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Application of Silver based nanoparticle for seed priming of medicinal and herbal plants

Abstract

Medicinal plants have been essential to the global evolution of human cultures. Many abiotic and biotic processes affect the growth and development of plants. Seedling establishment and germination are important phases in the plant life cycle and efficient seed germination encourages successful establishment and deep root system of plants. Through the production of various physiological and metabolic changes, priming has been shown to have a noteworthy impact. This review summarises the studies of various medicinal and herbal plants by using nano-priming of seeds.

A literature search was conducted with the help of electronic databases like Google Scholar, PubMed, Scopus, Sci Finder, and ONOS. The search was conducted using the keywords Medicinal Plants, Seed priming, nanotechnology, silver nanoparticle, germination.

The studies have shown that the use of silver nanoparticles (AgNPs) as nanopriming agent for enhancing seed germination is a step towards sustainable agriculture. This process is suitable for small seed plants.

This study gathered information on the seed germination using silver nanoparticles on various medicinal plants.

Introduction

Medicinal and herbal plants represent the most ancient form of medication, used for thousands of years in traditional medicine in many countries around the world (Marrelli, 2021). The empirical knowledge about their beneficial effects was transmitted over the centuries within human communities (Khan, 2014). Medicinal plants not only serve as complements or substitute for modern medical treatments, which are often inadequately available but also enhance the health and security of local people (Agidew, 2022). The medicinal importance of some plants is mainly due to the presence of active ingredients such as terpenes, flavonoids, coumarins, carotenoids, essential oils, and amino acids (Khalaki et al., 2021; Ahmad et al., 2018).

The cultivation of medicinal and herbal plants is currently confronted with several difficulties, such as changeable climatic circumstances that can negatively impact seedling development, seed germination, and crop output, such as drought, salt, heavy metal buildup in soil, and climate change (Shelar et al., 2023; Yadav et al., 2020; Imran et al., 2021; He et al., 2018). The uncertainty of the global climate with erratic rainfall patterns is the major cause of the frequent onset of drought stress around the world (Lobell et al., 2011). Over 6% of the world's land is spoiled by salinity which negatively affects crop survival by minimizing the growth and yield of staple food up to 70% (FAO, 2016; Schilling, 2016).

Drought-induced economic losses were estimated to be about 29 billion dollars during 2005 to 2015, and it is predicted to become more persistent and extensive in the coming decades (Schilling, 2016; Trenberth et al., 2014). By 2050, about 50% of arable lands are expected to be under drought stress (Marthandan et al., 2020). Drought can occur in all growth stages, but the first and foremost effect is on seed germination (Kasim et al., 2013; Kaya et al., 2006) where water entrance into the seed decreases due to hydraulic reduction; and thereby, all the physiological and metabolic germination processes are affected (Fahad et al., 2017). Impaired germination and establishment under drought stress have been studied in several crops viz., peas (Bareke, 2018), barnyard millet (Okcu et al., 2005), rice (Wu et al., 2019), and sunflower (Islam et al., 2018).

Efficient seed germination is important for increasing the production of forage and medicinal plants in rangeland fields (Humera Razzaq et al., 2017). The main role of the seed is to protect the embryo and sense environmental information to couple germination with seasons compatible with the completion of the plant life cycle (Azimi et al., 2014). The germination process is completed in three stages. In the stage 1, seed germinates upon absorption of water in the cells of the seed as they hydrate, and become rigid or turgid. In stage 2, the areas of growth, cell division, and differentiation at the root and shoot meristems or tips are where the sugars and amino acids are focused. In stage 3, the seed coat bursts due to the swelling in cells. The primary root or radicle emerges downward, and the stem grows upward (Carrera-Castaño et al., 2020).

