

**POTENTIAL ROLE OF JASMONIC ACID (JA) IN BIOMASS ACCUMULATION,
SECONDARY METABOLITES PRODUCTION IN ADVENTITIOUS ROOT
CULTURES OF *INDIGOFERA HETERANTHA***

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ABSTRACT

Medicinal plants are enriched with plenty of pharmaceutically important secondary metabolites, which are specific to different plant parts. Adventitious roots in this regard have their own importance. Enhancing biomass and secondary metabolite accumulation in the adventitious root culture of *Indigofera heterantha* is the main objective of the current study. For this purpose, the culture was elicited with Jasmonic acid (JA) with varying concentrations (0-3.0 mg L⁻¹) for a period of 7 weeks. Varying levels of JA positively influenced fresh as well dry biomass of adventitious root cultures. The biomass accumulation was noted at 7 days interval for a period of 49 days. Among the tested levels, adventitious roots cultured on medium fortified with 1.5 mg L⁻¹ JA accumulated significantly higher quantity of fresh (24.4 g L⁻¹) and dry (2.93 g L⁻¹) biomass than other concentrations. Similarly, by changing the levels of JA in the medium a significant variation in the accumulation of TPC was experienced. The highest TPC (131 µg g⁻¹DW) was synthesized in adventitious roots when the MS medium was supplemented with JA concentration at the rate of (1.5 mg L⁻¹). Regarding the total flavonoid contents, adventitious root cultures was observed with maximum TFC (380.00 µg g⁻¹DW) using the JA at 2 mg L⁻¹. Furthermore, cultures favoured 1.5 mg L⁻¹ JA concentrations and resulted in higher (67.78%) antioxidant activities in adventitious root cultures as compared to other JA levels. Hence, it is recommended that adventitious root culture of *Indigofera heterantha* could be developed potentially with more secondary metabolites in media having optimum amount of JA in culture media.

Keywords: Medicinal plant, *Indigofera heterantha*, Jasmonic acid, Biomass, Secondary metabolites.

INTRODUCTION

Secondary metabolites from higher plants, which have been employed as food additives, in the pharmaceutical and cosmetic industries, and for medical purposes, are abundant and of great importance. These valuable secondary metabolites can now be produced commercially without field cultivation because to recent advancements in plant cell, tissue, and organ culture techniques and applications. In addition, these in vitro culture techniques offer numerous options to increase the synthesis of desired metabolites, conveniently all year long (Rao and Ravishanakar 2002). Secondary metabolites produced by plants are low molecular weight compounds that plants most often use in stress conditions (Kennedy and Wightman 2011).

Zenk et al. (1991) used cell cultures of *Morinda citrifolia* to confirm that plants cultivated in-vitro are capable of producing the secondary metabolites. Techniques for secondary metabolite generation and propagation in vitro have been widely used (Mulabagal and Tsay 2004). In vitro techniques can be used year-round without seasonal restrictions, unlike field culture, which is time and season-restricted (Gaosheng and Jingming 2012). Additionally, by using such strategies, it is possible to plan to increase the synthesis of secondary metabolites using elicitors (Namdeo 2007).

The accumulation of secondary metabolites in various in-vitro plant cultures is influenced by a variety of factors, including microelements, phytohormones, and the presence of biosynthetic pathway precursors. However, the elicitation process is the most important step that initiates the synthesis. (Smetanska 2008). There are several strategies to improve the productivity of secondary products in in vitro cultures, some of which include the use of biotic and abiotic elicitors, have been widely recognized for the enhancement of a wide variety of secondary metabolite production. (Yu et al. 2005; Baskaran et al. 2012). One of the most practical methods for improving secondary metabolite production in plant tissue cultures is treatment with elicitors, which has just recently found commercial application. (Zhao et al. 2010; Smetanska 2008; Wu and Shi 2008; Sivanandhan et al. 2012; Baskaran et al. 2012). Recent research has demonstrated that a variety of elicitors can alter plant metabolism, resulting in increased production of some secondary compounds as well as accumulation of molecules that are not typically produced in the source plant. (Cui et al. 2012; Baskaran et al. 2012). By adding elicitors to the culture media, secondary metabolite accumulation can be induced. Jasmonic acid (JA) and its methyl ester (MeJA), which are signalling molecules, were used to stimulate the production of different metabolites such as phenylpropanoids and

saponins. (Kim et al. 2009), coumarin derivatives (Rhee et al. 2010), isoflavonoids (Korsangruang et al. 2010), bakuchiol (Lystvan et al. 2010), hypericins and hyperforin (Liu et al. 2007 and Coste et al. 2011). In different in vitro culture systems, jasmonic acid and MeJA have been widely used as elicitors to promote the formation of secondary metabolites. (Lijavetzky et al. 2008, Pauwels et al. 2008). In particular, the jasmonates have proven effective in improving the synthesis of anti-cancer drugs in a number of *Taxus* species and cell cultures. (Bonfill et al. 2006).

