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Callus Induction and Antifungal Studies on *Solanum surattense* Burm. F.

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Abstract: The young leaf explants of *Solanum surattense* were cultured on M S medium with different concentration of IAA, NAA with BAP and KIN. It was observed that the leaf explants showed growth response like enlargement followed by initiation of callus. Out of five IAA concentrations (0.50 mg/l - 2.5 mg/l) used, IAA 2.0 mg/l and BAP 0.50 mg/l induced maximum callus formation on leaf explants. The maximum percentage of callus was 84 and minimum percentage was 58 (3.0 mg/l IAA and 0.5 mg/l BAP). Colour of the calli was white and friable. Response of NAA compared with IAA was less. Fresh and dry weights also calculated. Calli dried and used for antifungal studies. To carry out antifungal studies, ethanol used as a solvent for both field grown leaves and leaf derived calli. Based on the concentration of leaf and callus derived extracts, inhibitory effect also varied for different microbes. Among the different fungi tested *Candida tropicalis* and *Trichophyton rubrum* showed maximum zone of inhibition and *Aspergillus flavus* showed minimum zone of inhibition. The MIC was effective and inhibitory effect increased by increasing the dilutions.

Key word: Plant tissue culture · *Solanum surattense* · Callus · Antifungal activity

INTRODUCTION

Many infectious microorganisms are resistant to synthetic drugs, hence an alternative therapy is very much needed [1]. Plants have been an important source of medicine for thousands of years [2]. The medicinal value of drug plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body [3]. Most of the medicinal plants are even today collected from the wild sources. The pressure on the natural population of medicinal herbs is so serious that several precious medicinal plants are likely to be designated as threatened, vulnerable, endangered, extinct, etc [4, 5]. Tissue cultures techniques are also being developed for the mass propagation of medicinal plants are of prime importance for mass production and conservation of the particular species or endangered species because of their medicinal properties [6, 7]. There is a need to employ biotechnological methods to improve the yield of metabolites for their medicinal purposes [6-8].

Solanum surattense Burm. F (Solanaceae) is a spiny perennial herb. It is usually found in India, Pakistan, Malaya and Australia. The solasodine and glycosides are rich in this plant. Besides, this plant widely used against inflammations, arthritis, colic, flatulence, rheumatoid arthritis, cough, fever, asthma, bronchitis, amenorrhea, low back pain, hemorrhoids, epilepsy and kidney stones [9-13]. Since this herb becoming a potential medicinal plant in South Asia, more advance investigations are needed concerning modification of characteristics including rapid growth, increase essential chemicals content and disease resistant in this plant [14, 15]. Various chemical constituents are reported to be isolated from *Solanum* species, which includes alkaloids, phenolics, flavanoides, sterols saponins and their glycosides [16].

MATERIALS AND METHODS

Solanum surattense Burm. F was the plant material used in the present study. From the field grown plants, the

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young leaves and nodal region were excised out and used for callus induction. The collected explants were washed thoroughly in running tap water for 30'. Then the explants were rinsed with 1% savlon solution containing 6-8 drops of tween 20 for 15' and again washed with double distilled water to remove the traces of detergent solution. Again, inside the laminar air flow chamber rinse once with autoclaved water. Then the explants washed with 70% alcohol for 30seconds. Next, the explants rinsed with 0.05% mercuric chloride solution for 5' and again washed with sterile distilled water for 6-8 times. Then the explants were placed in sterile petri plates for inoculation.

The Murashige and Skoog medium [17] consists of macronutrients, micronutrients, iron source and vitamins supplemented with sucrose (3%) as a carbon source and agar (1%) as a solidifying agent (6). The pH of the medium was adjusted to 5.8 before autoclaving at a pressure of 15lbs. The basal medium was supplemented with various auxins NAA (α - Naphthalene acetic acid), IAA (Indole - 3 - acetic acid) and cytokinins BAP (6 - Benzyl amino purine) and KIN (kinetin) at different concentrations and combinations. The cultures were incubated at 25±2°C with 16 hours photoperiod. Callus cultures were maintained on solid M S medium and sub cultured with frequent intervals and used for antifungal studies. Data were recorded in terms of the following parameters like explant response in percentage (concentration wise), fresh and dry weight of calli.

Leaves collected from field-grown plants and leaf derived calli of *Solanum surattense* were used for antifungal study. The leaves of *Physalis minima* were collected from field-grown plants and washed with distilled water to remove the adhering dust particles. Then they were dried in the shaded place. The dried leaves were fine powdered and stored in airtight bottles. 20-30g of leaf powder was placed in the soxhlet extractor by using the solvent ethanol at 60°C for the extraction of bioactive compound. For mass production of callus, 2.0mg/l IAA and 0.5mg/l BAP and1.50mg/l NAA and 0.50mg/l kinetin were used along with MS medium. Callus cultures were maintained on solid MS medium, sub-cultured every 35 days and maintained at 25±2°C with a daily photoperiod of 16/8 hours. Callus cultures were harvested on 40 days of inoculation. The collected calli were dried at 60°C for 48 hours. Susceptibility tests were performed using seven fungal cultures. These microorganisms were collected from Department of Microbiology, J.J College of Arts and Science, Pudukkottai, Tamilnadu.

Determination of Antifungal Activity: Potato Dextrose Agar was used as the medium for the antifungal assay by the agar well diffusion method. Swabbed plates were prepared with the proper concentration of inoculum. Five wells were made and extracts of leaf and callus were poured. Then the plates were incubated at 28°C for three days. Inhibition zones were measured after the incubation period. The tests were performed in duplicates for each microorganism evaluated and the final results were presented as arithmetic average.

