Tissue culture study of Eclipta alba (L) Hassk an important medicinal herb of traditional medicine.

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ABSTRACT:

Eclipta alba of *Asteraceae* is being used by the local people for the treatment of different diseases. In the present study attempts were made to induce callus from internodal and leaf explants of *Eclipta alba*. Both auxins alone, or in combination of cytokinin with auxins were supplemented in Murashige and Skoog medium to evaluate their callogenic potential. Picloram when supplemented in M S medium at different concentrations induced callus in both the internodal and leaf explants but with different rate of induction. Here maximum percentage of response 88.62 was obtained in nodal explants in the medium when 8.28 µM of picloram was supplemented. In this medium leaf explants revealed 66.45 percent of response for callus induction. The internodal explants as well as leaf explants revealed percentage of response, 86.78 and 75.26 respectively in the medium containing 11.33µM, 2,4-D. The time for induction of calli was 12 days and 15 days respectively for the above explants synergistic effect of auxins such as 2,4-D and NAA was also studied. It was observed that 11.33µM 2,4-D + 2.69µM NAA, induced callus in 83.54% of internodal explants, where as the leaf explants gave maximum percentage of response in this medium that was 73.24. Synergistic effect of Cytokinin (BAP) and Auxin (NAA) was also evaluated. Here internodal explants revealed highest percentage of response 92.28 in the medium supplemented with 13.72µM BAP + 5.37 µM of NAA. However, leaf explants revealed highest percentage of response in the medium fortified with 8.88µM BAP + 5.37µM NAA. Therefore, BAP at various concentrations and NAA at fixed concentration were considered as the most suitable plant growth regulators for the induction of calli in *Eclipta alba* an important medicinal plant.

KEY WORDS: *Eclipta alba*, Internodal explants, Leaf explants, Picloram, Auxin, Cytokinin, Callogenic.

INTRODUCTION:

Eclipta alba (L.) Hassk, of the family *Asteraceae*, commonly called as **Bhringraj** or **Bhengaria** in the local language, is a wild perennial herb, found in the moist, places, near the drainage of the houses, or in complete dry places. Due to its deep green leaves and white flowers, the species can be easily recognized among the other herbaceous plants which are found growing near it. This plant is familiar among the local people as an important medicinal plant. Local people use the extracts of the plant along with leaves to cure their wound or a fresh cut. Local vaidya suggest people suffering from liver disease, to use its fresh leaves in the empty stomach. *Eclipta alba* has got much importance in modern pharmacological activities. According to modern pharmacological research, the species contains various bioactivities such as anti-tumor, anti-HIV proteases and integrate, blood lipid reduction, prevents liver damage induced by carcinogenic chemicals (Santosh Kumar *et al*; 2006); anti snake venom (Walter *et al*; 1989)., It acts as bronchodialator, it is Hepato-protective (Defeng *et al*; 2014); It is also used in the treatment of enteritis, hepatitis, hyperlipidemia, atherosclerosis, skin diseases, spleen enlargement, gall bladder (Jae-Seung *et al*; 2015). It is also being used for the treatment of Jaundice, Peptic ulcers (Seyoung *et al*; 2012). An important secondary metabolite Coumestan-Wedelolactone has been reported to act as an anti-inflammatory agent, anti fibrotic agent, has anti osteroporotic effect, and as anti-cancer agent (Balakrishnan *et al*; 2018, Padma *et al*; 2005). Sunderamoorthy *et al*; (2014) reported that it has antibacterial activity so help in the wound healing. Franca *et al*; (1995) reported that it has anti liepatotoxic activity.

Tissue culture studies in different medicinal plants have been done by different workers. Some of them are being mentioned here such as Johari and Mitra (2001); Okudera (2009); Wani *et al*; (2010); Petrova *et al*; (2011); Deng (2012); Hamideh *et al*; (2012); Kaladhar (2012); Lakshami and Reddy (2012); Lein *et al*; (2012); Sharma *et al*; (2013); Niratkar *et al*; (2014). Sahraroo *et al*; (2014); Sen *et al*; (2014); Thirupathy *et al*; (2014); Han *et al*; (2015); Jayakuamr and Vivekanandan (2015); Sariram *et al*; (2015); Thakum and Megni (2015); Ahmad *et al*; (2016); Ayyadurai and Raman (2016); Varaporn (2016); Zamini *et al*; (2016); Islam and Alam (2018); Shikha and Ashwani Kumar (2018); and Ashokhan *et al*; (2020). Above workers have observed impact of plant growth regulators on induction of callus in *Eclipta alba* for regeneration and secondary metabolites production.