An efficient method for producing these important secondary metabolites on a large scale is the in-vitro culture of roots. Plant tissues from field-grown plants have been employed for the commercial synthesis of various secondary metabolites, although a number of biotic and abiotic variables may have a substantial impact on these products' quality. (Sivanandhan et al. 2012). Additionally, field cultivation is a labour and time-intensive process, therefore the use of plant cell, tissue, and organ cultures has been recognized as a potential substitute for more effectively producing beneficial secondary metabolites. (Yu et al. 2005; Jeong et al. 2009; Zhang et al. 2012). Among them, root cultures are thought to be a more efficient method for producing biomass because they grow quickly, are simple to manage, and exhibit steady metabolite productivity. (Yu et al. 2002; Kang et al. 2004; Subotic et al. 2009). In the same way, extensive root harvesting of naturally occurring medicinal plants to supply the demand for secondary metabolites has placed a heavy burden on the long term survival of numerous plant species. (Martin et al. 2008). As a result, the establishment and development of a rapid-growing root culture system would provide unique opportunities for the synthesis of such vital pharmaceuticals present in roots (Wasnik et al. 2009). Adventitious roots, which are post-embryonic roots, can sprout from the stem, leaves, and from non-pericyclic tissues in older roots (Li et al. 2009). These roots have exceptional capacities for secondary metabolite accumulation and are natural, rapidly proliferating, and fast-developing in phyto-hormone-supplemented media (Martin et al. 2008; Murthy et al. 2008; Wasnik et al. 2009).

As *Indigofera heterantha* is brutally reaped for root collection due to its high medicinal value, hence, root culture has the potential to multiply plant genetic resources for maximum production of secondary metabolites on commercial scale without any seasonal limitation and field cultivation. So keeping in view, the importance of *Indigofera heterantha* as medicinal plant, the research was initiated with the objective to enhance the biomass yield and secondary metabolites

production through elicitation with Jasmonic acid in adventitious root culture of *Indigofera heterantha*.

MATERIALS AND METHODS

Culture development and treatment application

For optimizing the potent role of Jasmonic acid (JA) in biomass accumulation and secondary metabolites production in adventitious root cultures of *Indigofera heterantha*, the experiment was design in Complete Randomized Design. Adventitious roots (30 days old) of uniform quantity taken from the stock were cultured in in 100 ml Erlenmeyer flasks comprising 50 ml liquid media having IBA (0.5 mg L⁻¹) as rooting plant growth regulators. Media containing IBA was further concentrated with several concentrations of Jasmonic acid (0, 0.5, 1.0, 1.5 and 2.0 mg L⁻¹). Each culture media was then autoclaved at 121 °C for 20 minutes before inoculation of adventitious roots to the medium. All the cultures were placed on orbital shaker at 80-120 rpm in dark condition at growth room (25 ±2°C) for 49 days.

Adventitious Root Biomass Determination

For fresh biomass (FB), adventitious roots were collected from liquid media. These roots were cautiously washed with sterile distilled water and pressed gently on filter paper (Whatman Ltd., England) to get rid of surplus water and finally weighted with Sortorius digital balance; Germany. Correspondingly, for dry biomass (DB) determination, roots were oven dried (Thermo Scientific; Germany) at 50 °C and finally weighted. Fresh and dry biomass of adventitious roots were expressed in gram/flask.

Analytical Methods

For the preparation of the extract, the dried biomass of the adventitious root cultures was ground in a mortar and pestle. Each sample's powdered component, 10 mg, was combined with 10 ml of ethanol in a test tube. For a week, the solution was vertixed every day. These solutions undergo a 15-minute centrifugation at 14,000 rpm after one week. The supernatant was used to measure various activities.

Quantification of TPC, TFC and DRSA

The total phenolic content in each sample was determined by implementing the approach used by Ahmad et al. (2014). Briefly, 2.55 ml of sterile distilled water, 0.03 ml of extract, and 0.1 ml (2N) Folin-Ciocalteus reagent were combined. The mixture was first centrifuged for 14 minutes at 10,000 rpm before being placed in a cuvette attached to a UV-visible spectrophotometer (Shimadzu-1650; Japan) and incubated for 6 minutes. The resulting mixture's absorbance was analysed at 760 nm. The standard calibration curve was generated using gallic acid (Sigma; 1.0–10 mg/ml; $R^2=0.9878$). The following equation was used to convert the results into gallic acid equivalent (GAE) mg g⁻¹ of dry root biomass (DRB) from percent TPC.

$$\% \text{ total phenolic content} = 100 \times (AS-AB) / (CF \times DF)$$

Where AS is the absorbance of the sample and AB is absorbance of blank, CF is the conversion factor from standard curve, and DF is the dilution factor.

