DETERMINATION OF RELATIVE ALLELOPATHIC VIGOUR OF THREE WEEDS OF WEST BENGAL USING THEIR LEAF EXTRACTS ON GERMINATION BEHAVIOUR OF *VIGNA RADIATA* SEEDS

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ABSTRACT

It was attempted to study the allelopathic potential of three weeds of West Bengal viz- *Desmostachya bipinnata* L. Stapf (Poaceae), *Parthenium hysterophorus* L. Asteraceae, *Alternanthera sessilis* L. R. Br. *ex* DC. (Amaranthaceae), on the seeds of species *Vigna radiata* (Fabaceae) ,the chosen bioassay material in this case. These were analyzed by some specific germination behaviour indices of of *Vigna radiata* measured in terms of percentage of seed germination , T_{50} (time required for 50% germination), TTC stainability of seeds and speed of seed germination exposed to various concentrations [1:5, 1:10 and 1:20 (w/v)] of leaf extracts of the weeds respectively. Obtained data reveal allelopathy as a dose and response phenomenon depending on the concentration of allelochemicals i.e. higher the concentration of extracts greater is the resistance to the percentage and speed of seed germination and seed respiratory activities as indicated by reduced stainability with TTC along with increase of the T_{50} values. Observed data and their coefficient of determination values indicate that the data is good fit and significant and also help to establish the relative allelopathic potential of the weeds as. *Alternanthera* < *Parthenium* < *Desmostachya* indicating that the monocotyledonous weed *Desmostachya* is more allelopathically vigorous than the other two dicotyledonous weeds *Parthenium* and *Alternanthera.* Further research might thus be relevant to know the interaction of other invasive dicotyledonous and monocotyledonous weeds on other weed and crop species opening new vistas of research.

Key words: **Allelopathy, *Alternanthera sessilis, Bioassay material, Desmostachya bipinnata,* Germination behaviour, *Parthenium hysterophorus, Vigna radiata*, weeds.**

I-INTRODUCTION:

Weeds pose major economic problems and threat to ecological balance and loss of biodiversity throughout the globe (Callaway, 2002). These also pose a serious threat to cultivated crops because of the phytochemicals they release into the rhizosphere by root exudation, leaf litter and bark that inhibit the growth of the cultivated crops (Muller, 1966). These chemical exudates are termed allelochemicals and their study is called allelopathy (Chou and Waller, 1983;Mominul Islam and Kato-Noguchi, 2013).Generally allelopathy refers to either beneficial or harmful effect of one plant upon another, both crop and weed species, by the release of phytochemicals (allelochemicals) from plant parts (Adler and Chase, 2007).

Keeping in conformity with the global context a large number of weeds have become endemic in India and consequently in West Bengal that has affected the distribution and diversity of native plant species to a large extent. The present allelopathic study therefore aims to know the inhibitory effect of three such growing weeds of West

Bengal *viz Desmostachya bipinnata* L. Stapf (Poaceae), *Parthenium hysterophorus* L.(Asteraceae*), Alternanthera sessilis* L. R. Br. *ex* DC. (Amaranthaceae) because of their vigorous growth, easy availability and better responses towards test plant material on the seeds of crop species *Vigna radiata.* This will enable us to understand weed-weed interaction much deeply, establish a relative allelopathic potential of the weeds and also to know the aspects of dose response relationships of allelochemicals on the bioassay plant seeds dependant on the concentrations of extracts. Experiments are designed to study the germination responses of seeds of *Vigna radiata* (Fabaceae) measured in terms of seed germination percentage, T_{50}(time required for 50% germination of seeds), TTC (2,3,5, triphenyl tetrazolium chloride) stainability and speed of seed germination exposed to various concentrations [1:5, 1:10 and 1:20 (w/v)] for each of the leaf extracts respectively of the above mentioned weeds.

