Aspergillus niger one of the most common and important fungal species. It is most commonly found in mesophilic environments such as decaying vegetation or soil and plants. Genome sequencing of A. niger as a biotic elicitor is important because of its involvement in producing citric acid as well as industrial enzymes, such as amylases, proteases, pectinases and lipases. The use of these enzymes is essential because of their importance for transformation to food enzymes. Other properties of A. niger include of pharmacologically significant secondary metabolites or phytopharmaceuticals such as alkaloids, glycosides, flavonoids, volatile oils, tannins, resins and aflatoxin. Metabolite production, involvement in food spoilage, and simply being a pathogen creates a great economic impact. Most of these secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is either extremely difficult or economically infeasible. Plants and/or plant cells in vitro, show physiological and morphological responses to microbial, physical or chemical factors which are known as ‘elicitors’. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness. Here, we discuss the classification of Aspergillus niger as a biotic elicitor, for the production of secondary pharmaceutical metabolites from medicinal plants in vitro.

Keywords: Aspergillus niger, biotic elicitors, secondary pharmaceutical metabolite, medicinal plants, in vitro.
**Elicitors**

Plants or plant cells *in vitro*, show physiological and morphological response to microbial, physical or chemical factors which are known as ‘elicitors’. An ‘elicitor’ may be defined as a substance which, when introduced in small concentrations to a living cell system, initiates or improves the biosynthesis of specific compounds. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival persistence and competitiveness. The application of elicitors, which is currently the focus of research, has been considered as one of the most effective methods to improve the synthesis of secondary metabolites or phytopharmaceuticals in medicinal plants, which include alkaloids, glycosides, phenols, flavonoids, volatile oils, etc. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavours and other industrial materials. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Commonly tested chemical elicitors are salicylic acid, methyl salicylate, bezoic acid, chitosan and so forth which affect production of phenolic compounds and activation of various defense-related enzymes in plants. Plants are challenged by a variety of biotic stresses like fungal, bacterial or viral infections. This lead to the great loss to a plant yield. Depending on this principle, some strategies were developed and used to encourage the *in vitro* production of secondary metabolites, these strategies include treatment with microbial, physical and chemical agents known as elicitors (Yue et al., 2016). It became known now that the use fungi is considered to be one of the best biotic elicitors. Because it stimulate the plant’s cells to produce the secondary metabolites such as flavonoids and phenols as in the case of using the fungus *A. niger* as a biotic elicitor (Shanker and Shanker, 2016; Ibrahim et al., 2019). It also plays an important role in the field of biotechnology to produce chemical substances, enzymes and medical drugs (Baker and Bennett, 2008). Classification of biotic elicitor for the production of secondary metabolites directly released by microorganisms and recognized by the plant cell (enzymes, cell wall fragments), formed by action of microorganisms on plant cell wall (fragments of pectins etc.), formed by the action of plant enzymes on microbial cell walls (chitosan, glucans) and compounds, endogenous and constitutive in nature, formed or released by the plant cell in response to various stimuli (Namdeo, 2007).

**Medicinal Plants**

Medicinal plants are the source of bioactive compounds with many blockbuster drugs derived directly or indirectly from plants having therapeutic value. The production of secondary metabolites in plants is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Rao et al., 2002; Thakur et al., 2013). To overcome this problem we can preserve the resulting loss of biodiversity by minimizing use of the product from the intact plant and synthesize secondary metabolite production using *in vitro* techniques by elicitors for enhancing their bio-production to meet commercial demands.

Medicinal plants of pharmaceutical interest whose secondary metabolite production is enhanced by the addition of the extract of biotic elicitor

