, p=0.002) ,(F=3.825, p=0.076) and significantly greater insoluble carbohydrates (F=7.155, p=0.021), (F=20.204, p=0.002) ,(F=2.522, p=0.154) (F=6.062, p=0.03) respectively among control group as compared to the three dry

leaf extract/ leachate groups. Soluble carbohydrate levels were found to be highest among seeds treated with *Desmostachya* LE/LL followed by *Parthenium* and *Alternanthera* (LE/LL.). Conversely, insoluble carbohydrate levels were found to be highest among seeds treated with *Alternanthera* (LE/LL) followed by *Parthenium* and *Desmostachya* (LE/LL). Also soluble carbohydrate level showed an increase, while insoluble carbohydrate levels decreased, with increase in concentration of extracts/leachates from 1:20 to 1:5. When correlation test were applied, soluble and insoluble carbohydrate levels showed a significantly strong negative correlation ($r=-0.902$, $p=0.000$) ($r=-0.84$, $p=0.002$). ($r=-0.843$, $p=0.002$) ($r=-0.71$, $p=0.021$) respectively.

The hydroxyl (–OH) groups in phenolic compounds might cause inhibitory action as these groups have the ability to interact with the cell membrane of seeds used for bioassay to disrupt membrane structures thereby causing leakage of cellular components. Active groups like –OH promote delocalization of electrons which consequently act as proton exchangers and reduce the gradient across the cytoplasmic membrane. This leads to collapsing of the proton motive force, depletion of the ATP pool, ultimately leading to death of the cells. Reports also exist that these –OH groups can very easily bind with the active sites of enzymes like dehydrogenase, catalase, peroxidase and amylase by altering the metabolism of the cells of the seeds.

The number and position of hydroxyl groups on the aromatic ring are the key determinants for their toxicity to the seeds. Purwar (2019) reported that caffeic and coumaric acids have similar structure with different number of –OH groups. Caffeic acid, has one more –OH

group in its phenolic ring than coumaric acid. So caffeic acid has better toxicity compared to coumaric acid.

The other important factor being the position of the –OH group within the phenolic ring. As an example, Thymol has better antibacterial activity than Carvacrol due to the presence of –OH group in ortho position (Purwar, 2019). According to the theory of electron transfer, the proper orientation of the substrates (reserved starch in the seeds) probably involve+s a short distance between the donor and acceptor site to enhance the e^- transfer rate4.

The reason behind seed deterioration is decrease in enzyme activity in seeds which lowers its respiratory potential. This in turn lowers both the energy (ATP) and food supply to the germinating seed. Several changes in the enzyme macromolecular structure may contribute to their lower effectiveness. They may undergo compositional as well as conformational changes by losing or gaining certain functional groups, by oxidation of sulfahydryl groups or by conversion of amino acids within the protein structure (Marthandan and Jerlin, 2017).

. The damage of the seed membrane by putative allelochemicals present in leaf extracts and leachates rendered the seed membranes leaky consequently enhancing membrane permeability, thereby increasing the levels of soluble carbohydrates in seed extracts and or leachates as the case may be. Increase of the soluble carbohydrate contents might be due to the enhanced activities of amylase enzyme which break down the insoluble carbohydrate level within the seeds thus enriching the soluble fraction of carbohydrate.

Tables 5, 12 and 19, demonstrated a significant association of amino acids (F=6.46, p=0.026), (F=14.004, p=0.004) and(F=8.968, p=0.012) and protein (F=30.447, p=0.001), (F=24.184,p=0.001) and (F=37.221, p=0.000),respectively, among different leaf extracts and or leachates and with control group. However, the difference was statistically non significant (F=4.366, p=0.059).in amino acids- from *Senna* seed kernels treated with DLE/DLL,PLE/PLL,ALE/ALL as seen in **Table-26**. Amino acid level was found to be highest among seeds treated with *Desmostachya* LE/LL followed by *Parthenium* LE/LL , *Alternanthera* LE/LL and the control group. Protein level was highest among Control group followed by seeds treated with the three test weeds in just the reverse order as mentioned above. With increase in concentration of leaf extracts or leachates amino acid was found to increase while protein level was found to decrease. A significantly strong negative correlation (r=-0.91, p=0.000)- T-5; (r=-0.963, p=0.000)-T-12 ; (r=-0.93, p=0.000)-T-19 and (r=-0.825, p=0.003)-T-26 was seen among amino acid and protein levels respectively.

