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COMPARATIVE ANALYSIS OF SOLASODINE FROM *IN VITRO* AND *IN VIVO* CULTURES OF *SOLANUM NIGRUM* LINN.

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ABSTRACT
An efficient protocol was devised for rapid callus induction of *Solanum nigrum* Linn. from young leaves. MS medium supplemented with different concentrations IAA (1-3 mg/l) with BAP (0.5 mg/l) and NAA (1-3 mg/l) with BAP (0.5 mg/l) for callus initiation. The growth of the calli derived from leaves increased with time of incubation and remained almost constant after 30 days. For solasodine estimation, the field grown plant part of young leaves and *in vitro* callus (0.5 g each) were weighed and extracted thrice with methanol and subjected to HPLC. The solasodine content of field grown leaves extracts was 0.0798 mg g^{-1} whereas the solasodine content in the in vitro callus extracts were 0.142 mg g^{-1} in 2.5 mgL^{-1} IAA + 0.5 mgL^{-1} BAP, followed by 0.1162 mg g^{-1} in 2 mgL^{-1} NAA + 0.5 mgL^{-1} BAP.

Key words: Callus induction, Solasodine, *Solanum nigrum*, *medicinal* plant

INTRODUCTION
Solanaceae family comprises a number of plants widely known for the presence of variety of natural products of medicinal significance mainly steroidal lactones, glycosides, alkaloids and flavanoids. *Solanum nigrum* L. (Black night shade) a member of the Solanaceae, has a wide range of medicinal values. The herb is antiseptic, antidysenteric and antidiuretic used in the treatment of cardiac, skin disease, psoriasis, herpsvirus and inflammation of kidney. The root bark is laxative, useful in the treatment of ulcers on the neck, burning of throat, inflammation of liver and chronic fever. Berries are bitter and pungent useful in the heart disease, piles, dysentery (Kritikar and Basu, 1935). Most prominent medicinal properties are presence of alkaloids, solamargin and solasonin, which yield solasodine as glycone has great demand in pharmaceutical industries. Callus production is one of the important steps of the meristem culture (Uddin *et al.*, 2004). These cultures represent clumps of unorganized parenchymatous tissues formed by the vigorous proliferation by the mitotic cell division from the small explants in culture, showing no polarity. Solasodine has embryotic, teratonic as well as antifungal, antiviral and molluscidal effects (Kim *et al*., 1996)

Several papers have described solasodine production in tissue cultures of *Solanum laciniatum*, and this has been reviewed by Macek (1989). The aim of the present work, to induce the callus formation from normal size of explants from young tissues and the comparative analysis of solasodine from field grown plant leaves and *in vitro* callus.

MATERIALS AND METHODS

Explants were collected from *in vivo* grown medicinal plants *Solanum nigrum* and its young leaves were used for establishing callus. The young leaves (1-2cm) were washed thoroughly under running tap water and then treated with a few drops of Tween-80 and 1% Savlon for 10 minutes with constant shaking. This followed by successive three washing with distilled water to make the material free from savlon. Again the explants were washed with 70% ethyl alcohol for few seconds and washed with distilled water for 3-4 times. After that, the explants were transferred to laminar air flow chamber and disinfected with 0.1% $HgCl_2$ for 2 minutes and washed with sterile distilled water for 5-7 times. Then, the explants were placed in sterile Petri plates before inoculation. The sterilized leaves were injured all over the surface and used for callus induction.

The excised explants were transferred in 250x150mm culture tubes with 15ml basal media (MS) supplemented with different hormone (IAA, NAA and BAP) concentrations for callus induction. Cultures were incubated at 25 ± 2^0C under the warm fluorescent light with intensity varied from 2000-3000 lux and 16 hours photoperiod.

For solasodine estimation, the field grown plant part of young leaves and *in vitro* callus (0.5 g each) were weighed and extracted thrice with methanol and subjected to HPLC.

