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

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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 (Kabera *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 (Droillard *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, H_2O_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.

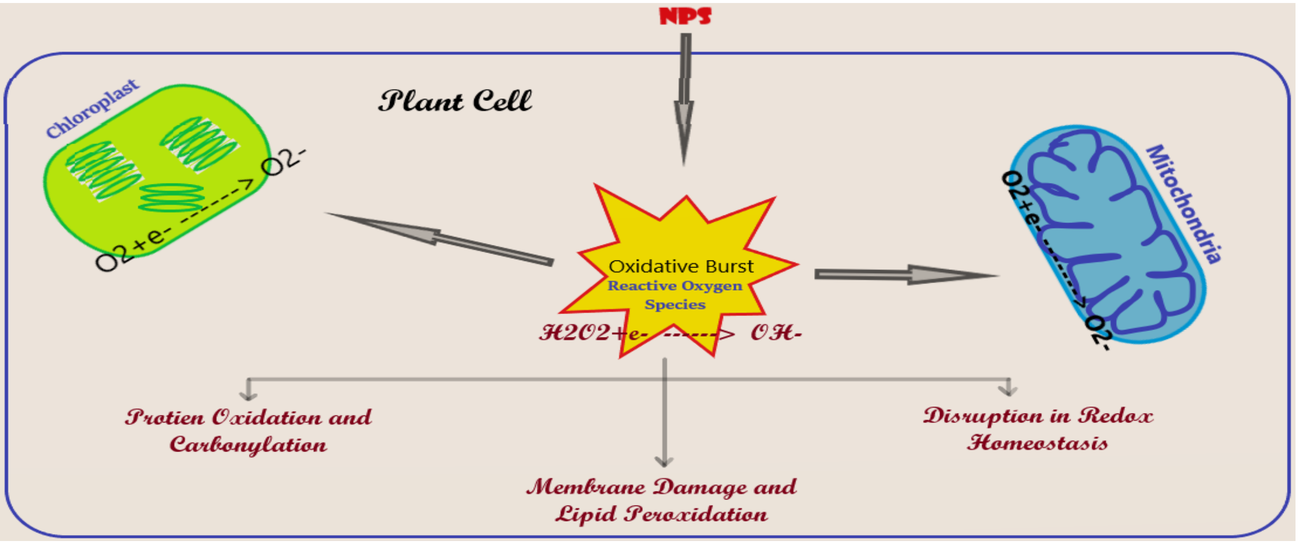

Fig. 1. Uptake of nanoparticles inside cell results in oxidative burst which stimulate cascade of various changes.

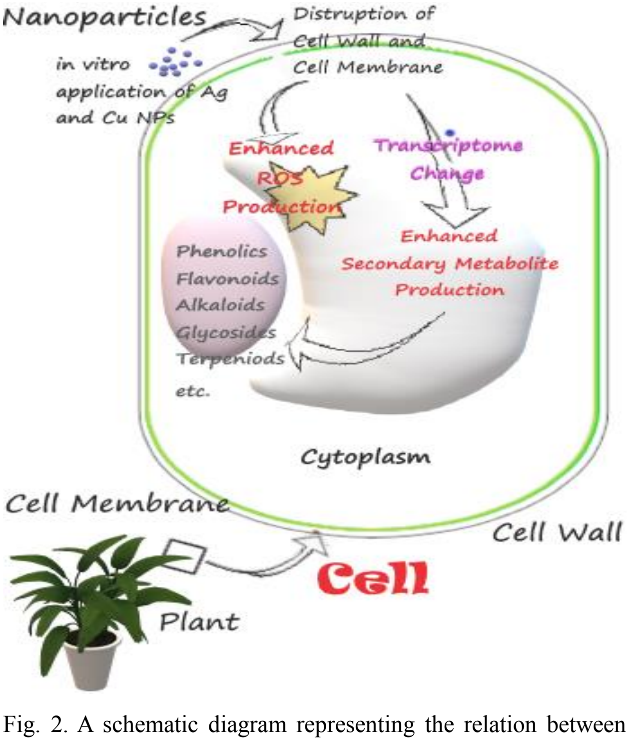

Fig. 2. A schematic diagram representing the relation between nanoparticles (NPs) and secondary metabolite production.

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).

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

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

S. No.	Plant species	Elicited Secondary Metabolites	Type of cultures	Reference (s)
1.	*Stevia rebaudiana*	Phenolics, flavonoids	Callus cultures	(Javed *et al*., 2018)
2.	*Brassica rapa* spp. *pekinensis*	Phenolics, flavonoids glucosinolates	Hairy root cultures	(Chung *et al*., 2018c)
3.	*Verbena bipinnatifida*	Phenolics	Cell suspension culture	(Genady *et al*., 2016)
4.	*Glycyrrhiza glabra*	Glycyrrhizin, phenolics, anthocyanins	Seedling cultures	(Oloumi *et al*., 2015)
5.	*Trigonella. foenum-graecum*	Phenolics, flavonoids	Seed and callus cultures	(ul Ain *et al*., 2017)
6.	*Brassica rapa* var. *rapa*	Anthocyanin, proline, glucosinolates, phenolics, flavonoids	Seedling culture	(Chung *et al*., 2019a)
7.	*Gymnema sylvestre*	Gymnemic Acid, Phenolics,	Cell suspension cultures	(Chung *et al*., 2019b)
8.	*Stevia rebaudiana*	Phenolics, flavonoids	Submerged root cultures	(Ghazal *et al*., 2018)
9.	*Stevia rebaudiana*	phenolics, flavonoids, steviol	Shoot cultures	(Javed *et al*., 2017)
10.	*Eruca sativa*	Phenolics, flavonoids	Shoot cultures	(Zaka *et al*., 2016)
11.	*Bacopa monnieri*	Bacoside	Shoot culture	(Sharma *et al*., 2015)
12.	*Digitalis lanata*	Cardiac glycoside, flavonoids	Cell suspension cultures	(Bota & Deliu, 2011)
13.	*Artemisia absinthium*	Phenolics, flavonoids	Seedling culture	(Hussain *et al*., 2017)

Examples of Ag NPs as elicitors in Plant tissue culture

1. *Vanilla palaniforia* 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 pharmaceutics 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 Tio2, sucrose, NH_4NO_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-ocimene (>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 (Golembiovska & 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, hydroxycinnamic, flavanols, 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, oleanolic 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 hydroxycinnamic 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 eilciter. The results of high-performance liquid chromatography presented substantial increase in lignans (sych as; secoisolariciresinol diglucoside, 252.75 mg/L; lariciresinol 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, cafeic acid, luteolin, quercetin, echinacin, echinacoside, germacrene D, borneol and alkamides (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 protocoal 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 elicit 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_2O_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 of *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* (Yücesan *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 *d*ifferent 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). Similarlay, 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 l−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. *Verbena 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 promoting 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} DW), reducing sugar content (0.116 mmol g^{-1} FW) and anthocyanins (0.0017 nmol 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 (Pasricha *et al*., 2014). It is a potential antioxidant because certain phytochemicals, such as vitamins, flavonoid compounds, terpenoids, carotenoids, curcumins, lignans, 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, callogenesis, 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, antidiabetic, 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.

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