. Allelochemicals are easily soluble in water and may persist in soil or leach out with water affecting surrounding plants within that seral stage, by a complex type mechanism which can involve the interaction of different classes of natural chemicals like benzoic and cinnamic acid derivatives, alkaloids, flavonoids, terpenoids, sesquiterpene lactones, steroids etc. (Rice, 1974; Inderjit, 1998). The mixture of allelochemicals sometime produces greater effect than an individual compound alone a mechanism called allelosynergism. It was reported that allelochemicals have no definite target site in physiological pathways or there is no common mode of action like synthetic herbicides (Khanh *et al*., 2007). Consolidated works on the inhibitory effects of invasive weeds and the use of allelopathic plants for bio-herbicidal development in replacement to conventional synthetic chemical herbicides are very few in weed management (Bhadoria, 2011). That is why allelopathy in recent years has received National and International importance.

II-MATERIALS AND METHODS:

Plant materials:

(a) Three weeds *Desmostachya bipinnata, Parthenium hysterophorus* and *Alternanthera sessilis* were taken into consideration for allelopathic studies. Fresh, mature and healthy leaves of each species were collected from actively growing populations.

(b) The bioassay materials viz green gram or *Vigna radiata,* seeds were collected from the sites as mentioned below.

Sites of collection:

Vidyasagar University Campus, Paschim Medinipur; Burdwan University campus, Burdwan; railway tracks around Bamangachi, North 24 parganas; Baruipur, South 24 parganas and Haldia, Purba Medinipur, West Bengal year round time to time during the period of investigations .

1. Healthy, young, mature and old dry leaves of the plants *Desmostachya bipinnata, Parthenium hysterophorus and Alternanthera sessilis* were collected (3 lots of 25g each) from each of the weed plants respectively and thoroughly homogenized using 500 ml double distilled water in 1000 ml beaker for 72 hours (h).
2. The homogenate was strained using a fine cloth and was stirred manually for five minutes and subsequently centrifuged at 5000 *g* for 15 min. Then it was filtered with the help of Whatman No.1 filter paper. The volume of the filtered solution was then made up to 125 ml, 250 ml and 500 ml in three sets respectively using double distilled water and this was considered as 1:5 w/v, 1:10 w/v and 1:20 w/v proportion of stock solution of leaf concentrations respectively and was used as the test sample for allelopathic studies.
3. Fully viable *Vigna radiata* seeds in ten lots of 25 g each were surface-sterilized with 0.1% $HgCl_2$ (Mercuric chloride) solution for 90 seconds. The seed lots were then separately pre-soaked in the three concentrations of leaf extracts of *Desmostachya, Parthenium* and *Alternanthera* or in distilled water (control) for 24 h and then allowed for germination tests.
4. To analyse the percentage germination, the individual seed lots in four groups of 100 seeds of each treatment were transferred to separate petri dishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded after 7 days of seed soaking following ISTA (1976) Rules.
5. The time for 50% germination of seeds (T_{50}) is determined using the method of Coolbear *et al* (1984).
6. For analysing TTC stainability, dehusked seeds of each treatment in four groups of 100 seeds were allowed to imbibe 0.5% TTC solution (w/v) in Petri dishes for 24 h in dark condition. The percentage TTC stained (red coloured) seeds were calculated from the total number of seeds of each treatment.
7. Speed of seed germination was recorded by analyzing percentage germination of seed lots of each treatment at an interval of 24 h in the laboratory up to 168 h of seed soaking following the International Seed Testing Association Rules (ISTA, 1996).

Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits (Panse and Sukhatme, 1967).

III-RESULTS AND DISCUSSION:

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	**1:05**	23.76	NA	39.62
	1:10	38.62	NA	47.21
	1:20	57.31	120.25	69.55
Parthenium	**1:05**	30.35	NA	42.65
	1:10	43.79	NA	62.42
	1:20	59.50	112.66	71.31
Alternanthera	**1:05**	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**

LSD= Least Significant Difference; NA: Non-attainment of 50% germination.