There is an urgent need to develop a sustainable technology that can contribute to the green revolution to address these growing concerns and to restore the damage caused to the ecosystem (Mahra et al., 2024). Seed priming is an innovative technique to improve seed germination rates, seedling growth, and crop yield, as well as provide resistance to various plant stresses like drought, salinity, and heavy metal toxicity in a sustainable way (Shelar et al., 2021). Priming is the process of pre-treating seeds before planting those plants using traditional methods such as pre-soaking and coating (Maroufi et al., 2011). Priming creates a physiological state in the seed that strengthens its growth capacity against biotic or abiotic stresses (Nile et al., 2022). However, many factors affect the performance of seed priming such as plant species, priming duration, temperature, priming media, and their concentration and storage conditions (Conrath, 2011; Rhaman et al., 2020).

Priming using nanoparticles (nano-priming) has been proven to be more promising than traditional priming approaches for achieving feasible agricultural yields (Rhaman et al., 2020). Nano-priming uses particles with a size of less than 100 nm (nanoparticles, NPs), and "priming" relates to the development of stress tolerance under moderate and recurring stress (Nile et al., 2022; Chandrasekaran et al., 2020). The literature search has shown that nanomaterials prepared with silver (Rajwade et al., 2020), gold (Prasad et al., 2017), copper (Usman et al., 2020), palladium (Kamle et al., 2020), selenium (Liu et al., 2021), zinc oxide (Pramanik et al., 2020), magnesium oxide (Jiang et al., 2020), titanium dioxide (Shang et al., 2019), and iron oxide (Pulizzi, 2019) have been proven to promote seed germination and improve crop yields (Figure 1).

Silver nanoparticles (AgNPs) are the most widely used nanoparticles with a variety of uses because of their unique properties (Yan and Chen 2019). The applications of AgNPs have received high focus and promotion in the medical and pharmaceutical fields (Khan et al., 2023). Among numerous monometallic NPs, biogenic AgNPs are frequently applied because of their characteristics such as electrical conductivity, optical polarization, and SER scattering (Abasi et al., 2022).The potential uses of Ag-NPs in catalysis (Kamat 2002), plasmonics (Maier et al. 2001), optoelectronics (Boncheva et al. 2002), biological sensors (Mirkin et al. 1996; Han et al. 2001), antimicrobial activities (Savithramma et al. 2011; Rai et al. 2009), DNA sequencing (Cao et al. 2001), climate change and contamination control

(Shan et al. 2009), clean water technology (Savage and Diallo 2005), energy generation (Z€ach et al. 2006), information storage (Carutherset al. 2007), and biomedical applications (Hullmann 2007) have all attracted a lot of interest in the formation of Ag-NPs. By showcasing its potential during the past ten years, the production of NPs has given us amazing advancements in the field of nanotechnology (Samberg et al. 2010).

Therefore, the current study's objectives are to investigate the potential utility of varying AgNPs priming concentrations on the morpho-physiological and biochemical characteristics of herbal and medicinal plants under salt stress, including growth, biomass, oxidative damage, and antioxidant system.

Basics of Seed Priming

Priming is the process of pre-treating seeds before planting those plants using traditional methods such as pre-soaking and coating (Torre-Roche et al., 2020; Waqas et al., 2019). Seed priming is controlled hydration of seeds to a level that allows pre-germinative metabolic activity to continue, but interrupts actual emergence of the radicle (Raj and Raj, 2019). Although seedlings raised from primed seeds have been reported to exhibit modifications in water contents, improved cell cycle regulation, management of oxidative stress and reserve food mobilization, the efficacy of seed priming highly depends on the plant species and the priming method (Raj and Raj, 2019; Johnson and Puthur, 2021). Several seed and agricultural firms have used the priming technology as a revolutionary strategy for achieving a consistent crop standby (Sivasubramaniam et al., 2011; Waqas et al., 2019; Raj and Raj, 2019).

Priming initiates cross-tolerance that assists improved germination and seedling establishment under harsh environmental conditions (Chen et al., 2012). This has been demonstrated in many crop plants, such as zucchini (*Cucurbita pepo* L.), onion (*Allium cepa* L.), pepper (*Capsicum annum* L.), tomato (*Lycopersicon esculentum* L.), zinnia (*Zinnia elegans* Jacq.) etc. (Anand et al., 2019; Szopinska and Polityvka, 2016; Silva et al., 2015; Zhao et al., 2018; Valivand et al., 2019). In mung bean plants, a faster seedling establishment resulting from priming may contribute to a total increase in yield up to 45% (Rashid et al., 2004). Increased seed vigour observed in primed seeds of Arabidopsis (Gallardo et al., 2001; Rajjou et al., 2006), alfalfa (*Medicago sativa* L.) (Yacoubi et al., 2013), wheat (Fercha et al., 2013; Fercha et al., 2014).

