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Evaluation of Allelopathic effects of *Lantana camara(Linn)* on regeneration of *Pogonatum aloides* in culture media

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ABSTRACT

Explants obtained from the apical, middle and basal parts of Pogonatum aloides were allowed to regenerate with half knop's liquid culture medium supplemented with Lantana camara(L) leaf,stem and root extract at various concentration(i.e.5,10,15,20,30,40,50%).Maximum regeneration was observed in control. The regeneration percentage decreased with increase in extract concentration of Lantana camara (L). The leaf extract was found to exhibit maximum inhibitory effect followed by the stem and root extracts. The apical explants showed the greatest potential for regeneration followed by the middle and basal explants.

Key Words – Pogonatum aloides, Lantna camra, Regeneration.

INTRODUCTION

Lantana camara L. (family Verbenaceae) is the among top ten invasive weeds on the earth (Sharma et.al.,2001). In its native range in Tropical America. Lantana camara generally remains to small thickets up to 1m diameter (Palmer and Pullen 1995). This weeds grow well on nutrient thickets and also well on nutrient deficient barren soils(Bhatt et.al.,1994) and light availability (Gentle and Duggin.,1997). There are allelochemicals present in all parts of shrub. When released in surrounding these chemicals interfere with germination of many species (Ambika et.al.,2003; Bais et.al. 2004; Mukesh Kumar et.al.2011) Its leaf, stem and root cotain some harmful allelochemicals which inhibited the germination and growth of Bryophytes (Mersie and Sing, 1987)

Epuipped with these features Lantana camara has potential to prevent to natural regernation of mosses (morton, 1994; Ambika et.al.2003). Allelopathy *of Lantana camara* may be the cause of its toxicity to living beings and its ability to cause shifts in species distribution and composition when it invades other ecosystems. *Lantana* contains about 50 species. Among them *Lantana camara* Linn, contain much

harmful alliochemicals (Chopra, and Kumar, 1961). Lantana is an aggressive invader of natural ecosystem (Choudhary& Bapna1995, S.R Satish kumar et. al.2011).

Lantana has allelopathic effect against agronomic crops and it is one of the most toxic weeds in the world (Giles, K.L.1971 Lalfakzuala R. et.al. 2006). Keeping this problem in mind an attempt has been made together information on various constituents of *Lantana camara Linn* and the effect of allelochemicals present in different plant parts which affect regeneration in Bryophytes. Very little work has been done on allelopathic effect of *Lantana* on Bryophytes. The objective of the present study is to study the Allelopathic effects of Lantana camara (Linn) on *Pogonatum aloides*.

MATERIALS AND METHODS

The material were collected from various localities of Dharmshala tehsil of Kangra District (H.P). The collected moss were carefully determined. Their identification was confirmed by matching the herbarium sheets at M.D.P.G. college, Sriganganager (Rajasthan).

Preparation of extracts

In the present experiment , Lantana camara Linn. Was used aqueous extract of Lantana camara leaves, stem , root, were separately prepared as under 200gm of fresh leavas , stem,root were separately soaked in 100ml distilled water and kept at a room temperature of $28-30^{\circ}$ C. After 24 hour , the aqueous extract was filtered through the sieve and than some of the extracts was diluted to make the concentration to 5,10,15,20,30,40,50% (on the basis of volume) and stored for explant treatment experiment.

Sterilization of glassware and culture media

Conical flask, plastic petri dishes, What man's filter paper No. I and test tubes were used for the experimental work. The glassware was wrapped in an aluminum foil and sterilized dry in an oven at 120°C for 48 hours. Plastic petri dishes and What man's filter paper No. 1 were wrapped in aluminium foil and sterilized in an autoclave. Half Knop's culture medium contained in corning glass flask were also sterilized by autoclaving.

