Plant Archives Vol. 26, Supplement 2, 2026 pp. 2307-2311 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.289

In vitro EFFICACY OF SILVER AND COPPER NANOPARTICLES AGAINST *Fusarium oxysporum* AND *Macrophomina phaseolina* ASSOCIATED WITH SOYBEAN SEEDS

Ajit D. Lokhande[1*], S.R. Zanjare[2], T.S. Godhavale[1], S. B. Latake[1], A.V. Suryawanshi[3], S.V. Kolase[1] and A.M. Navale[1]

[1]Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India- 413722.
[2]Seed Technology Research Unit, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India- 413722
[3]Seed Research Officer (Seed Pathology), Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India- 413722
*Corresponding author E-mail: ajilokhande234@gmail.com

(Date of Receiving : 18-04-2026; Date of Revision : 30-05-2026; Date of Acceptance : 25-06-2026)

ABSTRACT

Soybean, categorized as an oilseed crop rather than a pulse, is often referred to as the "golden" or "miracle" crop due to its significant contribution to India's oilseed economy. However, seed-borne pathogens pose a major constraint in soybean cultivation by adversely affecting seed germination and crop establishment. These pathogens cause seed rot and seedling mortality, leading to poor plant stand, reduced growth, and ultimately lower productivity. The present investigation was undertaken to evaluate the *in vitro* efficacy of silver and copper nanoparticles against two major seed borne pathogens of soybean, namely *Fusarium oxysporum* and *Macrophomina phaseolina* with the objective of developing a sustainable disease management strategy. The laboratory experiment was conducted using the poisoned food technique under a completely randomized design with three replications. Among the treatments, silver nanoparticles at 100 ppm were found to be the most effective, recording mycelial inhibition of (68.88%) and (65.18%), against *F. oxysporum* and *M. phaseolina*, respectively.

***Keywords*:** Soybean, Nanoparticles, Pathogens, *In vitro*.

Introduction

Soybean (*Glycine max*) is a major global oilseed crop, valued for its high protein (42%), oil content (20%), and health-promoting compounds like isoflavones and omega-3s" (Masuda and Goldsmith 2009). Soybean belongs to the family Fabaceae and grows under warm and moist climate during *kharif* season. More than 100 pathogens have been reported to infect soybean, of which nearly 35 are considered economically significant (Sinclair and Backman, 1989; Gupta, 2001). In most soybean-growing regions, disease incidence results in yield losses ranging from 10 to 30% (Sinclair, 1994). Seed-borne pathogens are responsible for substantial reductions in both seed quality and quantity and adversely affect germination and seedling vigour in soybean. These pathogens are associated with a range of diseases, including seed rot, seedling blight, bacterial pustule, root and stem rot, foliar infections, and pod blight (Agarwal *et al*., 1974).

Seed borne diseases not only affect the standard of the seed but also reduce potential crop production. Annual yield losses due to soybean diseases are estimated at approximately 12% of total production, with fungal pathogens alone accounting for 6–8% losses (Sarbhoy and Vishwadhar, 1992). Soybean serves as a host to numerous seed-borne fungal pathogens, many of which are efficiently transmitted through infected seeds. These pathogens impair seed germination and seedling vigour and are responsible for several important diseases, including anthracnose

(*Colletotrichum truncatum*), Rhizoctonia root rot (*Rhizoctonia solani*), Phomopsis seed decay (*Phomopsis sojae*), charcoal rot (*Macrophomina phaseolina*), Myrothecium leaf spot (*Myrothecium roridum*), Alternaria leaf spot (*Alternaria alternata*), and purple seed stain (*Cercospora kikuchii*) (Pawar *et al*., 2015).