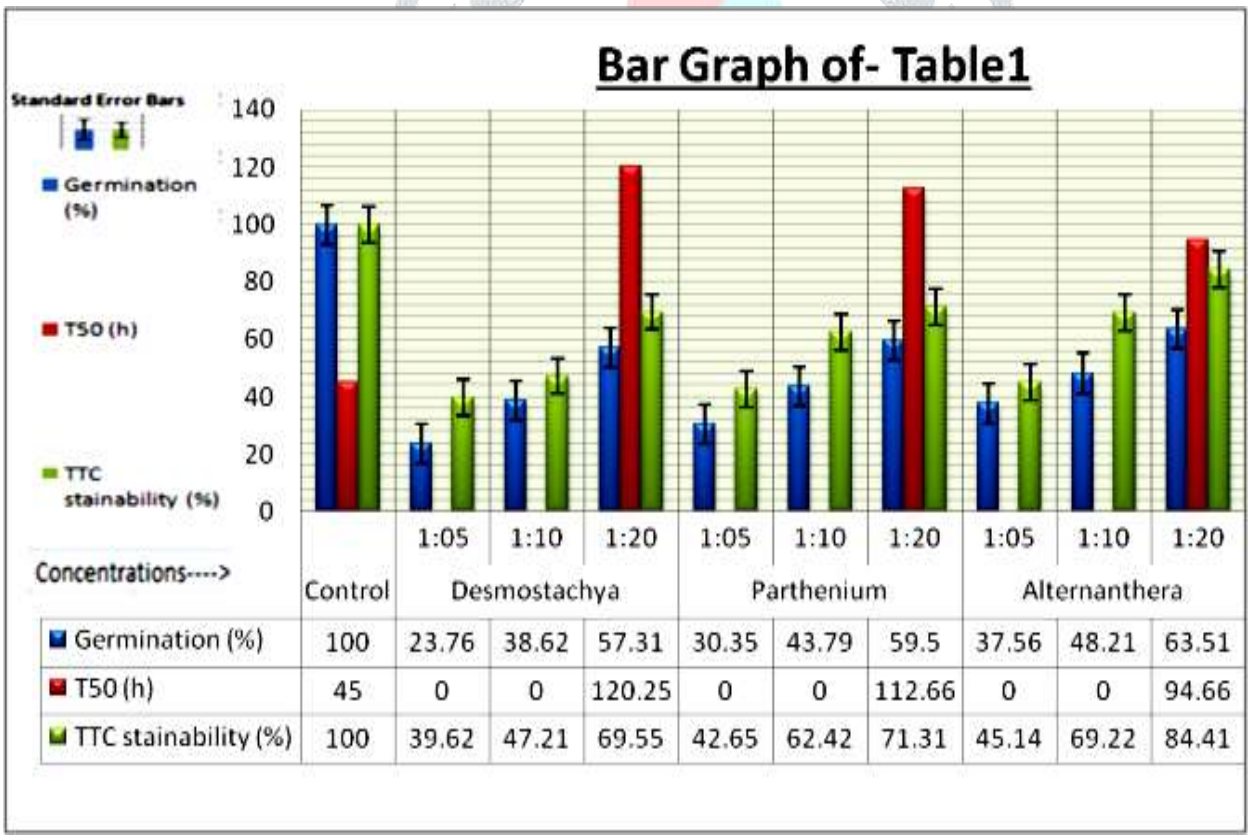

Fig.1. Bar Graph depicting the effect of seed pretreatment with dry leaf extracts of *Desmostachya, Parthenium and Alternanthera* on percentage of germination, Time (h) for 50% germination (T_{50}) and TTC stainability of *Vigna radiata* seeds.

Data clearly revealed that percentage germination of *Vigna* seeds were strongly inhibited by the three concentration grades of leaf extracts of each of the test weeds *Desmostachya*, *Parthenium* and *Alternanthera* respectively than control. More concentrated leaf extract of each of the three invasive weeds respectively was found more inhibitory than that of diluted leaf extract.

Also it is noted from table 1 that *Desmostachya* is more inhibitory to germination of *Vigna* seeds than *Parthenium* and *Alternanthera.*

On the other hand in treated *Vigna* seeds, time (h) required for 50% germination were noted significantly higher than control so much so that the leaf extracts of *Desmostachya, Parthenium* and *Alternanthera* treated seeds could not attain 50% germination at 1:5 and 1:10 concentrations. At 1:20 concentration of leaf extracts *Alternanthera* treated *Vigna* seeds attained T_{50} at 94.66 h proving lesser allelopathic as compared to the other two.

