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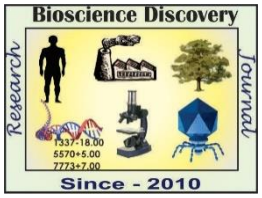
***Research Article***




# *In vitro* studies in *Tylophora asthmatica* (Burm.F) Merill

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**Abstract**

*Tylophora asthmatica* (Burm.F) Merill has recently been included as one of the important drug from natural source for the treatment of respiratory diseases. Traditionally, *Tylophora asthmatica* has been used in treatment of asthma, dermatitis and rheumatism. The plant has been described as bronchodilator, emetic, expectorant and diaphoretic. Clinical studies have shown effectiveness of the drug in bronchial asthma and thus modern research withstands the ancient claims of traditional medicine. The leaf extract of *Tylophora asthmatica* marketed by pharmaceutical companies is standardized to contain 0.1% of the total alkaloids. Recent studies have confirmed the anti-inflammatory activity of Tylophorine. Looking towards this possibility efforts have been made to grow callus in vitro for the extraction of active principle.

## INTRODUCTION

It is a perennial plant native to south and east India. It belongs to family Asclepidaceae and is commonly known as Indian ipecac. This name has been derived from two ancient greek words – '*Tylos*' meaning "knot" and '*phoros*' meaning "bearing". It was earlier placed in Asclepiadaceae which has now been sunk into Apocyanaceace. *Tylophora indica* is indigenous to India where it grows wild in the southern and eastern regions and has a long standing reputation in the treatment of asthma (Biswas and Ghosh, 1973). The leaves and roots of *Tylophora indica* have been included in Bengal Pharmacopoeia since 1884 (Nadkarni, 1976). In Ayurveda, *Tylophora asthmatica* is known as *antamool*. The drug is official in Bengal pharmacopoeia (Kirtikar and Basu, 2001). Traditionally, *Tylophora asthmatica* has been used in treatment of asthma, dermatitis and rheumatism. The plant has been described as bronchodilator, emetic, expectorant and diaphoretic (Shah and Kapoor, 1976).

From phytochemistry point of view, *Tylophora asthmatica* contains 0.2-0.3 % of alkaloids. Tylophorine and tylophornine are important alkaloids encountered and the percentage is not affected by seasonal variations. The extract of *Tylophora asthmatica* marketed by pharmaceutical companies is standardized to contain 0.1% of the total alkaloids. Recent studies have confirmed the anti-inflammatory activity of Tylophorine (Gupta *et al.*, 2010).

Plant rose through the seeds shows tremendous genetic vwariation which is not suitable for commercial cultivation. Vegetative propagation is difficult in *Tylophora* due to low seed viability and germination rate (Thomas and Philip, 2005). In addition, the destruction caused by harvesting the roots as a source of drug has threatened the survival of the plant. Thus, large-scale demand necessitates rapid multiplication of *Tylophora*. Plant tissue culture has been extensively utilized for the improvement of many medicinal plants. During the present investigations efforts have been made to raise plantlets form various explants *in vitro*.

## MATERIALS AND METHODS

Explant viz. shoot, leaves of *Tylophora asthmatica* were collected from the plant planted in botanical garden. Juvenile twigs were surface sterilized with $HgCl_2$ solution (0.1% w/v) for four - five min

followed by three washes with sterile distilled water. Nodal segments of twigs were cut (0.5 cm) and cultured on 8% (w/v) agar solidified MS supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kin) at different concentrations and combinations. Subculturing was done at an interval of 14 - 20 days. The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at 25 ± 2°C under 16/8 hr photoperiod. After 12 weeks, plantlets with roots were successfully planted in pot soil through gradual acclimation.

