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# Inhibitory Effect of Nano-fungicides and Fungicides on Mycelia Radial Growth of *Phytopthora infestans* under *in-vitro* condition

***Gajendra Pratap, Samir Kumar Biswas, Bipin Verma* and Naimish Kumar***
*Department of Plant Pathology,*
*Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (Uttar Pradesh), India.*

*(Corresponding author: Bipin Verma*)*
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**ABSTRACT: *Phytophthora infestans* (Mont) de Barry, causing Late blight of potato is one of the most devastating diseases of potato worldwide. The disease is distributed all over the world like North & South America, Europe, Asian continents. *P. infestans* is a destructive pathogen that is causing huge losses to potatoes in hilly as well as plains regions of India and can cause yield losses up to 95% in epidemic conditions. The present study is carried out to check inhibitory effect of nanofungicides and fungicides against *Phytophthora infestans*. Among nanofungicides, silver nanofungicide @ 100 ppm was found highly effective and inhibit the growth by75.70% followed by agritecnanofungicide (68.81%) and selenium nanofungicide (66.67%) during 2021-22 and silver nanofungicide with 72.25% followed by agritecnanofungicide (63.82%) and selenium nanofungicide (61.58%) during 2022-23. Among fungicides, maximum inhibition (80.25%) was observed in matco @ 0.2% followed by mancozeb (73.72%) and propiconazole (71.01%) during 2021-22 and matco with 75.95% followed by mancozeb (68.97%) and propiconazole (66.13%) during 2022-23 under *in vitro* condition.**

**Keywords:** Fungicide, Late blight, Radial mycelial growth and Growth inhibition.

## INTRODUCTION

Potato (*Solanum tuberosum* L.) popularly known as the "King of Vegetables" is a starchy, tuberous crop, belongs to family Solanaceae. The English word "Potato" comes from Spanish word "patata" (the name used in Spain). Food and Agriculture Organization of UN has declared potato as 'Food for future' (Hawkes, 1989). It is the world's fourth-largest food crop following rice, wheat and maize. United Nations (UN) declared the year 2008 as the *International Year of Potato* (IYP) in order to increase awareness about the importance of the potato in addressing issues of global concern, including hunger, poverty and threats to the environment. It is an annual herbaceous dicotyledonous plant. Potatoes play an important role in the daily human diet because having a copious source of minerals, vitamins, antioxidants and micronutrients (Ulger *et al*., 2018).

Potato also known as Alu and basically is a temperate crop that is grown under sub-tropical and tropical conditions in India. The potato crop is grown in almost all states of India, except Kerala, under a wide range of agro-climatic conditions (Kumar and Chandra 2018). Out of 100 % total area of potatoes, 90 % area is located in subtropical plains, 6 % in the hills and the rest 4 % located in the plate auregion of peninsular India (Chadha, 2009).

It was probably the Portuguese who brought the potato to India, the first mention of potato in India occurs in 'Terry's account of a banquet at Ajmer given by Asaph Khan to Sir Thomas Rao in 1615. The potato was grown in many gardens of Surat and Karnataka in about 1675 (Sharma, 2008; Singh and Rana 2014).

The potato is a basically temperate crop, but it grows under a wide range of climatic conditions (Haverkort and Verhagen 2008). The vegetative growth of the potato plant is best at a temperature of 24ºC. The most cultivated cultivars of potato higher tuber development in cool climates with night temperatures below 20°C and lower tuber formation occur at night temperatures more than 20°C (Malkawi, 2006). The soil pH for potato crops is ranged from 5.2 to 6.4 and is considered to be the best (Marques *et al*., 2021).

In India, potatoes are the most popular vegetable crop. Currently, 68% of domestic potato supply is consumed fresh, 7.5% is processed, 8.5% is used as seed, and 16% is lost for various reasons. In India, potatoes are used to brew alcoholic beverages like vodka, poitín, and akvavit. Worldwide, potatoes are used in various dishes, especially in India, where they are used for Aloo tikki, Batata vada, Bonda, Dabeli, Samosa, Aloo gosht, and Ragdapattice. Potato starch is used in the food industry as a thickener and binder for soups and sauces, and in the textile industry as an adhesive. Additionally, potatoes are used as fodder for livestock (Gopal and Khurana 2006).

Potato crop are generally affected by different types of biotic and abiotic diseases. Biotic diseases are mainly caused by a detrimental microorganism such as fungi, bacteria and viruses (Ashraf *et al*., 2012), nematodes and mycoplasma which adversely affected the potato quality as well as production in almost all over the world (Gul *et al*., 2013).