The application of nanoparticles in agriculture has emerged as a promising approach with considerable potential to enhance crop management practices over the long term. Rapid advancements in nanotechnology, particularly involving metal and metal oxide nanoparticles, have been driven by their extensive use across various industrial sectors, making it one of the fastest-growing areas of science and technology. Owing to their extremely small size, nanoparticles possess a high surface area, which leads to significant alterations in their physical, chemical, and biological properties. Their reduced size also allows lower application rates while improving penetration and targeted delivery to specific sites. Nanotechnology has facilitated the development of novel delivery systems and advanced agrochemicals, enabling increased agricultural productivity alongside reduced pesticide usage. The use of nanostructured fertilizers and pesticides offers a means to enhance crop yield efficiency while minimizing reliance on conventional plant protection chemicals. The continuous use of chemical fungicides poses environmental and health concerns, necessitating the development of eco-friendly alternatives. The present study aimed to evaluate the *in vitro* antifungal activity of green synthesized nanoparticles against two major seed-borne pathogens of soybean.

Material and Methods

The laboratory experiments for the present investigation were conducted in the Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. The efficacy of silver and copper nanoparticles were evaluated under *in vitro* at concentrations of 25, 50, 75 and 100 ppm by using poisoned food technique (Sukhwal *et al.*, 2014). Double strength PDA medium was prepared by doubling the amount of constituents except distilled water and the medium was sterilized at 15-20 psi pressure for 20 minutes. Simultaneously, different concentrations of silver and copper nanoparticles were also prepared in sterilized distilled water to get desired concentration of AgNPs and CuNPs after mixing the AgNPs and CuNPs solutions in the double strength media in the 250 ml flask. The PDA added with the AgNPs and CuNPs were shaken well to mix thoroughly and then poured in the Petri plates and allowed to solidify. A small culture bit of 4 mm size of the test pathogen cut with a sterile cork borer was picked up with the help of sterilized inoculation needle and placed in the center of each Petri plate under aseptic conditions in the laminar air flow. Petri plates poured with double strength PDA and inoculated with the pathogen served as control. Each concentration of silver and copper nanoparticles were replicated three times under Completely Randomized Design (CRD) and Petri plates were incubated at 27 ± 10^0 C for a period of 7 days after which growth of mycelium was measured and per cent inhibition calculated by formula given by Vincent (1947).

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Growth of the test fungus in untreated control plates.

T = Growth of the test fungus in treated plates

Experimental Details:

Treatments : Nine (9)

Replication : Three (03)

Design : Completely Randomize Design (CRD)

Treatment details:

T_1 : Silver Nanoparticles (AgNPs) @ 25 ppm
T_2 : Silver Nanoparticles (AgNPs) @ 50 ppm
T_3 : Silver Nanoparticles (AgNPs) @ 75 ppm
T_4 : Silver Nanoparticles (AgNPs) @ 100 ppm
T_5 : Copper nanoparticles (CuNPs) @ 25 ppm
T_6 : Copper nanoparticles (CuNPs) @ 50 ppm
T_7 : Copper nanoparticles (CuNPs) @ 75 ppm
T_8 : Copper nanoparticles (CuNPs) @ 100 ppm
T_9 : Control

Results and Discussion

In vitro efficacy of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on radial growth of *Fusarium oxysporum*

The *in vitro* efficacy of green synthesized silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) revealed a significant inhibitory effect on mycelial growth of the *Fusarium oxysporum* (Table 1 Figure 1 and Plate 1).

Table 1: *In vitro* efficacy of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on radial growth of *Fusarium oxysporum*

Tr. No.	Treatments	Mean colony diameter (mm)	Per cent growth inhibition
T_1	AgNPs @ 25 ppm	63.33	29.63
T_2	AgNPs @ 50 ppm	54.67	39.25

T_3	AgNPs @ 75 ppm	43.67	51.47
T_4	AgNPs @ 100 ppm	28.00	68.88
T_5	CuNPs @ 25 ppm	67.00	25.55
T_6	CuNPs @ 50 ppm	57.33	36.30
T_7	CuNPs @ 75 ppm	47.33	47.41
T_8	CuNPs @ 100 ppm	34.00	62.22
T_9	Control	90.00	0.00
	S.E(m) ±	0.57	-
	CD @ 1 %	2.31	-

The result revealed that all nanoparticles' treatments resulted in a statistically significant reduction in mean colony diameter, indicating a dose-dependent response. Among the silver nanoparticle treatments, AgNPs @ 100 ppm (T_4) recorded the minimum mean colony diameter (28.00 mm) and the maximum per cent growth inhibition (68.88%), which was significantly superior to all other treatments. This was followed by AgNPs @ 75 ppm (T_3) with a mean colony diameter of 43.67 mm and 51.47% growth inhibition, and AgNPs @ 50 ppm (T_2) showing 39.25% inhibition. The lowest inhibition among AgNP treatments was observed at 25 ppm (T_1) with 29.63% growth inhibition.