The TFC in DRB was calculated by using the protocol used by Ahmad et al. (2014) with little modification. Treatment sample methanolic extract (0.25 ml) was coupled with sterile distilled water (1.25 ml) and 0.075 ml of 5% (w/v) AlCl₃. The solution was combined with 0.5 ml of NaOH and incubated for five minutes before being centrifuged for 14 minutes at 10,000 rpm (1 M). Using a UV-visible spectrophotometer, the absorbance was estimated at 510 nm (Shimadzu-1650PC, Japan). The standard calibration curve was generated using rutin (Sigma; 1.0–10 mg/ml; $R^2=0.9866$). Rutin equivalent (RE) mg g⁻¹ DRB of extracts were used to express the total flavonoid content. With few alterations, Ahmad et al. (2013)'s approach was used to evaluate the DPPH radical scavenging activity (DRSA) in a variety of samples. Briefly, 2.0 ml of DPPH free radical solution (0.25 mg/ 20 mlx4) was combined with 1.0 ml of ethanolic extract (5 mg/20 ml) from each sample. For around 30 minutes, the mixture was incubated in the dark. Using a UV-visible spectrophotometer model, the absorbance of the resulting combination was measured at 517 nm at room temperature (Shimadzu-1650PC, Japan). The following equation was used to determine the radical scavenging activity as a percentage of DPPH discoloration

$$DRSA (\%) = 100 \times (1 - AP/AD)$$

Where AP represents absorbance of plantlets extract at 517 nm and AD is the absorbance of the DPPH solution without tissue extract.

Statistical Analysis

Analysis of replicated mean values and least significant difference (LSD) was carried out by using Statistix software (8.1 versions), and Origin Lab (8.5) software was used for graphical presentation.

RESULTS AND DISCUSSIONS

Effect of Jasmonic acid on biomass accumulation

Elicitation of *Indigofera heterantha* adventitious roots through Jasmonic acid (JA) was found effective in causing significant variations in biomass accumulation (Fig 1). The application of JA as elicitor showed considerable increase in biomass yield both fresh (24.4 g L⁻¹) and dry (2.93 g L⁻¹) with application of JA at 1.5 mg L⁻¹ concentration in culture media. However, root production was decreased with increasing JA concentration from 1.5 mg L⁻¹ (Fig 1). It was closely followed by the culture free of JA (control), which developed biomass of 21.4 g L⁻¹ fresh weight and 2.60 g L⁻¹ dry weight. When the basal culture was exposed to JA at a concentration of 2.5 mg L⁻¹, the adventitious root displayed the lowest biomass (6.60 and 0.40 g L⁻¹) formation in term of fresh and dry weight, respectively (Fig 2).

The effect of methyl jasmonates (MeJA) depends on the concentration of MeJA, the cells' ability to convert MeJA into jasmonate and isojasmonate isoleucine, a ligand for the inositol-phosphate-potentiated COL1-JAZ receptor complex, as well as the endogenous levels of jasmonates and other hormones (Wasternack et al. 2013). Jasmonates are known to cause eustress when present in low quantities, which activates transcriptional cascades and intracellular manufacturing of protective chemicals. In contrast, jasmonates present in high concentrations have been shown to accelerate senescence and the death of plant cells (Repka et al. 2013). Oxidative stress and an increase in reactive oxygen species (ROS) are also side effects of increased MeJA use, which appears to cause the production of antioxidants from a variety of sources, including antioxidant enzymes (Sasaki et al. 2005), ascorbate and glutathione (Sasaki et al. 2005; Pauwels et al. 2008).

The improvement in biomass production caused by JA treatment may be the result of an activating reaction persuaded by its presence on the production of endogenous auxins, which is crucial for plant roots (Yan et al. 2014). JA treatment resulted in the establishment of ROS due to

stress, which negatively influence root biomass (Ali et al. 2007). The accumulation of reactive oxygen intermediates (ROIs), which are activated as a result of elicitor treatment at lower concentrations, may be connected to the association of elicitor in root growth. NADPH Oxidase (NOX) typically produces ROIs in plant cells, such as (-OH), which cause cell extension, cell wall thinning, and ultimately root elongation and growth (Gapper and Dolan 2006; Liskay et al. 2004). As a result of JA's toxicity at greater doses, increasing levels of JA also limit root growth, which decreases biomass production (Suzuki et al. 2005).

