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RESEARCH ARTICLE

ALLELOPATHIC POTENTIAL OF CALLUS EXTRACT OF *LANTANA CAMARA*

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ABSTRACT

Callus cultures of *Lantana camara* L. (family verbenaceae) were induced from leaf and stem explants on Murashige and Skoog medium supplemented with α naphthalene acetic acid (NAA), Indole3-acetic acid (IAA) and 2,4 dichloro-phenoxyacetic acid (2,4-D). Callus cultures (4.04 g dry weight) developed from young leaf and shoot tips on medium containing 2, 4-D (0.02 mg/liter) after 25 days *in vitro*. Callus extract (1% conc.) showed toxicity to the growth of *Salvinia molesta* Mitchell after 7 days. The leaves and flowers of this species serve as for a source of allelopathic compounds, both exhibited high potential to act as strong biopesticide and suppressed a number of organism including bacteria, fungi, aquatic and terrestrial weeds in its aqueous leachate/extract in earlier studies. This study high lights the ability of callus of *L.camara* to produce allelochemicals under *in vitro*conditions that also exhibited phytotoxicity.

INTRODUCTION

Lantana camara L. (*Lantana*), family Verbenaceae, contains triterpenoids Lantadene A and Lantadene B, and essential oil as major constituent (Sharma and Sharma, 1989). Later 14 phenolic compounds including salicylic acid have also been identified (Jain *et al.*, 1989). The dried twigs of *L. camara* in the form of aqueous leachate inhibited the growth of terrestrial (Mersie and Singh, 1987; Sharma, 1989) and aquatic weeds (Sutton and Portier, 1989). The leachate of dried, fresh as well as decomposing residue of *Lantana* killed water hyacinth within a few days (Saxena, 2000). It also decreased the biodiversity in the field when its dry matter was incorporated in the soil (Saxena *et al*., 2010). Further, Kong *et al.* (2006) suggested that allelochemical of *L. camara* could potentially be used to improve the management of weeds and algae in aquatic systems. More than 62 secondary metabolites have been identified from this plant species (Chowdhury *et al.*, 2007). Essential oil from the fresh leaves of this plant exhibited larvicidal activity (Costa *et al.*, 2010). Methanolic extract of this plant exhibited strong toxicity to the growth *Staphyllococcus aureus* (Varma *et al.*, 2010). A number of studies indicated the toxicity of *Lantana* plant on a range of organism. This study was made to investigate the phytotoxicity of extract of *Lantana* callus using *Salvinia molesta* growth bioassay.

MATERIAL AND METHODS

Development of callus of Lantana

Explants of *Lantana* (leaf and stem segments) for *in vitro* studies were surface sterilized with 0.1% (w/v) mercuric chloride solution for 5 minutes and thoroughly washed 3 times with sterile distilled water. The explants (4 in each flask) were transferred on MS (Murashige and Skoog, 1962) medium containing 0.8% (w/v) agar, 3% (w/v) sucrose supplemented with different concentrations of 2,4-D, and a mixture of NAA and BAP as described in Table 1. The medium was autoclaved at 20 lbs pressure for 20 min before inoculation. Twelve replicates were prepared for each concentration. The culture were maintained at a temperature of 25±2 °C under continuous fluorescent light. Callus cultures thus obtained were collected and kept in a hot air oven at 80°C, dried, weighed and powdered in pestle and mortar.

Salvinia Growth Bioassay

Allelopathic impact of aqueous extract of *Lantana* callus was investigated using *Salvinia* growth bioassay. The aqueous leachate was prepared by immersing the 1g air dried *Lantana* twigs with inflorescence and dried callus in 100 ml of distilled water for 24h respectively and filtered twice through filter paper thereafter and referred as 1%. A growth experiment was set up in plastic disposable pots. Each pot was filled with 100 ml of each treatment. *Salvinia* had 12 leaves with an apical bud and 0.077±0.005g dry weight in each pot in all the sets. These pots were kept in an experimental chamber. The light intensity ranged from 1000-2500 lux measured by lux meter (model Aplab ML 4420). The mean minimum and maximum temperature ranged from22-26°C and 32-38°C respectively. The plants were harvested after 21 days .The leaves were counted weekly in all the five sets and dried to constant weight in a hot air oven at 80°C and weighed thereafter. Growth rate was calculated according to Hiliman (1961).

$$\frac{\text{Log 10 (Ffn) – Log 10 (Ifn)}}{\text{Number of days (d)}}$$

Where Ffn-final frond number and If n-initial frond number.

RESULTS

Induction of callus

The response of different growth hormones on *Lantana* explants is shown in Table 1. The highest yield of callus was obtained at

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0.2mg / l (mg l^{-1}) 2,4-D (Fig 1a), however, at 0.5 mg/l callus was not obtained. Callus was not developed in the presence of other hormones viz., BAP, IAA, KN+NAA and BAP+IAA at various concentrations studied except 2,4-D. Callus cultures were obtained only in 33% flasks. The total dry weight of callus was observed to be 4.04g (Table 1). Callus developed from stem explants was yellow-white greenish and friable (Table 2) whereas it was brown-white to greenish friable (Figure 1 a-b)

Salvinia growth bioassay

The growth of *Salvinia* was progressively increased (Table 3) in control set whereas the plants died in treated set having aqueous extract of callus and *Salvinia* died after 14 days. The number of leaves increased from 12±0.00 to 27±0.58 after 21 days whereas the dry weight increased from 0.077±0.005 to 0.103±0.030g in control at the end of the experiment (Table 3). The *Salvinia* fronds turned black and died within 14 days (Figure 1 c-d).