In India, late blight disease leads to varying losses, with approximately 10-20% in Uttar Pradesh, 10-15% in West Bengal and Punjab, and similarly 10-15% in Karnataka and Uttarakhand, as reported for the year 2013-14 by Lal *et al.* 2016. However, the extent of these losses is influenced by factors such as the potato variety cultivated and the protective measures adopted by farmers. *Phytophthora infestans* (Mont.) de Bary, a highly destructive pathogen, poses a significant threat to potato crops in both hilly and plains regions of India. Under epidemic conditions, it can result in staggering yield losses of up to 95%, as noted by Nagar *et al.* (2015). It can lead to complete tuber losses in susceptible varieties and significant crop losses, resulting in substantial crop protection expenses (Henfling, 1987).

Late blight epidemics are influenced by diverse pathogen genotypes and varying winter temperatures that promote pathogen survival. These epidemics depend on the presence of pathogens (sporangia, zoospores, mycelia, or oospores), favorable environmental conditions, and susceptible hosts during the crop season (Erwin and Ribeiro 1996).

The meteorological factors like: An optimum temperature (15-22°C), relative humidity in excess of (90%), and rainfall (irrigation) or leaf wetness duration are known to favor blight development (Krause *et al*., 1975).

The management of the disease can be done through cultural, mechanical, use of resistant variety, biological and chemical etc. In contrast to conventional application of fungicides antibiotics, nanoparticles are most important strategy to manage plant diseases. The use of silver nanoparticles as an alternative to chemical pesticides could make crop production more economical. Nano-biotechnology has emerged as one of the fastest growing modern areas of research in materials science and technology.

To assess the antimicrobial effectiveness, researchers conducted tests using colloidal silver particles against *Phytophthora infestans*, the most potent pathogen responsible for late blight disease in potatoes. While a wide range of chemicals and pesticides are available in the market for managing late blight, these options are often either less efficient or associated with significant environmental hazards due to residue buildup. Seeking an alternative to chemical fungicides, scientists have developed new molecules, specifically colloidal silver particles. These particles contain a lower concentration of ionic silver and fall within the size range of 100 to 1000 nm. Notably, these particles have demonstrated inhibitory effects on various fungal plant pathogens while maintaining safety for both human beings and the environment.

## MATERIAL AND METHODS

**Experimental site.** The experiments were conducted in the laboratory of the Department of Plant Pathology at C.S.A. University of Agriculture and Technology in Kanpur. These experiments were conducted over the period spanning 2021 to 2023.

**Isolation and purification of the pathogen.** In the investigation of late blight infection, late blight-infected leaves were carefully cut to include both affected and healthy portions. These leaf sections were then surface-sterilized by brief immersion in 70% alcohol. After the sterilization process, the leaf fragments underwent two rinses with sterilized water and were thoroughly dried using blotter paper. Medium-sized tubers, selected for their absence of rot, severe damage, or green discoloration, were chosen for the experiment. These tubers also underwent surface sterilization using 70% alcohol. A small incision was made on each tuber using a sterilized knife, and the previously prepared leaf fragments were inserted into the tuber. Subsequently, the tubers were incubated at a temperature ranging from 15 to 18°C for one week. Following the incubation period, they were examined under a microscope to identify any signs of sporulation. The white mycelial bits of *P. infestans* was removed from the margin of fungal colony and then transferred to another Petri-plate which was previously poured with sterilized tomato extract based medium. After purification, the pure culture of *P. infestans* was transferred on slant medium and incubated at 15-18°C in darkness till full growth. The culture was then transferred into the incubator at 10-12°C for further use.

**Identification of the pathogen.** The isolated pathogen was identified on the basis of its morphological, cultural characters and pathogenic behaviour towards the host. *P. infestans* belong to the class Oomycetes. The vegetation is mycelium characterized by the absence of cross walls, alongwith both a sexual and sexualre production occurs. The sporangiophores and sporangia emergeat as exualre production phase. The sporangia are lemon shape, measurement of 21-38µm×12-23µm. Sporangia develop at the end of these sporangiophores. The pathogen was found to produce the characteristics leaf blight symptoms on the affected plants. The isolation pathogen was identified on thebasisofits morphological and cultural characters and pathogenic behaviour towards the host.

**Maintenance of the culture.** After confirmation of isolated pathogen as *P. infestans*, the pure culture was transferred on media slant and maintain in the BOD at 10 - 12°C for further study.