Elicitation with JA often has a negative impact on biomass buildup, resulting in reduced productivity in in vitro cultures. For both fresh and dry biomass production in the current investigation, JA concentrations as low as 0.5 g L⁻¹ showed the best outcomes. The findings of previous research that demonstrated a decline in biomass accumulation as a result of the elicitation of MeJA and SA in *Panax ginseng* adventitious roots provide support for the findings (Kim et al. 2007), *Scopolia parviflora* adventitious roots (Kang et al. 2004) and *Eleuterococcus koreanum* adventitious roots (Lee et al. 2015). This was in harmony with the conclusion of Suzuki et al. (2005), who noticed JA toxicity possibly at high concentrations. Lu et al. (2000) and Sakunphueak and Panichayupakaranant (2010) witnessed browning in *Panax ginseng* and *Impatiens balsamina* root cultures, correspondingly, upon higher than 200 μM of MJ elicitation. Furthermore, Lee et al. (2015) stated that adventitious roots of *E. koreanum* exposed to high MeJA concentration exhibited superior growth than at high SA concentration.

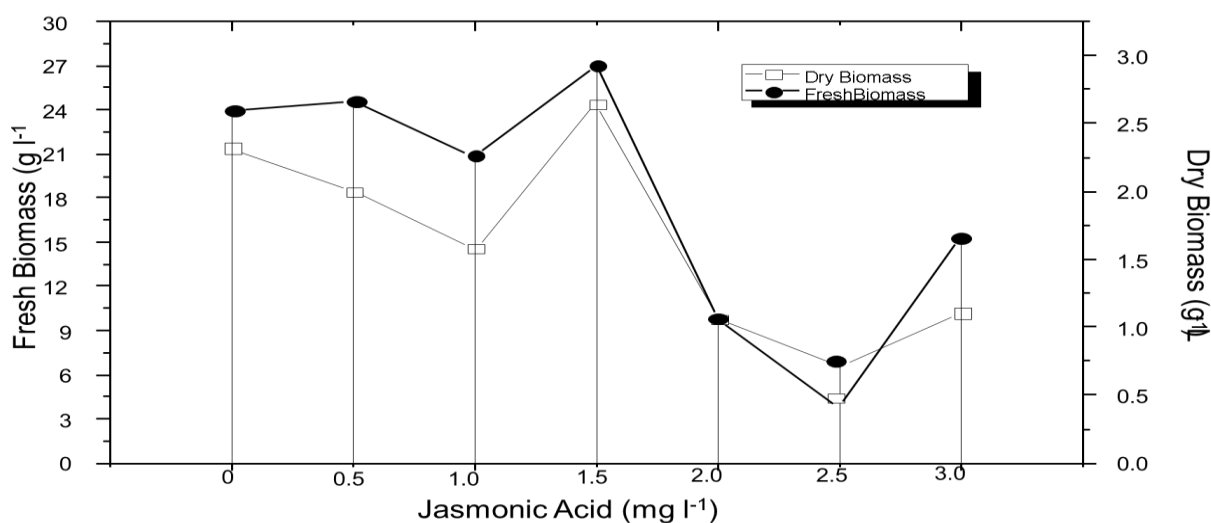


Fig: 1 Fresh and dry biomass (g L⁻¹) of *Indigofera heterantha* as affected by different concentration of Jasmonic acid.

