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Elicitation of gymnemic acid as gymnemagenin using biotic elicitors in cell suspension cultures of *Gymnema sylvestre* R. Br.

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Abstract
Gymnema sylvestre R. Br. (Family: Asclepiadaceae), is an important medicinal plant, rich in a group of oleanane triterpenoid saponins called Gymnemic acid, well known for its anti-diabetic activity. The vulnerability of the species emphasizes the need to exploit certain biotechnological strategy to maximize the production of its phytoconstituents. In recent times, developments in elicitation in plant tissue culture have opened new opportunities for the production of secondary metabolites. Thus, in the present study, elicitation for Gymnemic acid production in cell suspension culture was carried out using biotic elicitors *viz*. Yeast extract and Pectin. Cell suspension cultures were initiated using 500 mg friable callus in 50 ml MS liquid medium fortified with PGRs *viz*. 2,4-D (2 mg/l) + BAP (1mg/l) in 250 ml of Erlenmeyer flasks. The presence of Gymnemic acid was detected using HPTLC. Cell suspension cultures treated with 0.6 g/l of yeast extract resulted in the maximum production of Gymnemic acid as compared to other tested concentrations of Yeast extract, Pectin and control. The data obtained from the present study could be used for the enhancement of *in vitro* biosynthesis of commercially significant Gymnemic acid using cell suspension culture. In addition, it will help in reducing uncontrolled overexploitation of *Gymnema sylvestre* R. Br., which is declared as vulnerable plant in red data book.

Keywords: *Gymnema sylvestre* R. Br., gymnemic acid, biotic elicitors, yeast extract, pectin

Introduction

The importance of secondary metabolites is ever increasing due to its unique and highly valuable source of pharmaceuticals, food supplements and other industrial products (Narayani and Srivastava, 2017) [30]. They have complex structure, low molecular weight and are produced occasionally in living plant cells. The type and concentration of these metabolites is highly determined by the species, genotype, physiology, developmental stage and environmental factors during growth (Dixon, 2001; Oksman-Caldentey and Inze, 2004 and Isah, 2019) [10, 32, 19]. These dependencies lead to lower production rate and hence it becomes impracticable to fulfil the commercial needs of industries (Kolewe *et al*., 2008) [22].

In the last few decades, Plant Tissue Culture technique has emerged as an alternative approach in the field of biotechnology by serving as an indispensable source of secondary metabolites thereby conserving the species having slow growth rate (Verpoorte and Memelink, 2002) [40]. There are few examples where plant cell cultures were used for large scale production of secondary metabolites such as berberine from *Coptis japonica* (Galneder *et al*. 1988) [14] and Shikonin from *Lythospermum erythrorhizon* (Fujita, 1988) [13].

The production of valuable secondary metabolites from plant cell or tissue culture is more effective as compared to the production from the whole plant or plant material. However, it had limited success on commercial scale due to lack of understanding of how these metabolites are synthesized (Gaosheng and Jingming, 2012) [15]. Elicitation serves as one of the suitable means for commercial application in enhancing the production of secondary compounds using plant cell cultures (Pavlov *et al*., 2003) [34]. *In vitro* cultured plant cells show varied physiological as well as morphological changes to various biotic and abiotic factors *viz*. physical, chemical or microbial factors which are known as 'elicitors' (Patel and Krishnamurthy, 2013) [33].

Gymnema sylvestre R. Br. is a slow growing and vulnerable medicinal plant. It belongs to family Asclepiadaceae and is located in Southern India, Africa, Australia and China (Christopoulos *et al*., 2010) [7]. It is known by several names *viz*. Madhunashini in Sanskrit, Gudmaar and Medhasingi in Hindi and Periploca of the Woods in English (Anonymous, 2016) [1]. In ancient times, the plant was considered as one of the most important botanicals to treat diabetes and other major diseases like asthma, cancer, obesity, etc. (Singh *et al*., 2008) [35].