Proteome analyses of seed priming and germination have proven invaluable in identifying changes between primed and unprimed seeds in various plants (Wang et al., 2015). It is regulated by the interaction of the surrounding environmental conditions, the seeds' physiological state, and the germ (Khaeim et al., 2022; Vishal and Kumar, 2018). Temperature, light, pH, water availability, and soil moisture most affect seed germination among abiotic factors (Khaeim et al., 2022; Rizzardi et al., 2009). Figure 2 shows that plants produced from primed seeds often exhibit a faster growth than unprimed ones. The beneficial impact of priming on plant growth may be due to an improved nutrient use efficiency allowing a higher relative growth rate (Debbarma and Das, 2017; Muhammad et al., 2015). A higher growth of seedlings issued from primed seeds may also be analyzed in relation to a direct impact of pretreatment on cell cycle regulation and cell elongation processes (Debarma and Das, 2017; Chen and Arora, 2013). The growth parameters of chickpea were significantly affected by seed priming (Debbarma and Das, 2017; Vikas and Mahender, 2012).

Seed germination with Silver Nanoparticles

Nanotechnology utilizes-particles less than 100 nm in size, and it has a promising role in transforming agriculture (Fraceto et al., 2016). The two techniques for NP synthesis are "Top-down" and "Bottom-up." The most flexible method for producing metal nanoparticles top-down is evaporation-condensation (Rafique et al., 2017, Mathur et al., 2017). Nanoparticles exhibit high surface-area-to-volume ratios, enhanced reactivity, and controlled release capabilities, making them valuable for precision agriculture (Kędziora et al., 2018).

The excessive use of chemical fertilizers can be reduced by utilizing nanomaterials in agriculture (Upadhyaya et al., 2017). The mechanism behind high seed germination in nano-priming is the greater penetration *via* seed coat that improves nutrient and water uptake efficiency of the seed (Dutta, 2018). Studies reported that seed priming with calcium-phosphate, SiO_2, ZnO, and Ag nanoparticles enhanced germination and seedling development (Ghafari and Razmjoo, 2013).

One of the most promising methods for increasing agricultural output in both favourable and unfavourable environmental circumstances is the application of nanoparticles (NPs) (Biswas et al., 2023; Zulfiqar and Ashraf, 2021). It has been documented that nanopriming controls physio-biochemical reactions in abiotically stressed crops (Figure 3). AgNPs (Thiruvengadam et al., 2015; Baskar et al., 2015), AuNPs (Alshehddi and Bokhari, 2020), CuNPs (Chung et al., 2019; Nguyen et al., 2022), FeNPs (Kornarzyński et al., 2020), FeS_2NPs (Srivastava et al., 2014), TiO_2NPs (Gohari et al., 2020; Faraji and Sepehri, 2019), ZnNPs (Montanha et al., 2020), ZnONPs (Faizan et al., 2021; Taran et al., 2017; Abdel Latef et al., 2017), and carbon NPs like fullerene (Shafiq et al., 2019) and carbon nanotubes

(Rahimi et al., 2016) have all been used as seed pretreatment. In maize plants, seed priming using gold nanoparticles (AuNPs) frequently improves water absorption (Rajwade et al., 2020).

