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IMPACT OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES (ZNO NPS) AND SILVER NANOPARTICLES (AG NPS) ON SEED PRODUCTION OF ONION (*ALLIUM CEPA* L.)

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ABSTRACT

The zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs) were synthesized through biological method using leaf extract of *Ocimum sanctum.* The characteristic absorbance peak was observed on on UV-Visible spectrophotometer at 385 and 450 nm of ZnO NPs and Ag NPs, respectively to confirm synthesis of nanoparticles. The particle size of ZnO NPs was found 30 - 80 nm and Ag NPs was 25-75 nm, respectively by transmission electron microscope (TEM). The efficacy of biosynthesized ZnO NPs and Ag NPs was attributed on seed production of onion. The foliar application of ZnO NPs and Ag NPs was undertaken at 45, 60 and 75 days after planting of the bulbs @ ZnO NPs 50 ppm (S_1), 75 ppm (S_2), 100 ppm (S_3), 150 ppm (S_4) and Ag NPs @ 50 ppm (S_5), 75 ppm (S_6), 100 ppm (S_7), 150 ppm (S_8) along with untreated control (S_9). The treatment ZnO NPs 150 ppm (S_4) and Ag NPs 100 ppm (S_7) was found significant on plant height, number of leaves, days to 50 per cent flowering, number of flower stalks, height of flower stalk, stem thickness of main flower stalk, diameter of umbel of main flower stalk, days to maturity, number of seeds per umbel, seed weight per umbel, 1000 seed weight and seed yield than untreated control (S_9).

The treatment ZnO NPs 150 ppm (S_4) was found superior on plant height (49.00, 57.87 and 60.07 cm at 30, 60 DAP and at harvest), number of leaves (31.00, 50.87 and 54.13 cm at 30, 60 DAP and at harvest), days to 50 per cent flowering (at 80 DAP), number of flower stalks (1.73, 5.27 and 3.20 of Primary, Secondary and Tertiary), height of flower stalk (57.00, 53.67 and 44.67 cm of Primary, Secondary and Tertiary), stem thickness of main flower stalk (1.67 cm), diameter of umbel of main flower stalk (8.15 cm), days to maturity (139 DAP), number of seeds per umbel (1383, 1079 and 909 of Primary, Secondary and Tertiary umbels), seed weight per umbel (5.66, 4.77 and 3.47 g of Primary, Secondary and Tertiary umbels),1000 seed weight umbel (4.10, 4.41 and 3.81 of Primary, Secondary and Tertiary umbels) and seed yield (941.00 kg/ha) than untreated control (S_9).
Keywords : Onion, zinc oxide nanoparticles, silver nanoparticles, seed production.

Introduction

Onion (*Allium cepa* L.), one of the most significant commercial vegetable crops in the world, it occupies an important position among vegetables because of its high food preference, high price and regular market demand. The farmers cultivate it extensively over the nation and it is widely consumed worldwide. It is both a vegetable crop and a condiment. Both mature and immature bulbs are used as spices and as vegetables. Although India is the world's second-biggest producer and the country with the largest total area under onion production, its yield is significantly lower than China. The United States, Turkey, Pakistan, Russia, Indonesia, Vietnam, Korea and Myanmar are following in position. Due to decreased acreage, a lack of high-quality seeds and their high cost, the nation's onion production is predicted to drop by 4.5% this year compared to 21.4 million tones in 2018-19 (based on data given by the ministry of agriculture in January 2019).

Seed is the primary and most important input in agricultural production. Maintaining an adequate amount of high-quality seeds for producers, creating genetically pure seeds and maintaining seed qualities from harvest to subsequent sowing are the most important factors in this situation. Since onion seeds lose their viability after a few months of being stored in ambient condition, they are categorized as poor storer seeds. The way in which the reserve food is kept leads to low viability, poor germination and storability. The buildup of free radicals caused by lipid peroxidation during storage is another factor contributing to the rapid decline in seed viability. The poor yield of onions is largely caused due to lack of high-quality seed. In the cultivation of onions, high-quality seed is considered as the most important input, upon which all other inputs can be managed for maximum yield of onion. A recently developed field in nanoscience and technology is the use of materials with at least one dimension in the 1-100 nanometer range. Because of their extraordinary surface-to-volume ratio, the nanomaterials exhibit remarkable physical, chemical and electrical properties (Abbas *et al.,* 2019). Due to their unique characteristics, such as a high reaction activity and a large specific surface area, nanomaterials have drawn more and more interest (Khorsand *et al,* 2011). Additionally, nanomaterials have been employed in a number of basic and useful applications. Plant protection, plant growth monitoring and plant disease detection, increased global food production, improved food quality and waste reduction for "sustainable intensification" are all possible with nanotechnology (Locke *et al.,* 2000). Zinc enhances the ability to absorb and transport water, improves seed viability and radical development in germination and minimizes the negative impacts of heat, drought and salt stress. Furthermore, zinc actively assists in the synthesis of gibberellins and auxins (Tsonev *et al.,* 2012 and Sedghi *et al.,* 2013). Likewise, a wide range of plant diseases can be controlled by using ZnO NPs, which have a broad antifungal and antibacterial effect (Sabir *et al.,* 2014, Helaly *et al.,* 2014 and Raskar and Laware, 2014). Because of their small size, ZnO-NPs can penetrate different plant tissues through the stomata and cuticle of plant leaves and root hair cells, improving plant development and yield (Chanu and Upadhyaya, 2019 and Su *et al.,* 2019).