**Aspergillus niger**

*Ruta graveolens* L. is perennial medical plant belongs to the family Rutaceae (Al-Kateb, 2000), widely distributed and has a long history in traditional medicine, as it had been used in medicine for more than 1500 year ago (Bowen et al., 1988). Poutaroud et al. (2000) mentioned that the plant has a strong smell and produce different types of secondary metabolites, that give it the medical and pharmaceutical importance, like essential oils, alkaloids, flavonoids and furanocoumarins (Al-Mahdawe, 2018a). It was also reported that it contains carotenoids, chlorophylls and a number of compounds that have antimicrobial activities such as acridone (Wessner et al., 1999). Talfan et al., (2020), indicated that the addition of *A. niger* extract, has a strong effect on increasing the concentration of Psoralen, Xanthotoxin and Bergapten in the callus cultures. The effect of the addition of *A. niger* extract on increasing the production and accumulation of furanocoumarins in callus culture. Could be explained according to the fact that plant cells give their highest production when they are under stressful conditions or at study state or clumped together. There is a number of important factors that affect callus production and accumulation of secondary metabolites, such as, the elicitor concentration, the length of exposure to the elicitor, the formation of nutrients, the age and the culture status. Ibrahim et al. (2019), mentioned that the addition of *A. niger* extract at a concentration of 2.0 ml L⁻¹ to callus culture of the plant *Calendula officinalis* L., produced the highest concentration of salicylic acid 1.147 mg g⁻¹ in comparison of the control treatment which reached 0.428 mg g⁻¹. Manjula and Mythili (2012), also reported that the addition of *A. niger* extract at a concentration of 2.0 ml L⁻¹ to callus cultures of the plant *Marsilea quadrifolia*, resulted in an increment in the growth of the plant and the concentration of carbohydrate and protein as primary metabolites, which reflected later on increment of the accumulation of the secondary metabolites, such as, the phenolic substances and flavonoid in the roots and the branches.

*Psoralea corylifolia* L. is an important medicinal plant found in the tropical and subtropical regions of the world. It synthesizes diverse phenylpropanoids such as furanocoumarins, isoflavonoids etc. (Boardley et al., 1986). Psoralen is the furan ocoumarin and commercially important for having broad range of pharmacological activities such as photosensitizing, photobiological and phototherapeutic properties (Frank et al., 1998). Psoralen has been used for the photochemotherapy of vitiligo and skin diseases such as psoriasis, mycosis fungoides and eczema (Khashboo et al., 2010; Ozkan et al., 2012). It also shows antitumor (Szliszka et al., 2011), antibacterial (Chanda et al., 2011) and antifungal properties (Srinivasan and Sarada, 2012). The addition of *A. niger* elicitor to the cultured cells of *P. corylifolia* L. increased the psoralen accumulation. The maximum increase in psoralen accumulation was recorded in 20 days old culture and it decreased with increase in age of the culture thereafter. The accumulation of psoralen increased with increase in age of cell culture up to 16 days and thereafter the increase was inconsistent (Syed and Mirza 2014). The stimulation of psoralen accumulation by biotic elicitors such as *A. niger*, *Penicillium notatum*, yeast extract and chitosan has also been observed in the cell cultures of plant species viz. *Calendula officinalis* (Wiktorowska et al.,
2010), Sorbus aucuparia (Gaid et al., 2011) and Abrus precatorius (Karwasara et al., 2010, 2011). The cell wall extract preparation of A. niger possessed an oligosaccharide elicitor that induced high level of shikonin (Wen and Riqiang, 1996). Another significant effect of the elicitors observed in the experiments was the rapid increase in psoralen accumulation with elicitor dosage. Thus, the accumulation of psoralen is a dose elicitor dependent response of P. corylifolia L. cell cultures. The growth and accumulation of secondary metabolites were influenced by the type and mode of elicitor preparation (Karwasara et al., 2011). Syed and Mirza, (2014), mentioned that the extract of A. niger (1.0% v/v) was found to be the best for maximum metabolite elicitation influenced the accumulation of psoralen in the cultured cells.

Calendula officinalis L. is one of the plant of Asteraceae family, it is an aromatic plant that is classified in terms of its growth into annual winter ornamental plants (Alexopoulous, 1962). The plant has extensive uses in the field of herbal medicine flowers are used in the treatment of smallpox, measles, jaundice, constipation and reduced bleeding during menstruation (Al-Taha and Al-Mazine, 2016). Ibrahim (2019), indicated that addition of different concentrations of A. niger fungus extract, it has an effect on increasing the accumulation of salicylic acid in the callus, of Calendula officinalis which increased by increasing the concentration of fungus extract, and reached the highest content at the concentration 2.0 mg. L⁻¹.

Andrographis paniculata Nees. is commonly known as ‘Kalmegh’ in India and as a medicinal plant belongs to the family Acanthaceae. The plant is recommended for its drug utility in Indian Pharmacopoeia and widely used in Ayurveda, Unani, Siddha and Homeopathy systems of medicines. The plant is reported to possess terpenoids and flavonoids. The major terpenoids viz., 14-deoxy-11-oxandrographolide, 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide andrographolide are the active constituents of this plant. The main active constituent is andrographolide which is reported to possess liver stimulant, astrangent, anodyne, tonic and alexipharmic properties and useful in dysentery, chola, diabetes, consumption, influenza, bronchitis, swellings, itches, piles and gonorrhea (Zhao and Frang 1991). The most significant pharmaceutical properties of this plant are anticancerous (Kumar et al. 2004) and anti-HIV (Calabrese et al. 2000). Moinuddin and Vijay (2013), showed that elicitation of andrographolide by Aspergillus niger elicitors in cell suspension culture of Andrographis paniculata, in 4 days and 7 days treatment duration, 1 ml of A. niger extract was found to be most positive concentration for eliciting andrographolide compound. The estimated quantity of andrographolide was 52.0 µg/g and 331.0 µg/g in 4 days and 7 days treatment duration, respectively, which showed 2.47 and 3.76 fold increase over their respective controls.