**Comparative evaluation of different fungicides and nano fungicides on radial mycelial growth of *Phytophthora infestans***

The Efficacy of four suitable fungicides (Matco @ 0.2%, Metalaxyle @ 0.2%, Propiconazole @ 0.2% and Mancozeb @ 0.2%,) and four suitable nano-fungicides (Nickel 100 ppm, Agritec 100 ppm, Selenium 100 ppm and Silver 100 ppm) on the growth of *P. infestans* was evaluated using Poison Food Technique (Schmitz,

1930). First of all, a stock solution of 1000 ppm of each fungicide was made. The desired concentrations were obtained by adding the appropriate amount of stock solution of fungicides to tomato dextrose agar (TDA) medium in a separate flask and thoroughly mixed them by shaking prior to pouring in sterilized Petri plates. The medium was allowed to solidify and then 5 mm bits of fungal culture from seven days old culture of *P. infestans* were placed at the centre of Petri plates. The fungal disc was reversed so that the pathogen could come in direct contact with the medium. Three replications were kept for each treatment. The inoculated Petri plates were sealed with paraffin wax strips and incubated at 18±2°C in B.O.D. incubator. One set of control was maintained in which the medium was not mixed with any fungicide but simply inoculated with the pathogen. The data on radial growth of the fungal colony was measured in mm after seven days. Per cent inhibition of growth will be calculated by following formula as given by Horsfall (1956).

$$I = \frac{C - T}{C} \times 100$$

where,
I = Per cent growth inhibition
C = Growth of fungus in control (mm)
T = Growth of fungus in treatment (mm)
Treatments were as follows: $T_1$-Matco @ 0.2%, $T_2$-Metalaxyle @ 0.2%, $T_3$ Mancozeb @ 0.2%, $T_4$ Propiconazole @ 0.2%, $T_5$- Agritec 100 ppm, $T_6$-, Selenium 100@ ppm, $T_7$ Nickel @100 ppm, $T_8$ Silver @100 ppm, $T_9$ Control

**Statistical analysis.** Completely Randomized Design (CRD) used for the experiments will be conducted in laboratory in Petri plates. The data will be statistically analysis by means of critical difference (CD) at five per cent level of significance.

## RESULTS AND DISCUSSION

***In vitro* impact of fungicides and nano fungicides on radial mycelial growth of *Phytophthora infestans.***

The efficacy of the suitable concentration of four nano fungicides (Nickel 100 ppm, Agriteck 100 ppm, Selenium 100 ppm and Silver 100 ppm) and four fungicides ( Matco @ 0.2%, Metalaxyl @ 0.2%, Propiconazole @ 0.2%, and mancozeb @ 0.2%,) against *P. infestans* (Table 1, Fig. 1 and 2) and the result showed that among the nanofungicide, the minimum radial growth of mycelium was found in Silver nanofungicide @ 100 ppm with (75.70 and 72.25) per cent inhibition was observed in treatment and it was followed by treatment Selenium nano fungicide @ 100 ppm with (68.81 and 63.82%) and among four fungicides tested, Matco @ 0.2 % was found most effective with significantly least mycelial growth of the test pathogen (80.25 and 75.95) per cent inhibition followed by Mancozeb @ 0.2 % (73.72 and 68.97) ) per cent inhibition during 2021-22 and 2022-23, respectively. Propiconazole @ 0.2 % was found comparatively less effective with minimum mycelial growth inhibition (71.01 and 66.13) per cent. Similar findings were also reported by several workers against several pathogenic fungi (Kone *et al.*, 2009; Alex 2019; Ziv and Zitter 1992). The bicarbonates salts have been shown to have a profound inhibitory effect on several fungi and causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan 1982; Abd-El-Kareem and Abd-El-Latif Faten 2012). Fungicides of different formulations of inorganic and organic materials which have the potentials of growth inhibition, killing of zoosporangia/zoospores and mycelium of the causative organism (Song *et al.*, 2003). Results also showed that the most significant inhibition of plant pathogenic fungi was observed on PDA and 100 ppm of AgNPs (Amardeep and Gyanika 2021; Ashok Somalraju *et al.*, 2021). The results are in agreement with the finding of Shantamma *et al*. (2022) Tested the efficacy of synthesized colloidal silver particles under *in vitro* conditions (exhibited minimum mycelium growth inhibition 44.57 % at 100 ppm concentration. The growth of *Alternaria alternata*, *Penicillium digitatum* and *Alternaria citri*, was greatly suppressed by silver nanoparticles in a concentration dependent way and the inhibitory 73.33%, 66.66% and 68.88% effect were recorded concentration of 100 ppm (Abdelmalek and Salaheldin 2016).