Gymnemic acid, the major active phytoconstituent of *G. sylvestre* R. Br. is a complex mixture of oleanane triterpenoid saponins. Gymnemagenin, a common aglycone of Gymnemic acid is produced after the hydrolysis of Gymnemic acid (Trivedi *et al*., 2011) [38]. The pharmaceutical properties of the plant are believed to be due to presence of Gymnemic acid. Among all plant parts *viz*. stem, root, leaves, flower and fruit, leaves are considered to be the rich source of Gymnemic acid. Hence, the leaves of *G. sylvestre* R. Br. have been reported to be effective against

diabetes (Singh *et al*., 2008) [35], rheumatism, diuretic, anemia, ulcer, jaundice, dyspepsia, constipation, etc. (Tiwari *et al*., 2014) [37]. In the present study, cell suspension cultures of *Gymnema sylvestre* R. Br. were treated with biotic elicitors to check the production of Gymnemic acid. Subsequently, Gymnemic acid (as Gymnemagenin) was quantified using HPTLC to assess the effect of biotic elicitors *viz*. Yeast extract and Pectin and to optimize the elicitor concentrations that could significantly enhance the concentration level of Gymnemic acid (Gymnemagenin).

Materials and methods

Collection of Plant material

Plants of *Gymnema sylvestre* R. Br. were collected from Alibaug, Raigad district, Maharashtra, India. The plant was authenticated from Blatter Herbarium, St. Xavier's college, Mumbai. The fresh and healthy leaves of *Gymnema sylvestre* R. Br. were selected as explant for the present *in vitro* cell culture study.

Callus induction and initiation of cell suspension cultures

Callus was successfully induced from the four segments of *G. sylvestre* R. Br. leaves *viz.* Apical Leaf Segment (ALS), Middle Leaf Segment (MLS), Basal Leaf Segment (BLS) and Petiole (P). The explants were inoculated on MS medium (Murashige and Skoog 1962) [28] and Woody Plant Medium (WPM) (Lloyd and Mc Cown, 1980) [24] fortified with plant growth regulators (PGRs) *viz.* 2,4-D, IAA, IBA, NAA, BAP and KIN (0.5 - 2.5 mg/l) in various combinations.

Cell suspension cultures were initiated by inoculating 500 mg of callus derived from ALS and MLS in 50 ml of MS liquid medium (Murashige and Skoog, 1962) [28] and Woody Plant Medium (WPM) (Lloyd and Mc Cown, 1980) [24] fortified with various concentrations and combinations of Auxins *viz*. 2,4-D and NAA and Cytokinin *viz*. BAP.

The cultures were incubated for 40 days at 110 rpm on rotatory shaker-incubator at 25 ºC. To study the growth of cells, optical density was measured at regular time intervals at 0, 5, 10, 15, 20, 25, 30, 35 and 40 days.

Quantification of Gymnemic acid

500 mg of dried biomass derived from cell suspension cultures was extracted using 10 ml of Methanol: Water (1:1 v/v) and 2 ml of 11% Potassium hydroxide (KOH) solution by refluxing for 1 hour. 1.8 ml of HCl was added to extract and refluxed again for an hour. The mixture obtained was then cooled to room temperature and the pH was adjusted in between 7.5 to 8.5 with 1% NaOH solution. Further, the mixture was diluted to 50 ml with 50% of Methanol in volumetric flask and filtered through 0.45 µm nylon filter (Millipore). The volume was made up to 50 ml with Methanol and the clear supernatant was used for HPTLC analysis (Anonymous, 2016 and Manohar *et al*., 2009) [1, 27].

Elicitor preparation

Biotic elicitors *viz*. yeast extract (0.3, 0.6, 0.9 and 1.2 g/l) and Pectin (0.2, 0.4, 0.6 and 0.8 %) were used for eliciting Gymnemic acid production in suspension cultures. These biotic elicitors were dissolved in sterile distilled water separately and the solutions thus obtained were filter-sterilized before use. The pH of these elicitor solutions was adjusted to 5.8 and then added aseptically to the cell suspension culture.