It has been reported that commercially available silver nanoparticles (AgNPs) with sizes ranging from 50 to 100 nm can lessen the negative effects of stress caused by 120 and 150 mM NaCl in *Pennisetum glaucum* (Parveen and Rao 2014). Additional instances of plants that show improved development and a favorable physiological response after being exposed to silver nanoparticles include *Phaseolus vulgaris*, Zea mays, (Salama 2012) *Phytolacca Americana*, *Panicum virgatum* (Yin et al., 2012). In *Boswellia ovalifoliolata* (Savithramma et al., 2012), treatment with AgNPs has been demonstrated to have a favorable effect on seed germination. In *Brassica nigra* seeds and seedlings, it was similarly discovered that an AgNP dose ranging from 0.2 to 1.6 mg/L inhibited lipase activity, soluble and reducing sugar content, and seed germination (Amooaghaiea and Tabatabaeia 2015). Additionally, it has been discovered that 10 mg/L AgNPs hinder *Hordeum vulgare* seed germination and shorten the shoot length of barley (*Hordeum vulgare*) and flax (*Linum usitatissimum)* (Temsah and Joner 2012).

To increase plant tolerance to salinity, AgNPs enhanced plant height, proline contents, ion homoeostasis, antioxidant enzyme activities, and phenolic and flavonoid contents (Biswas et al., 2023; Khan et al., 2020). While higher and lower concentrations of AgNPs may have a negative impact on plant growth, exposure to particular concentrations may improve plant growth when compared to non-exposed plants (Kaveh et al., 2013; Geisler-Lee et al., 2013; Qian et al., 2013). The optimum concentration of AgNPs to induce a growth response in *Brassica juncea* seedlings was found to be 50 ppm, out of the utilized concentrations of 0, 25, 50, 100, 200, and 400 ppm (Almutairi and Alharbi, 2015). The root length, fresh weight, shoot length, and vigour index of seedlings had positive impact at this concentration. The treated seedlings' vigour index increased by 133% and their root length increased by 326% as a result of this dosage (Sharma et al., 2012). Seedling biomass was shown to increase when Arabidopsis thaliana plants were treated with 1 or 2.5 mg/L of AgNPs, while seedling biomass decreased when larger doses were applied (Kaveh et al., 2013).

1 In order to investigate drought stress on lentil germination five levels of silver nanoparticles (0, 10, 20, 30 and 40 µg mL^{-1}) on Lentil (*Lens culinaris Medic*) were taken
1 AgNP enhanced the radical length and the highest length (4.45 cm) observed in severe stress by applying 10 µg mL^{-1} of the nanoparticle. When plants were treated with 20 µg/mL of

silver nanoparticles (AgNPs), the radicle (embryonic root) had the highest dry weight, which was 0.07 grams (Hojjat and Ganjali 2016).

Studies also showed that the addition of 10 or 50 mg·dm^{-3} of AgNPs changes the composition of essential oils produced by lavender plant (Jadczak et al., 2020). For instance, broad bean（*Vicia faba L*.) seeds exposed to citrate-coated silver nanoparticles (ca. 40 nm) at 10 ppm for 6 h showed higher growth, physiological, and biological traits than non-nano treatments (Alhammad et al., 2023). With alfalfa (*Medicago sativa L*.), the results were also encouraging when exposed to AgNPs (approximately 50 nm) for 3 h (Song et al., 2022). AgNPs exhibited strong antimicrobial activity against a variety of diseases. For example, they significantly limit the growth of fungi that cause rice blast disease and the colony formation of *Magnaporthe grisea* (Vishwanathan and Negi 2021). (Matras 2022) reported that AgNPs had antifungal efficacy against *Alternaria alternata* and *Botrytis cinerea*. According to the study, the highest inhibition of fungal hyphal development was caused by the 15 mg/L concentration of AgNPs. However, it was demonstrated that AgNPs had no discernible effects on the castor bean plant, *Ricinus communis L*., in terms of seed germination, root length, or shoot length (Yasur and Rani 2013). *Cucumis sativus* and *Lactuca sativa* seed germination was observed to be unaffected by 100 mg/L AgNPs (Ingle et al.,2020). M. incognita invasive larvae viability was 100% reduced and the egg hatch process was 100% inhibited after incubation with a very low quantity of silver compounds (0.05 ppm) (Furmanczyk et al., 2025).