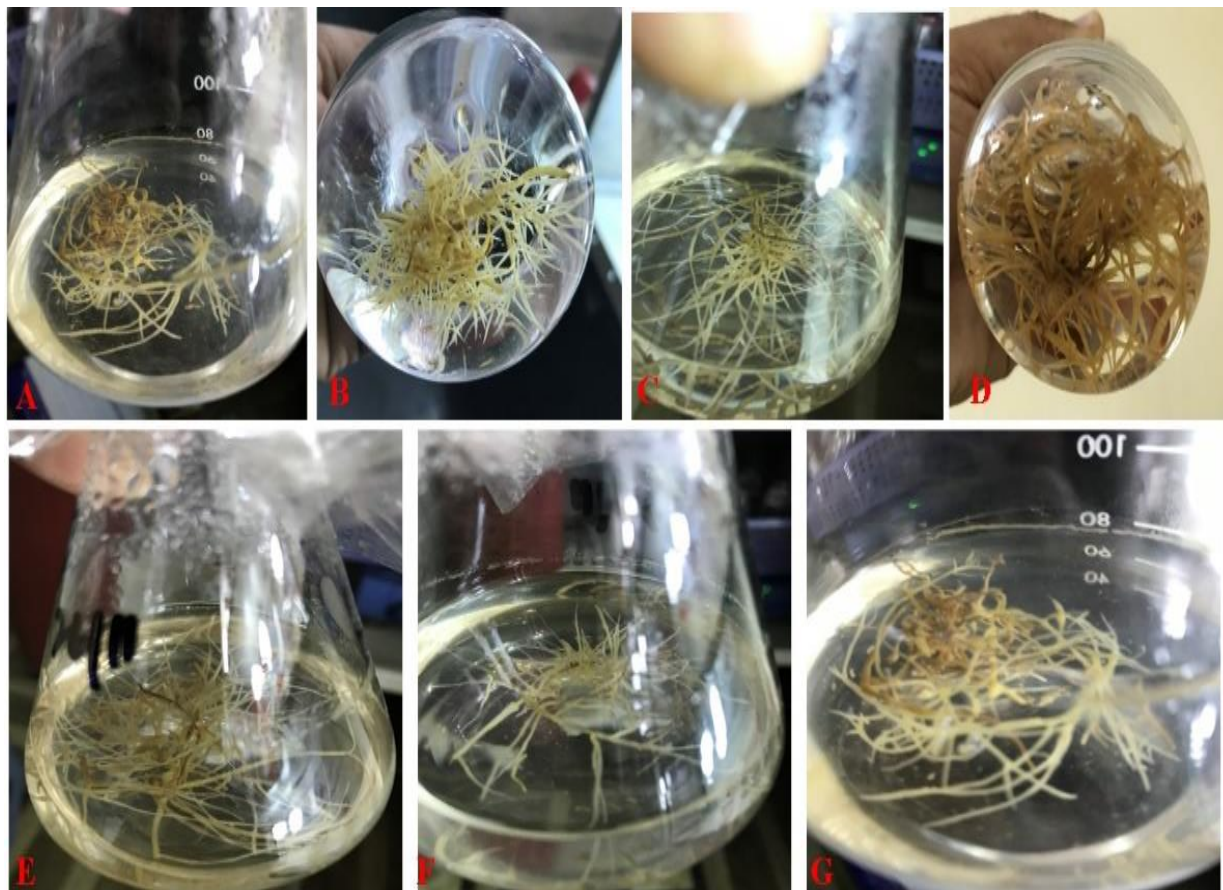


Fig 2. The influence of JA levels on adventitious root cultures of *Indigofera heterantha*. (a; 0, b; 0.5, c; 1.0, d; 1.5, e; 2.0, f; 2.5, g; 3.0 mg L⁻¹)

Total phenolics content (TPC)

The statistical analysis of the data confirmed that elicitation with JA lead to a significant increase in accumulation of total phenolic contents (TPC) compared with the control in adventitious roots of *Indigofera heterantha* (Fig 3). Among the treatments, the highest (131 $\mu\text{g g}^{-1}$ DW) TPC were synthesized in adventitious roots cultured in media supplemented with JA at the rate of 1.5 mg L⁻¹ in comparison with the control medium which yielded TPC of 76.67 $\mu\text{g g}^{-1}$ DW. It was followed by the media fortified with 2.0 mg L⁻¹ resulting in total phenolic contents of 113.70 $\mu\text{g g}^{-1}$ –DW. Further rise in the JA concentration reduced the TPC in the adventitious root culture. The adventitious roots developed in the medium having Jasmonic acid at the concentration of 0.5 and 1 mg L⁻¹ produced TPC (90.56 $\mu\text{g g}^{-1}$ DW) and (104. 4 $\mu\text{g/g}$ –DW), respectively with statistical similarités. The minimum TPC (50. 30 $\mu\text{g g}^{-1}$ DW) were obtained under Jasmonic acid

fortification of the basal medium at of 3 mg L⁻¹ in adventitious roots culture of *Indigofera heterantha*.

Jasmonates are considered to be the plant signalling molecule, which plays an important role in plant growth, development and defence mechanism (Reymond and Farmer 1998). Through signal transduction, jasmonates treatments in the culture medium result in the establishment of secondary metabolites, which in turn accelerates enzyme catalysis and results in the synthesis of substances like polyphenol, flavonoids, alkaloids, and terpenoids (Mueller et al. 1993; Zhao et al. 2005). These signalling molecules, play a role in signal transduction system, which activate specific enzymes of the secondary metabolite pathway to synthesize defence compounds such as phenolics (Ding et al. 2002). Exogenous supplementation of methyl jasmonates in plants encourages expression of certain defence genes (Ding et al. 2002). Glucose-6-phosphate dehydrogenase (G6PDH), shikimate 5- dehydrogenase (SKDH), phenylalanine ammonia lyase (PAL) and carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) enzymes are accountable for the synthesis of phenolics. By synthesizing the suitable precursors both G6PDH and SKDH enzymes induces the production of phenolics (Ratledge 1982; Randhir et al. 2006). As a result, in roots that have been fortified with methyl jasmonates, the expansion of both G6PDH and SKDH activities causes the synthesis of phenolics. This could be a result of the ROS-facilitated response since ROS could promote the endogenous generation of MJ in the roots (Brader and Palva 2004). The L-phenylalanine ammonia lyase (PAL) enzyme, which is typically considered to be the major enzyme biosynthesis of phenolic compounds, causes the creation of phenolics and flavonoids. There was a clear correlation between the JA and increased PAL activity, which resulted in the highest accumulation of TPC. It is evident from the research that MJ-augmented roots culture encourages increased PAL activity because it acts as a signal molecule to promote PAL activity, which in turn increases the phenolic accumulation (Rao et al. 2000). The encouraged PAL activity not only boosts accumulation of phenolics, but also plays an significant part in plant defence (Ding et al. 2002).