In the copper nanoparticle treatments, CuNPs @ 100 ppm (T_8) exhibited the highest antifungal activity with a mean colony diameter of 34.00 mm and 62.22% growth inhibition, followed by CuNPs @ 75 ppm (T_7) and CuNPs @ 50 ppm (T_6), which recorded 47.41% and 36.30% growth inhibition, respectively. The least inhibition among CuNP treatments was observed at 25 ppm (T_5) with 25.55% growth inhibition. The control treatment (T_9) recorded the maximum colony diameter (90.00 mm) with no growth inhibition, confirming the efficacy of nanoparticle treatments.

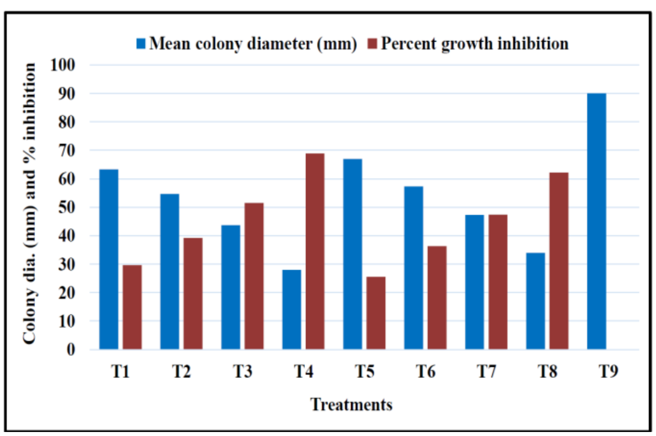

Fig. 1: Effects of silver and copper nanoparticles against *Fusarium oxysporum*

Plate 1: Effects of silver and copper nanoparticles against *Fusarium oxysporum*

In vitro efficacy of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on radial growth of *Macrophomina phaseolina*

The efficacy of green synthesized silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) against *Macrophomina phaseolina* was assessed under *in vitro* conditions. The result is presented in Table 2 Plate 2 and Figure 2. Results revealed that all nanoparticle treatments exhibited a statistically significant reduction in mean colony diameter, indicating a concentration-dependent inhibitory effect on fungal growth. Among the silver nanoparticle treatments, AgNPs @ 100 ppm (T_4) recorded the minimum mean colony diameter of 31.33 mm with the maximum per cent growth inhibition of 65.18%, and was found to be statistically superior over all other treatments. This was followed by AgNPs @ 75 ppm (T_3), which resulted in a colony diameter of 46.00 mm and 48.00% growth inhibition, and AgNPs @ 50 ppm (T_2) with 36.66% inhibition. The lowest inhibition among AgNP treatments was observed at 25 ppm (T_1), recording 27.03% growth inhibition.

In the copper nanoparticle treatments, CuNPs @ 100 ppm (T_8) was the most effective treatment, recording a mean colony diameter of 36.00 mm and 60.00% growth inhibition, followed by CuNPs @ 75 ppm (T_7) and CuNPs @ 50 ppm (T_6) with 44.81% and 31.47% growth inhibition, respectively. The least inhibitory effect among CuNP treatments was observed at 25 ppm (T_5), which recorded 21.47% growth inhibition.

Table 2: *In vitro* efficacy of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) on radial growth of *Macrophomina phaseolina*

Tr. No.	Treatments	Mean colony diameter (mm)	Per cent growth inhibition
T_1	AgNPs @ 25 ppm	65.67	27.03
T_2	AgNPs @ 50 ppm	57.00	36.66
T_3	AgNPs @ 75 ppm	46.00	48.00
T_4	AgNPs @ 100 ppm	31.33	65.18
T_5	CuNPs @ 25 ppm	70.67	21.47
T_6	CuNPs @ 50 ppm	61.67	31.47
T_7	CuNPs @ 75 ppm	49.67	44.81
T_8	CuNPs @ 100 ppm	36.00	60.00
T_9	Control	90.00	0.00
	S.E(m) ±	0.42	-
	CD @ 1 %	1.69	-