Elicitor treatment

The cell suspension cultures with maximum biomass production and Gymnemic acid content were selected for the further study. Biotic elicitors were added to the suspension cultures and the cultures were incubated at 110 rpm on rotatory shaker-incubator at 25 ºC. The biomass was determined after filtration at a 5-day interval and oven dried at 40 ºC until a constant weight was observed. The amount of Gymnemic acid was quantified based on the dry weight of callus using high Performance Thin Layer Chromatography method.

Determination of cell growth

The growth of cell cultures was determined in terms of optical density (O.D.) and in terms of fresh and dry weight of cell biomass.

Optical density was measured using colorimeter where MS liquid medium without inoculum was considered as blank and the readings for the cell suspension cultures were recorded at 600 nm. The readings for optical density (O.D.) were recorded at regular time intervals *viz.* 0, 5, 10, 15, 20, 25, 30, 35 and 40 days and growth curve was plotted.

At the interval of 5, 10, 15, 20, 25, 30, 35 and 40 days after elicitation, the suspension cultures were filtered and the fresh weight was determined by centrifugation. The supernatant was discarded and sediment was washed 2 - 3 times with distilled water and excess of water was removed by placing it between the folds of blotting paper. Further, dry weight was determined after drying the filtered biomass in an oven at 40 ± 2 °C for 3 - 4 days till the constant weight was obtained.

Results

Different combinations of plant growth regulators *viz*. auxins and cytokinins were studied for the induction of callus from the leaf explants of *G. sylvestre* R. Br. The callus cultures of *G. sylvestre* R. Br. were successfully established from the different segments of leaf lamina. Among the different treatments used, the best callus response was observed from apical and middle leaf segment on MS medium fortified with 2 mg/l – 2,4–D in combination with 1 mg/l – BAP.

Similar to callus culture, cell suspension cultures were successfully initiated and established from the apical segment of leaf lamina on MS medium fortified with 2 mg/l – 2,4-D in combination with 1 mg/l – BAP. Therefore, the present study on elicitation was carried out using MS liquid medium fortified with 2 mg/l – 2,4-D in combination with 1 mg/l – BAP.

Gymnemic acid production after elicitation

The Gymnemic acid was quantified from the cell suspension cultures using HPTLC. The production of Gymnemic acid increased with the increase in cell biomass content i.e., reaching its peak after 20 days of elicitation. The presence of Gymnemic acid as Gymnemagenin was confirmed by comparing Retention factor (Rf) and color with standard Gymnemagenin.

Effect of biotic elicitors on biomass production of cell suspension cultures

Effect of biotic elicitors on Gymnemic acid production in

cell suspension culture (MS liquid medium fortified with 2 mg/l 2,4-D in combination with 1mg/l BAP) was observed for 40 days.

Effect of biotic elicitors

Yeast extract

The growth rate of cells in cell suspension cultures treated with different concentrations of yeast extract (YE) *viz*. 0.3, 0.6, 0.9 and 1.2 g/l was initially slow which gradually increased till 20th day of elicitation and was found maximum. Eventually, the growth rate decreased after 20th day of elicitation (Table 1 and Fig.1).

Further, it was observed that YE showed a positive effect on biomass production at all concentrations tested compared to respective control after 20 days of elicitation. YE at 0.9 g/l concentration produced maximum fresh weight (3.502 ± 0.014 gm) and dry weight (0.350 ± 0.005 gm) followed by 0.3 g/l; 0.6 g/l and 1.2 g/l concentrations of YE as compared to control (Table 2).