Sterilization of explants

Before setting the experiments the gametophyte of *Pogonatum aloides* were washed repeatedly under slow running tap water to remove soil particles. Then apical, middle and basal explants were surface sterilized for 2-3 minutes in 2% solution of calcium hypochlorite or some time with chlorine water and again washed with double distilled water.

Experimental design

Explant obtained from the apical, middle and basal parts of *Pogonatum aloides* were allowed to regenerate in Petri dishes containing filter paper moistureed with half- strength knop's liquid culture Supplemented with *Lantana camra* root, stem, and leaf extracts at various concentration (i.e. 5,10,15,20,30,40and 50 %). The regeneration percentage were recorded on 10th 20th and 30th day.

RESULTS

1.Regeneration from Apical Explants of Pogonatum aloides with Leaf, Stem and Root Extract of L. camara in Half Knop's Liquid Culture Medium on 10th, 20th and 30th day (Table 1.1a&1.1b): On 10th day, control shows 60.00 per cent regeneration which was the highest. In leaf, stem and root extract, the regeneration was observed up to 30 % (6.67) concentration only. The mean values for regeneration per cent was maximum for root extract (30.00), followed by stem extract (25.00) and leaf extract (20.83). Regeneration occurred from 5 % concentration to 30 % concentration.

On 20th day, the maximum 66.67 per cent regeneration occurred in control. The percentage of regeneration was observed only up to 30 % (6.67) concentration of leaf extract, up to 40 % (13.33) concentration of stem extract whereas root extract shows regeneration in all the concentration up to 50 % (6.67). The mean value for regeneration per cent was maximum for root extract (40.00) followed by stem (35.00) and leaf extract (27.50). Further it is seen from the mean value that regeneration occurred up to 50 % concentration of the various extracts.

On 30 day, control resulted in the maximum per cent of regeneration i.e. 80.00 %. In leaf extract, the percentage of regeneration was observed up to 40 % (6.67) concentration. In stem extract, the percentage of regeneration was recorded up to 50 % (6.67) concentration and in root extract it was up to 50 % (13.33) concentration. The mean value for regeneration per cent was maximum for root extract (46.67), followed by stem (40.83) and leaf extract (34.17). Mean value also shows that regeneration took place up to 40 % leaf extract and up to 50 % of stem and root extracts.

Statistical analysis shows that difference between the different extracts was not significant, difference between different concentrations was always significant, but their interaction was not significant.

2.Regeneration from Middle Explants of Pogonatum aloides with Leaf, Stem and Root Extract of L. camara in Half Knop's Liquid Culture Medium on 10th, 20th and 30th day (Table 1.2a&1.2b):

.On 10th day, Table shows that the highest regeneration was recorded in control, which was 46.67 per cent. The percentage of regeneration was recorded only up to 15 % (6.67) concentration of leaf extract, up to 20 % (6.67) concentration of stem and up to 20 % (20.00) of root extract. The mean value for regeneration percentage was minimum for root extract (20.83), followed by stem (16.67) and leaf extract (11.67). There was complete inhibition of regeneration in 30 % of concentration and above.

On 20th day, the maximum per cent of regeneration was observed in control i.e. 53.33 %. In leaf extract the percentage of regeneration was recorded up to 20 % (20.00) concentration, up to 30 % (13.33) concentration of stem and root. The mean value for regeneration percentage was maximum for root extract (34.17), followed by for stem (29.17) and for leaf extract (25.00). Regeneration occurred up to 30 % concentration of various extracts.

On 30th day, it was concluded by Table (1.2a) that the percentage of regeneration increased with the passage of time. Control shows the maximum per cent of regeneration i.e. 66.67 %. In leaf extract, the percentage of regeneration was recorded up to 30 % (13.33) concentration, whereas up to 30 % (20.00) concentration of stem extract and up to 40 % (13.33) concentration of root extract. The maximum mean value for regeneration percentage was recorded for root extract (34.17) followed by stem extract (29.17) and leaf extract (25.00). Increases in concentration of each extract resulted in respective decrease of regeneration per cent.