Gawde and Paratkar (2012) observed enhanced production of Wedelolactone in shoot cultures of *Eclipta alba*. Zhao *et al*; (2014) observed effect of *Eclipta prostrata* on lipid metabolism in hyper lipidemic animals. Fang *et al*; (2015) extracted identified and determined its quantity from *Eclipta prostrata*. Zhang *et al*; (2015) also performed experiments for qualitative and quantitative analysis of secondary metabolites in *Eclipta prostrata*. Khurshid *et al*; (2018); reported biosynthesis fo precious metabolites in callus cultures of *Eclipta alba*. Balakrishnan *et al*; (2018) reported distinctive pharmacological activities of *Eclipta alba* an its Coumestan-Wedelolactone. Salma *et al*; (2018) reported elicitor mediated enhancement of Wedelolactone in cell suspension culture of *Eclipta alba*. Keeping all these ideas in mind the present work was taken to select better plant growth regulators for efficient callus induction in *Eclipta alba* an important medicinal herbs.

MATERIALS AND METHODS:

Healthy branches of *Eclipta alba* were collected from the campus of University Department of Botany, B.R.A.B.U. Muzaffarpur. From the branch of *Eclipta alba*, healthy leaves were taken. Similarly, internodal segments were prepared. Both the above explants were taken in 1 L Conical flask and mouth was covered with muslin cloth with the help of rubber bands. This protected the explants from going out of the flask along with circulating tap water when washing of the explants was done under running tap water. This was allowed for 45 min. Explants were taken out and rinsed with 0.2% tween 20; This was followed with surface sterilization with 0.1% aqueous solution of $HgCl_2$ for 1 min. All the explants were immediately rinsed with pre-sterilized distilled water. This was repeated thrice so that even the trace of $HgCl_2$ was removed from the surface of the explants. If the chemical is left attached on the surface this may cause toxic effect. All this explants were preserved in pre-sterilized and moist cloths in the aseptic condition of Laminar air flow chamber. Before this the chamber was itself sterilized by Ultraviolet radiation.

Preparation of culture medium:

In the present work MS (Murashige and Skoog, 1962) medium was used for the induction of callus from internodals and leaf explants of *Eclipta alba* L. Above medium was supplemented with different concentrations of auxins alone or in combinations of auxin and cytokinin. For the entire experiments full strength MS medium with 30 g/l sucrose and 8 g/l agar were used. pH of the medium was adjusted to 5.8 with 1 N NaOH or 1 N HCl before autoclaving at 15 lb pressure, for 20 min.

Inoculation of the explants:

Single internode was inoculated in the culture medium under aseptic conditions of Laminar flow air chamber, following all steps of inoculation. Similarly, segment of leaf was also inoculated in the culture flasks. The cultures were transferred in the culture room, where the temperature was maintained at 26 ± 1^0C relative humidity of the room was maintained at 60-64%. Constant light intensity 3000 lux, was provided by cool, white fluorescent tubes. Light was provided at 16/8 hours photo periods. Observation was made on an alternate day. Cultures showing contamination were discarded after autoclaving. Observations were made for percentage of response for callus induction, days after which callus induction was observed, growth rate of callus, and texture of the calli. All the experiments were done in triplicate and means of all the data were tabulated in the tables 1,2& 3 for further analysis and discussion.

Results and Discussion:

In the present study experiments were done to induce callus on internodal and leaf segment explants of *Eclipta alba*, inoculated in MS medium supplemented with 3% sucrose, different concentrations of auxins alone and with fixed concentration of cytokinin, here 6-benzylaminopurine at the rate of 2.22µM/l. The mean of the data was placed in talbe-1 and table-2. From the table-1, it may be noted that picloram at its all concentrations used here such as 2.07, 4.14, 6.21, 8.28, 10.35 and 12.42µM/l induced callus in both the internodal as well as

leaf segment explants. Here higher percentage of response for callus induction in internodal segment, 88.62 was observed at 8.28µM/l, the minimum time taken for callusing was 14 days and the colour of callus was white and texture friable. This was followed by 6.21µM/l of picloram where the percentage of response was 66.48, time taken for callus induction 16 days, the colour of the callus was white and texture friable. It was also noted form the table, that both the lowest and highest concentrations of picloram, that is 2.07 µM/l and 12.42µM/l had no promising impact on callogenesis in internodal explants. It may further be noted that leaf segment used as an explants, revealed 66.45% response for callus induction, the time taken was 17 days and the colour of the callus white while texture friable when inoculated in MS + 8.28µM /l picloram. This was followed by 6.21µM/l of picloram where the percentage of response was 48.64, time taken for callus induction 18 days and colour of the callus was creamy white while the texture was loose. Here also neither the lowest nor the highest concentration of picloram gave better response for callus induction.

