ENHANCING THE DEFENSIVE POWER OF PLANTS THROUGH THE APPLICATION OF SOME POTENTIAL ELICITORS, SILVER (AG) AND COPPER (CU) NANO PARTICLES: REGULATION OF SECONDARY METABOLISM

AFIFA ZAEEM1*, RABBIA MUDASSER2 AND MATIU LLAH KHAN2

1Institute of Plant Biology and Biotechnology, University of Münster, Schlossplatz 8, 48143 Münster, Germany
2Department of Biotechnology, Virtual University of Pakistan, Rawalpindi Campus, Pakistan
*Corresponding author's email: zaem.afifa@gmail.com

Abstract

Secondary metabolites are biologically active compounds synthesized by plants necessarily for defense. These secondary metabolites are very vital because of their tremendous use in the pharmaceutical industry. They have wide-ranging applications in drug development and medical science. However, the production rate of these compounds in plants is limited and increasing demand in medicine requires enhanced production of secondary metabolites. To compact this low potential, the secondary metabolite production is manipulated by In vitro practice of plant tissue culture. In these procedures, plant cells are grown in various biotic and abiotic stressors as elicitors. From them, the most recently used are nanoparticles (NPs), because of their interesting and unique nature. They are of different types depending on the nature of the composition element. This review article mainly focuses on silver (Ag) and copper (Cu) NPs as active plant elicitors in stimulating essential In vitro secondary metabolite production. Ag and Cu NPs are being extensively explored in recent researches. The review basically outlines and summarizes the major recent studies conducted on different plant species for industrial level metabolite productions by Ag and Cu NPs during the last years. The enhanced production of secondary metabolites via Ag and Cu NPs is not reported previously, thus this review will help in further understanding of the role of specific NPs in triggering vital secondary metabolites in plants.

Key words: Elicitation, Copper nanoparticles, Silver nanoparticles, Plant tissue culture.

Introduction

Secondary metabolites are the chemical complexes synthesized by plants to better adapt to the surrounding environment, but they have little essential role in maintenance of natural life processes. However, various attempts are made to predict their important role in plant growth. Many varieties of secondary metabolites are produced after primary metabolites (fat, nucleic acids, carbohydrates etc.). These secondary metabolites are important for defense system of plants to counteract environmental stresses (Akula & Ravishankar, 2011). Secondary metabolites are very beneficial for humans because of their use in medicine, drug production and flavoring. These metabolites have three main classes: phenolics, terpenoids and alkaloids (Kabra et al., 2014). Moreover, plants have applications in cardiovascular, central nervous, antidiabetic, antiparasitic, antimicrobial, immunological, antioxidative and many other pharmaceutical industries (Al-Snaﬁ, 2019). Many plants producing high valued secondary metabolites are at verge of extinction, and their productive ability is limited. In addition to this, chemical synthesis of secondary metabolites is not possible because of complex and specific stereochemistry of secondary metabolites (Namdeo, 2007). To combat this problem biotechnological interventions are the paramount solution. Plant tissue culture (PTC) is the best In vitro technique to overproduce the secondary metabolites. PTC uses explants from plants to grow under aseptic conditions on artificial media supplemented with different elicitors. In this way important plant secondary metabolites are overproduced compared to natural production rate for commercial purpose (Namdeo, 2007).