## RESULTS AND DISCUSSION

Within fifteen days of culture, callus was induced at the cut end of nodal explants. Explants like leaf, stem segment, grown on MS supplemented with 1, 2, 3 and 5 mg/1 BAP and Kin either alone or in combination with 0.1 - 1.0 mg/1 NAA, IAA and IBA (Table 1). Maximum (80%) callus was induced on MS fortified with 5.0 mg/1 BAP with 0.5 mg/1 NAA after two successive subcultures. In this combination light yellowish green and nodular callus developed. Callus was also induced in BAP and Kin supplemented medium (Plate a, b). However, BAP was found to be more effective than Kin for callus induction (Table 1). According to Faisal and anis 2005, callus forms frequently at the basal cut ends of nodal explants on cytokinin enriched medium in species exhibiting strong apical dominance.

**Table 1. Effect of different concentrations of growth regulators on MS for the adventitious shoot regeneration from the nodal callus of *Tylophora asthamatica.***

| Growth regulators(mg/l) | | | | | % explants producing callus | No of Shoots induced |
|---|---|---|---|---|---|---|
| **BAP** | **Kin** | **NAA** | **IAA** | **IBA** | | |
| Growth regulator Free Medium | | | | | - | - |
| 1 | | | | | 20 ± 3.84b | 12±1.52a |
| 2 | | | | | 51 ±1.22a | 12 ± 1.52a |
| 5 | | | | | 40 ± 1.21ab | 12 ± 2.08a |
| | 1 | | | | 18 ± 2.22b | 15 ± 3.21a |
| | 2 | | | | 31 ± 4.44ab | 14 ± 3.03a |
| | 5 | | | | 20±1.21b | 14 ± 3.46a |
| 5 | | 0.1 | | | 40 ± 3.84bc | 10±1.0bc |
| 5 | | 0.5 | | | 82 ± 2.22a | 7±1.15c |
| 5 | | 1.0 | | | 55 ± 0.92b | 8 ± 1.52c |
| 5 | | | 0.1 | | 20 ± 3.84d | 15 ± 2.51a |
| 5 | | | 0.5 | | 44 ± 4.44bc | 10 ± 1 be |
| 5 | | | 1.0 | | 27±1.13cd | 15 ± 3.5a |
| 5 | | | | 0.1 | 31± 5.87bcd | 14 ± 3.51ab |
| 5 | | | | 0.5 | 40+ 7.69bcd | *14* ±2,0ab |
| 5 | | | | 1.0 | 20±1.21cd | 15 ± 3.05a |

Mean values within columns followed by the same letter are not significantly different at 5% level. There were significant differences in regeneration frequencies of callus depending upon the number of shoots/leaf. As stated by Martin (2002) the high morphogenic efficiency of node segments derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds were also developed from callus culture elongated. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days. In *Tylophora indica* many tissue culture studies have reported for successful representation protocols. Somatic embryogenesis has been reported from mature leaves of *Tylophora indica* (Das and Rout, 2005, **Choudhary** et.al. 2005). Another protocol has been developed for high-frequency shoot regeneration and plant establishment of *Tylophora* from petiole-derived callus (Faisal and Anis, 2005).

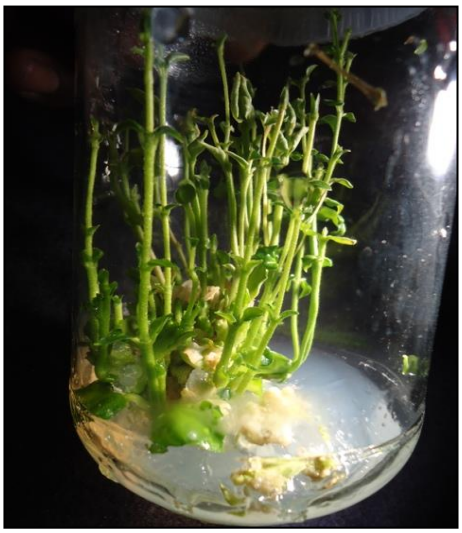

**Fig. 1: a) Callus from stem as an explant b) Callus from leaf as an explant**

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