In adventitious root cultures, the supplementation of MeJA promotes several defence mechanisms. Parallel to the present conclusions, addition of MeJA to the culture medium encouraged the defence system and motivated the biosynthesis of bioactive compounds in adventitious roots (Ali et al. 2007). The outcomes are consistent with those of Murthy et al. (2016)

who employed MeJA as an elicitor to increase the synthesis of phenolic chemicals in *Polygonatum multiflorum* adventitious root cultures. Similar to the current findings, it was previously noted that increased elicitor augmentation had negative impacts on metabolite accumulation in adventitious root culture (Ali et al. 2007; Wu et al. 2014; Lee et al. 2015).

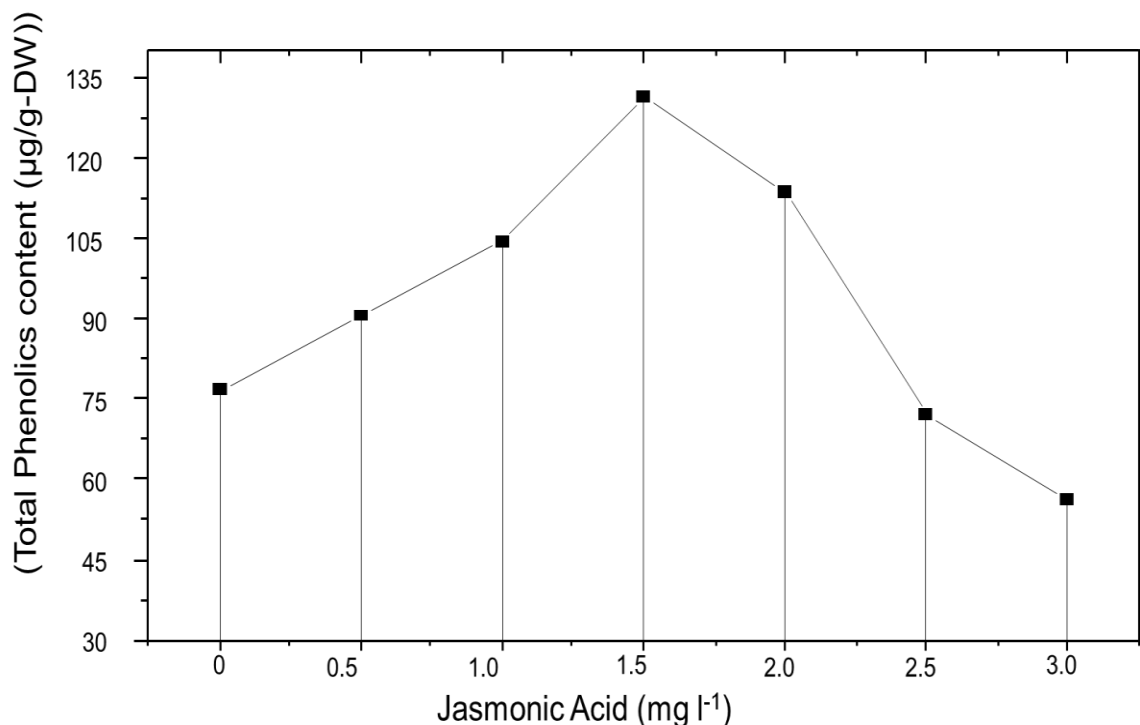


Fig 3: Effect of jasmonic acid concentration on the accumulation of total phenolic contents in adventitious root culture of *Indigofera heterantha*.

Total flavonoid contents (µg g-1DW)

The flavonoids are vital compounds with abundant pharmaceutical properties. Varying Jasmonic acid levels significantly influenced the total flavonoid contents (TFC) in adventitious root cultures of *Indigofera heterantha* (Fig 4). Among all the treatments, the adventitious root culture augmented with JA (2.0 mg L⁻¹) synthesized maximum amount of TFC (380.00 µg g-1DW). It was followed by the medium having JA (2.5 mg L⁻¹), which produced (292.12 µg g-1DW) TFC. However, the adventitious root culture grown on half strength MS medium without JA resulted in the lowest (156.76 µg g-1DW) accumulation of total flavonoid contents.