Internodal and leaf segment explants were also inoculated in MS+ six different concentrations of 2,4-D such as 2.27, 4.53, 6.80, 9.06, 11.33 and 13.59 µM/l. The mean of the data was presented in table-1. From the table-1, it was noted that highest percentage of response 86.78 for callus induction and minimum time 12 days was taken for it in MS + 11.33µM/l of 2,4-D. Here the colour of the callus was white and texture friable. This was followed by the explants inoculated in MS + 9.06µM/l of 2,4-D where percentage of response was 72.74, time taken for callogenesis, 14 days. The colour of callus was white and the texture friable. It was further noted that in the similar concentrations of 2,4-D, the highest percentage for callogenesis in leaf explants was 75.26, time taken 15 days and the colour of callus was white, while the texture friable followed by percentage of response 66.54, time taken 16 days, with white coloured and friable texture callus in the same concentration of 2,4-D as was used for internodal explants.

MS medium was also supplemented with six different concentrations of 2,4-D (2.27, 4.53, 6.80, 9.06, 11.33 and 13.59 µM/l) along with 2.69 µM/l of NAA. The internodal and leaf segment explants were inoculated in the above medium and mean of the data for percentage response for callus induction; time taken for callusing was tabulated in table-2. From the table-2, it was noted that highest percentage of callogenetic response for nodal explants was 83.54, and for leaf explants 73.24 when inoculated in MS + 11.33µM/l 2,4-D + 2.69µM/l NAA medium. Time taken for callus induction in nodal explant was 14 days, while for leaf segment it was 15 days. The colour was white and texture friable in both the cases, as was noted from the table. Above percentage of response was followed when the explants were inoculated in MS + 9.06µM/l 2,4-D + 2.69µM/l of NAA, where the percentage of response for internodal explants was 76.32, time taken 15 days and for leaf segment explants 68.32 and time taken 17 days respectively. From the table it was also noted that in both the explants there were gradual increase in the percentage of callogenic response, decrease in time taken for callusing along with increasing concentrations of 2,4-D from 2.27µM/l to 11.33µM/l. However, there was sudden decrease in the percentage response for callusing at higher concentration that was 13.59µM/l in both the explants.

To observe the synergistic effects of cytokinin and auxin on callus induction in internodal and leaf segment explants, MS medium was supplemented with six different concentrations of (2.22, 4.44, 6.66, 8.88, 11.10 and 13.32 µM/l) of BAP along with 5.37µM/l of NAA. The mean of the data for percent response for callusing and tie taken for callusing in both the explants was tabulated in table-3. From the table-3, it was found that highest percentage for callogenetic response in internodal explant 92.78 and leaf segment explants 90.25, and time taken for callusing in internodal explants 12 days and for leaf segment explants 14 days were in MS + 13.32µM/l BAP + 5.37µM/l NAA respectively. This was followed by 90.34% for internodal explants 89.25% in leaf segment explants, while the time taken was 12 days for the former and 15 days for the later. Here in all the above culture medium the colour of callus was white & texture friable.

In the present study internodal and leaf explants of *Eclipta alba* were inoculated in MS medium supplemented with various concentrations of picloram, 2,4-D alone and with NAA and BAP + NAA to observe their impact on callus induction. It was observed here that picloram at its all concentrations, induced callusing, but the percentage of response was different. It was also noted that along with the increasing concentrations of picloram there was increase in the percentage response. However, after 8.28µM/l of picloram, there was again reduction in the percentage of response for callusing and increase in the time taken for callogenesis.

This situation was true for both the explants used here. Similar situations were observed among the explants inoculated in MS medium fortified with different concentrations of 2,4-D alone or with NAA. Here from 2.27µM of 2,4-D to 11.33µM of 2,4-D, there was gradual increase in percent response for callogenesis but at 13.59µM there was sudden decrease in the callogenic response. However, when BAP at various concentrations, with NAA at 5.37µM/l of NAA was supplemented in MS medium maximum percent of callogenic response in both the explants was observed at highest concentration 13.32µM BAP + 5.37µM of NAA.

In the present work, experiments were done to induce callus on MS medium supplemented with different concentrations of auxins alone or in combinations and cytokinin with auxin or using internodal and leaf explants of *Eclipta alba* 8.28µM, Picloram supplemented in MS medium, induced callus in internodal and leaf explants where the percent response was 88.62 and 66.45% for the above explants.

Similarly, MS +11.33µM of 2,4-D was most suitable for induction of callus in internodal explants, as well for leaf explants. Maximum percentage 86.78, for internodal explants, and 72.26% for leaf explants were obtained here. It was further observed that synergistic effect 2,4-D and NAA was not promising with respect to callogenic activities for both explants. However, synergistic effect of cytokinin (BAP) and auxin (NAA) was more promising with respect to callogenic response in both the explants used here.