Table 26 demonstrated a significant association of protein (F=72.895, p=0.000) among different leaf extracts/leachates and control group. Protein level was highest among Control group followed by seeds treated with *ALE/ALL*, *PLE/PLL and DLE/DLL*. Amino acid level was found to be highest among seeds treated with *Desmostachya* LE/LL followed by *Parthenium* LE/LL and *Alternanthera* LE/LL and finally control group. However, the difference was statistically non significant (F=4.366, p=0.059).With increase in concentration from 1:20 to 1:5 of LE/LL, amino acid was found to increase while protein level was found to decrease. A significantly strong negative correlation (r=-0.825, p=0.003) was evident among Amino acid and Protein levels.

Tables 6 and 13 showed significantly (p=0.000) highest level of DNA (108.56) and RNA (857.98) among Control group. **Table 20 and 27** showed significantly (p<0.05) highest level of DNA (101.33) and RNA (830) among control group. Among the seeds treated with three dry leaf extracts (T-6 and T-13) or leachates (T-20 and T-27), DNA and RNA was found to be highest among *Alternanthera* LE/LL followed by *Parthenium* LE/LL and *Desmostachya* LE/LL. Also, the levels of DNA and RNA were reported to decrease with increase in concentrations of all the three leaf extracts or leaf leachates. A significantly strong positive correlation [(r=0.949, p=0.000; T-6; (r=0.955, p=0.000); T-13(r=0.939, p=0.000 ;T-20 and (r=0.933, p=0.000) ;T-27)] in each case was observed among RNA and DNA levels when correlation test was applied.

Total protein, nucleic acids (DNA and RNA) levels in cotyledons of seeds declined in treated seeds than the control . The total protein, DNA and RNA levels in cotyledonary tissue of macerated seeds of both *V.radiata* and *S. occidentalis* was highest when its seeds underwent treatment with the leaf extracts/leachates of *A. sessilis* followed by *P.hysterophorus* and *D. bipinnata* giving testimony to their relative allelopathic potential in an ascending order. The reduction in total protein ,DNA and RNA content in treated seeds with increasing order of concentraton ,i.e.(1:5>1:10>1:20) of leaf extracts/leachates of each of the three plants may be attributed to the effect of allelochemicals like quercetin, quercitrin, kaempferol common to the three plants which can inhibit topoisomerase I and II enzyme activity and thus interfere with the replication and transcription process , hampering the process of the religation of DNA, induce double strand breaks , increasing the formation of DNA- enzyme complexes which are cleavable (Matthews et al., 2006;

Srividya et al. 2013). There is also possibility of transformation of nucleotides by intercalation with nucleic acids with the help of ionic bonding with their negatively charged phosphate groups. Also at the highest concentraton of allelochemicals there is possibility of accumulation of phenolic glycine along with other phenolics like p-coumaric acid, ferulic and vanillic acids that might interfere with the cytoplasmic ribosomes and production of RNA. In this respect, it has been reported that cinnamic acid derivatives depressed translation activity of polysomal m RNAase of bean cells that reduced the protein synthesis (Bolwell *et al*., 1988). *Vigna* and *Senna* seeds might be affected in a similar way.

Tables - 7, 14, 21 and 28 depict a significant association ($p<0.05$) of enzymatic activities in kernels of *Vigna and or Senna* seeds as the bioassay material may be upon pretreatment with dry LE or LL of *D. bipinnata, P. hysterophorus* and *A. sessilis.*

The level of the enzymes Dehydrogenase, Catalase and Peroxidase showed an upward trend with increase in dilution (from 1:5 to 1:20) of all the three dry leaf extracts/leachates in each case. The level of Amylase enzyme showed a decreasing trend with increase in dilution for each case.

A significant variance of dehydrogenase enzyme level as seen from tables:T-7($F= 47.888$, $p=0.000$);T-14($F= 43.415$, $p=0.000$);T-21($F= 27.715$, $p=0.001$); T-28($F= 26.057$, $p=0.001$) was observed when compared according to groups (Control, *Desmostachya, Parthenium, Alternanthera*). The dehydrogenase level was significantly high in each case among Control group when compared to other three groups in which seeds were pretreated with different

LE/LL. The enzyme level was highest among *Alternanthera* group followed by *Parthenium* group and *Desmostachya* group.