Datur metel L. (Solanceae), a small branched perennial herb with purple coloured flowers, is distributed in the tropical and sub-tropical regions of the world. This medicinal plant has been traditionally used as intoxicant, emetic, digestive and healing since ancient times (Anonymous, 1952; Muthukumar et al., 2004). The main active constituents of the plant are medicinally important tropane alkaloids-hyoscyamine and scopolamine.D. Metel showed very high content of hyoscyamine (Knopp et al., 1988) and scopolamine (Hiraoka et al., 1996). Being anticholinergic agents, these are used in medicine as antispasmodics, preoperative medication, analgesics, narcotics, sedatives and in treatment of asthma, Parkinson’s disease and motion sickness (Pitta-Alvarez et al., 2000). The use of elicitors is one of the effective strategies employed to increase the production of important alkaloids in cell and organ culture. L. Ajungla et al. (2009) mentioned that root cultures treated with 1.0 g L⁻¹ of A. niger homogenate resulted in higher hyoscyamine (1.77 mg/g dw) and scopolamine (0.087 mg/g dw) production than that the Alternaria sp. and Fusarium monoliforme and the results indicate that the A. niger homogenate is favourable for promoting of tropane alkaloids in D. metel.

Hypericum perforatum L. (Hypericaceae) known as St John’s wort is an important medicinal plant. A number of pharmacological studies and clinical trials have shown that H. perforatum extracts possess an astounding array of pharmacological properties including antidepressant, anti-inflammatory, antiviral, anticancer and antibacterial activities. These medicinal properties are related to the composition of the secondary metabolites present in the extract, particularly hypericins, hyperforins, flavonoids, xanthones and other valuable compounds (Wang et al., 2012). Xu et al. (2005), indicated that A. niger cell walls induced hypericin biosynthesis production in H. perforatum cell suspension cultures and found that, fungalelicitor prepared from the A. niger induces multiple responses of Hypericum perforatum cells, including nitric oxide (NO) generation, jasmonic acid (JA) biosynthesis. Also, Gadzovska et al. (2015) reported a significant increase in naphthodianthrones, total phenolics, flavonoids, and anthocyanins in Hypericum perforatum cell suspensions by adding A. niger extract.

Gymnema sylvestre, native to central and western India, tropical Africa, and Australia, is a perennial woody climber rich in triterpenoid saponins belonging to the oleane (gymemic acids) and dammarene (gymemasesides) classes (Parijat et al., 2007). The gymemic acids are a group of closely related molecules isolated from the leaves of G. sylvestre (Liu et al. 1992; Manni and Sinsheimer 1965). The anti-diabetic, anti-sweet and anti-inflammatory activities of G. sylvestre have been attributed to the presence of gymemic acids; the other phytoconstituents include flavones, anthraquinones, hentriacontane, resins, d-quercitol, lupeol, b-amyrin-related glycosides, and stigmasterol. The cell wall acts as a chemical messenger with specific regulatory properties. The results obtained by Bhuvanweswari (2013), showed that the maximum accumulation of gymemic acid was observed with A. niger, i.e. 11.2-fold (98.65 ± 0.93 mg/gDCW). This is 1.6-fold higher than the abiotic elicitor CdCl₂. On the other hand, Subathra and Mohana (2011), mentioned that when A. niger cell extract was used as a elicitor, the productivity of gymemic acid increase was considerably greater than that obtained with non-elicited cultures.

