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IN VITRO CALLUS INDUCTION IN PHARMACOLOGICALLY SIGNIFICANT HERB: *ECLIPTA ALBA*

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ABSTRACT

A competent protocol was developed for *in vitro* plant regeneration and multiplication through callus culture of *Eclipta alba*. Perfect medium for callus establishment through nodal tissue was observed in 2,4-D supplemented media. The callus formed was creamish yellow in colour, showed a growth period of three to four weeks. A moderate quality callus was obtained with different gradients of BAP supplemented medium. A white to creamy callus with rapid growth was observed in the medium supplemented with NAA. Callus was subjected for multiple shoot induction.

Figures : A-D References : 06 Table : 01
KEY WORDS :Callus culture, *Eclipta alba*, *In vitro*, Regeneration.

Introduction

India serves as a home for a vast variety of herbal plants which are truly beneficial for curing a lot of diseases in humans without causing any harm. The trend of using natural products is increasing and hence causing great extinction risks to herbal plants and also depletion of genetic divergence. *Eclipta alba*, also known as *Bhringraj* is a member of Asteraceae/Compositae family is one of the very important medicinal plant. It grows worldwide as a weed and is frequently found in tropical and subtropical regions. It is widely used in hair care products like hair oils and shampoos. It is also extensively used in pharmaceutical and cosmetic industry. The vast range of secondary metabolites showing antimytotoxic, antimicrobial, antihepatotoxic, antioxidant, antihyperglycemic, rejuveniser and antivenom action etc. has been investigated and reported in *Eclipta alba*[2]. The bioactive compounds responsible for these activities are alkaloids, flavonoids, Phenolic compound, Tannins, Saponin **etc**[1].

Material and Method

Nodal tissue segments collected from tissue cultured plants were used as explants for the present study. The tissue cultured plants were cut into smallest segments with at least one node per segment under sterilised conditions in triplicate. These segments were then cultured on MS media supplemented with different concentrations of 2, 4-D, BAP and NAA. All experimental manipulations were carried out under aseptic conditions. Cultures were incubated at 25±2°C under 16 hrs, photo-period and 50-60% relative humidity for upto four weeks. The cultures were regularly sub cultured on fresh medium at four weeks intervals and observations were recorded. Seven replicates per treatment were taken and experiments were