The various ways that silver nanoparticles reveal antibacterial properties are not yet completely understood. According to numerous studies, AgNPs can bind to cell walls, the S-H group of proteins and the N7 atom of guanine, which can disrupt the cell wall, increase intercellular ROS production, oxidative stress and photocatalytic activity and prevent cell division and replication (Franci *et al.,* 2015 and Gudkov *et al.,* 2022). Due to their low use of resources and energy, as well as their environmental safety and biocompatibility, green synthesis of silver nanoparticles has attracted interest from all over the world (Devatha and Thalla, 2018 and Khan *et al.,* 2022).The present research, titled "Impact of biosynthesized zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs), on seed production of onion (*Allium cepa* L.)," aims to take into consideration the potential of biosynthesized nanoparticles.

Material and Methods

Preparation of leaf extract of Tulsi (*Ocimum sanctum* L.)

The fresh tulsi leaves were taken in a beaker and washed with water to remove the dust. The leaves were kept for shade drying at room temperature. 10 g of shaded dried leaves were crushed in mortar and pestle with 100 ml distilled water. After grinding, the aqueous extract was taken in a 250 ml beaker and boiled at 80 °C temperature for 10 minutes in a hot water bath.The plant extract was allowed to cool at room temperature and then filtered with Whatman filter paper. The filtrate was centrifuged at 10,000 rpm for 20 to 25 min., the supernatant light yellow in colour was collected and stored in a refrigerator at 4 °C for further synthesis of zinc oxide nanoparticles and silver nanoparticles.

Biosynthesis of zinc oxide nanoparticles (ZnO NPs)

The 30 ml of tulsi leaf extract and 70 ml of 1mM zinc acetate solution were mixed in beaker. The mixture was heated at 80°C for 10 min in a hot water bath. The colour of the solution turns from light yellow to dark yellow indicating the synthesis of zinc oxide nanoparticles. The synthesized ZnO NPs were separated by the process of centrifugation from the reaction mixture (10,000 rpm for 25 min.) and further obtained sediments were washed with distilled water and dried in a hot air oven at 100 to 130 °C for 40-45 min. The powder obtained from ZnO NPs was light yellow in colour.

Biosynthesis of silver nanoparticles (Ag NPs)

The 10 ml of tulsi leaf extract and 90 ml 1 mM silver nitrate solution ($AgNO_3$) were mixed in beaker. The mixture was heated up at 70 °C for 10 min in a hot water bath. The colour of the solution turns from light yellow to reddish brown which indicates the formation of silver nanoparticles (Ag NPs). The biosynthesized Ag NPs were separated by the process of centrifugation (10,000 rpm for 25 min) and further obtained sediments were washed with distilled water and dried in a hot air oven at 100 ^{0}C for 40-45 min. The silver nanoparticles (Ag NPs) obtained were sparkling black in colour.

Characterization of nanoparticles

The biosynthesized zinc oxide nanoparticles and silver nanoparticles (ZnO NPs and Ag NPs) were confirmed by sharp peaks shown by the absorption spectrum in Nanodrop 1000 spectrophotometer (Thermo Scientific) at 385 and 450 nm, respectively at the State Level Biotechnology Centre, MPKV, Rahuri (Fig. 1 a & b). The size of biosynthesized zinc oxide nanoparticles and silver nanoparticles (ZnO NPs and Ag NPs) was characterized by Transmission Electron Microscopy (Jeol Asia Pvt., Ltd., Singapore) at Eco-friendly Disease Management and Beneficial Microbes Research Laboratory, MPKV, Rahuri. The size of biosynthesized zinc oxide and silver nanoparticles (ZnO NPs and Ag NPs) were found 30 - 80 nm and 25 - 75 nm, respectively (Fig. 1 c & d).

Experimental details

The field experiment on the "Impact of biosynthesized zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs) on seed production of onion (*Allium cepa* L.)" were laid down during *Rabi,* 2022 in randomized block design (RBD) with three replications at Seed Technology Research Unit, MPKV, Rahuri.

Treatments Details

The foliar application of biosynthesized zinc oxide and silver nanoparticles (ZnO NPs and Ag NPs) was undertaken at 45, 60 and 75 days after planting of bulbs.

S_1: Zinc oxide nanoparticles (ZnO NPs) @ 50 ppm
S_2: Zinc oxide nanoparticles (ZnO NPs) @ 75 ppm
S_3: Zinc oxide nanoparticles (ZnO NPs) @ 100 ppm
S_4: Zinc oxide nanoparticles (ZnO NPs) @ 150 ppm
S_5: Silver nanoparticles (Ag NPs) @ 50 ppm
S_6: Silver nanoparticles (Ag NPs) @ 75 ppm
S_7: Silver nanoparticles (Ag NPs) @ 100 ppm
S_8: Silver nanoparticles (Ag NPs) @ 150 ppm
S_9: Untreated control.

Observations recorded:

Plant height

The five plants from each treatment were selected randomly, labelled and plant height was recorded in centimeterat 30, 60 and 90 DAP.

Number of leaves

The numbers of leaves were counted from the randomly selected plants and were recorded at 30, 60 and 90 DAP.

Days to 50 per cent flowering

The number of days for 50 per cent flowering were noted when 50 per cent of plants in each plot were flowered.

Number of flower stalks per bulb

The number of primary, secondary and tertiary flower stalks per bulb were recorded at 75 DAP.

Height of flower stalk at maturity

The height of primary, secondary and tertiary flower stalks was measured at the time of maturity and recorded in centimeter.

Stem thickness of main flower stalk

The stem thickness of the main flower stalks of five randomly selected plants were recorded in centimetre by digital vernier calliper at 75 DAP.

Diameter of umbel on main flower stalk

The diameter of the umbel from the main flower stalks of five randomly selected plants were recorded in centimetre by digital vernier calliper at maturity.

Days to maturity

The number of days required to maturity of seed from each treatment was recorded.

Number of seeds per umbel

The numbers of seeds from primary, secondary and tertiary umbel were recorded at maturity.