Glycyrrhiza glabra L. plant belongs to Fabaceae family and is the inhabitant of Central and Southwest Asia. G. glabra is commonly known as Jothi-madhu, Mulhatti (Hindi), licorice, liquorice, sweet wood (English) (Jatav et al., 2011). G. glabra plant is blessed with many medicinal properties. The use of licorice is more than 4000 years old. It is considered under important medicinal plants mentioned in...
Assyrian herbal (2000 BC). This plant is used in the treatment of dyspepsia, gastric ulcers, fevers, liver ailments, asthma, bronchitis, sore throats, Addison’s disease, and rheumatoid arthritis. It is also useful as an antitussive, expectorant, and laxative. In ancient times, this plant was also suggested in cases of women sterility. Licorice root is considered under top five herbs, which are recommended for the treatment of fatigue. This herb decreases temptation for sugars and increases cortisol activity in the human body. Glycyrrhizin is present in a very high amount in licorice roots. The roots of licorice contain a large amount of glycyrrhizin (up to 15%) and oleanane-type triterpene saponins. These saponins are used in various foods and industrial, cosmetic, and pharmaceutical applications. Saponins are commercially used in food industry as foaming, detergent, emulsifying, wetting, and sweetening agents (Hostettmann and Marston, 2005; Shibata, 2000). The pharmacological properties of triterpenes have been broadly studied which showed that these compounds have significant medicinal properties. Besides this, they also showed involvement in plant defense responses. Glycyrrhizin is also efficient against several viruses, such as HIV (Ito et al., 1987, 1988) and severe acute respiratory syndrome (SARS caused by corona virus-like viruses) (Cinatl et al., 2003). It is used for curing acute respiratory problems, gastritis, gastric ulcers, inflammatory conditions in general, and adrenal exhaustion. Compounds found in licorice roots possess both estrogenic and antiestrogenic activity, and due to these properties, this important herb is used for treating the female hormonal problems (Jatav et al., 2011). Although some side effects are also associated, due to high doses and prolonged use of this, such as hypokalemia, hypertension, mineralocorticoid effects, myoglobinuria, lethargy, quadriplegia, etc. (Nasrollahi et al., 2014). To improve the yield of glycyrrhizin, some fungal elicitors prepared from Aspergillus niger and Rhizopus stolonifer were tested at different concentrations in transformed cell suspension cultures of A. precatorius. The maximum enhancement of 4.9- and 3.8-fold in glycyrrhizin contents was obtained with A. niger (7.5% v/v) and R. stolonifer (5.0% v/v), respectively, on the fifth day after elicitor treatment (Karwasa et al., 2011).

_Bacopa monnieri_ is a medicinal plant belongs to Plantaginaceae family commonly known as Brahmi, found throughout the Indian subcontinent in wet, damp and marshy areas. It is used in traditional Indian medicine and Ayurveda for the treatment of anxiety and improving intellect memory in several countries. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative free radical scavenging and anti-lipid peroxidative activities. The use of whole plant system for medicine, poor replenishment efforts and untrained plucking of the plant material leads this medicinal plant towards endangered. The major chemical entity shown to be responsible for neuropharmacological effects and the nootropic action or antiamaesic effect of _Bacopa monnieri_ is Bacopasaponins A, B, and C which are dammarane-type triterpenoid saponins. Since the supply is limited and faces constraints in meeting the increasing demand of these biochemical. Pharmacological properties of _Bacopa monnieri_ were studied extensively and the activities were attributed mainly due to the presence of characteristic saponins called as Bacosides. Bacoside have been indicated for memory-enhancing properties while Bacoside A assists in release of nitric oxide that allows the relaxation of the aorta and veins, to allow the blood to flow more freely through the body making this exceptional plant a nootropic drug. According to Central Drug Research Institute (CDRI) situated in Lucknow, the saponins, Bacosides A and B are responsible for repairing damaged neurons; furthermore _Bacopa monnieri_ has been studied clinically for its acute and chronic effects on cognitive function. Plants have been found to elicit the same response as the pathogen itself when challenged by compounds of pathogenic origin (elicitors). Biotic elicitors have biological origin derived from the pathogen or from the plant itself. Misal et al. (2020), resulted that the _Aspergillus niger_ filtrate significantly influenced the Bacoside production in Brahmi. After seven days elicitation, maximum enhancement in Bacoside (1.62% DW) was reported at lower concentrations of _Aspergillus niger_ filtrate (0.5 mL/L) over control. Higher concentrations of _A. niger_ filtrate also showed increasing but undulating results in Bacoside production over the control. Elicitation effects were might be due to the fungal cell wall works as a polysaccharide elicitor, which induces calcium concentration in the cell and activates various defense responsive pathways leading to the accumulation of phytoalexins and low molecular weight antimicrobial compounds (Cordell, 1997).