Treatments of the *Vigna* seeds with leaf extracts of all three concentration grades are shown to alter gross TTC stainability of the seeds indicating reduced respiratory activities at higher concentrations. Here also, as evident from the values in the table a more concentrated leaf extract of each of the three invasive weeds respectively was found to be more inhibitory to the percentage of seed staining than that of diluted extract i.e.(1:5>1:10>1:20), indicating reduced respiratory activities at higher concentrations.

Also it is noted from table-1 that *Desmostachya* are more inhibitory to TTC staining of *Vigna* seeds indicating reduced respiratory activities than when treated with *Parthenium* followed by *Alternanthera* again giving testimony to the relative allelopathic potential of the weeds in a descending order respectively i.e. *Desmostachya bipinnata* > *Parthenium hysterophorus* > *Alternanthera sessilis.*

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

Treatments	Speed of germination at 24 h 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	**1:05**	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	**1:05**	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	**1:05**	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**

LSD= Least Significant Difference; NC= Not Calculated.

Effect on speed of germination of Vigna radiata seeds:

Data showed that in control samples of *Vigna* seeds all seeds have germinated in 96 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=0.727, 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 even 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 23.76% in both the cases. *Parthenium* treated seeds also cease germination with a constant value of 30.35% in both the cases. Whereas *Alternanthera* treated seeds germinate an extra 1.01% with very less speed of 36.55% to 37.56% only.Notably during the first 24 h *Vigna* seeds have totally failed to germinate when treated with *Desmostachya, Parthenium and Alternanthera* leaf extracts. After

48 h *Vigna* seeds have still totally failed to germinate when treated with *Desmostachya* and *Parthenium* leaf extracts except for *Alternanthera* treated seeds which shows only 8.71% indicating that they are less affected than the other two. After 72 h only 8.25% germination occurs in *Desmostachya* treated *Vigna* seeds while *Parthenium* and *Alternanthera* treated seeds show germination of 10.25% and 19.71% respectively. 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.