Elicitors

Plant elicitors are molecules which trigger different morphological and physiological responses for enhanced production of metabolites including the secondary ones. They are divided as biotic (bacteria, fungi or herbivores) and abiotic (ionic metals and inorganic chemicals) elicitors. It has been reported that treatment of plants with elicitors trigger defense mechanisms which result in defensive secondary metabolite production (Namdeo, 2007). Many reports are available on plant elicitation but very few reports are found highlighting the accurate metabolic mechanism behind elicitation. Various roughly sketched metabolic mechanisms are hypothesized like inhibition/activation of intracellular pathways, loss of integrity of cell membrane and changes in osmotic stress, etc. (Garcia-Brugger et al., 2006). Moreover, some research groups proved that there was a rapid change in activation of protein kinase and patterns of protein phosphorylation as a elicitation response (Neelofer et al., 2021). Other researchers reported the activation in the production of G-proteins and mitogen-activated kinases (Drouillard et al., 2000; Ofoe, 2021). Similarly, a research also helped in clarifying the role of reactive oxygen species (ROS) in cross-linking proline-rich proteins on cell wall for activating defense genes (Torres et al., 2006). So, elicitors are those compounds which trigger various pathways through the production of different enzymes.

Nanoparticles as elicitors: Nanoparticles (NPs) have both toxic and eliciting effects on plants as far their nature is concerned (Ma et al., 2010). There are many methods of preparing NPs. In laboratory, they are synthesized both chemically and biologically. Biological synthesis, also known as green synthesis, is more appreciated because of eco-friendly nature (Sengani et al., 2017). Moreover, NPs can be used as potential elicitors in plants when employed at
specific optimized concentrations (Javed et al., 2018; Zahir et al., 2019). The eliciting effect of NPs on plants is because of their unique physiochemical nature which directs the metabolic pathways to operate in a more conductive way (Abbasi et al., 2019; Fazal et al., 2016). The elicitation effect on plants varies as it is dependent on the NP’s concentration and the type of plant species (Khan et al., 2016; Nadeem et al., 2018; Thuesombat et al., 2014). Generally, the presence of NPs in growth medium creates oxidative stress by producing reactive oxidation species (ROS) as shown in (Fig. 1) (Wilson et al., 2002). Because of the nanometer size of NPs, they are easily able to pass through cell wall and plasma membrane to target different metabolic processes at higher rates (Nair et al., 2010).

ROS are normally synthesized in plants as a result of one-electron reductions of molecular oxygen. Mainly, \( \text{H}_2\text{O}_2 \) is produced which is mainly involved in developmental processes such as the formation of lignins. Extensive production of ROS is observed in wounded cells accompanied with oxidative burst (Olson & Varner, 1993). Regulation of the ROS signals and multiple redox, require a high-grade coordination between signaling and metabolic pathways in various cellular compartments of plant cells (Suzuki et al., 2012).

Plant cells are able to neutralize the ROS-induced stress by actively producing some special compounds like superoxide dismutase, peroxidase, phenolics, flavonoids etc. (Fig. 2) (Valko et al., 2007). These metabolites are commercially very important and applicable in the pharmaceutical industry in curing different types of diseases (Rasool et al., 2009). In this review, the role of silver (Ag) and copper (Cu) NPs is discussed in plant secondary metabolite elicitation because of their emerging applications in industry.