*Panax ginseng* is a perennial herb of the Araliaceae family, is well known traditional medicine plant and its roots have been used as a herbal remedy for various disorders (Akerele, 1992). The herb is of pharmacological importance because of the presence of major bioactive compound triterpene saponin called Ginsenoside (Rahimi et al., 2015). Ginsenoside Rg3 is not naturally produced in ginseng. Of particular note are the anti-tumor effects produced by ginsenosides. Ingredients of ginsenosides, such as Rb1, Rg1, Rg3, Rh2, PPT, and compound-K, have shown pharmacological effects through a variety of mechanisms (Li et al., 2008; Liu et al., 2000). In regards to Rh2 production, which has been shown to produce anticancer effects, β-glycosidase purified from _Aspergillus niger_ was shown to effectively produce ginsenoside F2 at a good yield (F2: 305 mg/g) (Youl et al., 2012). This process fully converted the compounds to F2 and prevented there action from proceeding further. Ginsenosides F1 and F2 produced by β-glycosidase with high yield and the production of Rh2 from F2 are more favorable because the pathway from F2 to Rh2 was predominant as opposed to the pathway creating compound-K. (Yan et al., 2008; Yuet al., 2007).

_Helicteres isora_ L. (Indian screw tree, a plant with traditional medicinal usages) has been reported as a cleaner source of diosgenin, where the compound is not admixed with other steroidal sapogenins (Barik et al., 1998; Deshpande and Bhalsing, 2014; Kumar et al., 2014). Exploration of this plant for diosgenin production is therefore advantageous. One more advantage of choosing this plant is that it is abundantly found in almost all parts of the country in forests as undergrowth, especially as secondary growth. This makes it a natural choice for exploration as a source of diosgenin. However, the diosgenin content is low in _H. isora_ as compared to other traditional plant sources of diosgenin (Barik et al., 1998), which needs to be enhanced before commercial exploration of this plant as an alternative source
of diosgenin. As stated earlier, plant cell cultures have been established as potent alternative sources for the production of high-value secondary metabolites of industrial importance in a holistic (without causing destruction to the natural sources) and sustainable way (Rao and Ravishankar, 2002; Mulabagal and Tsay 2004; Hussain et al., 2012). Diosgenin, one of the most important plant secondary metabolites, is a steroidal sapogenin traditionally derived from the tubers of Dioscorea species (yams). It is a precursor of sex hormones (progestrone), corticosteroids (cortisone) and contraceptives as well as other important steroids (Zhang et al., 2009; Zhu et al., 2010; Wang et al., 2011; Selim and Al Jaouni, 2015; Sethi et al., 2018). It has also showed pharmacological activities such as anti-lipoperoxidative and anti-aging effects, cognitive impairment, hypoglycaemic effect, antifungal and antiviral activities (Jayachandran et al., 2009; Chiu et al., 2011; Wang et al., 2011; Patel et al., 2012; Hao et al., 2015; Sethi et al., 2018). In India, steroidal drug production is almost 100% based on diosgenin and diosgenin accounts for two-thirds of the total world consumption of steroids (Chaturvedi et al., 2007). Its annual global demand is 3000 tonnes; while in India, 150 tonnes of diosgenin are required per year, however, total production of diosgenin in India is only 30 tonnes annually and rest is met by imports (Dangi et al., 2014; Deshpande and Bhalsing, 2015). The traditional sources of diosgenin are under threat due to their over-exploitation for extracting diosgenin, consequently, some of the species with high diosgenin contentsuch as Dioscorea zingiberensis and D. deltoidea are fast depleting (Chaturvedi et al., 2007; Li et al., 2012). This necessitates new alternative diosgenin sources and to develop strategies for its maximum, cost-effective production. In an attempt to identify an alternative and potent source of diosgenin, Samrin et al. (2020), study and focused on exploration of suspension cultures of H. isora for optimal production of diosgenin via biotic elicitation. Fungal elicitors from cultures of Aspergillus niger (ATCC10578) and Saccharomyces cerevisiae (NCIM3050) were prepared using fresh biomass. The results indicated that elicitor prepared using A. niger was responsible for significant increment in biomass production at all the applied concentrations (1%, 1.5% and 2%). Highest DW was observed at 1.5% fungal elicitor-treatment with a twofold increase over control. The same treatment was found responsible for highest diosgenin production (1.42-fold higher over controls). Fungal elicitors are considered as surface structures and/or fungal cell-secretions, with fungal mycelia or degraded fungal mycelial-products, and fermentation broth which may also contain fungal secretions. The said fungal elicitorhence may contain sugars (polysaccharides, oligosaccharides), proteins (glycolipid proteins, glycoprotein, and peptides), fatty acids and other substances. Fungal elicitorsoften result in biomass and secondary metabolite enhancement, as well as improved enzymatic activities in plants (Chen et al., 2015).