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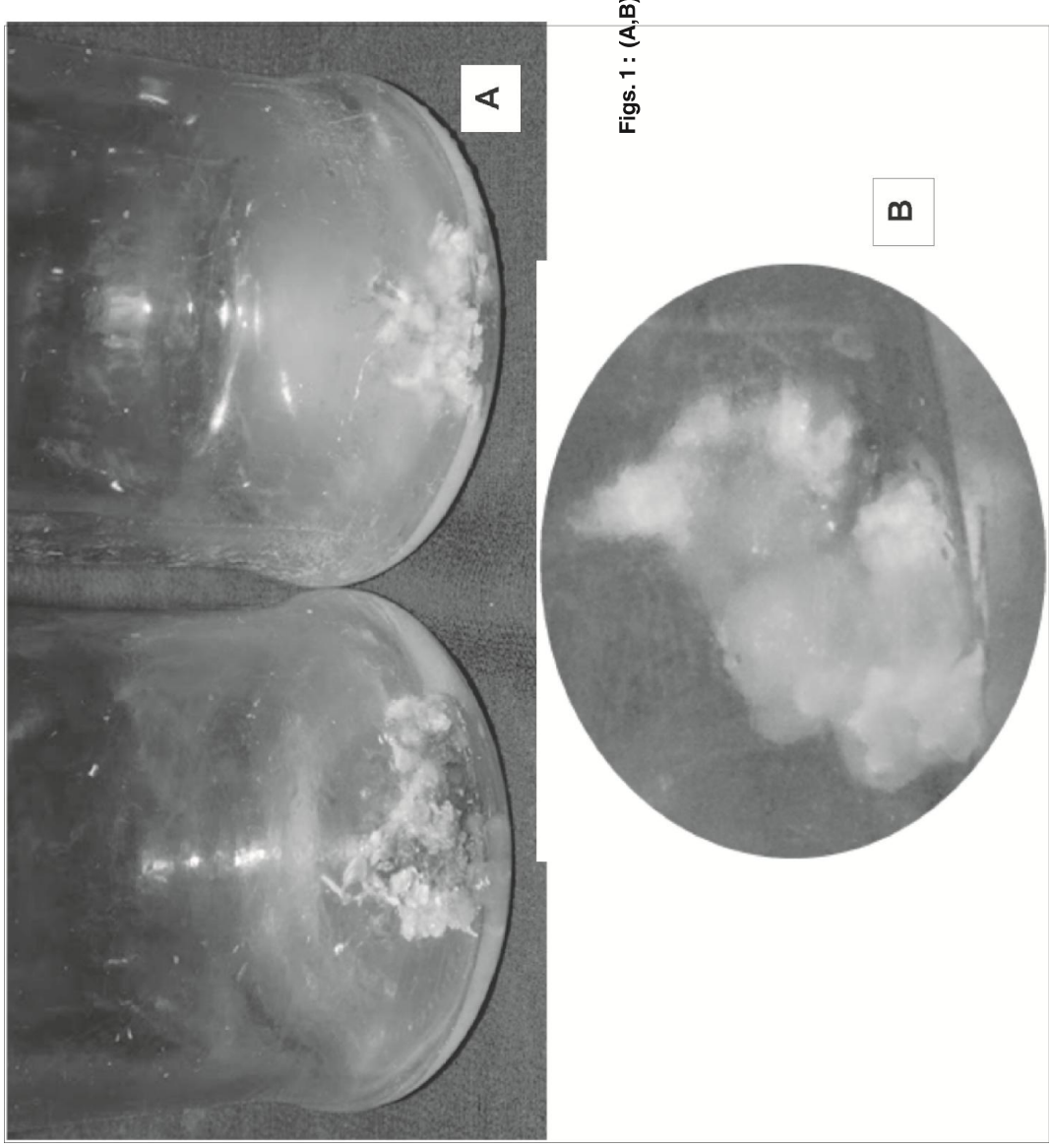

Figs. 1 : (A,B) : Various stages of callus establishment from nodal stem segments on MS medium + 2,4-D (2.5mg/l)/NAA (2.0mg/l).

TABLE-1: Effect of different concentrations of hormone and 2, 4-D on callus induction of explants of *Eclipta alba* using MS medium

MS Medium with 30g/l Sucrose Explant used: Nodal Stem Tissue		
Hormone	**Hormone Concentration (mg/l)**	**Response**
Control	**No hormone**	———
2,4-D	0.5	C^{+}
	1.0	C^{+}
	1.5	C^{++}
	2.0	C^{++}
	2.5	**C^{+++}**
	3.0	C^{+++}
	3.5	C^{+++}
	4.0	C^{+++}
BAP	0.5	C^{+}
	1.0	C^{+}
	1.5	C^{+}
	2.0	**C^{++}**
	2.5	C^{++}
	3.0	C^{++}
	3.5	C^{+}
	4.0	C^{+}
NAA	0.5	C^{+}
	1.0	C^{++}
	1.5	C^{++}
	2.0	**C^{+++}**
	2.5	C^{+++}
	3.0	C^{+++}
	3.5	C^{++}
	4.0	C^{++}

Note : C^{+} - Minor callus formation; C^{++} - Moderate callus formation; C^{+++} - Good Callus Formation