Seed weight per umbel

The seed weight of primary, secondary and tertiary umbel was recorded from five randomly selected plants in grams.

1000 seed weight

A thousand seeds in each treatment from primary, secondary and tertiary umbel were counted by seed counter and the weight was recorded in grams.

Seed yield per plant

The seed yield obtained from five randomly selected plants were weighed and recorded ingrams.

Seed yield per plot

The seeds obtained from each net plot was weighed and recorded in kilograms.

Seed yield per ha

The seed yield per hectare was calculated by multiplying the seed yield per plot by hectare factor and recorded in kilograms.

Results and Discussion

Plant height

It was observed that the plant height of onion gradually increased with the advancement of crop growth period irrespective biosynthesized nanoparticles treatment. The significant differences were recorded among the different concentrations of biosynthesized nanoparticles at 30, 60 and 90 days after planting of onion bulbs. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest plant height (49.00 cm) at 30 days after planting of bulbs followed by foliar application with biosynthesized silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (48.27 cm) thanun treated control (S_9) (45.27 cm), respectively. The highest plant height (57.87 and 60.07 cm) were recorded of foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) at 60 and 90 days after planting of onion bulbs, followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (56.93 and 59.20 cm), respectively. The lowest plant height (48.33 and 54.87 cm) was exhibited in untreated control (S_9) at 60 and 90 days after planting of onion bulbs (Table 1).

Both the biosynthesized nanoparticles showed growth promoting effects on onion plants as compared to the untreated control. It is concluded that foliar application of both biosynthesized nanoparticles significantly enhances the growth of onion plants as compared to the untreated control.

According to Zeidan *et al.* (2010), zinc promotes plant development by increasing enzymatic activity, promoting photosynthesis and translocating assimilates from source to sink. When 125 ppm of nanoscale ZnO was introduced to peanut shoots and pod output increased. In comparison to chelated zinc sulfate, foliar application of nanoscale ZnO resulted in high zinc uptake by the leaf and kernel (Prasad *et al.,* 2012). According to Benzon *et al.,* (2015), nano zinc's ability to permeate and migrate through plant roots and leaves could indicate why it outperforms bulk zinc particles at photosynthesis. According to studies, application of 100 ppm ZnO NPs to wheat increased plant height by 16% over the control treatment (Munir *et al.,* 2018). Sun *et al.,* (2020) found that foliar spraying with 100 mg/L ZnO NPs produced the highest plant height of tomato. As reported by Sadak and Bakry (2020), applying 40 mg/L of nano ZnO to flax seed significantly increased plant height compared to the untreated control. When compared to standard $ZnSO_4$ fertilization, Singh *et al.,* (2023) demonstrated that foliar application of ZnO NPs increased rice plant height by 17.9% at harvest time. As reported by Ahmed *et al.,* (2023), foliar spraying of ZnO NPs at 100 ppm on tomato plants enhances plant height compared to the control.

Seif *et al.,* (2011) found that the application of Ag NPs raised the height of Borago plants, which supports our findings. According to Salama (2012), Ag NPs improved plant growth features in *B. juncea*, *P. vulgaris* and *Z. mays*, such as root and shoot length and leaf area. In comparison to the control, the application of Ag NPs significantly boosted the lotus (*Nelumbo nucifera*) plant height, leaf diameter, fresh leaf weight, dry leaf weight and other biochemical features (Nguyen *et al.,* 2021). Similar findings regarding the application of silver nanoparticles to onions, tomatoes and green gram were reported by Adhikari *et al.,* (2016) and Dimkpa*et al.,* (2017) in Soybean, Mahdieh *et al.,* (2018) in *Phaseolus vulgaris* L. and Acharya *et al.,* (2019) in Onion, Noshad *et al.,* (2019) in *Solanum lycopersicum* and Prazak *et al.,* (2020) in Chilli.

Number of leaves

It was observed that the number of leaves in onion, gradually increased with the advancement of crop growth period irrespective treatments of biosynthesized nanoparticles. The significant differences in number of leaves were recorded among the different concentrations of biosynthesized nanoparticles at 30, 60 and 90 days after planting of onion bulbs. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest number of leaves (31.00) at 30 days after planting of bulbs followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (30.93) than untreated control (S_9) (27.80). The highest number of leaves (50.87 and 54.13) were recorded by foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) at 60 and 90 days after planting of onion bulbs, followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (50.17 and 53.30), respectively. The lowest number of leaves (38.13 and 39.93) was exhibited in untreated control (S_9) at 60 and 90 days after planting of onion bulbs (Table 1).

The biosynthesized nanoparticles showed growth promoting effects on onion plants as compared to the untreated control. It is concluded that foliar application of both biosynthesized nanoparticles significantly enhance the growth of onion plant compared to the untreated control. The use of nano zinc increased the number of leaves in savories (*Satureja hortensis* L.), as stated by Vafa *et al.,* (2015). Zinc oxide nanoparticles work in combination with metabolic processes and meristematic cells to enhance growth characteristics (Faizan and Hayat, 2019). When Ag NPs at 100 ppm were applied topically to onions 45, 60 and 75 days after bulb planting, the greatest number of leaves was recorded by Das *et al.,* (2018).As noted by Peiman *et al.,* (2022), when compared to the untreated control, *Withania* plants treated with ZnO NPs at a dosage of 3 g L^{-1} produced the highest number of leaves per plant. In the study by Singh *et al.,* (2023), foliar ZnO NP application raised the leaf-area index by 69.4% at 90 days after transplanting (DAT) of ricein comparison to soil application.