**Ephedra alata** L. is an Egyptian natural plant species found mainly in Sinai desert and Eastern Mediterranean coastal region (Boulos, 2009). It is a pharmaceutically important plant, which belongs to the Ephedraceae family of gymnosperms and is known to have a number of medicinal properties. *Ephedra alata* shows antimicrobial, antioxidant, and hypoglycemic activities (Soltan and Zaki, 2009; Parsaeimehr et al., 2010; Chebouat et al., 2014; Al-Snafi, 2017). In general, plants in the genus *Ephedra* have been used in traditional medicine to treat allergy, bronchialasthma, chills, cold, cough, edema, fever, flu, nasalcongestion, and headache (Parsaeimehr et al., 2010). Phytochemical analysis of *E. alata* indicated the presence of tannins, cardiac glycosides, alkaloids, phenolics, reducing sugars, and flavonoids (Jadadat et al., 2015). Additionally, *Ephedra* species contain alkaloids such as ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, methylpseudoephedrine, ephedroxane, and ephedradine A-D. Phenolic compounds including chlorogenic acid, rutin, catechin, quercetin, and coumaric acid and various flavonoids have also been isolated from *E. alata*. The total amount of alkaloids isolated from *E. alata* aerial parts was 0.2–0.22% (Al-khateeb et al., 2014), and the amount of ephedrine and pseudoephedrine was 0.05–0.19% and >0.5%, respectively (Al-Snafi, 2017). Ephedrine is a naturally occurring alkald in different species of *Ephedra*. It is used as a drug and has structure and activity similar to those of adrenaline, which raises blood pressure, heart rate, and respiratory capacity (Limberger et al., 2013). Ephedrine produces several pharmacological effects such as cardiovascular effect (it increases the arterial pressure by peripheral vasoconstriction and cardiac stimulation), bronchodilatation, nasal decongestion, mydriasis, nocturnal enuresis, spinal anesthesia, appetite suppressant and weight loss, cytotoxic effects, and many other (Al-Snafi, 2017). Most of the ephedrine produced today for medicinal use is obtained by chemical synthesis, because the process of extracting and isolating from the plant is difficult and economically costly (Limberger et al., 2013). Therefore, it is necessary to find an effective, practical, and economically feasible method to provide a continuous, natural, and pure source of ephedrine in large quantities as an alternative to the synthetic one that causes negative side effects. Therefore, attention should be given to isolate natural compounds instead of producing synthetic ones (Khan et al., 2017). In the present study, the A. niger extract was found to be useful for the enhancement of ephedrine accumulation. It was observed that treatment with the lowest concentration of A. niger extract caused the highest elicitation; thus, elicitor concentration and the exposure time are very critical for the elicitation of ephedrine production. This result agrees with that reported by Taha et al. (2009), who found that 0.25% of *A. niger* extract resulted in the production and accumulation of the highest amount of total alkaloids, vinblastine, and vincristine in the callus culture of *Catharanthus roseus*. However, as reported in the present study, increasing the concentration of *A. niger* extract showed less ephedrine accumulation. This may be because the mycotoxins have hindered ephedrine production and its biosynthetic pathway was negatively affected. Similar results were reported by Mathur (2018), who founda considerable decrease in secondary metabolite production and growth of *Commiphora wightii*, *Zingiber officinale*, and *Daucus carota* cell suspension cultures when *A. niger* extract was used as an elicitor. In general, the accumulation of ephedrine was promising and varied largely according to the applied elicitors. Ephedrine accumulated in huge amounts as compared to that in the mother plant (7-fold) and the control treatment without elicitation after 24 days of exposure (6,427-fold). These results are very promising for the large-scale production of this valuable bioactive compound. The present investigation could be scaled up for the production of commercially feasible levels of ephedrine by using suspension cultures of *E. alata*.
Atropa belladonna L. (Solanaceae) is one of the most important medicinal plants and is a source of tropane alkaloids such as hyoscyamine and scopolamine. Medicinally, A. belladonna is used for the use of its alkaloids in the treatment of Parkinson's disease for its anti-inflammatory properties, for relief of bronchial asthma and motion sickness and its ability to counteract toxic agents. Belladonna extract is used as an antimuscarnic agent, which accounts for its use as a spasmylocic drug. Also, it is used as a concomitant therapy in the treatment of peptic ulcer and functional digestive disorders, including spastic, mucous, and pancreatitis. Taha, (2003) showed that The effect of different concentrations (0%, 5%, 10%, 15% and 20%) of 0.1P.C.V. of A. niger (0, 2.5, 5.0, 7.5 and 10 mg/mL of liquid cell cultures), which were added to the MS medium containing 1 mg/L of each of NAA and BA on cell number (105) and total alkaloid production from different types of cell cultures were investigated. Data shows that leaf explants of A. belladonna gave the optimum value for cell number (5.92 x105) with the blank elicitor treatment, as compared with elicitor treatments. The increase in elicitor levels reduced cell growth, but stimulated the accumulation of total alkaloids (calculated as scopoline %). Stem cell cultures showed a low cell number (1.25 x 105) as well as low total alkaloid production. A. niger at 10 %after 10 days from the duration of incubation (21 days) showed the highest value for tropine alkaloid accumulation, in comparison to the other concentrations The highest values for total tropine alkaloids were 0.048 %, 0.035 % and 0.018% for leaf, root and stem cell cultures, respectively. The obtained results are in agreement with those of Harkes et al. (1985), who indicated that anthraquinone content can be increased to 500 µg/g fresh weight (as compared with the control) by the addition of 0.5 mg/mL of A. niger as a biotic elicitor to the culture medium of Cinchona ledgeriana cell cultures. Most plant secondary products are produced in the stationary phase of cell growth. It may be concluded that cultivation of leaf explants of A. belladonna in liquid MS-medium containing 1mg/L of each of NAA + BA in the presence of 5 mg/mL of A. niger for 10 days was the most favourable condition for stimulating total tropane alkaloids production.