**Silver nanoparticles and elicitation:** Ag NPs are among the most commonly used NPs in industry. On worldwide basis almost 320 tons of Ag NPs are produced in one year (Nowack et al., 2011). Ag NPs can have eliciting effects on plants as far secondary metabolite production is under consideration (Rezvani et al., 2012). In the last few years, Ag NPs have been used to increase the growth of plants, activate production of reactive oxygen species (ROS), enhance rooting, and increase accumulation of proteins of cell cycle, carbohydrate digestion and chloroplast production. In the process of cell cycle, the proteins called cyclins are central controllers that activate the action of cyclin dependent protein kinases (Kitsios & Doonan, 2011). In addition to this it has been reported Arabidopsis in that the production of ROS is linked with cell cycle genes of meristematic cells of roots (Tsukagoshi, 2012). Conclusively, it is said that the root growth proliferation may be linked with ROS formation resulting in the excessive elongation of roots (Syu et al., 2014). Moreover, silver nanoparticles induce jacalin related lectin expression which is involved in the release of nitrile. This nitrile is a future precursor in the synthetic pathway of auxin production (Vannini et al., 2013). Due to this, Ag NPs are known for interfering with different signaling pathways which ultimately enhance the release of medically vital secondary metabolites as represented in (Table 1).
### Table 1. Elicitation of Silver (Ag) Nanoparticles (NPs) on the production of Secondary Metabolites in Plant Tissue Culture (PTC).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant species</th>
<th>Elicited Secondary Metabolites</th>
<th>Type of cultures</th>
<th>Reference (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Datura metel</em></td>
<td>Atropine</td>
<td>Hairy root cultures</td>
<td>(Shakeran et al., 2015)</td>
</tr>
<tr>
<td>2.</td>
<td><em>Prunella vulgaris</em></td>
<td>Phenolics, flavonoids</td>
<td>Callus cultures</td>
<td>(Fazal et al., 2016)</td>
</tr>
<tr>
<td>3.</td>
<td><em>Capsicum frutescens</em></td>
<td>Capsaicin</td>
<td>Cell suspension</td>
<td>(Bhat et al., 2016)</td>
</tr>
<tr>
<td>4.</td>
<td><em>Artemisia annua</em></td>
<td>Artemisinin</td>
<td>Hairy root cultures</td>
<td>(Zhang et al., 2013)</td>
</tr>
<tr>
<td>5.</td>
<td><em>Corylus avellana</em></td>
<td>Taxol</td>
<td>Cell suspension culture</td>
<td>(Jamshidi et al., 2016)</td>
</tr>
<tr>
<td>6.</td>
<td><em>Arabidopsis</em></td>
<td>Anthocyanins</td>
<td>Seedling cultures</td>
<td>(Sya et al., 2014)</td>
</tr>
<tr>
<td>7.</td>
<td><em>Vanilla planifolia</em></td>
<td>Phenolics</td>
<td>Regenerated shoots</td>
<td>(Spinoso et al., 2017)</td>
</tr>
<tr>
<td>8.</td>
<td><em>Aloe vera</em></td>
<td>Aloin</td>
<td>Cell suspension</td>
<td>(Raei et al., 2014)</td>
</tr>
<tr>
<td>10.</td>
<td><em>Achillea millefolium</em></td>
<td>Phenolics, flavonoids, alkaloids, essential oils</td>
<td>Cell culture</td>
<td>(Ghanati et al., 2014)</td>
</tr>
<tr>
<td>11.</td>
<td><em>Momordica charantia</em></td>
<td>Phenolics, flavonoids</td>
<td>Cell suspension cultures</td>
<td>(Chung et al., 2018b)</td>
</tr>
<tr>
<td>12.</td>
<td><em>Cucumis anguria</em></td>
<td>Phenolic compounds (flavanols, hydroxycinnamic, hydroxybenzoic acids)</td>
<td>Hairy root cultures</td>
<td>(Chung et al., 2018a)</td>
</tr>
<tr>
<td>14.</td>
<td><em>Linum usitatissimum</em></td>
<td>Lignans, neolignans, phenolics, flavonoids</td>
<td>Cell suspension cultures</td>
<td>(Zahir et al., 2019)</td>
</tr>
<tr>
<td>15.</td>
<td><em>Echinacea purpurea</em></td>
<td>Cichoric acid</td>
<td>Cell suspension cultures</td>
<td>(Ramezannezhad et al., 2019)</td>
</tr>
<tr>
<td>16.</td>
<td><em>Lavandula angustifolia</em></td>
<td>Phenolics, flavonoids</td>
<td>Seedling cultures</td>
<td>(Jadczak et al., 2019)</td>
</tr>
<tr>
<td>17.</td>
<td><em>Trigonella foenum graecum</em></td>
<td>Phenolics, flavonoids</td>
<td>Seedling cultures</td>
<td>(Jasim et al., 2017)</td>
</tr>
</tbody>
</table>