Days to 50 % flowering

The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded the lowest days to 50 % flowering (80 days), followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (81 days) and zinc oxide nanoparticles (ZnO NPs) @ 100 ppm (S_3) (83). The highest days to 50% flowering (88 days) were exhibited in the untreated control (S_9) days after transplanting onion bulbs. Both the biosynthesized nanoparticles showed positive effects on days to 50% flowering as compared to the untreated control. However, the biosynthesized ZnO NPs generally resulted induced flowering earlier compared to the biosynthesized Ag NPs. It is observed that foliar application of both biosynthesized ZnO NPs and Ag NPs significantly enhances the onion seed production compared to the untreated control (Table 1).

The development of plants from nanoparticle-treated seeds that achieve 50% flowering in a short duration of time may be due to the beneficial effects of nanoparticles on germination, which encourage early establishment and allow early flowering by a few days over control (Hafeez *et al.,* 2013). According to Laware and Raskar (2014), all ZnO NP treatments resulted in a considerable reduction in the number of days needed for onion flowering. The findings are consistent with studies conducted on groundnuts by Prasad *et al.,* (2012), sorghum by Poornima and Koti (2019), okra by Keerthana *et al.,* (2021) and feeder maize by Tondey *et al.,* (2022), where early flowering was noted following the application of ZnO NPs as a seed treatment. The Ag NPs seed treatment had similar results in tomato by Noshad *et al.,* 2019 and bean by Prazak *et al.,* 2020, as well as fodder crops, oats and berseem.

Number of flowering stalk per bulb

The significant differences in number of flowering stalk per bulb were recorded among the different concentrations of biosynthesized nanoparticles at primary, secondary and tertiary flowering stalk per onion bulbs. The foliar application **o**f biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest number of primary flowering stalk (1.73) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (1.53). The lowest number of primary flowering stalk (1.00) was exhibited in untreated control (S_9), respectively.

The highest number of secondary flowering stalks (5.27) were recorded by foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4), followed by(4.93) silver nanoparticles (Ag NPs) @ 100 ppm (S_7) than (3.33) untreated control (S_9)**,**respectively. The highest number of tertiary flowering stalks (3.20) were observed in foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) followed by (2.87) silver nanoparticles (Ag NPs) @ 100 ppm (S_7) than (1.53) untreated control (S_9)**,** respectively (Table 1).

It is concluded that, foliar application of biosynthesized nanoparticles (ZnO NPs & Ag NPs) showed positive growth promoting effects on onion plants as compared to the untreated control in onion seed production.

Zinc plays an important role in photosystem II, is required for various essential enzymatic activities and is an essential component of transcription factors that belong to the zinc finger family (Sharma *et al.,* 2013). Rostami *et al.,* (2019) found that increased levels of soluble protein, chlorophyll, relative water and peroxidase and catalase activities resulted to increased flowering in saffron (*Crocus sativus* L.). When 40 mg/L of nano ZnO was applied to flax seed, the number of fruiting branches and capsules per plant significantly increased when compared to the untreated control, according to Sadak and Bakry (2020). The improved uptake of nutrients such as calcium, potassium and sulfur as well as increased water uptake during the early stages of seedling growth can be largely attributed to the increase in yield-attributable traits caused by silver and zinc oxide nanoparticles, which results from the beneficial effects of the nanoparticles during germination (Landa, 2021). Accordingly, increased plant metabolic activity and overall plant development affected by nanoparticles result in a higher number of pods (Benzon *et al.,* 2015, Singh, 2017 and Babajani *et al.,* 2019). When ZnO NPs at 100 ppm were applied topically to tomato plants, Ahmed *et al.,* (2023) noted an increased number of primary branches.

Similar responses to ZnO nanoparticles treatment were observed in the following crops: groundnut (Prasad *et al.,* 2012), sorghum (Poornima and Koti, 2019), soybean (Yacoub *et al.,* 2020 and Yusefi-Tanha *et al.,* 2020), pomegranate (Amer *et al.,* 2020), fodder maize (Tondey *et al.,* 2022), okra (Keerthana *et al.,* 2021), lentil (Kolencik*et al.,* 2022) and pea (Labeeb *et al.,* 2023). Zari *et al.,* (2015) reported similar outcomes with the application of silver nanoparticles in safflower, Acharya *et al.,* (2019) in onion, Noshad*et al.,* (2019) in tomato and Acharya *et al.,* (2020) in watermelon.

Height of flowering stalks

It was recorded that the height of flowering stalks gradually decreased with the advancement of primary, secondary and tertiary flowering stalks irrespective biosynthesized nanoparticles treatment. The significant variations in the height of flowering stalks were observed among various concentrations of biosynthesized nanoparticles at the primary, secondary and tertiary flowering stalks per onion bulb at maturity.

The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest height of primary flowering stalk (57.00 cm) followed by (55.53) silver nanoparticles (Ag NPs) @ 100 ppm (S_7) than untreated control (S_9)(50.53), respectively. The highest height of secondary flowering stalks (53.67 cm) were recorded by foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4), followed by(52.13 cm) silver nanoparticles (Ag NPs) @ 100 ppm (S_7), respectively. The lowest height of secondary flowering stalks (44.73 cm) was exhibited in untreated control (S_9).The highest height of tertiary flowering stalks (44.67 cm) were observed in foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (42.80 cm) than, untreated control (S_9) (34.00), respectively (Table 2).

It is concluded that, foliar application of biosynthesized nanoparticles (ZnO NPs and Ag NPs) showed increase height of primary, secondary and tertiary flowering stalks than untreated control.

In accordance with Sun *et al.,* (2020), foliar spraying with 100 mg/L ZnO NPs produced highest-growing plants. Noshad *et al.,* (2019) reported similar results, observing that foliar application of Ag NPs considerably improved tomato growth metrics such as plant height, yield/plant, fresh biomass, number of shoots/plant, root weight and dry biomass. The plant height, root numbers, leaf length and rice yield all significantly improved when Ikhajiagbe and Musa (2020) sprayed rice with biosynthesized Ag NPs.