Several researchers have demonstrated that silver and copper nanoparticles are highly effective in suppressing plant pathogenic fungi, and their activity increases with concentration. Kim *et al*. (2012) who reported that silver nanoparticles applied at 10–100 ppm were effective against eighteen different plant pathogenic fungi, with the highest inhibition observed at 100 ppm, particularly on potato dextrose agar. Similarly, Teimoori *et al*. (2017) also reported antifungal activity of silver nanoparticles synthesized using *Amaranthus retroflexus* leaf extract, demonstrating the potential of green synthesis approaches in plant disease management. The antifungal activity of the biosynthesized AgNPs was assessed using a modified poisoned food technique, wherein different concentrations of AgNPs (50, 100, 200 and 400 mg ml^{-1}) were incorporated into autoclaved culture media prior to solidification. This method ensured uniform dispersion of nanoparticles within the growth medium and allowed direct interaction between the fungal mycelium and AgNPs. The results revealed a significant inhibition of mycelial growth of *Macrophomina phaseolina*, *Alternaria alternata* and *Fusarium oxysporum*, with the degree of inhibition increasing progressively with increasing AgNP concentration. This dose-dependent response clearly indicates that higher concentrations of AgNPs exert stronger antifungal effects, likely due to increased availability of active nanoparticle surfaces interacting with fungal cells.

More recently, Ashraf *et al*. (2025) investigate the antifungal properties of silver nanoparticles at various concentrations in controlling *Fusarium* wilt for tomato crop improvement under laboratory, greenhouse, and field conditions and reported that antifungal activity of AgNPs against *Fusarium oxysporum*, with 80.9–95.4% inhibition at 150 µg/mL under laboratory conditions. Overall, these studies clearly indicate that both silver and copper nanoparticles exhibit strong, concentration dependent antifungal activity and can effectively control the fungal pathogens.

Some other researcher also finding similar findings such as, Elumalai and Vinothkumar (2013), Shende *et al*. (2016) and Shikha *et al*. (2023).

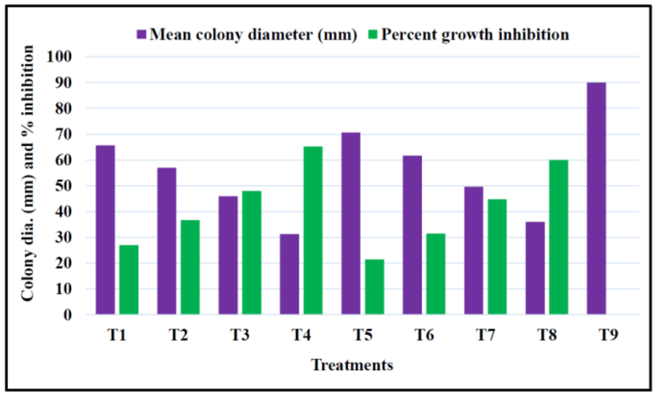

Fig. 2: Effect of silver and copper nanoparticles against *Macrophomina phaseolina*

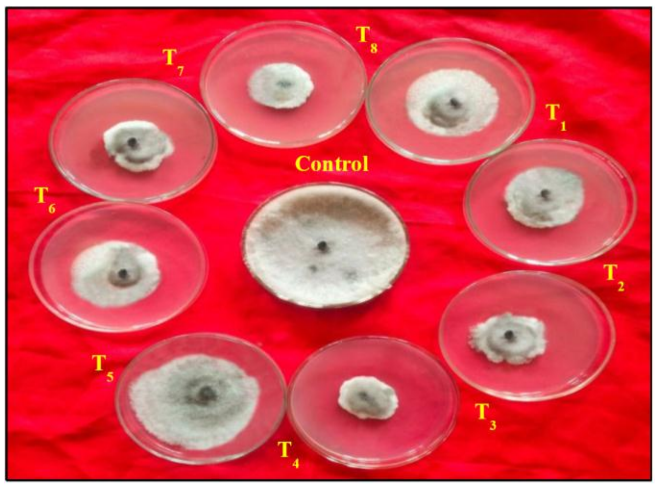

Plate 2: Effects of silver and copper nanoparticles on *Macrophomina phaseolin*

Conclusion

The antifungal activity of silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) were evaluated under *in vitro* conditions against two major seed borne pathogens of soybean. The result revealed that both nanoparticles significantly inhibited mycelial growth in a dose-dependent manner. Among the treatments silver nanoparticles at 100 ppm exhibited slightly greater inhibitory activity as compared to copper nanoparticles.