### Table 2. Elicitation of Copper (Cu) Nanoparticles (NPs) on the production of Secondary Metabolites in Plant Tissue Culture (PTC).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant species</th>
<th>Elicited Secondary Metabolites</th>
<th>Type of cultures</th>
<th>Reference (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Stevia rebaudiana</em></td>
<td>Phenolics, flavonoids</td>
<td>Callus cultures</td>
<td>(Javed et al., 2018)</td>
</tr>
<tr>
<td>2.</td>
<td><em>Brassica rapa spp. pekinensis</em></td>
<td>Phenolics, flavonoids glucosinolates</td>
<td>Hairy root cultures</td>
<td>(Chung et al., 2018c)</td>
</tr>
<tr>
<td>3.</td>
<td><em>Verbena bipinnatifida</em></td>
<td>Phenolics</td>
<td>Cell suspension culture</td>
<td>(Genady et al., 2016)</td>
</tr>
<tr>
<td>4.</td>
<td><em>Glycyrrhiza glabra</em></td>
<td>Glycyrrhizin, phenolics, anthocyanins</td>
<td>Seedling cultures</td>
<td>(Okum et al., 2015)</td>
</tr>
<tr>
<td>5.</td>
<td><em>Trigonella foenum-graecum</em></td>
<td>Phenolics, flavonoids</td>
<td>Seed and callus cultures</td>
<td>(ul AIn et al., 2017)</td>
</tr>
<tr>
<td>6.</td>
<td><em>Brassica rapa var. rapa</em></td>
<td>Anthocyanin, proline, glucosinolates, phenolics, flavonoids</td>
<td>Seedling culture</td>
<td>(Chung et al., 2019a)</td>
</tr>
<tr>
<td>7.</td>
<td><em>Gymnema sylvestre</em></td>
<td>Gymnemic Acid, Phenolics,</td>
<td>Cell suspension cultures</td>
<td>(Chung et al., 2019b)</td>
</tr>
<tr>
<td>8.</td>
<td><em>Stevia rebaudiana</em></td>
<td>Phenolics, flavonoids</td>
<td>Submerged root cultures</td>
<td>(Orazal et al., 2018)</td>
</tr>
<tr>
<td>9.</td>
<td><em>Stevia rebaudiana</em></td>
<td>Phenolics, flavonoids, steviol</td>
<td>Shoot cultures</td>
<td>(Javed et al., 2017)</td>
</tr>
<tr>
<td>10.</td>
<td><em>Eruca sativa</em></td>
<td>Phenolics, flavonoids</td>
<td>Shoot cultures</td>
<td>(Zaka et al., 2016)</td>
</tr>
<tr>
<td>11.</td>
<td><em>Bacopa monnieri</em></td>
<td>Bacoside</td>
<td>Shoot culture</td>
<td>(Sharma et al., 2015)</td>
</tr>
<tr>
<td>12.</td>
<td><em>Digitalis lanata</em></td>
<td>Cardiac glycoside, flavonoids</td>
<td>Cell suspension cultures</td>
<td>(Bota &amp; Deliu, 2011)</td>
</tr>
<tr>
<td>13.</td>
<td><em>Artemisia absinthium</em></td>
<td>Phenolics, flavonoids</td>
<td>Seedling culture</td>
<td>(Hussain et al., 2017)</td>
</tr>
</tbody>
</table>
Examples of Ag NPs as elicitors in Plant tissue culture

1. **Vanilla planiforia** an orchid is a native plant of Southern Mexico (Bory et al., 2008; Gallage & Møller, 2015). It is used as a potential source of energy. It has wide ranging uses in the pharmaceutical and food industry (Bory et al., 2008; Gallage & Møller, 2015). Vanilla planiforia has some drawbacks such as, it is over exploited because of its tremendous need, it has comparatively less seed viability and reduced germination rate (Rodolphe et al., 2011). To combat this issue, the scientists used the nanotechnology to accumulate elevated levels of proteins, nucleic acids, chlorophyll and various hormones. Experiments revealed that concentration of Ag NPs activated the defense mechanism of V. planiforia. A study of PTC concluded that the treatment with Ag NPs (200 mg l\(^{-1}\)) enhanced the level of phenolics (150 mg GAE/g DW) and lipid peroxidation (950 nmol g/DW) (Spinoso-Castillo et al., 2017).