Stem thickness of main flowering stalks

The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest stem thickness of main flowering stalks (1.67 cm) at 75 days after planting of onion bulbs followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (1.57 cm). The lowest stem thickness of main flowering stalks (1.33 cm) was exhibited in untreated control (S_9) at 75 days after planting of onion bulbs (Table 2).

The biosynthesized nanoparticles showed desirable quality seed production parameter of onion plants as compared to the untreated control. However, the biosynthesized ZnO NPs generally resulted in higher stem thickness of main flowering stalks as compared to biosynthesized Ag NPs.

Diameter of main flowering stalk

The significant differences in diameter of main flowering stalk were recorded among the different concentrations of biosynthesized nanoparticles at maturity during seed production of onion. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest diameter of main flowering stalk (8.15 cm) followed by foliar application of silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (7.62 cm) at maturity during seed production of onion, respectively. The lowest diameter of main flowering stalk (5.63 cm) was observed in untreated control (S_9).The biosynthesized ZnO NPs generally resulted in higher diameter of main flowering stalk compared to biosynthesized Ag NPs. Both biosynthesized nanoparticles were exhibit the enhancement in yield contributing characteristics on onion plants as compared to the untreated control (Table 2).

In the opinion of Harris and Mathuma (2015), the zinc nutrients have a significant effect on fruit quality, including fruit size. Tomato fruit set, fruit length and fruit diameter all depend on zinc, as does RNA metabolism and the promotion of DNA, protein and carbohydrate synthesis. Their study found that using zinc oxide nanoparticles was the most efficient way to increase the size and diameter of tomato fruit. The largest strawberry fruit diameter and length were produced by ZnO NPs at 150 ppm, as per Kumar *et al.,* (2017).

Days to maturity

The days to maturity were recorded significant differences among the different concentrations of biosynthesized nanoparticles during seed production of onion.The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded lowest days to maturity (139 DAP) followed by foliar application of silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (141 DAP) and zinc oxide nanoparticles (ZnO NPs) @ 100 ppm (S_3) (142 DAP) of onion umbels, respectively. The highest days to maturity (148 DAP) was observed in untreated control (S_9)(Table 2).

It is concluded that, the foliar application of biosynthesized ZnO NPs resulted in earlier maturity as compared to biosynthesized Ag NPs than untreated control.

As previously documented, early flowering and early maturity were the results of faster seed establishment and germination caused by treatment with silver and zinc oxide nanoparticles. ZnO NPs in peanuts (Prasad *et al.,* 2012), sorghum (Poornima and Koti, 2019) and okra (Keerthana *et al.,* 2021) showed comparable outcomes. The findings of treatments with silver nanoparticles (Ag NPs) were equivalent to those of Prazak *et al.,* 2020 (bean cultivars) and Noshad *et al.,* 2019 (tomato).

Number of seeds per umbel

It was recorded the number of seeds per umbelgradually decreased with the primary, secondary and tertiary umbels, irrespective of biosynthesized nanoparticles treatments. The significant variations in the number of seeds per umbel were recorded among various concentrations of biosynthesized nanoparticles on primary, secondary and tertiary umbels of onion.

The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest number of seeds per primary umbel (1383) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (1324) than untreated control (S_9) (1012), respectively. The highest number of seeds per secondary umbel (1079) were recorded by foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4), followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (1026), than the untreated control (S_9) (895)**,** respectively.

The number of seed per tertiary umbel was recorded (909) highest in foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (883), than untreated control (S_9), respectively (Table 3).

It is concluded that, foliar application of biosynthesized nanoparticles **(**ZnO NPs & Ag NPs) enhances seed setting and increases number of seed per umble in onion.

According to the study by Laware and Raskar (2014), the foliar application of zinc oxide nanoparticles at a concentration of 30 µg ml^{-1} resulted in the greatest number of seeded fruits per umbel (228.68) and the lowest number (220.14) in untreated control The largest number of fruits per pomegranate tree was recorded by Amer *et al.,* (2020) when ZnO NPs were applied topically at a concentration of 200 ppm. According to Das and Dutta (2022), primed chickpea seed with 100 ppm ZnO NPs produced the highest number of seeds per pod.

Seed weight per umbel

It was recorded that, the seed weight per umbelgradually decreased with the primary, secondary and tertiary umbels irrespective of biosynthesized nanoparticles treatment. The significant variations in the seed weight per umbel were recorded among various concentrations of biosynthesized nanoparticles on primary, secondary and tertiary umbels of onion.

The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest seed weight of primary umbel (5.66 g) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (5.15 g) than (S_9) untreated control (3.41 g), respectively. The highest seed weight of secondary umbel (4.77 g) were recorded by foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4), followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (4.29 g) than (S_9)untreated control (2.91 g), respectively. The seed weight of tertiary umbel was recorded (3.47 g) highest in foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (3.23 g) than untreated control (S_9) (2.31 g), respectively (Table 3).

The foliar application of biosynthesized nanoparticles showed positive effects on seed weight of umbels as compared to the untreated control. It is concluded that foliar application of both biosynthesized nanoparticles significantly enhance the weight of seed compared to the untreated control.

The zinc oxide nanoparticles interaction with biochemical and enzyme activities may promote enhanced mobilization of photosynthates for the growth of tomato fruits (Kumar *et al.,* 2017 and Khan *et al.,* 2019). Adil *et al.,* (2022) found that when ZnO NPs were applied externally to wheat under salt stress, the wheat's fresh root weight, plant height and amount of chlorophyll significantly increased, resulting in a higher yield. The similar results was found that, foliar application of zinc oxide nanoparticles @ 20 µg ml^{-1} produced the highest seed weight per umbel (2.34 g) than untreated control (1.94 g) by Laware and Raskar (2014). According to Amer *et al.,* (2020) foliar application of ZnO NPs @ 200 ppm recorded maximum pomegranate fruits weight.