HPLC: The model used for HPLC analysis was Shimadzu, Japan. All samples were filtered through 0.2µm membranes. Main column used was analytical-shim-pack CLC-OCTA DECYL SILANE (ODS-C18) (46mm10x25cm) and guard column was shim-pack G-ODS (4mm 10x1cm), flow rate: 1ml per minute, run time: 15 minutes, detector wavelength: 254nm, stationary phase: Silica gel (reversed phase) and mobile phase: Methanol (100% HPLC grade). Column head pressure was 125Kg+1cm^2 20 microlituce sample was injected to the column using Hamilton Microlituce Syringe, made in Japan. UV spectra were recorded with a diode array spectrophotometer coupled online. The analysis was carried at Characterization and measurement lab, Central Electro Chemical Research Institute, CECRI, Karaikudi, Tamilnadu, India. The experimental results were calculated as follows (mg/100g D.wt) =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard weight}}{\text{Dilution}} \times \frac{\text{Dilution}}{\text{Sample weight}} \times \text{Standard purity} \times 100$$

RESULTS AND DISCUSSION

Callus induction was observed in MS media containing different concentrations and combinations of IAA, NAA and BAP. Within 15-17 days of incubation the leaves explants depending upon the concentrations and combination of hormone were induced calli. There was a wide range of variation in percentage of callus formation and average fresh weight of callus. The highest percentage of callus induction (86.66±0.84) was observed in MS medium containing 3mg/L NAA and 0.5mg/L BAP and followed by (80.00±0.97) in MS medium containing 2.5 mg/L NAA and 0.5 mg/L BAP (Table 1 and Plate 1). Highest callus growth in terms of fresh weight (1818.20±0.37) was observed in MS medium fortified with 3 mg/L IAA and 0.5 mg/L BAP and highest dry weight of calli 1317.18±0.83 was observed in MS+3 mg/L IAA and 0.5 mg/L BAP (Table 2 and Plate 2). The color of calli was mostly greenish white. The results obtained by Shahzad (1999) were similar to our findings.

Table 1: Effect of IAA on callus induction and callus growth of young leaves explants of *Solanum nigrum* in combination with BAP (Mean ± S.D).

Hormones mg/l IAA+BAP	Percentage of Callus induction	Fresh weight (mg)	Dry weight (mg)
1.0+0.50	46.66±1.96	985.10±29.13	486.16±24.410
1.5+0.50	53.33±0.66	1016.21±36.13	686.98±28.40
2.0+0.50	66.66±0.53	1325.28±48.01	899.13±29.13
2.5+0.50	73.33±0.42	1540.18±55.17	1098.14±33.17
3.0+0.50	80.00±0.37	1818.20±63.30	1317.18±42.83

Table 2: Effect of NAA on callus induction and callus growth of young leaves explants of *Solanum nigrum* . in combination with BAP (Mean ± S.D).

Hormones mg/l NAA+BAP	Percentage of Callus induction	Fresh weight (mg)	Dry weight (mg)
1.0+0.50	42.62±1.91	486.10±23.41	384.28±22.81
1.5+0.50	60.00±1.53	638.13±28.31	465.31±23.04
2.0+0.50	73.12±1.12	893.41±31.89	534.17±24.05
2.5+0.50	80.02±0.97	998.58±33.07	786.98±26.38
3.0+0.50	86.62±0.84	.1128.17±35.13	895.18±27.01

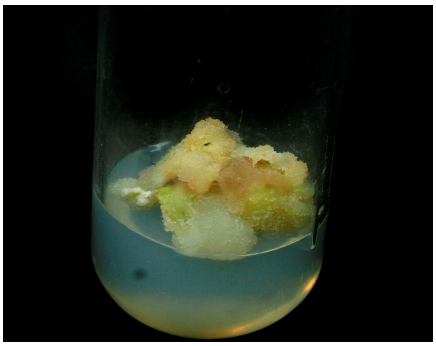


Plate 1 Callus induction (NAA + BAP)