2. **Aloe vera**, a medicinal plant of the family Aloaceae is found in Africa (Mirza et al., 2008). In A. Vera, a clear white gel is present which has significant biological properties such as, antibacterial, antifungal, cosmetic and therapeutic. This plant is more famous for inhibiting the AIDS virus (Ramachandra & Rao, 2008). The most important secondary metabolites, anthraquinone and aloin, are found in A. vera which makes them a potential plant of anticancer and anti-inflammatory properties (Ramachandra & Rao, 2008). A successful experiment was done on A. vera, where the aloin production was elicited by the use of different elicitors (nano TiO\(_2\), sucrose, NH\(_4\)NO\(_3\)) including Ag NPs (0.625 mg/ml). This experiment proved that the In vitro cultures could be a best way to optimize aloin production (43.7%) to meet the industrial demands (Raei et al., 2014).

3. **Achillea millefolium** is another medicinal herbaceous plant of the Asteraceae family. It has more than 100 different compounds of antimicrobial and antifungal activity. This plant contains important secondary metabolites including sesquiterpenes, essential oils, phenolics, flavonoid compounds, apigenin, luteolin, camphor and coumarin (Benedek et al., 2007; Potrich et al., 2010). Because of the presence of these important complexes scientists decided to apply Ag NPs on A. millefolium to increase their normal production rate. It has been observed that methyl jasmonate (MeJA) and Ag NPs significantly increased the flavonoid content but decreased the anthocyanin content in the plant. One interesting result of elicitation of A. millefolium with Ag NPs (0.4, 0.8, and 1.2 mM) and MeJA (0, 50, and 100 µM) was the induction of Allo-oicimene (>2.88%), a compound which was resistant to fungal attacks. Along this the different concentrations of Ag NPs triggered terpenoid pathways of plants toward biosynthesis of certain anti-inflammatory compounds like trans-caryophyllene which can selectively bind to the cannabinoid receptor type-2 and increase cannabimimetic properties (Ghanati et al., 2014).

4. **Prunella vulgaris** is a non-aromatic herbaceous plant of the Lamiaceae family (Chen et al., 2013). This plant is well known for its innate wound healing abilities (Chen et al., 2013). It proved that P. vulgaris also have anti-cancerous, antiseptic, anti-rheumatic and anti-viral activities (Golembiowska & Tsurkan, 2013; Huang et al., 2013; Rasool et al., 2009). Because of the increasing therapeutic demand, the rapid and optimized growth of this plant is very necessary. For this purpose, the cell suspension and callus cultures are used as the most reliable method (Ali & Abbasi, 2013). A study of PTC concluded that the treatment with Ag NPs (30 µg l\(^{-1}\)) and Au NPs (30 µg l\(^{-1}\)) enhanced the production of phenolics (9.57 mg/g DW) and flavonoids (6.71 mg/g DW) (Fazal et al., 2016).

5. **Cucumis anguria** is an important nutritious plant with various potential phytochemicals which have many medicinal applications in treating different diseases (Yoon et al., 2015). Previously, this plant was exploited using agrobacterium mediated gene transformation to induce hairy roots for higher production of bioactive compounds. The biomass, hydroxybenzoic acids, hydroxyccinnamic, flavonols, phenolics (33.25 mg/g) and flavonoid were augmented compared to control when Ag NPs (1.0 mg/L) were used as abiotic elicitor. So, PTC could be a one good way to overexploit this plant for meeting the industrial needs. In this study, it could be predicted that Ag NPs are oxidized on roots as Ag\(^+\) ions which enter directly into the root tissues to target the metabolic pathways for elicitation (Chung et al., 2018a).