Table 1: Effects of yeast extract (YE) on growth of cells in suspension culture [MS liquid medium fortified with 2,4-D (2 mg/l) + BAP (1 mg/l)]

YE (g/l)	Number of Days								
	0	5	10	15	20	25	30	35	40
	O.D. (600 nm)								
0.3	0.013±0.001	0.113±0.002	0.291±0.018	0.382±0.016	0.443±0.015	0. 321±0.005	0.242±0.009	0.165±0.007	0.033±0.002
0.6	0.024±0.002	0.092±0.003	0.263±0.005	0.391±0.007	0.512±0.006	0.425±0.004	0.296±0.003	0.175±0.003	0.026±0.001
0.9	0.035±0.002	0.113±0.003	0.293±0.004	0.402±0.012	0.551±0.009	0.432±0.008	0.293±0.005	0.143±0.003	0.042±0.002
1.2	0.024±0.002	0.082±0.004	0.224±0.007	0.362±0.002	0.486±0.002	0.392±0.003	0.281±0.006	0.140±0.003	0.065±0.001
Control (without YE)	0.032±0.001	0.114±0.002	0.216±0.002	0.353±0.001	0.412±0.006	0.331±0.001	0.242±0.001	0.150±0.001	0.067±0.002

Values are mean of three sets of determinants

Keywords: YE= yeast extract and ± = Standard Deviation (S.D.)

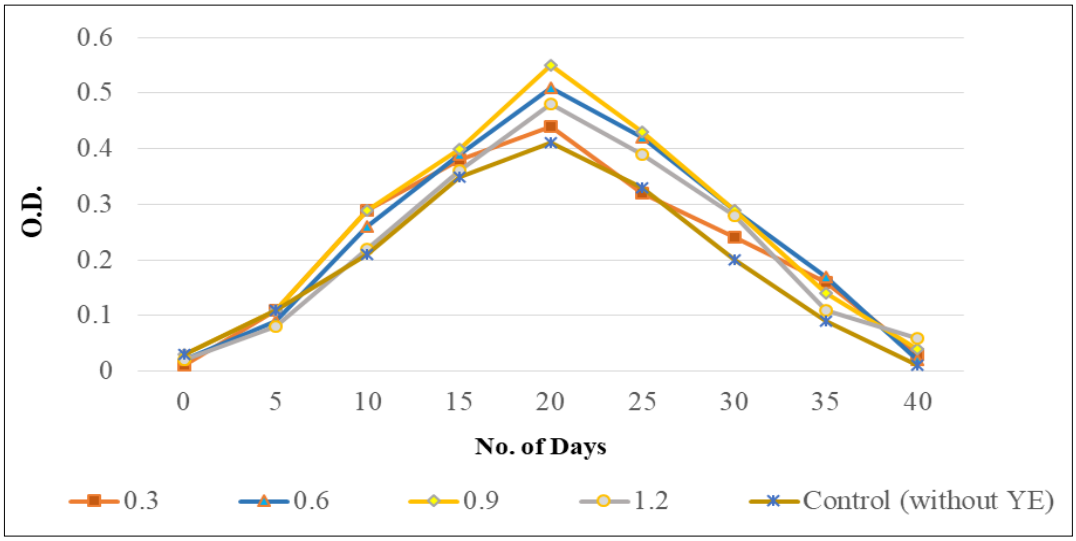


Fig 1: Effect of various concentrations of yeast extract on growth of cells in cell suspension culture [MS liquid medium fortified with 2, 4-D (2 mg/l) + BAP (1 mg/l)]

Table 2: Effect of Yeast extract on fresh weight (FW) and dry weight (DW) of cell biomass [MS liquid medium fortified with 2,4-D (2 mg/l) + BAP (1 mg/l)] obtained after 20 days of elicitation

Yeast extract (g/l)	Fresh Weight (gm)	Dry Weight (gm)
0.3	3.252 ± 0.027	0.325 ± 0.006
0.6	3.285 ± 0.032	0.321 ± 0.003
0.9	3.502 ± 0.014	0.350 ± 0.005
1.2	3.250 ± 0.023	0.320 ± 0.004
Control (without YE)	3.243 ± 0.021	0. 319 ± 0.006

Values are mean of three sets of determinants

Keywords: ± = Standard Deviation (S.D.)