The plants produce secondary metabolites and stimulate enzymes to deal with stress conditions (Hahlbrock and Scheel 1989). Methyl Jasmonates trigger a variety of metabolic defense

responses in plants against various stimuli, such as physical injury and pathogen attack (Conrath et al. 2002; Tan et al. 2004). Low molecular weight compounds like flavonoids and phenolics are produced by plants as a defence response to stress (Ali et al. 2006). The largest and most diversified class of natural substances is flavonoids, which are used to manage stressful situations (Santos and Mira 2004). MeJA affects plants' secondary metabolism (Ali et al. 2007). Usually, phenolics and flavonoids are anti-oxidative in nature and are shaped in the phenyl-propanoid pathway (Heller and Forkmann 1988; Lister et al. 1996). The proposed enzyme phenylalanine ammonia-lyase is responsible for the deamination of L-phenylalanine to trans-cinnamic acid and ammonia, which is necessary for the biosynthesis of phenolics and flavonoids in plants (Khan et al. 2015a). MeJA's interaction with plant cell surface receptors triggers the plant defense response, which causes the de novo transcription of several essential genes involved in secondary metabolism, including the PAL gene (Gundlach et al. 1992). MeJA's role as a signaling molecule in plant cells is well known in literature due to its morphological and physiological properties (Jalalpour et al. 2014).

Using a variety of secondary metabolites, Hayashi et al. (2003) investigated the effects of jasmonic acid on their biosynthesis and established the participation of vital enzymes that hasten this process. Our findings are consistent with those of Saeed et al. (2017), who showed a substantial increase in the total phenolic and flavonoid content of *Ajuga bracteosa* root suspension. Exogenous application of jasmonates significantly aided the biosynthesis of secondary metabolites in cell suspension cultures, according to numerous other researchers who have also demonstrated an association between the effects of jasmonic acid on the accumulation of flavonoids and other significant metabolites in various cultures (Van der Fits and Memelink 2000), anthocyanin accumulation in *Arabidopsis thaliana* (Peng et al. 2011) strawberry fruits (Perez et al. 1997), *Vaccinium pahalae* (Fang et al. 1999) and *Vitis vinifera* (Zhang et al. 2002).

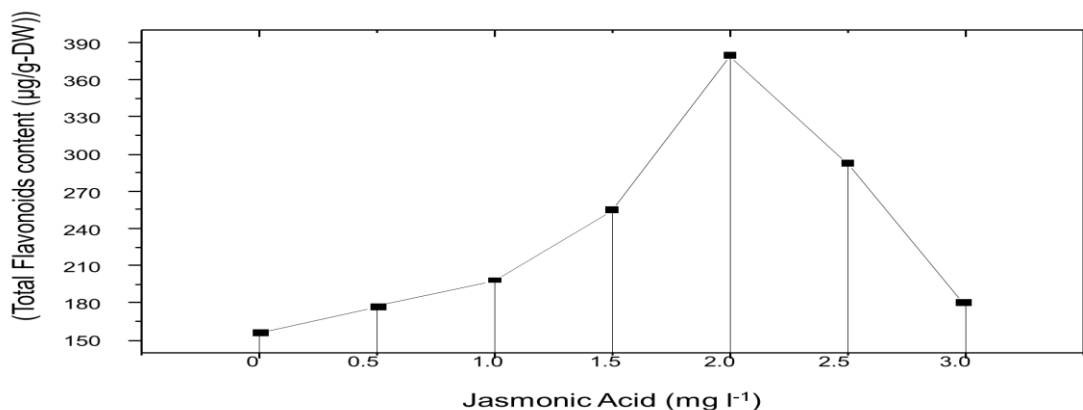


Fig 4: Effect of jasmonic acid concentration on the accumulation of total flavonoids contents in adventitious root culture of *Indigofera heterantha*

DPPH free radical scavenging activity

To improve the production of bioactive compounds and check the level of free radical scavenging activities in adventitious root culture, the media was augmented with varying concentration of JA. The overall results clarified that the supplementation of JA to *Indigofera heterantha* adventitious root cultures triggered the antioxidant activities compared to untreated roots cultures (Fig 5). In the current study, the antioxidant activities were presented as percent (%) DPPH free radical scavenging activity (DRSA). The augmentation of various concentration of Jasmonic acid was effective in causing variations in DPPH free radical scavenging activity of *Indigofera heterantha*. Among the different tested concentrations of JA, maximum antioxidant potential (67.78%) was found in adventitious roots cultured in media augmented with 1.5 mg L⁻¹ JA. The culture medium supplemented with JA at a concentration of 1.0 and 2.0 mg L⁻¹ behaved alike and showed 56.43 and 48.81 %, respectively DRSA. However, the lowest DPPH-radical scavenging activity (DRSA; 31.90 %) was documented in the control medium.