6. **Momordica charantia** known as bitter gourd belongs to the family of Cucurbitaceae (Grover & Yadav, 2004). It has wide medicinal applications such as anti-inflammatory, antidiabetic, antiseptic and insecticidal properties (Grover & Yadav, 2004). It is a vital source of proteins, minerals (such as; iron, calcium), carbohydrates, vitamin C and fibers. Various secondary metabolites such as phenolics, charantin, momordin, momorcharin, triterpenesvicine, oleoanic acids, saponin, triterpene glycosides and alkaloids are characteristic productions of this plant (Grover & Yadav, 2004). PTC techniques are employed to enhance the secondary metabolite productions of these important compounds. One study was done to see the impact of Ag NPs (5 mg/L) on M. charantia. This study elicited hydroxybenzoic (1713.40 µg/g), flavonols (1822.37 µg/g) and hydroxyccinnamic acids (1080.10 µg/g) in suspension cultures of M. charantia (Chung et al., 2018b).
7. *Linum usitatissimum* belonging to family Linaceae is an important historical medicinal plant. It is commercially famous for linen fiber as potential phytomedicine (Muir & Westcott, 2003). This plant is widely employed in pharmaceutical industry because of significant polyphenol productions (lignans and neolignans) (Wallis, 1998). Both of these secondary metabolites are demanded on commercial scale. A recent research was conducted on suspension cultures of *L. usitatissimum* using Ag NPs (30 µg/L) as abiotic elicitor. The results of high-performance liquid chromatography presented substantial increase in lignans (such as; secoisolariciresinol diglucoside, 252.75 mg/L; larciresinol diglucoside, 70.70 mg/L) and neolignans (such as; dehydrodiconiferyl alcohol glucoside, 248.20 mg/L; guaiacylglycerol-β-coniferyl alcohol ether glucoside, 34.76 mg/L) in optimized suspension cultures of *L. usitatissimum* (Zahir et al., 2019).

8. *Echinacea purpurea* is a very prominent medicinal plant commonly known and demanded for citric acid production. It is vital in producing many secondary metabolites such as, cafec acid, luteolin, quercetin, echinacin, echinascoside, germacrene D, borneol and alkalamides (Gupta et al., 2012). To enhance the natural synthesis of these secondary metabolites, suspension cultures of *E. purpurea* were employed on Ag NPs (2 mg/L). This research study optimised a protocol for increased cichoric acid (9.54 mg/g DW) production to meet the industrial demands (Ramezannezhad et al., 2019).

Copper nanoparticles and elicitation: Copper (Cu) is a very important micronutrient for plant development (Huang et al., 2017; Javed et al., 2017). It plays an important part in various biological and physiological processes including the process of cellular transportation, hormone signaling, mitochondrial respiration, antioxidative potential and protein signaling (Huang et al., 2017; Javed et al., 2017). However, the same Cu can be dangerous to plants if it is present in excessive amount resulting in retarded growth, necrosis and intolerable antioxidative response. If Cu is supplied to plants in optimal concentration, it can result in the elicitation of amino acids and important phenolic compounds (Martins et al., 2016). NPs have increased surface to volume ratio and their size differs from 1 nm to 100 nm. Moreover, engineered NPs are designed to enhance properties. NPs are employed on an industrial scale because of their unique physical, chemical and biological nature. Their productivity is increasing tremendously because of industrial and household applications (Javed et al., 2017; Martins et al., 2016). Therefore, because of these reasons Cu NPs have been employed to elic the productivity of essential phytochemicals through callus, hairy root, and seedling and suspension cultures (Table 2). Mainly, in the cell there is clear transition of Cu$^{2+}$ to Cu$^{+}$ in redox cycle. This process activates the interaction of superoxide ion (O$^{2-}$) and hydrogen peroxide (H$_2$O$_2$) to produce hydroxyl radicals. These products cause oxidative stress resulting in the altering of metabolic pathways (Halliwell & Gutteridge, 1984). That is why, Cu NPs are used to augment the production of medical secondary metabolites (Table 2).