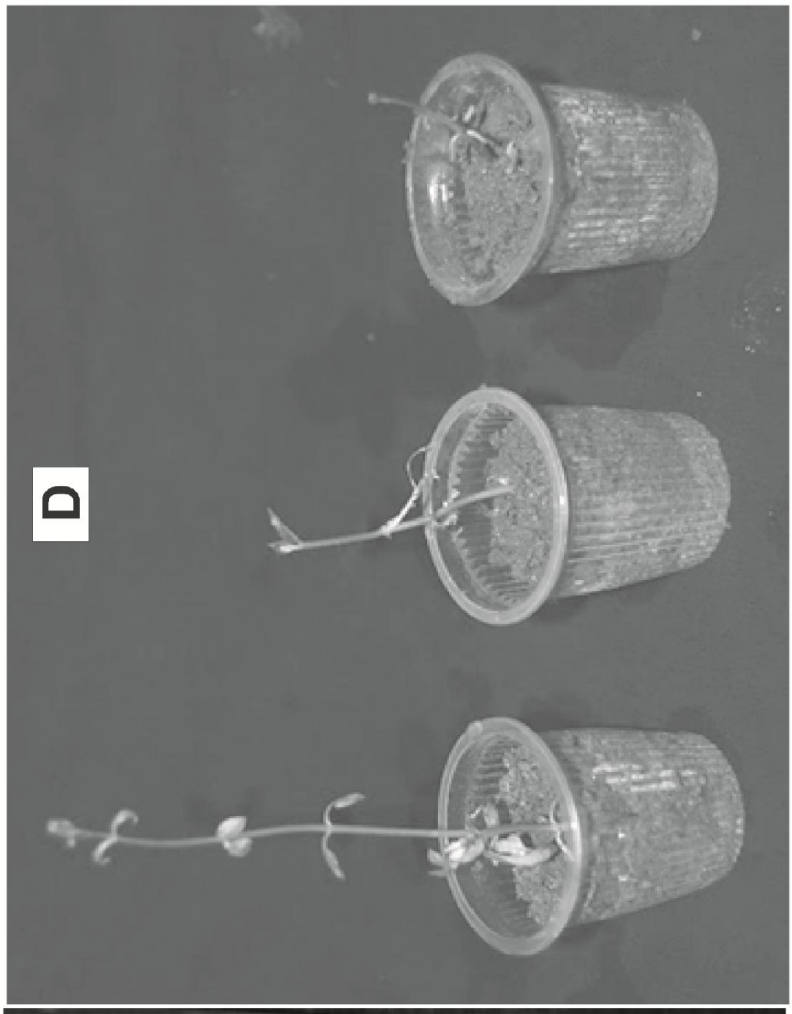

Fig. D : Hardening of Plants

Fig. C : Development of shoot from shoot buds

repeated thrice for confirmation of results. Periodic observations were recorded and the results were subjected to statistical analysis.

Results and Discussions

A good callus formation was observed in 2,4-D supplemented media. Explants from nodal tissues were cultured on MS medium supplemented with different concentration of growth hormones (auxins and cytokinins) alone or in combination for callusing. A MS medium consisting 30g/l sucrose supplemented with 2.5mg/l 2,4-D showed a very good callusing. The callus formed was creamish yellow in colour, showed a growth period of three to four weeks. A moderate quality callus was obtained with different gradients of BAP supplemented medium. The best callus was observed when the MS medium consisting 30g/l sucrose was supplemented with 2.0mg/l NAA. A white to creamy callus with rapid growth was observed in the medium supplemented with NAA. The callus produced was healthy, nodular and green with fast growth.[5] MS medium supplemented with 2, 4-D (1.0 mg/l) proved best for callus initiation which good callus with rapid growth was reported[5]. Similar results in *Eclipta alba* was shown[3,4,6]. Effect of different concentrations of hormone and 2, 4-D on callus induction was summarised (Table-1). Budding, elongation and further development of shoot buds into shoots was achieved using MS medium supplemented with BAP (1.0mg/l) and Kn (1.0mg/l.

Ideal medium for callus establishment through nodal stem segment explant was MS medium consisting 30g/l sucrose supplimented with 2.5mg/l 2,4-D and also good callus was observed when the MS medium consisting 30g/l sucrose was supplimented with 2.0mg/l NAA. Maximum shoot bud differentiation from callus culture was achieved on MS-medium supplemented with BAP (1.0 mg/l) and Kn (1.0 mg/l). Elongation and further development of shoot buds into shoots was also achieved on the similar hormonal concentration in MS medium.

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