Oldenlandia umbellata L. is one of the important members of Rubiaceae known for its dyeing and medicinal properties. This plant is used in traditional medicine and Siddha for its styptic property (Seydel and Dornenbug, 2006). The leaf and root extracts were considered as good expectorants and used for treatment of asthma, bronchitis, and bronchial catarrh (Gupta et al., 2007). The decoction prepared from its leaves is used as a rinse to treat poisonous bites (Rekha et al., 2006), and also used as a febrifuge. A novel pH indicator dye was reported from this plant (Siva et al., 2009). Extract of the whole plant shows significant antitumor activity (Sethuramani et al., 2014). The major dyeing property depends on anthraquinone content of roots and used to impart red color to the textile materials (Siva, 2007; Siva et al. 2012). The multipurpose usage has made increased usage of this plant and a reliable protocol was developed for enhanced growth of the plant through tissue culture technique. Saranya and Velayutham, (2019) mentioned that the treated calli were further subcultured on solid media with respective fungal elicitors and obtained large amount of calli. Of the three fungal elicitors treated, large amount of green compact callus was obtained on medium treated with the extract of A. niger followed by T. viride. However, the callus treated with M. prayagensis showed an equal response for callus growth to that of control at all tested concentration. The maximum shoot regeneration response was obtained from 100 µg 1−1 A. niger elicitor treated calli. Maximum number of 78.8 shoots with shoot length of 11.4 cm was achieved on the callus treated with A. niger. The maximum root regeneration response was obtained from 100 µg 1−1 A. niger elicitor treated shoots followed by 50 µg 1−1 M. prayagensis and 25 µg 1−1 T. viride with an average number of 46.6, 28.6 and 30.8 respectively from root induction medium containing 6 µM IBA. The root regeneration frequency and number of roots were decreased whenthe shoots were treated with above optimal concentration.

Blumea lacera (Burm.f.) DC. is a medicinal plant with strong odour of terpentine and it belongs to Asteraceae family. In Ayurveda, Blumea lacera is described as anthelmintic, liver tonic, expectorant, thermogenic, anti-inflammatory, ophthalmic, digestive, antipyretic and memory enhancer (Warrier et al., 1996). The plant is astringent, diuretic and useful in catarrhal affections (Quisumbing, 1998). Essential oil has analgesic, hypothermic, tranquilizing and antimicrobial activity (Dixit and Verma, 1976; Bharnagar et al., 1977). Campstrol, triterpenoid and prenylated phenol glycosides are the main active constituents of B. lacera (Pal et al., 1972; Agarwal et al., 1995). The other important constituents are flavonoids (Rao et al., 1997), monoterpen glycoside (Ragasa et al., 2007). The essential oil of the plant include ß-caryophyllene, thymol hydroquinone dimethyl ether, caryophyllene oxide, α-humulene and E-β-farnesene (Laakso, 1989) and coniferal alcohol derivative (Bohlmann and Zdero, 1969). Vijay et al., (2016), resulted that Aspergillus niger treatment with 1.5 ml concentration for 4 days duration revealed 3.3 fold enhancement in flavonoid content (0.036 mg/g) as compared to control (0.011 mg/g). The results indicate that for flavonoid elicitation in Blumea lacera, Aspergillus niger is more responsive than Salicylic acid.