Concentration	$10^{\rm th}$ day			20 th day			30 th day					
	LLE	LSE	LRE	Mean	LLE	LSE	LRE	Mean	LLE	LSE	LRE	Mean
Control	60.00	60.00	60.00	60.00	66.66	66.66	66.66	66.66	80.00	80.00	80.00	80.00
5%	40.00	46.66	53.33	46.00	46.66	53.33	60.66	53.33	53.33	60.00	66.66	60.00
10%	26.66	40.00	46.66	37.77	40.00	46.66	53.33	46.66	46.66	53.33	60.00	53.33
15%	20.00	26.66	40.00	28.88	33.33	40.00	46.66	40.00	40.00	53.33	53.33	48.88
20%	13.33	20.00	33.33	22.22	26.66	33.33	40.00	33.33	33.33	40.00	46.66	40.00
30%	6.66	6.66	13.33	6.66	6.66	26.66	26.66	20.00	13.33	20.00	33.33	22.22
40%	0.00	0.00	6.66	0.00	0.00	13.33	20.00	1.11	6.66	13.33	20.00	13.33
50%	0.00	0.00	0.00	0.00	0.00	0.00	6.66	2.22	0.00	6.66	13.33	6.66
Mean	20.83	25.00	30.00	25.27	27.50	35.00	40.00	34.09	34.16	40.83	46.66	40.55
SEm±	6.8	6.8	6.8	3.93	7.45	7.45	7.45	4.3	7.33	7.33	7.33	4.23
CD 5 %	19.35	19.35	19.35	11.17	21.19	21.19	21.19	12.14	24.84	20.84	20.84	12.03
Extract medium (A)SEm±	2.41			2.64			2.59					
CD 5 %		6.	84		7.49			7.36				

Table1.1a.: Showing the effect of different	nt concentration of le	af, stem and root	extract of Lantana ca	mara on
apical explants of Pogonatum aloides	on 10 th , 20 th and 30 th	' day in Half Knop	o's Liquid culture me	dium.

Table1.1b:Mean	square of dif	ferent davs fo	r regeneration in	anical explants.
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Source	d.f	10 th day	20 th day	30 th day
Between extracts (A)	2	505.55	938.88	938.88*
Between centrations (B)	7	4456.35**	4149.21**	5647.62**
A X B	14	80.15	56.34	30.952
Error	48	138.88	166.66	161.11

* Significant at 5 % level of significance, ** Significant at 1 % level of significance LLE= Lantana Leaf Extract LSE= Lantana Stem Extract LRE= Lantana Root Extract

It is revealed from statistical analysis that difference between the different extracts was significant on 10th day. Difference between concentrations was also significant at all time periods, but their interaction was not significant.

3.Regeneration from Basal Explants of Pogonatum aloides with Leaf, Stem and Root Extracts 'of L. camara in Half Knop's Liquid Culture Medium on 10th, 20th and 30th day (Table 1.3a&1.3b): On 10th day, Table indicates that addition of different extracts of Lantana resulted in decreases in regeneration percentage as compared to control (53.33 %). The per cent of regeneration was observed only up to 20 % (6.67) concentration of leaf extract, up to 30 % (6.67) concentration of stem extract whereas up to 30 % (6.67) of root extract. The mean value for regeneration percentage was maximum for root extract (25.83), followed by stem extract (22.50) and leaf

extract (15.00), It is evident from mean values that 40 % and above concentrations of various explants show total inhibition of regeneration.

On 20th day, the maximum percentage of regeneration was observed in control i.e. 60.00 %. In leaf extract regeneration per cent occurred up to 30 % (13.33) concentration, up to 30 % (20.00) concentration of stem extract and 40 % (13.33) concentration of root extract. The mean value for regeneration per cent was maximum for root extract (33.33), followed by stem (28.33) and leaf extract (22.50). It was observed from mean value that leaf and stem extract shows regeneration upto 30 %, concentration, while root extract shows regeneration upto 40 % concentration.