Satureja hortensis L., is a medicinal plant endemic to Iran, which is well-known in the folk medicine for its therapeutic uses as herbal tea and as an analgesic and antiseptic substance due to the presence of secondary metabolites including terpenoids, phenolics, flavonoids, steroids, and tannins (Hosainzadegan and Delfan, 2009). During recent years, antibacterial, antioxidant, antifungal, antidiabetic, antinociceptive, antihyperlipidemic, antibiofilm, anti-inflammatory, antispasmodic and antidiarrhea effects and as well as triglyceride-lowering potential have been reported for *S. hortensis* (Hosainzadegan and Delfan, 2009). Priming of *S. hortensis* L. (summer savory) seeds before planting had a positive physiological and biochemical impact. This likely resulted in improved seed germination, enhanced stress tolerance, and better overall plant growth by activating beneficial metabolic processes before the seeds were sown in the seedbed. In *S. hortensis*, the highest seed germination percentage was observed when 80 ppm of AgNPs were applied, particularly under conditions of low salinity. Conversely, the lowest germination percentage was recorded under high salinity conditions 120 MmL^{-1} when AgNPs were not applied (Nejatzadeh, 2021).

According to some studies, a high concentration of AgNPs had detrimental effects on photosynthesis, lowered the amount of chlorophyll overall, and dramatically raised the parameters of oxidative stress (Song et al., 2022; Abasi et al., 2022). The aquatic plant *Lemna gibba* has been shown to exhibit growth inhibition, as evidenced by a notable decline in frond numbers that is dependent on AgNPs concentration (Oukarroum et al., 2013). According to (Stampoulis et al., 2009), the biomass and transpiration rates of *Cucurbita pepo* (zucchini) were reduced by 41% and 57%, respectively, when exposed to 100 nm AgNPs at 100 and 500 mg/L. AgNPs dramatically reduced plant biomass, plant tissue nitrate-nitrogen content, chlorophyll a/b, and chlorophyll fluorescence (Fv/Fm) in an aquatic macrophyte (*Spirodela polyrhiza,* larger duckweed), according to a study by Jiang et al. (2012). Plant toxicity was solely examined in pure cultures in any of these investigations.

AgNPs seed priming has become a viable method for improving crop development and physiological responses to abiotic stressors, such as salinity. In order to help seeds overcome environmental obstacles and improve germination rates and seedling establishment, AgNPs enhance water uptake, enzyme activity, and oxidative stress tolerance. Optimizing the concentration and application techniques is essential to avoiding possible toxicity, though. However, concerns regarding nanoparticle toxicity, environmental persistence, and regulatory challenges necessitate further research to optimize their safe and sustainable application in modern agriculture.

A simple way to improve seed germination and seedling establishment and consequently field performance of medicinal plants is seed priming (Dalil, 2014). Most of the medicinal plants have some problems in seed germination and stand establishment in the field (Zare et al., 2011). Since germination and seedling establishment are critical stages in the plant life cycle (Cheng and Bradford, 1999), offering the solutions for improvement of seed germination and seedling establishment will help to better performance in cultivation of medicinal plants. One of the simple techniques which can improve seedling vigor and establishment and consequently field performance of plants is seed priming or physiological advancement of the seed (Mc Donald, 2000). In comparison to the control seedlings, AgNPs increased the amount of chlorophyll and enhanced the cellular electron exchange and photosynthetic quantum efficiencies in medicinal plant (Sharma et al., 2012).

According to several reports, flavonoids are in charge of the environmentally friendly synthesis of AgNPs (Mustapha et al. 2022). AgNPs produced synthetically have been utilized a food packaging, wastewater treatment, and biological uses as an antibacterial agent (Vanlalveni et al. 2021). This method is successful in small seed plants such as many medicinal plants that have great economic value with quick and uniform emergence requirements (Ellis and Roberts, 1981). Majority of studies focus on agricultural plants and some focus on flowering/medicinal plants have been compiled in Table 1 (*Psophocarpus tetragonolobus (L.), Vigna mungo (L.) Hepper, Satureja hortensis L., Sanguisorba mino*r, *Lavandula angustifolia, Allium cepa L, Phaseolus vulgaris L., Echinop*s *macrochaetus, Medicago sativa L., Linum usitatissimum L., Ocimum basilicum L., Helianthus annuus L., Solanum lycopersicum, Vicia faba, Capsicum annuum L.)*.