Conclusion

The present review reports the information about the use of Aspergillus nigeras biotic elicitorsin medicinal plants for the enhancement of their bioactive compounds by different in vitro culture techniques to meet the commercial demands of pharmaceuticals. It is found that medicinal plants are used across the globe to cure various diseases like, Parkinson's disease, motion sickness, hypertension, tumor, depression, constipation, malaria, asthma, jaundice, vitiligo, skin diseases such as psoriasis, mycosis fungoides, eczema, rheumatism, cancer and diabetes, etc. These plants have medicinal properties due to presence of a bioactive compound in them. The bioactive compound in the intact plant is less in quantity so to synthesize secondary metabolites in desired quantity. Aspergillus niger was used as biotic elicitorsin vitro using different cultures.
Table 1: Effect of *Aspergillus niger* as biotic elicitors on secondary metabolites production of medicinal plants *in vitro* culture.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Elicitors</th>
<th>Secondary Metabolites</th>
<th>Type of Culture</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruta graveolens</td>
<td><em>A. niger</em></td>
<td>furanocoumarins</td>
<td>callus culture</td>
<td>Talfan et al.</td>
</tr>
<tr>
<td>Psoralea corylifolia</td>
<td><em>A. niger</em></td>
<td>psoralen</td>
<td>cultured cells</td>
<td>Syed et al.</td>
</tr>
<tr>
<td>Calendula officinalis</td>
<td><em>A. niger</em></td>
<td>salicylic acid</td>
<td>callus</td>
<td>Ibrahim et al.</td>
</tr>
<tr>
<td>Andrographis paniculata</td>
<td><em>A. niger</em></td>
<td>andrographolide</td>
<td>in cell suspension culture</td>
<td>Moinuddin et al.</td>
</tr>
<tr>
<td>Datur metel</td>
<td><em>A. niger</em></td>
<td>alkaloids-hyoscyamine and scopolamine</td>
<td>cell and organ culture</td>
<td>L. Ajunghla et al.</td>
</tr>
<tr>
<td>Hypericum perforatum</td>
<td><em>A. niger</em></td>
<td>hypericin</td>
<td>cell suspension cultures</td>
<td>Xu et al.</td>
</tr>
<tr>
<td>Gymnema sylvestre</td>
<td><em>A. niger</em></td>
<td>gymnemic acid</td>
<td>Cell suspension cultures</td>
<td>Bhuwaneswari et al.</td>
</tr>
<tr>
<td>Glycyrrhiza. glabra</td>
<td><em>A. niger</em> &amp; <em>Rhizopus stolonifer</em></td>
<td>glycyrrhizin</td>
<td>transformed cell suspension cultures</td>
<td>Karwasara et al</td>
</tr>
<tr>
<td>Bacopa monnieri</td>
<td><em>A. niger</em></td>
<td>Bacoside</td>
<td>full culture</td>
<td>Misal et al.</td>
</tr>
<tr>
<td>Panax gingseng</td>
<td><em>A. niger</em></td>
<td>ginsenoside</td>
<td>purified from <em>A. niger</em></td>
<td>Youl et al.</td>
</tr>
<tr>
<td>Helicteres isora</td>
<td><em>A. niger</em></td>
<td>diosgenin</td>
<td>cultures</td>
<td>Samrin et al.</td>
</tr>
<tr>
<td>Ephedra alata</td>
<td><em>A. niger</em></td>
<td>Ephedrine</td>
<td>suspension cultures</td>
<td>Ghada et al.</td>
</tr>
<tr>
<td>Atropa belladonna</td>
<td><em>A. niger</em></td>
<td>alkaloid</td>
<td>cell culture</td>
<td>Taha</td>
</tr>
<tr>
<td>Oldenlandia umbellata</td>
<td><em>A. niger</em></td>
<td>anthraquinone</td>
<td>callus</td>
<td>Saranya et al.</td>
</tr>
<tr>
<td>Blumea lacera</td>
<td><em>A. niger</em></td>
<td>flavonoid</td>
<td>cell culture</td>
<td>Vijay et al.</td>
</tr>
</tbody>
</table>

References


acid products in callus culture of Calendula officinalis L. Plant J. of Physics, 1294.