Acknowledgement

The authors sincerely acknowledge the Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India, for providing the necessary facilities to conduct this research. Deep gratitude is extended to the research guide and colleagues for their invaluable encouragement, guidance, and constructive suggestions throughout the course of this work. The financial support provided by the BARTI Institute is gratefully acknowledged.

References

Agarwal, V. K., Singh, O. V., and Nene, Y. L. (1974). Influence of fungicidal seed treatment on the mycoflora of stored seed and seedling emergence. *Indian Journal of Agriculture Science.* **43**(8), 820-824.

Ashraf, H., Anjum, T., Ahmad, I. S., Ahmed, R., Aftab, Z. and Rizwana, H. (2025). Phyto fabricated silver nanoparticles unlock new potential in tomato plants by combating wilt infection and enhancing plant growth. *Scientific Reports*. **15**, 10527.

Elumalai, E. K and Vinothkumar, P. (2013). Role of silver nanoparticles against plant pathogens. *Nano Biomedical Engineering*. **5**(2), 90-93.

Gupta, A. (2001). Role of seed mycoflora in determination of soybean during storage under ambient conditions. *Indian Phytopathology*. **54**, 503.

Kim, S. W., Jung, J. H., Lamsal, K., Kim, Y. S., Min, J. S. and Lee, Y. S. (2012). Antifungal Effects of Silver Nanoparticles (AgNPs) against Various Plant Pathogenic Fungi. *Mycobiology.* **40**(1), 53-58.

Masuda, T. and Goldsmith, P. D. (2009). World soybean production, Area harvested, yield, and long-term projections. *International Food and Agribusiness Management Review*. **12**(4), 143–161.

Pawar, K., Mishra, S. P. and Singh, R. K. (2015). Efficacy of bioagents and fungicides against seedborne fungi of soybean. *Annals of Plant and Soil Research*. **17**(1), 77-81.

Sarbhoy, A. K. and Vishwadhar, P. (1992). Soybean rust in India and its management, farming system. (Eds. J.P. Verma and A. Verma). India, Malhotra Publishing House, New Delhi-64. 117-120.

Shende, S., Gaikwad, N. and Bansod, S. (2016). Synthesis and evaluation of antimicrobial potential of copper nanoparticles against agriculturally important phytopathogens. *Synthesis.* **1**(4), 41-47.

Shikha, S., Sharma, G. and Kumari, K. (2023). *In vitro* Evaluation of the Antifungal Effect of Nanoparticles against *Fusarium sacchari* causing Pokkah Boeng Disease of Sugarcane. *Biological Forum – An International Journal.* **15**(5), 647-650.

Sinclair, J. B. 1994. Reducing losses from plant diseases. World Soybean Research Conference V. Abstracts. 10.

Sinclair, J. B. and Backman, P. A. (1989). Compendium of Soybean Diseases (3rd Edn.) St. Paul, Minnesota, USA, *American Phytopathological Society*. 106.

Sukhwal, A., Joshi, A., Rawal, P. and Jain, D. (2014). Assessment of antimicrobial activity of biogenic silver nanoparticles against plant pathogens. *Journal of Pure and Applied Microbiology*. **8**(6), 4593- 4600.

Teimoori, B. B., Nikparast, Y., Hojatianfar, M., Akhlaghi, M., Ghorbani, R. and Pourianfar, H. R. (2017). Characterization and antifungal activity of silver nanoparticles biologically synthesized by *Amaranthus retroflexus* leaf extract. *Journal of Experimental Nanoscience***.** **12**(1), 129-139.

Vincent, J. H. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature. **150**, 850.