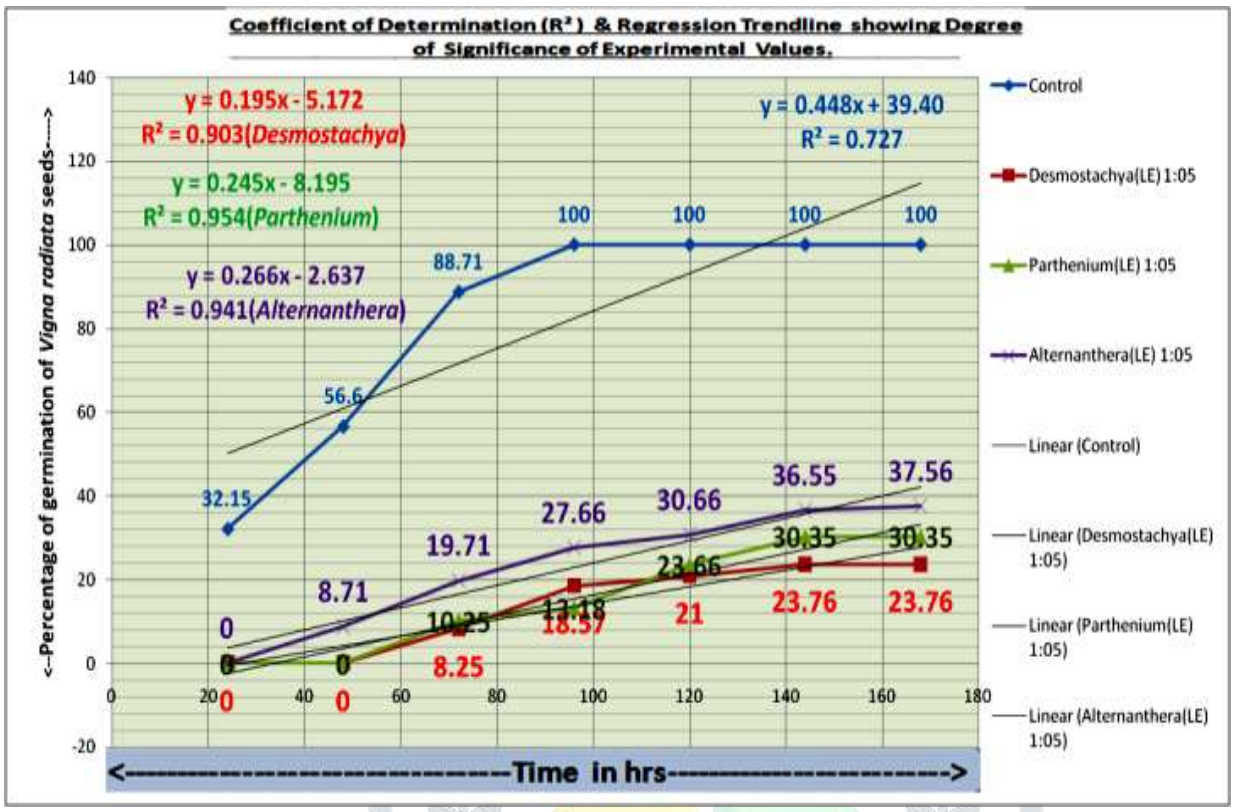

Fig2. Graphical presentation of coefficient of determination (R^2) of control and 1:5 leaf extract of *Desmostachya, Parthenium* and *Alternanthera,* using MS Excel Spreadsheet.

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.954 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 *Vigna* seeds, for a fixed concentration of 1:5. R^2 for the other two viz, 0.941 in case of *Alternanthera* and 0.903 in case of *Desmostachya* treated *Vigna* 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 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 show least germination with values of 37.36% and 38.62% respectively. *Parthenium* treated seeds also germinate with very less speed of 42.65% to 43.79% whereas *Alternanthera* treated seeds germinate an extra 0.93% i.e. 47.28% to 48.21% only, indicating that germination speed of *Vigna* seeds will become negligible or stop after 168 h in all the three cases.

Also it is notable that during the first 24 h *Vigna* 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 *Vigna radiata*. After 48 h *Desmostachya* treated *Vigna* seeds show a meagre 3.65% germination. *Parthenium* shows 10.93% while *Alternanthera* leaf extract treated *Vigna* seeds show 16.92% giving testimony to the relative allelopathic potential of the three weeds in a descending order as *Desmostachya* > *Parthenium* > *Alternanthera.* After 72 h only 13.11% germination occurs in *Desmostachya* treated *Vigna* seeds while *Parthenium* and *Alternanthera* treated seeds show germination of 18.45% and 22.86% respectively. 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.