The MICs were estimated by broth dilution technique. The conical flasks were incubated at 28°C for each type of fungal cultures for three days. Minimum inhibitory concentration (MIC), which was determined as the lowest concentration of plant extracts inhibiting the growth of the organism, was determined.

RESULT

Out of the different ten hormonal combinations, 2.00mg/l IAA and 0.50mg/l BAP induced maximum callus formation. The maximum percentage of callus was 84 and minimum percentage was 58 (3.00mg/l IAA and 0.50mg/l BAP). Colour of the calli was white and friable. 2.00mg/l IAA and 0.50mg/l BAP and 1.50mg/l NAA and 0.50mg/l kinetin used for the mass production of callus. Time taken for the initiation of callus was seven days. Out of ten different concentrations, 2.00mg/l IAA and 0.50mg/l BAP produced maximum growth in terms of fresh and dry weight of young leaflets callus were 5947.0mg and 2752.00 mg respectively (Fig - 1 and Table - 1). IAA at 3.0mg/l and 0.50mg/l BAP concentration gave minimum growth in terms of fresh and dry weight was 4071.25 mg and 2091.37 mg respectively.

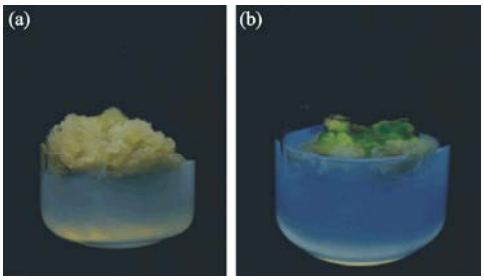

Fig. 1: Callus induction of *Solanum surattense Burm.F.* (a) IAA +BAP; (b) NAA+ kinetin

Table I: Effect of plant growth regulators on callus induction from leaf segments of *Solanum surattense* in MS medium after five weeks of culture

Sl.No.	Hormones (mg/l) IAA + BAP	Percentage of callus induction	Fresh weight (mg) (Mean±S.D)	Dry weight (mg) (Mean±S.D)
1.	0.50 + 0.50	59	4587.50±82.87	2341.25±190.06
2.	1.00 + 0.50	73	5347.50±306.80	2547.25±370.47
3.	1.50 + 0.50	69	4876.43±73.86	2976.54±147.26
4.	2.00 + 0.50	84	5947.0±147.66	2752.00±58.78
5.	2.50 + 0.50	63	5070.75±307.32	2578.50±345.37
6.	3.00 + 0.50	58	4071.25±150.81	2091.37±117.87
NAA + Kinetin				
7.	0.50 + 0.50	70	4987.32±83.25	2316.00±191.05
8.	1.00 + 0.50	67	5692.02±101.25	2303.43±324.83
9.	1.50 + 0.50	81	5862.14±72.11	2724.01 ±158.76
10.	2.00 + 0.50	59	4843.74±36.46	2098.14±96.01
11.	2.50 + 0.50	62	4893.78±66.25	2487.24±84.27
12.	3.00 + 0.50	71	4587.39±36.00	2646.13±68.41

Table II: Antifungal activity of leaf and calli extracts of *Solanum surattense*

Sl.No	Name of the microorganism	Concentration of leaf/callus extract (µg)	Zone of inhibition against leaf extract (mm)	Zone of inhibition against callus extract (mm)
1.	*Aspergillus flavus*	25	4	5
		50	9	9
		100	13	14
		250	13	15
		500	14	17
2.	*Aspergillus fumigatus*	25	4	4
		50	3	5
		100	8	9
		250	11	12
		500	14	13
3.	*Aspergillus niger*	25	4	5
		50	8	8
		100	14	13
		250	19	19
		500	23	21
4.	*Candida albicans*	25	7	7
		50	12	13
		100	15	15
		250	18	19
		500	21	21
5.	*Candida tropicalis*	25	4	4
		50	8	10
		100	12	13
		250	17	18
		500	20	22
6.	*Penicillium oxalicum*	25	6	7
		50	11	12
		100	15	15
		250	18	19
		500	19	19
7.	*Trichophyton rubrum*	25	6	8
		50	11	13
		100	14	18
		250	18	22
		500	21	24

The antifungal activity results are shown in Table- 2. The extracts showed varying degree of inhibitory effect. The inhibitory effect of extracts was directly proportional to increasing concentration of field grown leaf and callus extracts. The leaf and calli extracts inhibit the growth of fungi. Maximum zone of inhibition was obtained in 500ìg concentration of leaf and calli extracts of all fungi screened.Calli extract of *Solanum surattense* was more effective against *Candida tropicalis* and *Trichophyton rubrum.*

DISCUSSION

Solanum surattense is the most potent plant against microorganisms. Callus cultures and field grown plant extracts are useful for controlling various infections. Based on the concentrations of the extracts, the zone of inhibition was changed (John Britto, *et al*., 2001). Agar well diffusion method was effective against eight strains of microorganisms (Marcos Salvador *et al*., 2004). Ethanol was suitable solvent for the extraction (Kumar *et al*, 2005). However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *Solanum surattense* extracts looking toward a pharmaceutical use. Herbal medicines are gaining growing interest because of their cost effective and eco- friendly attributes, this is an urgent need to meet the ever growing demand of medicinal plants in the market.

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