Numerous biotic and abiotic stress situations are faced by plants. However, plants have their own defense mechanisms that are aided by the buildup of essential secondary metabolites. As a signal transducer for the creation of secondary metabolites, jasmonic acid has been identified. These signals serve as elicitors and activate the oxidative defense system, which removes ROS via enzymatic processes or the production of antioxidant molecules (Thulke and Conrath 1998; Zhao et al. 2005). Enzymes such as SODs are in front line against these ROS and is key scavenger of the superoxide that can help in the synthesis of H₂O₂ (Hu and Zhong 2008; Rahimi et al. 2014).

POD is also identified as it breaks H_2O_2 into H_2O and O_2 (Hu and Zhong 2008). Besides enzymes, plant cultures also accumulate compounds to detoxify the activities of ROS (Takahashi and Asada 1983). Ali et al. (2006) also reported that MJ and SA improve the activities of POD, SOD, and the accumulation of secondary metabolites of *Panax ginseng* root culture.

As antioxidant activities are due to both enzymatic and non-enzymatic factors like phenols and flavonoids (Ahmad et al. 2014). Therefore, it is suggested that increasing antioxidant activities with increasing concentrations may be caused by higher antioxidant enzyme activities or synthesis of phenolics and flavonoids. Therefore, flavonoids and phenol prevent oxidative damage (Pieta et al. 1998). In adventitious root cultures of numerous medicinal plants, the impact of phenols on antioxidant activities has been proven (Jayasinghe et al. 2003; Canadanovic-Brunet et al. 2005; Ali et al. 2006; Ali et al. 2007; Sengul et al. 2009; Bidchol et al. 2011; Diwan et al. 2012). Numerous sources of information are also available that link the antioxidant capacities of various plants' phenol and flavonoid contents (Canadanovic-Brunet et al. 2005; Wong et al. 2006; Mahmoudi et al. 2009; Asghar et al. 2011).

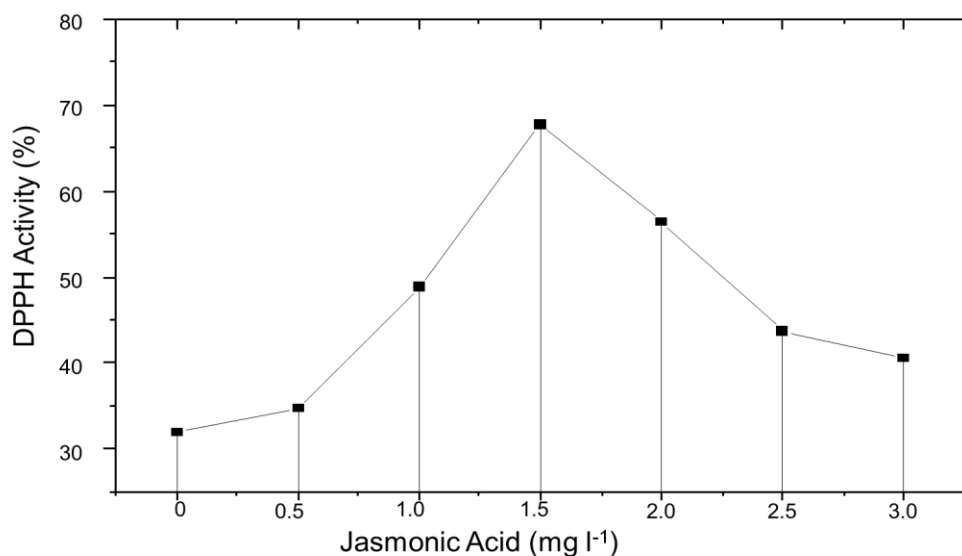


Fig 5. DPPH free radical scavenging activity of adventitious root culture of *Indigofera heterantha* as affected by various concentration of Jasmonic acid.

Conclusions

Significant differences were experienced in biomass accumulation and secondary metabolites production in response to varying levels of JA (0-3.0 mg L⁻¹) in adventitious root cultures of *Indigofera heterantha*. The supplementation of MS medium with 1.5 mg l⁻¹ JA was optimized for superior fresh (24.4 g L⁻¹) and dry (2.93 g L⁻¹) biomass as well as TPC (131 µg g⁻¹DW) and DRSA (67.78%). in adventitious root cultures. However, Greater TFC (380.00 µg g⁻¹DW) was resulted in the adventitious root cultures of *Indigofera heterantha* when the culture was augmented with the concentration of 2 mg L⁻¹ Jasmonic acid.

Recommendations

For higher accumulation of biomass (fresh and dry) and enhanced TPC and DRSA the medium should be augmented with JA at 1.5 mg L⁻¹. While, for maximum production of TFC in the adventitious root culture of *Indigofera heterantha*, the basal medium should be additionally supplemented with JA at the rate of 2 mg L⁻¹.

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Declarations

Conflict of interest the authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Informed consent both the authors have approved this manuscript, agree to the order in which their names are listed, declare that no conflict of interest exist and deny any commercial affiliation.

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