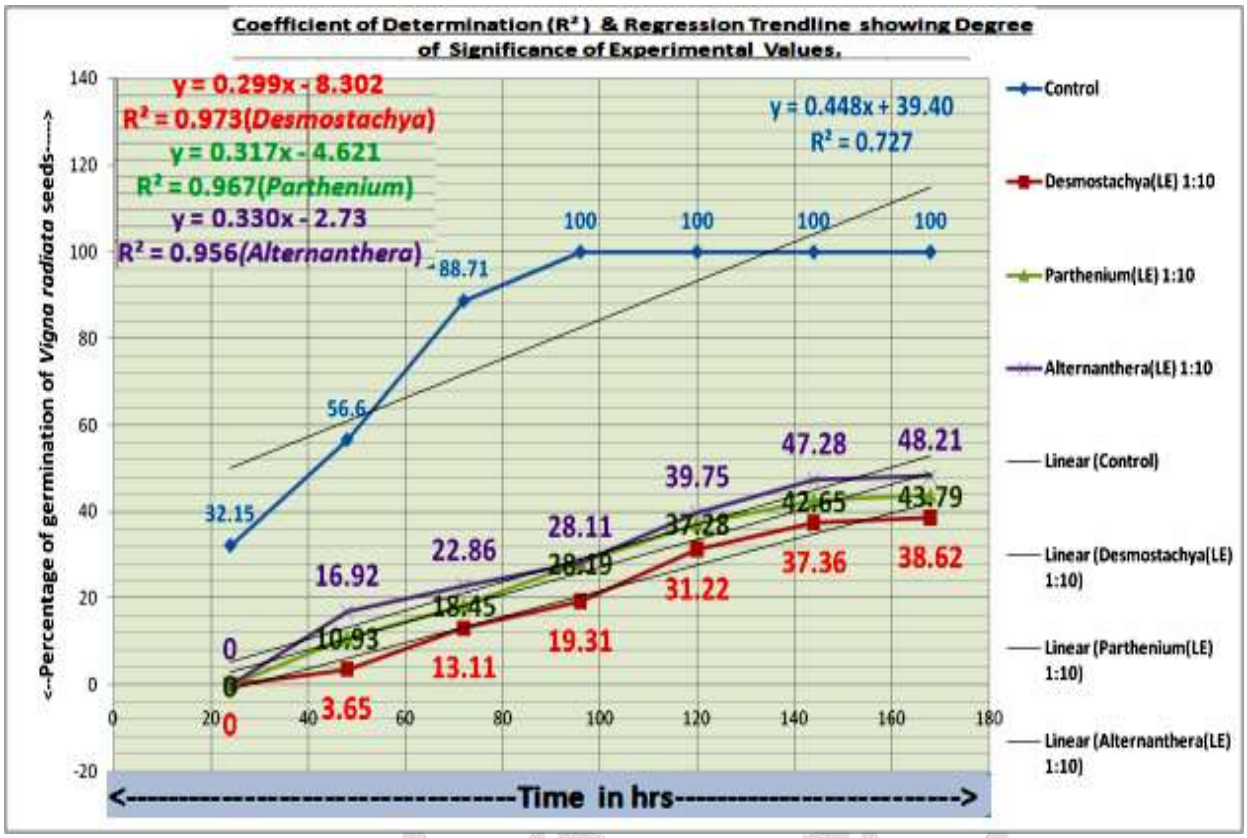

Fig3. Graphical presentation of coefficient of determination (R^2) of control and 1:10 leaf extract of *Desmostachya, Parthenium* and *Alternanthera,* using MS Excel Spreadsheet.

The analysis of coefficient of determination (which attempts to predict how well the regression line fits the actual data), i.e. (R^2=0.973 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 in terms of speed of germination of *Vigna* seeds, for a fixed concentration of 1:10. R^2 for the other two viz, 0.956 in case of *Alternanthera* and 0.967 in case of *Parthenium* treated *Vigna* 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 extracts germination speed at 96 h has only crossed 50%, i.e. 52.65% 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. 39.95% and 38.21% respectively 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. 50.35%. *Parthenium* and *Alternanthera* treated seed samples comparatively show greater germination speed than *Desmostachya* as evident from table 2. At 144 h and 168 h *Desmostachya* treated seeds show least germination with values of 55.77% and 57.31% respectively with 1.54% increment only. *Parthenium* treated seeds also germinate with very less speed of 0.84% (58.66% to 59.50%) but greater than *Desmostachya* treated seeds whereas *Alternanthera* treated seeds germinate an extra 0.63% (62.88% to 63.51%) only lower than expected may be due to the fact that its speed has reached its germination threshold level crossing the 60% mark indicating that there will be cessation of seed germination after this.
Also it is notable that during the first 24 h *Vigna* 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 *Vigna radiata*. After 48 h *Desmostachya* treated *Vigna* seeds show a 14.96% germination. *Parthenium* shows 16.35% while *Alternanthera* leaf extract treated shows 30.45%. Similar results are obtained after 72 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 bipinnata* > *Parthenium hysterophorus* > *Alternanthera sessilis.*