Pectin

The growth rate of cells in cell suspension cultures treated with different concentrations of pectin *viz*. 0.2, 0.4, 0.6 and 0.8 % was initially slow which gradually increased till 20th day of elicitation and was found maximum. Eventually, the growth rate decreased after 20th day of elicitation (Fig. 2 and Table 3).

Pectin, like YE also increased the fresh biomass production at all concentrations tested compared to respective control, except at 0.8 % (2.032 ± 0.019 gm FW and 0.189 ± 0.008 gm DW) after 20 days of elicitation, where a significant decrease was observed in biomass production as compared to control (3.243 ± 0.021gm FW and 0. 319 ± 0.006 gm DW). At 0.4% concentration, pectin induced maximum fresh weight (3.262 ± 0.028 gm) and dry weight (0.326 ± 0.009 gm) followed by 0.6% and 0.2% concentration of pectin after 20 days of elicitation as compared to control (Table 4).

Table 3: Effects of Pectin on growth of cells in cell suspension culture [MS liquid medium fortified with 2, 4-D (2 mg/l) + BAP (1 mg/l)]

Pectin (%)	Number of Days								
	0	5	10	15	20	25	30	35	40
	O.D. (600 nm)								
0.2	0.024±0.003	0.072±0.004	0.123±0.013	0.303±0.012	0.432±0.014	0. 274±0.001	0.157±0.006	0.115±0.003	0.043±0.001
0.4	0.015±0.002	0.096±0.005	0.167±0.005	0.358±0.003	0.498±0.011	0.334±0.004	0.193±0.005	0.107±0.007	0.412±0.002
0.6	0.021±0.002	0.060±0.003	0.139±0.007	0.347±0.007	0.444±0.006	0.373±0.004	0.254±0.003	0.142±0.003	0.026±0.003
0.8	0.013±0.002	0.050±0.001	0.098±0.009	0.216±0.002	0.285±0.009	0.183±0.003	0.101±0.006	0.050±0.003	0.035±0.001
Control (without pectin)	0.035±0.001	0.114±0.002	0.217±0.002	0.354±0.001	0.416±0.006	0.338±0.001	0.240±0.001	0.154±0.001	0.063±0.002

Values are mean of three sets of determinants
Keywords: ± = Standard Deviation (S.D.)