Examples of Cu NPs as elicitors in plant tissue culture (PTC)

1. *Stevia rebaudiana* is an herb and natural non calorie sweetener plant belonging to the family *Asteraceae* having economic and ecological importance. It is also known as “candy leaf” (Shivanna et al., 2013). Diabetes mellitus, hypertension and obesity are treated with the use of *S. rebaudiana* (Yucesan et al., 2016). Large-scale *In vitro* production of *S. rebaudiana* is used to cultivate enough plants within a short period of time to reduce the effect of factors that result in poor germination and reduced efficiency of stem cuttings (Rafiq et al., 2007). Various abiotic elicitors are used to elicit secondary metabolites in *S. rebaudiana*, such as different concentrations of Zn and Cu NPs in MS medium are employed as elicitors (Gupta et al., 2015; Hendawey et al., 2014; Khalil et al., 2015). It has been reported that the production of flavonoids (TFC; 2.85 µg/mg of DW), total antioxidant capacity (TAC; 9.69 µg/mg of DW) and 1,1-diphenyl-2-picrylhydrazyl (DPPH; 85.91%) free radical scavenging activity enhanced when callus cultures of *S. rebaudiana* were exposed to 100 mg/L of Cu NPs nanoparticles (Javed et al., 2018). Similar, a previous study (Ghazal et al., 2018) predicted that the adventitious roots of *S. rebaudiana* have the potential to produce important biological contents. Using elicitation method, that biomass accumulation (1.447 g/flask), phenolics (16.17 mg/g DW), flavonoids (4.20 mg/g DW) and DPPH activity (79%) were enhanced in cultures. So, it could be said the Cu NPs (30 µg–1) greatly enhanced the biomass accumulation as well as secondary metabolite production in *In vitro* adventitious roots of *S. rebaudiana* (Ghazal et al., 2018).

2. Chinese cabbage is the common name of *Brassica rapa* that belongs to the family *Brassicaceae*, one of the well-known family for medicinal plants (Kim et al., 2010). In Asian countries, it is consumed as green leafy vegetable because of high levels of glucosinolates, carotenoids, phenolics and tocopherols. Moreover, it has many important health-promoting activities (Kim et al., 2010). Glucosinolates and phenolic compounds are the vital secondary metabolites used in treating heart disorders, inflammation, neurodegeneration, obesity, diabetes and gastrointestinal infections by acting as an antioxidant, anticancer and healing agent (Chung et al., 2016). Previously, elicitation by copper has increased amino acids, phenolics and glucosinolates in plants of *B. rapa* (Jahangir et al., 2008). Moreover, it has also been reported that the use of Cu NPs (100 mg/L) as abiotic elicitors causes enrichment of flavonols (1293.09 µg/g), hydroxycinnamic acid (922.9 µg/g) and hydroxybenzoic acid (889.23 µg/g) in Chinese cabbage root cultures (Chung et al., 2018c). The effect of Cu NPs on the physiology and metabolite changes in *B. rapa* was also evaluated when their seedlings were treated with different concentrations of Cu NPs. The results stated that the higher concentrations of Cu NPs (500 mg/L) stimulated the phenolic (250 mg/g) and...
flavonoid (5.4 mg/g) productions in *B. rapa* seedlings (Chung et al., 2019a).

3. *Verbenae* *bipinnatifida* is a small herbaceous plant with small purple flowers that belongs to the family Verbenaceae (Genady et al., 2016). In Egypt, it is cultivated as an ornamental plant (Genady et al., 2016). It is a potential antioxidant plant which helps in preventing sleep and hepatotoxicity (Genady et al., 2016). It is reported that effect of different concentrations of Cu NPs (5 μM L-1) on *In vitro* suspension culture of *V. bipinnatifida* Nutt. increase the phenolic production (42.7 mg GAE/g DW) (Genady et al., 2016). Various medicinally important secondary metabolites are found in *V. bipinnatifida* Nutt., such as methoxyflavone glycosides, iridoids and phenylethanoid glycosides (El-Hela et al., 2000; Michael et al., 2001).