Catalase enzyme level revealed a significant association,T-7(F=23.595, p=0.001);T-14(F=23.038, p=0.001);T-21(F=27.698, p=0.001); T-28(F=12.583, p=0.005) with different groups (Control, *Desmostachya, Parthenium, Alternanthera*). The catalase level was found to be significantly highest among Control group for each case as evident from the tables followed by *Alternanthera, Parthenium* and *Desmostachya* LE/LL as the case may be.

When Peroxidase enzyme level was compared among different groups, a significant association was seen i.e T-7(F= 21.052, p=0.001);T-14(F= 21.557, p=0.001);T-21(F= 24.626, p=0.001); T-28(F= 13.54, p=0.005) . It was observed that Control group had highest Peroxidase level in each case as evident from the tables followed by *Alternanthera, Parthenium* and *Desmostachya* seed kernels.

Amylase enzyme also showed a significant association with groups T-7(F=18.011, p=0.002); T-14(F=12.313, p=0.006);T-21(F=25.818, p=0.001); T-28(F= 13.54, p=0.001) . However the level of Amylase enzyme was found to be lowest among control group as evident from the tables followed by *Desmostachya, Parthenium* and *Alternanthera* LE/LL as the case may be.

Pandey (1996) reported that parthenin caused the inhibition of the growth of water hyacinth by damage to cellular membranes, due to loss of Dehydrogenase activity in the roots and chlorophyll in leaves. Moreland and Novitzky (1987), advocated that allelochemicals inhibit electron transport in mitochondria and impair enzyme activity as a primary target. This in

turn may result in reduced ability of seeds to metabolize reserve materials. The putative allelochemicals present in leaf extracts and leachates of the test weed samples taken for experimental study, might have inhibited the synthesis of enzymes, growth hormones and other related metabolic parameters responsible for germination to seedling establishment in a similar way.

Dehydrogenase enzyme plays a significant role in the biological oxidation of organic matter by transferring hydrogen from organic matter to inorganic acceptors (Zhang et al. 2010). Allelochemical induced stress can cause oxidative damage, as evidenced by enhanced activity of ROS (Reactive Oxygen Species). ROS scavenging enzymes like dehydrogenase are impaired and subsequently cause an increased amount of membrane lipid peroxidation in the seeds (Lara Nunez *et al*., 2006; Ye *et al*., 2006). It has also been postulated that the allelopathic effect might lead to a disbalance between antioxidant defenses and the quantity of Reactive Oxygen Species (ROS), thus resulting in an oxidative stress. The intracellular level of hydrogen peroxide (H_2O_2) is regulated by a wide spectrum of enzymes like catalase (CAT). The allelochemicals impairing enzymatic activities might have regulated the energy metabolism and thus consequently resulted in impairment of germination and metabolism of the test seed samples. The modified physiological and biochemical processes inhibit and delay the germination as well as metabolism of mung bean seeds under the influence of allelochemicals present in leaf extracts and leachates (Islam and Kato-Noguchi 2013, Li et al. 2014). (Chauhan et al., 2011) studied the level of various enzymes of seeds under natural ageing and found that Amylase is an important hydrolytic enzyme synthesized during seed germination in plants. This enzyme is abundant in the germinating seeds and

catalyses a random hydrolysis of α-1, 4 glucosidic linkage in the starch component. Seed development is closely associated with seed metabolism and transport processes (Weber et al., 1998). It is involved in the mobilization of starch reserves which are transported as sugars and utilized by the growing embryo.The enzymes most commonly endorsed with the initial attack on starch granules are α-amylase and β-amylase, responsible for breakdown and initiating the mobilization of starch in germinating seeds (Trethewey & Smith, 2000). The study indicates that some allelochemicals are present in extracts and leachates of these three weeds taken for study and they worked as enzyme amylase regulators. It has also been reported that even lower concentrations of allelochemicals were shown to stimulate amylase activity and therefore increased solublization of starch during the germination process (Singh et al., 2009).Tannins inactivate α-amylase, dehydrogenase and other enzymes. Plant hormones are very important for regulation of seed dormancy and germination. ABA is positive regulator of dormancy induction and maintenance, while it is negative regulator of germination. GA3 reduces the dormancy, promotes germination, leads to amylase synthesis and counteracts ABA effects. Allelopathy interactions are frequently manifested through inhibited or delayed seed germination influenced by changes in production of plant hormones and their perturbed balance.