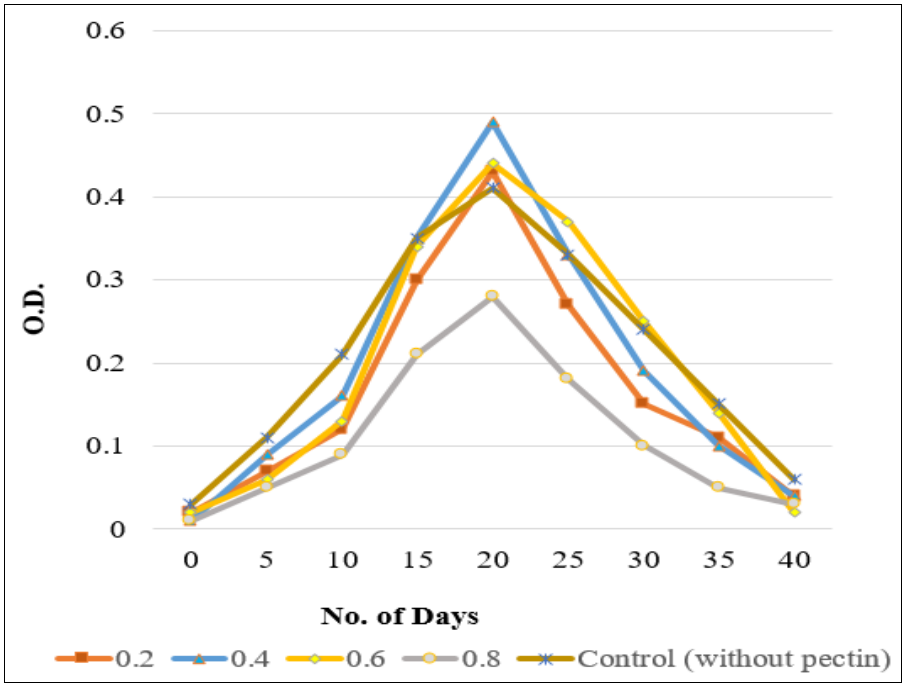


Fig 2: Effect of various concentrations of pectin on growth of cells in suspension culture [MS liquid medium fortified with 2, 4-D (2 mg/l) + BAP (1 mg/l)]

Table 4: Effect of pectin on fresh weigh (FW) and dry weight (DW) of cell biomass [MS liquid medium fortified with 2, 4-D (2 mg/l) + BAP (1 mg/l)] obtained after 20 days of elicitation

Pectin (%)	Fresh Weight (gm)	Dry Weight (gm)
0.2	3.250 ± 0.033	0.322 ± 0.005
0.4	3.262 ± 0.028	0.326 ± 0.009
0.6	3.251 ± 0.043	0.324 ± 0.002
0.8	2.032 ± 0.019	0.189 ± 0.008
Control (without pectin)	3.243 ± 0.021	0. 319 ± 0.006

Values are mean of three sets of determinants
Keywords: ± = Standard Deviation (S.D.)

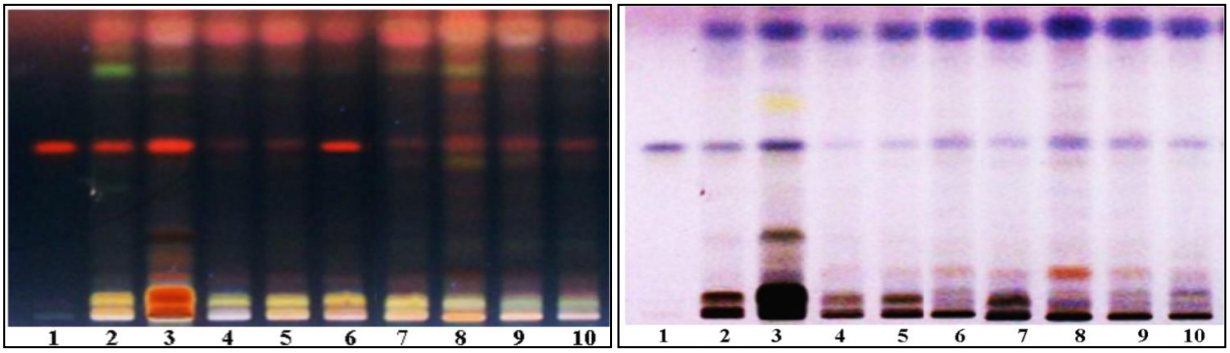
Effect of yeast extract and pectin on production of Gymnemic acid

Cell suspension cultures when treated with 0.6 g/l concentration of yeast extract enhanced the highest production of Gymnemic acid which was found to be maximum (0.86 ± 0.04 mg/g) after 20 days of elicitation. Other concentrations *viz*. 0.3 g/l concentration (0.83 ± 0.02 mg/g), 0.9 g/l concentration (0.72 ± 0.03) and 1.2 g/l concentration (0.71 ± 0.02 mg/g) also enhanced the production of gymnemic acid after 20 days of elicitation (Table 5 and Plate 1).

The content of Gymnemic acid increased with the decreasing concentration of Pectin. However, maximum content of Gymnemic acid was obtained when cell suspension cultures were treated with 0.2% of pectin concentration (0.84 ± 0.05 mg/g). Other concentrations *viz*. 0.4 % concentration (0.81 ± 0.01 mg/g), 0.6 % concentration (0.76 ± 0.06 mg/g) and 1.2 g/l concentration (0.72 ± 0.04 mg/g) also enhanced the production of gymnemic acid after 20 days of elicitation (Table 5 and Plate 1).