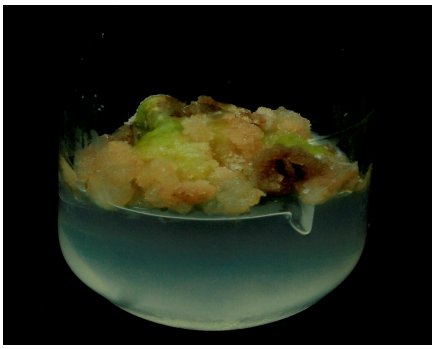
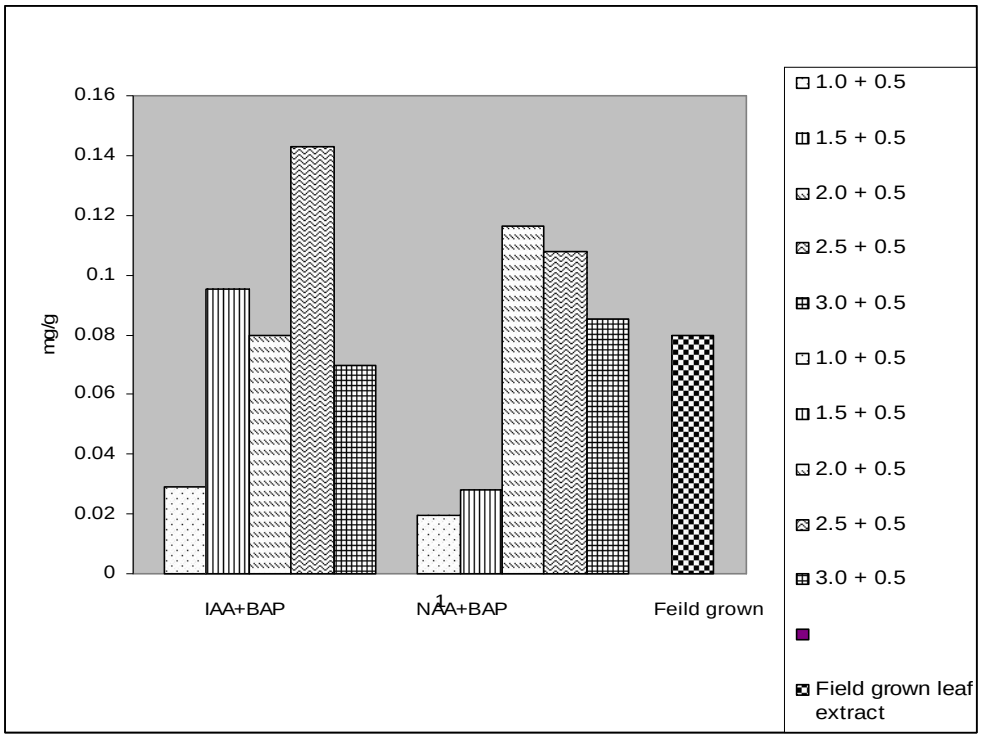


Plate 2 Callus induction (IAA + BAP)

The solasodine content in callus extracts of *Solanum nigrum* L. were higher compared to that the field grown leaves extracts. The solasodine content of field grown leaves extracts was 0.0798 mg g^{-1} whereas the solasodine content in the *in vitro* callus extracts were 0.142 mg g^{-1} in 2.5 mgL^{-1} IAA + 0.5 mgL^{-1} BAP, followed by 0.1162 mg g^{-1} in 2 mgL^{-1} NAA + 0.5 mgL^{-1} BAP (Fig. 1). Similar observation was reported in earlier studies on *Solanum jasminoides* and *Solanum verbascifolium* by Jain *et al*., 1995, *Solanum platfolium* by Jaggi and Singh, 2001 and *Solanum Khasianum* by Bhalsing, 2000.

Figure 1. Solasodine production in Field grown leaf extract and Callus extract of *Solanum nigrum* L.



We have standardized a repeatable protocol for callus induction and solasodine production. The callus cultures are useful for continuous production of solasodine and used for controlling different human diseases.

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Abbreviations:

MS	:	Murashige and Skoog
2, 4-D	:	2,4-dichlorophenoxy acetic acid
IAA	:	Indole -3- acetic acid
NAA	:	α-naphthalene acetic acid
BAP	:	N^6-Benzylaminopurine
Kin	:	Kinetin
D. wt.	:	Dry weight.