Table 1: Various plants studied under silver nanoparticles seed priming:

Plant studied	Seed condition	Concentration of NPs	Aspect studied	Impact of NPs	References
Psophocarpus tetragonolobus (L.) (Legume)	Seedling growth stress	50 mg	Seed germination	Increased upto 88.33%	(Kumar et al., 2020)
Vigna mungo (L.) Hepper	Poor seed vigour, Low germinatio n rates	1.5 mM	Germination percentage Root length Shoot length Vigour index	Increased upto 21% Increased upto 19% Increased upto 23% Increased upto 35%	(Krishnas-amy et al., 2024)
Satureja hortensis L.	salinity stress	80 ppm	Seed germination Salinity stress	Improved salinity tolerance with increased seed germination	(Nejatzade h, 2021)

Sanguisorba minor	Drought stress	30 mg/l	Biomass production	Increased by 25%	(Farmahini Farahani et al., 2022)
Lavandula angustifolia	Lack of enhanced metabolic and defensive activities	2 mg·dm^{-3}	activity of antioxidant enzymes and total polyphenolic capacity	Increased activity of APX and SOD	(Jadczak et al., 2020)
Allium cepa L.		25 ppm	Seed germination and vigour index	Stimulated early germination and significant increase in Vigour Index	(Patidar et al., 2024)
Phaseolus vulgaris L.		25 mg/L 50 mg/L	Root length Flavonoids Protein Chlorophyll content Shoot length	Maximum shoot and root length elongation was observed at 25 mg/L of AgNPs concentration. Secondary metabolites increased	(Ahmed and Murtaza, 2024)
Echinops macrochaetus	Salinity stress	40 µmol/L	Biomass Shoot length Chlorophyll content	Faster germination with increase in biomass, shoot length and chlorophyll	(Khan et al., 2024)

				content was observed at 40 µmol/L AgNPs	
Medicago sativa L.		25 mg/l	Seed germination Root and shoot length	Significant increase in water absorption rate. Thus, increasing seed germination along with increased root and shoot length.	(Song et al., 2022)
Linum usitatissimum L	Salinity stress	AgNPs combined with *C. testosteroni*	Overall growth and yield in saline condition.	increases production of photosynthetic pigments, proteins, sugars, and proline	(Khalofah et al., 2021)
Ocimum basilicum L.		200 mg/L	germination percentage and root length	Germination increased upto 85% and root length increased by 19.4 mm.	(Sencan et al., 2024)
Helianthus annuus L.		25 mg L^{-1}	Overall growth and plant characteristics	Improved morphological characteristic, seed quality,	(Haq et al., 2024)

				and oil content, secondary metabolites	
Solanum lycopersicum		25–50 µg/mL	seed germination, seedling growth	increase in the seed germination percentage	(Sonawane et al., 2021)
Vicia faba	Aged seeds	100 ppm	vigor and mitotic index	Increased mitotic cell cycle and seedling growth	(Younis et al., 2019)
Capsicum annuum L.		30-50 ppm	root length, shoot length, seedling length, and germination vigour index	primed seedlings increased root and shoot length, seedling length, and germination vigour index	(Mawale and Giridhar, 2024)

Conclusions

Medicinal and herbal plants have been essential to the global evolution of human cultures. Their proper growth helps in development of socio-economic aspects of any society. Seedling establishment and germination are important phases in their life cycle. The efficient seed germination encourages successful establishment and deep root system. Seed priming is the process of treating seeds with chemical or biological priming agents to display better growth and to amplify abiotic stress resistance. The use of nanopriming has been proven to be more promising than traditional priming approaches for achieving feasible agricultural yields. This article discussed the use of silver nanoparticles (AgNPs) as nanopriming agent to enhance the seed germination of many medicinal and herbal plants. The future lies in the utility of silver nanoparticle in seed priming with proper applications, appropriate 'stop' time, and better prior standardization. The seed priming adoption at field level is the need for future studies.

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