Table 1 Response of different growth hormones on *Lantana camara* explants

	Different concentrations of growth hormones								
Hormone	**BAP**	**IAA**		**KN+NAA**		**2,4-D***		**BAP+IAA**	
Concentration	0.05	0.01	0.05	0.05+0.02	0.02+0.02	0.02	0.5	0.01+0.05	0.02+0.02
Callus	-	-	-	-	-	+	-	-	-
Growth	-	-	-	-	-	+	-	-	-
Dry weight, g	4.04±07								

*Four flasks showed positive result out of 12 replicates of each concentration, - negative response of hormones, + moderate response of hormones, BAP 6- Benzylaminopurine, KN Kinetin, IAA Indole-3 acetic acid, 2,4-D 2,4-dichlorophenoxy acetic acid, NAA Napthalene acetic acid

Table 2 Response of different growth hormones on *Lantana camara* explants

	Different concentrations of growth hormones								
Hormone	**BAP**	**IAA**		**KN+NAA**		**2,4-D***		**BAP+IAA**	
Concentration	0.05	0.01	0.05	0.02+0.02	0.05+0.02	0.02	0.5	0.01+0.05	0.02+0.02
Callus	-	-	-	-	-	+	-	-	-
Growth	-	-	-	-	-	+	-	-	-
Dry weight, g	4.04±07								
Callus Morphology	Explants turned brown	Explants turned brown		Explants turned brown		Stem explant Yellow-white Greenish Friable Callus	Leaf explant Brown-White Greenish friable Callus	Explants turned brown	Explants turned brown

*Four flasks showed positive result out of 12 replicates of each concentration, - negative response of hormones, + moderate response of hormones, BAP 6-Benzylaminopurine, KN Kinetin, IAA Indole-3 acetic acid, 2,4-D 2,4-dichlorophenoxy acetic acid, NAA Napthalene acetic acid

Table 3 Phytotoxicity of aqueous extract of callus of *L. camara* using *Salvinia bioassay*

Treatments	**Number of fronds**				**Dry weight (g)**		**Growth rate**
	Intial	**7 Days**	**14 Days**	**21 Days**	**Initial**	**Final**	
Control	12.00 ±00.00	23.00 ±00.58	27.00 ±00.58	26.66 ±00.68	0.077 ±0.005	0.103 ±0.030	10.00 ±00.03
Treated Callus extract, 1%	12.00 ±00.00	Death	Death	Death	0.077 ±0.005	Death	Death

Means ± SE,

Table 4 Phytotoxicity of aqueous extract of leaves of *Lantana camara* using *Salvinia bioassay*

Treatments	**Number of fronds**				**Dry weight (g)**		**Growth rate**
	Intial	**7 Days**	**14 Days**	**21 Days**	**Initial**	**Final**	
Control	12.00 ±00.00	23.00 ±00.58	27.00 ±00.58	26.66 ±00.68	0.077 ±0.005	0.103 ±0.030	10.00 ±00.03
Treated Leaf extract,1%	12.00 ±00.00	21.33 ±00.33	22.00* ±01.52	06.66** ±00.88	0.077 ±0.005	0.02** ±00.00	Death

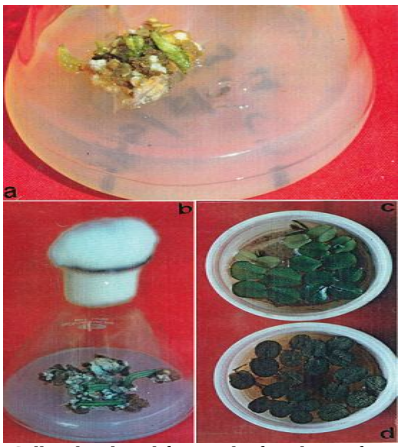

Fig. 1 Callus developed from a- leaf explants of *Lantana camara*, b- stem explants; *Salvinia* growth bioassay- C- control, D-. 1% aqueous extract of callus of *L. camara*

DISCUSSION

Lantana plant is known to exhibit strong allelopathic interaction in a number of studies (Sharma *et al*., 1988). The larvicidal, fungicidal, insecticidal and weedicidal properties of *Lantana* have been reported (Sharma and Sharma, 1989; Verma and Saxena, 2012). Saxena (2000) reported that it completely killed water hyacinth within few days in experimental conditions. The present study shows that the fronds of *Salvinia molesta* completely turned black in the presence of extract of *Lantana* callus. However, only marginal portions of *Salvinia* fronds turned black when it was grown in the leachate of fresh or dried leaves of *Lantana* plant (Gupta, 1998). The toxic agents were identified as phenolic acids (Jain *et al*., 1989). Earlier, Rice (1986) has reported lesser amounts of production of allelochemicals inside the green house in comparison to outdoor plants. The present study confirmed high allelopathic potential of extract of callus. It also indicates that

callus cultures of *L. camara* are capable of producing allelochemicals under *in vitro* conditions exactly similar to *Lantana* plant growing in the field. These findings open up new vistas for production of biopesticides from this plant under *in vitro* conditions for commercial use.

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