Table 5: Quantification of Gymnemic acid in cell suspension culture (MS liquid medium + 2 mg/l- 2, 4-D + 1mg/l -BAP) treated with biotic elicitor (Yeast extract and Pectin) of *Gymnema sylvestre* R. Br.

Sr. No.	Elicitors	Content of Gymnemic acid (mg/g)
1.	Yeast extract (0.3 g/l)	0.83 0.02
2.	Yeast extract (0.6 g/l)	0.86 0.04
3.	Yeast extract (0.9 g/l)	0.72 0.03
4.	Yeast extract (1.2 g/l)	0.71± 0.02
5.	Pectin (0.2 %)	0.84 0.05
6.	Pectin (0.4 %)	0.81 0.01
7.	Pectin (0.6 %)	0.76 0.06
8.	Pectin (0.8 %)	0.72 0.04
9.	Control (without Yeast extract and Pectin)	0.80 ± 0.03



A. At 366 nm B. At 540 nm

Plate 1: HPTLC chromatogram of biotic elicitors treated cell suspension cultures (MS + 2 mg/l 2,4-D + 1 mg/l BAP) of *G. sylvestre* R. Br. with standard Gymnemic acid and Control

Keywords: 1. Standard Gymnemic acid as Gymnemagenin; 2.Yeast extract (0.3 g/l); 3. Yeast extract (0.6 g/l); 4. Yeast extract (0.9 g/l); 5. Yeast extract (1.2 g/l); 6. Pectin (0.2 %); 7. Pectin (0.4 %); 8. Pectin (0.6 %); 9. Pectin (0.8 %); 10. Control (without Pectin and Yeast extract)

Discussion

Plant cell culture is evolving fast with the development of innovative treatment strategies in the field of highly valuable secondary metabolite regulation. Production of such metabolites on large scale has now become feasible by applying several strategies that generates stress in the culture medium by altering the culture conditions *viz*. light, carbon source, photoperiod, pH, precursor, plant growth regulators, etc. (Hagimori *et al*., 1982) [16].

Elicitor treatment or elicitation is one such strategy to achieve large scale production of target compounds for industrial applications. Since long time, several researchers have been working on establishment of different culture systems of numerous medicinal plants for the production of valuable compounds at commercial scale. The approach will not only facilitate the high yield production of target compounds, but will also help to conserve the natural resources (Gaosheng and Jingming, 2012) [15].

Kikowska *et al*. (2012) [21] and Huang *et al*. (2013) have reported the production of various secondary metabolites using elicitors in *in vitro* cultures of medicinal species *viz*., *Eryngium planum* L. and *Panax ginseng* respectively. Elicitation of *in vitro* culture system may be promising as it reveals positive outcomes for the production of various valuable secondary metabolites with pharmacological properties. Though, the process is involved in the enrichment of secondary metabolism in plant cell culture system, the regulatory aspects and biosynthesis mechanism of elicitation is still not properly known (Namdeo, 2007) [29].

Also, many researchers have reported the successful elicitation of gymnemic acid through cell suspension cultures, using either biotic or abiotic elicitation. Netala *et al*. (2016) [31] reported the enhancement of Gymnemic acid in cell suspension using the endophytic fungi *viz*. *Polyancora globosa* and *Xylaria* species as elicitors. Chodisetti *et al*. (2015) [5] have reported the elicitation of Gymnemic acid in the cell suspension cultures of *Gymnema sylvestre* R. Br. using the signaling molecules like salicylic acid and methyl jasmonate as elicitors. In addition, Chodisetti *et al*. (2013) [6] have improved Gymnemic acid production in the suspension cultures using biotic elicitors like *Agrobacterium rhizogenes*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Bacillus subtilis* and *Escherichia coli*. Veerashree *et al*. (2012) [39] have reported the elicitation of Gymnemic acid through cell suspension cultures using biotic elicitors such as methyl jasmonate, yeast extract, pectin and chitin.