On 30th day, control resulted in the maximum per cent of regeneration i.e. 73.33 %. In leaf extract regeneration was observed upto 40 % (6.67) concentration, in stem up to 50 % (6.67) concentration and in root extract up to 50 % (13.33) concentration. The mean value for regeneration per cent was maximum for root extract (41.67) followed by stem extract (36.67) and leaf extract (30.00). Regeneration per cent increased with the passage of time.

It is revealed from statistical analysis that difference between the different extracts was significant on 10th day. Difference between different concentrations was always significant, but their interaction was not significant.

Table1.2a: Showing the effect of differen	t concentration of le	eaf, stem and root extract of Lan	tana camara on
middle explants of Pogonatum aloides	on 10 th , 20 th and 30 ^t	th day in Half Knop's Liquid culf	ure medium.

Concentration	10^{th} day			20 th day			30 th day					
	LLE	LSE	LRE	Mean	LLE	LSE	LRE	Mean	LLE	LSE	LRE	Mean
Control	46.66	46.66	46.66	46.66	53.33	53.33	53.33	53.33	66.66	66.66	66.66	66.66
5%	26.66	33.33	40.00	33.33	33.33	40.00	46.66	40.00	40.00	46.66	53.33	46.66
10%	13.33	26.66	33.33	24.44	26.66	33.33	40.00	33.33	33.33	40.00	46.66	40.00
15%	6.67	20.00	26.66	17.77	20.00	26.66	33.33	26.66	26.66	33.33	40.00	33.33
20%	0.00	6.66	20.00	8.88	20.00	20.00	26.66	22.22	20.00	26.66	26.66	24.44
30%	0.00	0.00	0.00	0.00	0.00	13.33	20.00	11.11	13.33	20.00	26.66	20.00
40%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.33	4.44
50%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	11.66	16.66	20.83	16.38	19.16	23.33	27.50	23.33	25.00	29.16	34.16	29.44
SEm±	5.09	5.09	5.09	2.94	6.53	6.53	6.53	3.71	6.53	6.53	6.53	3.77
CD 5 %	14.48	14.48	14.48	8.35	18.56	18.56	18.56	10.71	18.56	18.53	18.53	10.71
Extract medium	1.8			2.31			2.21					
(A)SEm±							2.51					
CD 5 %	5,11			6.56			6.56					

Table1.2b:Mean square of different days for regeneration in apical explants

Source	d.f	10 th day	20 th day	30 th day
Between extracts (A)	2	505.55*	416.66	505.55
Between centrations (B)	7	2742.06**	3250.79**	4390.48**
AXB	14	80.15	48.41	35.71
Error	48	77.77	127.77	127.77

^{*} Significant at 5 % level of significance, ** Significant at 1 % level of significance LLE= Lantana Leaf Extract LSE= Lantana Stem Extract LRE= Lantana Root Extract

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Concentration	10 th day				20 th day			30^{th} day				
	LLE	LSE	LRE	Mean	LLE	LSE	LRE	Mean	LLE	LSE	LRE	Mean
Control	53.33	53.33	53.33	53.33	60.00	60.00	60.00	60.00	73.33	73.33	73.33	73.33
5%	26.66	40.00	46.66	37.77	33.33	46.66	53.33	44.44	46.66	53.33	60.00	53.33
10%	20.00	33.33	40.00	31.11	26.66	40.00	46.66	37.77	40.00	46.66	53.33	46.66
15%	13.33	26.66	33.33	24.44	20.00	33.33	40.00	33.33	33.33	40.00	46.66	40.00
20%	6.66	20.00	26.66	17.77	13.33	26.66	33.33	26.66	26.66	33.33	40.00	33.33
30%	0.00	6.66	6.66	4.44	6.66	20.00	20.00	17.77	13.33	26.66	26.66	22.22
40%	0.00	0.00	6.66	0.00	0.00	0.00	13.33	4.44	6.66	20.00	20.00	13.33
50%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.66	13.33	6.66
Mean	15.00	22.50	25.83	21.11	22.50	28.33	33.33	28.06	30.00	36.66	41.66	36.11
SEm±	5.44	5.44	5.44	3.14	6.53	6.53	6.53	3.77	7.07	7.07	7.07	4.08
CD 5 %	15.48	15.48	15.48	8.93	18.56	18.56	18.56	10.71	20.11	20.11	20.11	11.61
Extract medium	1.02			2.21			2.50					
(A)SEm±	1.92			2.51			2.50					
CD 5 %	5.42			6.56			7.10					