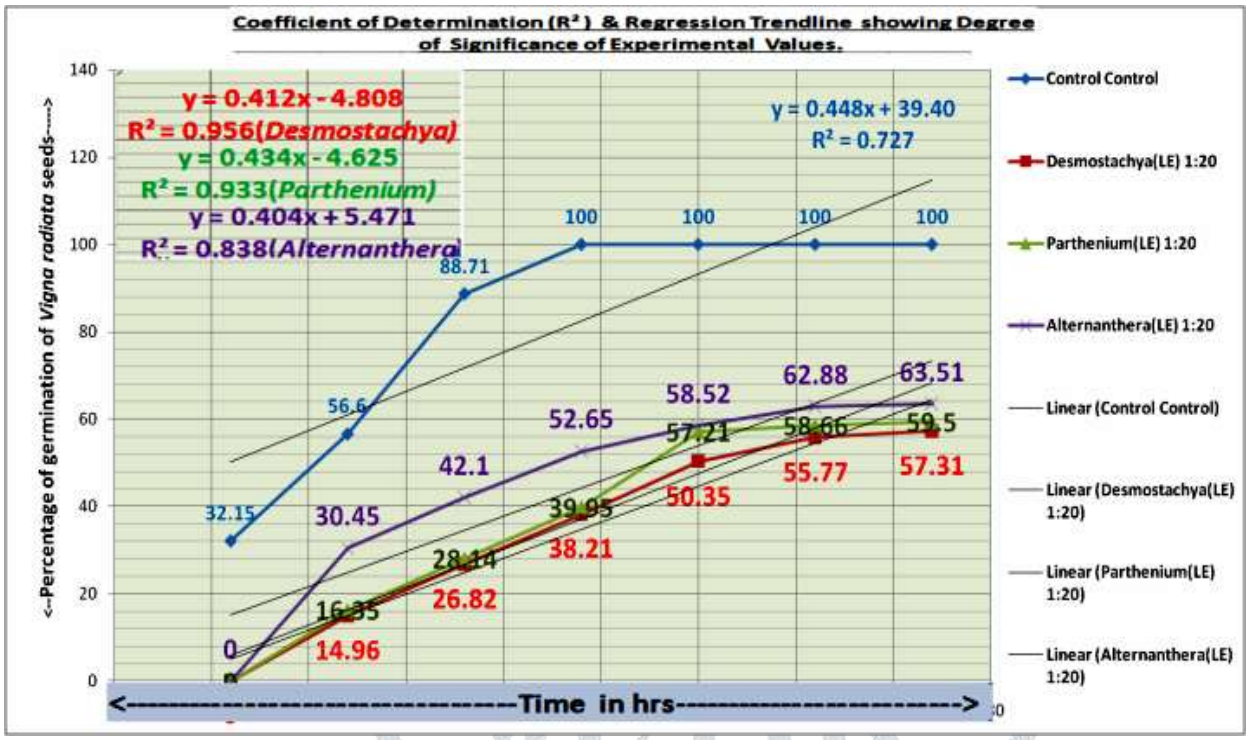

Fig4. Graphical presentation of coefficient of determination (R^2) of control and 1:20 leaf extract of *Desmostachya, Parthenium* and *Alternanthera,* using MS Excel Spreadsheet.

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 *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, 0.933 in case of *Parthenium and* 0.838 in case of *Alternanthera* treated *Vigna* seeds also indicate a high level of goodness of fit, indicating that for all the three cases, the experimental data is highly significant.

IV- CONCLUSIONS :

A critical analysis help reveal that all the weeds taken for study potentially render allelopathic action on *Vigna radiata* seeds. Allelopathy is thus a dose and response phenomenon depending on the concentration of allelochemicals i.e. higher the concentration of extracts greater is the resistance to the percentage and speed of seed germination and seed respiratory activities as indicated by reduced stainability with TTC along with increase of the T_{50} values clearly evident from table 1. Also the observed data on the percentage speed of *Vigna* seeds germination with elapsed time each twenty four hours interval till 168 hours (Table 2) help to establish the relative allelopathic potential of the weeds in an ascending order i.e. *Alternanthera sessilis <Parthenium hysterophorus <Desmostachya bipinnata.* Interestingly it is thus concluded that the monocotyledonous weed *Desmostachya bipinnata* is more allelopathically vigorous than the other two dicotyledonous weeds *Parthenium hysterophorus* and *Alternanthera sessilis* raising questions as to whether monocotyledonous weeds are more allelopathic or dicotyledonous weeds? Further research might thus be relevant to know the interaction of other dicotyledonous and monocotyledonous weed species on both wild weed species and domesticated crop species for a better comparative understanding of the allelopathic mechanisms of wild versus domesticated, dicotyledonous versus monocotyledonous crop plants opening new vistas in allelopathy research.

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