Use of biotic elicitors in cell suspension cultures has been of great significance and widely used by many researchers for enhancing the production of secondary metabolite (Lystvan *et al*., 2009 and Junge *et al*., 2011) [25, 20]. The influence of an elicitor whether biotic or abiotic is based on several factors *viz*. concentration and specificity of elicitor, the growth phase of the culture and the treatment time (Holden *et al*., 1988) [17]. Also, the sensitivity of such factors differs according to the plants used in the culture study (Mangas *et al*., 2006) [26].

In the present study, effect of two different biotic elicitors *viz*. yeast extract and pectin was assessed for the production of Gymnemic acid in cell suspension cultures of *Gymnema sylvestre* R. Br. It was found that concentrations used for both the biotic elicitors *viz*. yeast extract (0.3, 0.6, 0.9 and 1.2 g/l) and Pectin (0.2, 0.4, 0.6 and 0.8 %) influenced the cell growth and biomass content in cell suspension cultures

of *Gymnema sylvestre* R. Br. Moreover, it was observed that the elicitors showed varied effects for the production of Gymnemic acid as Gymnemagenin.

Since long, yeast extract are frequently used by many researchers as one of the biotic elicitors to enhance the production of various secondary metabolites. Several reports emphasizing the importance of yeast extract in eliciting secondary metabolite production in *in vitro* cultures includes rosmarinic acid and lithospermic acid B (Chen *et al*., 2001) [4], plumbagin (Komaraiah *et al*. 2002) [23] and camptothecin (Deepthi and Satheesh kumar, 2016) [9]. Also, there are reports on use of yeast extract for bacterial resistance in *Phaseolus vulgaris* (Stangarlin *et al*., 2011) [36] and ethylene biosynthesis in tomato (Felix *et al*., 1991) [11].

Pectin, one of the important components of the primary cell walls in dicotyledonous plants, helps the plants in numerous ways like regulation of organogenesis, cell surface reactions, defense mechanism, etc. (Darvill *et al*., 1992) [8]. There are several reports on use of pectin as elicitors for example: elicitation of triterpene acids from *Uncaria tomentosa* cell suspension cultures (Flores-Sanchez *et al*., 2002) [12], menthol from *Mentha piperita* cell culture (Chakraborty and Chattopadhyay, 2008) [3], etc.

In the present study, it was found that cell suspension cultures treated with 0.6 g/l of yeast extract gave the best result for the production of Gymnemic acid when compared to other tested concentrations of yeast extract and control. At 0.9 g/l and 1.2 g/l concentration, YE showed negative influence on gymnemic acid production after 20 days of elicitation. The negative effect of YE at higher concentrations on secondary metabolite (catechin and caffeic acid) production was also reported in *Hypericum triquetrifolium* Turra (Azeez and Ibrahim, 2013) [2].

Besides the yeast extract, pectin also stimulated the production of Gymnemic acid. Among the various concentrations of pectin *viz*. 0.2, 0.4, 0.6 and 0.8 % tested for elicitation of Gymnemic acid, 0.2% of concentration gave best results in terms of maximum gymnemic acid production, as compared to other tested concentrations and control. However, cultures treated with 0.6% and 0.8% of pectin showed lower production of gymnemic acid after 20 days of elicitation which revealed that, with increased pectin concentration, content of gymnemic acid decreased. Our results are in agreement with the previous findings of Veerashree *et al*. (2012) [39] who reported lower production of gymnemic acid with 0.8 % pectin concentration.

Thus, in the present study it can be concluded that addition of two different elicitors *viz*. YE and pectin to cell suspension cultures of *G. sylvestre* R. Br. increased the Gymnemic acid content. Of the two elicitors tested, YE (0.6 g/l) induced maximum production of gymnemic acid as compared to Pectin. It was therefore quite evident from the study carried out that yeast extract was more effective in the Gymnemic acid production as compared to pectin.

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