Table1.3a: Showing the effect of different concentration of leaf, stem and root extract of Lantana camara on basal explants of *Pogonatum aloides* on 10th, 20th and 30th day in Half Knop's Liquid culture medium.

Table1.3b:Mean se	quare of different	days for re	egeneration in	apical explants

d.f	10 th day	20 th day	30 th day
2	738.88	705.55	822.22*
7	3352.38**	3681.75**	4365.08**
14	78.57	57.93	22.22
48	88.88	127.77	150.00
	d.f 2 7 14 48	d.f 10 th day 2 738.88 7 3352.38** 14 78.57 48 88.88	d.f 10 ^h day 20 ^h day 2 738.88 705.55 7 3352.38** 3681.75** 14 78.57 57.93 48 88.88 127.77

* Significant at 5 % level of significance, ** Significant at 1 % level of significance LLE= Lantana Leaf Extract LSE= Lantana Stem Extract LRE= Lantana Root Extract

DISCUSSION

Regeneration process affected by allelochemicals is in the decreasing order of leaf, stem and root extracts of Lantana camara . Process of regeneration was very little affected by Lantana camara root extract, while leaf extract affected the process more adversely. Similar result were observed by Rahbar, and Chopra(1982) who studied the allelopathic effect of L. camara on regeneration of a liverwort Asterella angusta. Bhansali (2002) also concluded that leaf, stem and root extract of L. camara proved inhibitory for the regeneration of moss Physcomitrium japonicum. Chaudhary and Agrawal (2003) reported that leaf, stem and root extracts of Lantana camara prepared in Half Knop's liquid culture medium and water extracts inhibited the spore germination of Plagiochasma appendiculatum. Leaf extract is the most potent inhibitor followed by stem and root extracts.

The phytotoxicity of Lantana leaf extracts was the maximum due to complex interactions between its 14 phenolic compounds (Gedenas. 2001). Glandular trichomes present on the leaf surface might be storing these chemicals is the second reason, and third reason was the more solubility of allelochemicals present in leaves as compared to stem and root. Absence of glandular and non glandular trichomes on the root may result in lesser solubility of these allelochemicals content as cellular constituents of the root cells. According to Schuster(1980) allelopathy partially provides protection against decay and imparts dormancy to weed seeds

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present in the soil and thus they remain viable for several years. The weeds affect plants through release of phytotoxins from seeds decomposing residues may exert allelopathic effect on neighbouring plants due to (a) Inhibition of biological nitrogen fixation, (b) Inhibition of seed germination, growth and yield.

The inhibition of regeneration process in different explant of Pogonatum aloides was found in the decreasing order of apical, basal and middle explant. The results are in conformity with Patidar, and Kaul (1993) and Agarwal (2003) in liverwort Riccia billardieri and Chohan(2002) in liverwort Asterella angusta. Apical explants least affected by all three extracts due to presence of higher concentration of auxin in this region. Basal explants has higher concentration of growth hormones in comparison to middle part, so basal part shows higher regeneration as compared to control and middle explants. Fulford,M (1956) found out the synthesis of auxins in the apical region of leaves. Polar transport of auxins is a well established phenomenon. The capacity of regeneration increases with passage of time which may be due to degradation of allelochemicals present in different exracts of Lantana.

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