1000 Seed weight

It was recorded that, the 1000 seed weight gradually increased with the primary and secondary umbels, whereas decrease with the tertiary umbel irrespective of biosynthesized nanoparticles treatment. The significant variations in the 1000 seed weight were recorded among various concentrations of biosynthesized nanoparticles on primary, secondary and tertiary umbels of onion.

The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest 1000 seed weight of primary umbel (4.10 g) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (3.89 g). The lowest 1000 seed weight of primary umbel (3.36 g) was exhibited in untreated control (S_9), respectively. The highest 1000 seed weight of secondary umbel (4.41 g) were recorded by foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4), followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (4.18 g), respectively. The untreated control (S_9) was recorded lowest 1000 seed weight of secondary umbel (3.25 g). The 1000 seed weight of tertiary umbel was recorded (3.81 g) highest in foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (3.66 g), respectively. The lowest 1000 seed weight of tertiary umbel (3.02 g) was recorded in untreated control (S_9) (Table 3).

The foliar application of biosynthesized nanoparticles showed positive effects on 1000 seed weight as compared to the untreated control. It is concluded that foliar application of both biosynthesized ZnO NPs and Ag NPs significantly enhance the 1000 seed weight compared to the untreated control. The 1000 seed weight of secondary umbel recorded higher seed weight than primary umbel and tertiary umbel of onion.

Similar findings were made by Laware and Raskar (2014), who reported that foliar application of zinc oxide nanoparticles at 30 µg ml^{-1} yielded the highest 1000 seed weight per umbel (3.52 g) compared to the untreated control (3.18 g). When 40 mg/L of nano ZnO was applied to flax seeds, the weight of the seeds increased significantly in comparison with the control (Sadak and Bakry, 2020). According to Adil *et al.,* (2022), there were notable differences in the impact of ZnO NPs at different concentrations on thousand-grain weight and grain yield per plant. The maximum 1000 seed weight was observed in primed chickpea seed at 100 ZnO NPs (Das and Dutta, 2022).

Seed yield (g/plant)

It was recorded the seed yield was gradually increased with biosynthesized nanoparticles treatment. The significant differences in seed yield were recorded among various concentrations of biosynthesized zinc oxide nanoparticles and silver nanoparticles. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest seed yield per plant (18.07 g) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (16.83 g) and zinc oxide nanoparticles (ZnO NPs) @ 100 ppm (S_3) (16.11 g). The lowest seed yield per plant (9.57 g) was exhibited in untreated control (S_9), respectively (Table 3).

The foliar application of biosynthesized nanoparticles showed positive effects on seed yield per plant as compared to the untreated control. It is observed hat foliar application of both biosynthesized nanoparticles significantly enhances the seed yield per plant compared to the untreated control.

As reported by Del *et al.,* (2022), hybrid materials composed of ZnO-containing lignin nanoparticles (ZnO-L NPs) significantly enhanced the chlorophyll content and antioxidant properties of treated maize seedlings. The chlorophyll A, B, carotenoids and total pigments were highest in plants treated with 50 mg L^{-1} ZnO NPs compared to the control (Asmaa and Rania, 2022). Singh *et al.,* (2023) discovered that the foliar application of ZnO NPs increased rice grain and straw yield by 16.9% and 17.3%, respectively, in comparison to conventional $ZnSO_4$ fertilizer. Silver nanoparticles (Ag NPs) improved yield consequences, including pod height, in soybean (Sheykhbaglou *et al.,* 2010). The growth and yield characteristics of rice under ferruginousity and low salinity conditions were substantially enhanced when Ikhajiagbe and Musa (2020) sprayed the rice with biosynthesized silver nanoparticles.

Seed yield (g/plot)

The seed yield was gradually increased with biosynthesized nanoparticles treatment. The significant differences in seed yield per plot were recorded among various concentrations of biosynthesized zinc oxide nanoparticles and silver nanoparticles. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest seed yield per plot (994g) followed by silver nanoparticle (Ag NPs) @ 100 ppm (S_7) (875 g) and zinc oxide nanoparticles (ZnO NPs) @ 100 ppm (S_3) (816 g), respectively. The lowest seed yield per plot (399 g) was exhibited in untreated control (S_9)(Table 3).

The foliar application of biosynthesized nanoparticles showed positive effects on seed yield per plot as compared to the untreated control. It is concluded that foliar application of both biosynthesized nanoparticles significantly enhances the seed yield per plot compared to the untreated control.

Del *et al.,* (2022) observed that when maize seeds were treated with hybrid materials consisting of ZnO-containing lignin nanoparticles (ZnO-L NPs), the amount of chlorophyll and the antioxidant properties were significantly increased.

Seed yield (kg/ha)

It was recorded the seed yield was gradually increased with biosynthesized nanoparticles treatment. The significant differences in seed yield per hectare were recorded among various concentrations of biosynthesized zinc oxide nanoparticles and silver nanoparticles. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) recorded highest seed yield per ha (941.0 kg) followed by silver nanoparticles (Ag NPs) @ 100 ppm (S_7) (828.5 kg) and zinc oxide nanoparticle**s** (ZnO NPs) @ 100 ppm (S_3) (773.1 kg), respectively. The lowest seed yield per ha (377.7 kg) was exhibited in untreated control (S_9)(Table 3).

The foliar application of biosynthesized nanoparticles showed positive effects on seed yield per ha as compared to untreated control. It is concluded that foliar application of both biosynthesized nanoparticles significantly enhance the seed yield per ha compared to the untreated control.