4. *Glycyrrhiza glabra* Licorice of the family Fabaceae, and is commonly found in south-east Europe and south-west Asia, including the regions of Iran (Irani et al., 2010). It is a moist soothing herb and sweet in taste. It has anti-inflammatory and expectorant properties which reduce cough, treat ulcer, detoxify and protect the liver (Irani et al., 2010). Hairy roots of *G. glabra* produce many secondary metabolites, including phenolics and glycyrrhizin. These compounds have various applications in the pharmaceutical sciences and food industry (Shibata, 2000). The effects of Zn and Cu NPs (1 and 10 μM) were studied in *G. glabra* seedlings in synthesizing secondary metabolites. It was seen that the NPs of Cu and Zn increased the contents of glycyrrhizin (28 mg g⁻¹ FW), reducing sugar content (0.116 mmol g⁻¹ FW) and anthocyanins (0.0017 mmol g⁻¹ FW) in *G. glabra* seedlings (Oloumi et al., 2015).

5. *Trigonella foenum-graecum* belongs to the family Fabaceae and is commonly known as fenugreek (methi). It is a self-pollinating leguminous crop cultivated in central Asia, Europe, Africa, America, India, and some parts of Australia (Pashricha et al., 2014). It is a potential antioxidant because certain phytochemicals, such as vitamins, flavonoid compounds, terpenoids, carotenoids, curcumin, lignins, saponins etc, are commonly present in this plant. It is eaten as a vegetable in many parts of the world. Moreover, it is also important in treating many medicinal illnesses. It has antibacterial activity and prevents diseases such as atherosclerosis, rheumatism, diabetes mellitus and other autoimmune diseases (Akbari et al., 2012; Al-Asadi & Naeem, 2014). The effects of Cu NPs on morphology, calllogenesis, seed germination as well as biochemical screening of *T. foenum-graecum* plant was reported previously which stated that Cu NPs (5 mg/L) enhanced the production of phenolics (4 μg GAE/mg DW) and flavonoids (3 μg QE/mg DW) (ul Ain et al., 2017). These results provide an understanding of Cu NPs as abiotic elicitors for the production of important metabolites in bioreactors (Ul Ain et al., 2017).

6. *Gymnema sylvestre* is a valuable plant of the family Asclepiadaceae. It is a potential source of bioactive compounds such as gymnemic acid and phenolics. These plants are used in the treatment of many diseases and they also have anticancer activities (Pothuraju et al., 2014). This plant is commonly applied in many pharmaceutical and food industries. *In vitro* plant tissue or suspension culture is used to enhance the metabolite production in *G. sylvestre* (Narayani et al., 2017). It has been reported that Cu NPs (3 mg/L) were used as abiotic elicitors to improve biomass and bioactive compound production (gymnemic acid; 89.25 mg/g DW and phenolic compounds; 245.10 mg/g DW) in cell suspension culture of *G. sylvestre*. These compounds have antioxidant, antibacterial, anti-diabetic, antifungal, anti-inflammatory and anticancer activities, which are also elicited by the supplementation of Cu NPs in media (Chung et al., 2019b).

**Conclusion**

Conclusively, silver and copper nanoparticles are potential abiotic elicitors for medicinally important plant species. Plant tissue culture techniques could be used together with NPs to meet commercial level demands. Experiments on various plant species are already done. In this review, we have listed various elicitation-based studies done on different plant species. Results of these studies conclude that silver and copper nanoparticles could be used as a potential abiotic elicitor for essential secondary metabolite productions. In future, these studies could be scaled up for bioreactor level secondary metabolite productions.

**References**


(Received for publication 17 August 2020)