Irvani *et al*; (2010) reported that MS + 1 mg/l NAA + 2.0 mg/l BAP gave the highest response for callus induction in *Dorem ammoniacum* an endangered medicinal plant. Hemideh *et al*; (2012) reported that auxins like NAA, 2,4-D alone can induce calli, but when supplemented together in MS + 1.0 mg/l NAA + 0.5 mg/l BAP, gave better results with respect to callus

induction in *Falcaria vulgaris* an important medicinal plant. Similarly, Sahraroo *et al*; (2014) reported that synergistic action of cytokinin and auxin revealed better response for callus induction in *Satureja khuzistanica* an important medicinal plants. Findings of present work therefore, corroborate with the findings of the above as here also most suitable culture was MS + 13.32µM BAP + 5.37 µM NAA, for the internodal explants where the percentage of response was 92.78% and for leaf explants MS + 8.88µM BAP + 5.37µM NAA, where maximum percentage of response was 89.38.

It may be concluded that among the explants for induction of callus in *Eclipta alba*, internodal segments were most suitable than that of the leaf explants. Furthermore among the plant growth regulators, the synergistic action of cytokinin and auxin was found more suitable than the synergistic action of auxins. Induction of callus in medicinal plants has got more importance these days. There are reports that with the help of suitable elicitors, rate of synthesis of desired secondary metabolites can be enhanced many fold that of the rate of synthesis of same metabolite *in vivo*. This protocol of induction of callus in *Eclipta alba* may be exploited for the production of secondary metabolites found in the plant in general and Wedelolactone in particular, as it bears much importance in pharmaceutical companies.

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Table:-1

Showing impact of different plant growth regulator on callus induction on internodal and leaf explant of *Eclipta alba*.

	PGR (µM)	Internodal explants			Leaf explants		
S.N.	Picloram	% Response	Callus initiation day	Callus colour and texture	% Response	Callus initiation day	Colour & Texture
1	2.07	26.84	18	Dark G, C	19.32	20	YWC
2	4.14	38.75	18	Creamy W C	31.78	20	Dark GC
3	6.21	66.48	16	W Friable	48.64	18	Creamy W L
4	8.28	88.62	14	W Friable	66.45	17	W Friable
5	10.35	52.18	18	Y G C	42.26	19	Dark GC
6	12.42	28.34	19	Grey W C	22.55	21	Grey WC
	PGR	Internodal explants			Leaf explants		

	(µM)						
S.N.	2,4-D	% Response	Callus initiation day	Callus colour and texture	% Response	Callus initiation day	Colour & Texture
1	2.27	32.42	17	G C	24.38	19	GWC
2	4.53	60.35	16	W GC	33.16	19	Dark GC
3	6.80	68.52	16	W Friable	51.62	18	W C
4	9.06	72.74	14	W Friable	66.54	16	W Friable
5	11.33	86.78	12	W Friable	75.26	15	W Friable
6	13.59	63.26	15	W C	52.16	17	Y C

C = Compact, G = Green PGR = Plant Growth Regulator

W = White Y = Yellow L= Loose

Table:-2

Showing impact of plant growth regulator on *in vitro* induction of callus from different explants of *Eclipta alba*.

	PGR (µM/l)	Internodal explant			Leaf explant		
S.N.	2,4-D + NAA	% Response	Days Taken	Texture & Colour of the callus	% Response	Time Taken	Texture & Colour of the callus
1	2.27 +2.69	34.64	16	GWC	26.54	18	Dark GC
2	4.53+2.69	65.18	16	WC	36.45	18	Creamy WC
3	6.80+2.69	74.66	15	W Friable	54.66	17	W Friable
4	9.06+2.69	76.32	15	W Friable	68.32	17	W Friable
5	11.33+2.69	83.54	14	W Friable	73.24	15	W Friable
6	13.59+2.69	64.22	16	W C	54.38	18	Grey WC

C = Compact, G = Green PGR = Plant Growth Regulator

W = White Y = Yellow L= Loose

Table:-3

Showing synergistic impact of cytokinin and auxin on callogenesis in internodal and leaf explants of *Eclipta alba*.

	PGR (µM/l)	Internodal explant			Leaf explant		
S.N.	BAP+ NAA	% Response	Days Taken	Texture & Colour of the callus	% Response	Time Taken	Texture & Colour of the callus
1	2.22+5.37	42.45	15	Light YC	40.34	17	Light GC
2	4.44+5.37	76.62	14	YGC	71.56	18	YGC
3	6.66+5.37	81.26	12	W Friable	78.28	15	W Friable
4	8.88+5.37	87.18	12	W Friable	85.38	14	W Friable
5	11.10+5.37	90.34	12	W Friable	89.25	15	W Friable
6	13.32+5.37	92.78	12	W Friable	90.25	14	W Friable

C = Compact, G = Green PGR = Plant Growth Regulator

W = White Y = Yellow L= Loose

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