When ZnO NPs were applied externally to wheat under salt stress, the wheat's fresh root weight, plant height and chlorophyll content are significantly improved and yield increased (Adil *et al.,* 2022). ZnO NPs also improve plant resilience to drought and salt stress by stabilizing photosynthetic pigments, regulating stress-related proteins, antioxidant enzyme activity and nitrogen transport and total nitrogen content. Del *et al.,* found in 2022 that hybrid materials composed of ZnO-containing lignin nanoparticles (ZnO-L NPs) significantly increased the chlorophyll content and antioxidant properties of treated maize seeds.

Conclusions

1. The foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) @ 150 ppm (S_4) at 45, 60 and 75 days after planting of the bulbs, improved the seed production of onion *viz.,* plant height, number of leaves, days to 50 % flowering, number of flowering stalk per bulb at 75 DAP (primary secondary and tertiary), height of flowering stalk at maturity (primary secondary and tertiary), stem thickness of main flowering stalk at 75 DAP, days to maturity, number of seed per umbel (primary secondary and tertiary), seed weight per umbel (primary secondary and tertiary), 1000 seed weight (primary secondary and tertiary), seed yield per plant, seed yield per plot and seed yield per ha followed by biosynthesized silver nanoparticles (Ag NPs) @ 100 ppm (S_7) than untreated control.
2. It was concluded that foliar application of biosynthesized zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs) enhances onion seed quality during seed production.

Table 1 : Effect of biosynthesized zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs) on seed production of onion.

Treatments	Plant height (cm)			Number of leaves			Days to 50 % flowering	Number of flowering stalk per bulb at 75 DAP		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP		Primary flowering stalk per bulb	Secondary flowering stalk per bulb	Tertiary flowering stalk per bulb
S_1(ZnO NPs @ 50 ppm)	47.07	52.53	58.00	29.73	43.00	46.43	86	1.20	3.67	1.67
S_2(ZnO NPs @ 75 ppm)	47.33	54.47	58.53	29.60	45.67	47.60	85	1.13	4.33	1.87
S_3(ZnO NPs @ 100 ppm)	47.60	55.80	58.27	30.53	47.40	52.60	83	1.20	4.40	1.87
S_4(ZnO NPs @ 150 ppm)	49.00	57.87	60.07	31.00	50.87	54.13	80	1.73	5.27	3.20
S_5(Ag NPs @ 50 ppm)	46.67	53.93	57.60	30.13	45.47	47.60	85	1.07	4.27	1.87
S_6(Ag NPs @ 75 ppm)	47.00	54.07	57.60	29.33	48.93	50.37	85	1.13	4.00	2.20

S_7(Ag NPs @ 100 ppm)	48.27	56.93	59.20	30.93	50.17	53.30	81	1.53	4.93	2.87
S_8(Ag NPs @ 150 ppm)	46.40	53.20	56.67	29.20	50.60	52.43	86	1.07	3.47	1.87
S_9(Untreated control)	45.27	48.33	54.87	27.80	38.13	39.93	88	1.00	3.33	1.53
SE(+)	0.284	0.644	0.493	0.514	1.230	0.452	0.66	0.069	0.210	0.174
CD @5%	0.853	1.932	1.480	1.542	3.686	1.36	1.991	0.207	0.629	0.520

NS – Non significant **DAP**- Days after planting of bulbs

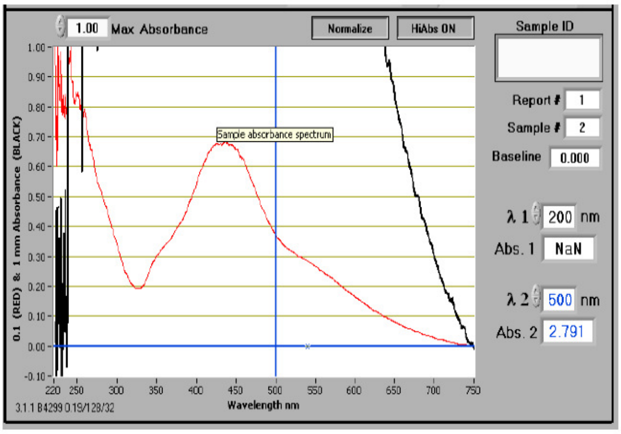
Table 2 : Effect of biosynthesized zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs) on on seed production of onion.

Treatments	Height of flowering stalk at maturity (cm)			Stem thickness of main flowering stalk (cm) at 75 DAP	Diameter of main flowering stalk (cm) at maturity	Days to maturity	Number of seeds per umbel		
	Primary flowering stalk	Secondary flowering stalk	Tertiary flowering stalk				Primary umbel	Secondary umbel	Tertiary umbel
S_1(ZnO NPs @ 50 ppm)	53.53	48.53	38.47	1.46	5.96	144	1248	985	838
S_2(ZnO NPs @ 75 ppm)	54.33	49.60	39.80	1.50	6.13	143	1271	1001	853
S_3(ZnO NPs @ 100 ppm)	54.47	50.67	41.60	1.54	7.15	142	1298	1010	868
S_4(ZnO NPs @ 150 ppm)	57.00	53.67	44.67	1.67	8.15	139	1383	1079	909
S_5(Ag NPs @ 50 ppm)	53.87	48.40	37.73	1.42	6.11	143	1243	967	825
S_6(Ag NPs @ 75 ppm)	53.33	50.00	38.40	1.47	5.91	144	1264	981	840
S_7(Ag NPs @ 100 ppm)	55.53	52.13	42.80	1.57	7.62	141	1324	1026	883
S_8(Ag NPs @ 150 ppm)	52.20	47.80	36.80	1.46	5.81	144	1225	957	814
S_9(Untreated control)	50.53	44.73	34.00	1.33	5.63	148	1012	895	765
SE(+)	0.213	0.391	0.430	0.017	0.081	0.403	19.010	8.410	5.802
CD @5%	0.639	1.172	1.29	0.035	0.242	1.207	56.988	25.213	17.390

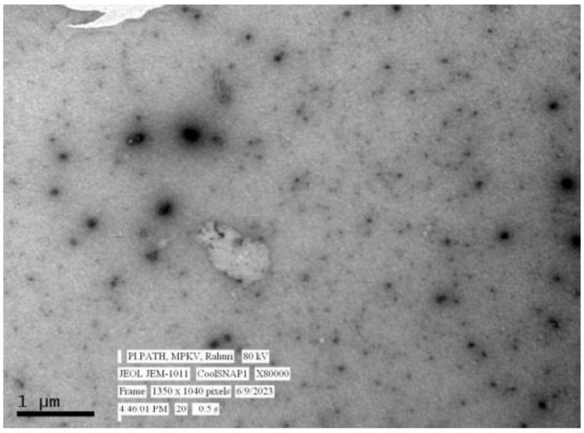
NS – Non significant **DAP**- Days after planting of bulbs *Figures in parenthesis are arcsine transformed values*

Table 3 : Effect of biosynthesized zinc oxide (ZnO NPs) and silver nanoparticles (Ag NPs) on seed production of onion.

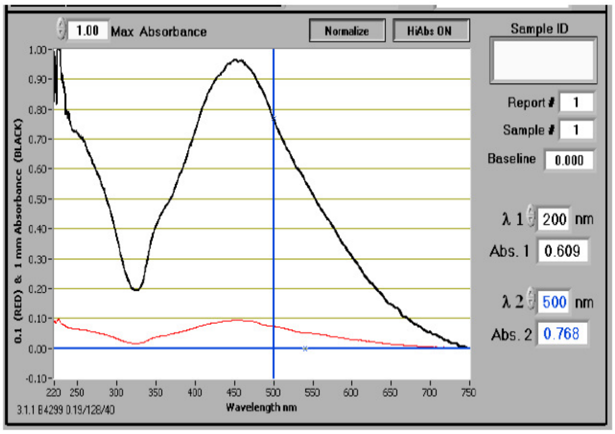
Treatments	Seed weight per umbel (g)			1000 seed weight (g)			Seed yield per plant (g)	Seed yield per plot (g)	Seed yield per ha (kg)
	Primary umbel (g)	Secondary umbel (g)	Tertiary umbel (g)	Primary umbel (g)	Secondary umbel (g)	Tertiary umbel (g)			
S_1(ZnO NPs @ 50 ppm)	4.56	3.79	2.89	3.65	3.85	3.45	10.20	486	460.46
S_2(ZnO NPs @ 75 ppm)	4.71	3.88	2.98	3.70	3.87	3.49	14.99	729	690.66
S_3(ZnO NPs @ 100 ppm)	4.91	4.06	3.08	3.79	4.02	3.55	16.11	816	773.10
S_4(ZnO NPs @ 150 ppm)	5.66	4.77	3.47	4.10	4.41	3.81	18.07	994	941.00
S_5(Ag NPs @ 50 ppm)	4.49	3.70	2.83	3.60	3.82	3.43	13.55	636	602.72
S_6(Ag NPs @ 75 ppm)	4.58	3.78	2.91	3.61	3.85	3.46	14.04	665	629.50
S_7(Ag NPs @ 100 ppm)	5.15	4.29	3.23	3.89	4.18	3.66	16.83	875	828.55
S_8(Ag NPs @ 150 ppm)	4.30	3.60	2.72	3.51	3.76	3.34	9.79	450	426.59
S_9(Untreated control)	3.41	2.91	2.31	3.36	3.25	3.02	9.57	399	377.70
SE(+)	0.070	0.045	0.030	0.030	0.019	0.016	0.176	14.73	13.94
CD @5%	0.211	0.133	0.09	0.0908	0.058	0.047	0.528	44.16	41.81



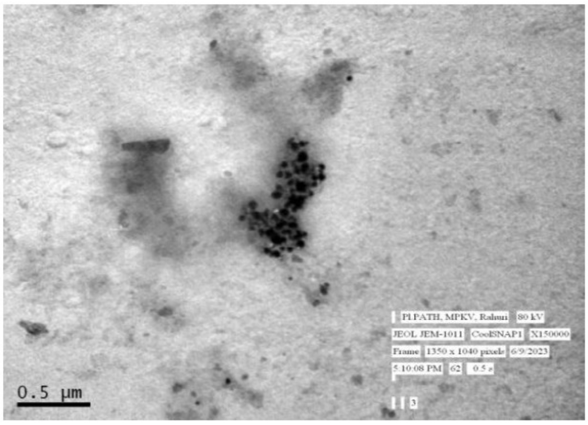
a) Absorbance of biosynthesized zinc oxide nanoparticles (ZnO NPs) at **385 nm**



c) Size of biosynthesised zinc oxide nanoparticles (ZnO NPs) **30-80 nm**



b) Absorbance of biosynthesized silver nanoparticles (Ag NPs) at **450 nm**



d) Size of biosynthesised silver nanoparticles (Ag NPs) = **25-75 nm**

The biosynthesized zinc oxide nanoparticle (ZnO NPs) and silver nanoparticles (Ag NPs) were confirmed by sharp peaks shown by the absorption spectrum in Nanodrop 1000 spectrophotometer (Thermo Scientific) at 385 and 450 nm, respectively at the State Level Biotechnology Centre, MPKV, Rahuri.

Fig. 1 : Characterization of biosynthesized of zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (Ag NPs).

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