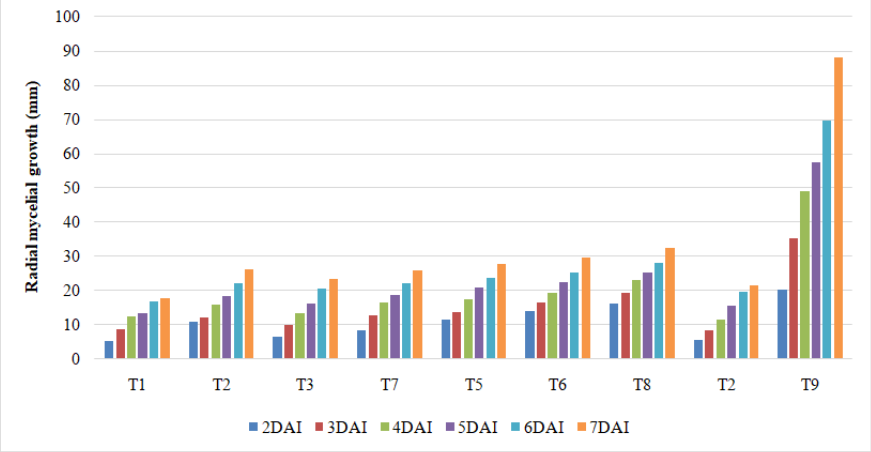


**Fig. 1.** Comparative effect of nano-fungicides and fungicides on radial mycelial growth (mm) of *Phytophthora infestans* during 2021-22.

**Table 1: Comparative effect of nano-fungicides and fungicides on radial mycelial growth (mm) of *Phytophthora infestans* during 2021-22.**

| Sr. No. | Treatment | Radial mycelia growth(mm) | | | | | | Per cent inhibition over control |
|---|---|---|---|---|---|---|---|---|
| | | 2DAI | 3DAI | 4DAI | 5DAI | 6DAI | 7DAI | |
| $T_1$ | Matco @ 0.2 % | 05.10 | 08.60 | 12.30 | 13.20 | 16.70 | 17.47 | 80.25 |
| $T_2$ | Metalaxyle @ 0.2 % | 10.68 | 12.05 | 15.72 | 18.34 | 21.92 | 26.20 | 70.53 |
| $T_3$ | Mancozeb @ 0.2 % | 06.40 | 09.60 | 13.10 | 16.10 | 20.50 | 23.25 | 73.72 |
| $T_7$ | Propiconazole @ 0.2 % | 08.10 | 12.50 | 16.40 | 18.70 | 22.10 | 25.65 | 71.01 |
| $T_5$ | Agritecnanofungicide @ 100 ppm | 11.43 | 13.52 | 17.35 | 20.61 | 23.70 | 27.60 | 68.81 |
| $T_6$ | Selenium nanofungicide @ 100 ppm | 13.82 | 16.27 | 19.20 | 22.48 | 25.30 | 29.50 | 66.67 |
| $T_8$ | Nickel nanofungicide @ 100 ppm | 16.00 | 19.13 | 22.86 | 25.26 | 27.86 | 32.50 | 63.27 |
| $T_2$ | Silver nanofungicide @ 100 ppm | 05.30 | 08.20 | 11.40 | 15.50 | 19.35 | 21.50 | 75.70 |
| $T_9$ | Control | 20.10 | 35.10 | 48.90 | 57.50 | 69.90 | 88.50 | - |
| | **CD** | 1.127 | 1.793 | 2.390 | 2.812 | 3.403 | 2.275 | - |
| | **SE (m)±** | 0.376 | 0.599 | 0.798 | 0.939 | 1.136 | 0.760 | - |

**Table 2: Comparative effect of different nano-fungicides and fungicides on radial mycelial growth (mm) of *Phytophthora infestans* during 2022-23.**

| Sr. No. | Treatment | Radial mycelia growth(mm) | | | | | | Per cent inhibition over control |
|---|---|---|---|---|---|---|---|---|
| | | 2DAI | 3DAI | 4DAI | 5DAI | 6DAI | 7DAI | |
| $T_1$ | Matco @ 0.2 % | 5.88 | 7.6 | 9.3 | 12.5 | 15.35 | 20.50 | 75.95 |
| $T_2$ | Metalaxyle @ 0.2 % | 8.68 | 13.05 | 15.72 | 18.80 | 23.92 | 29.20 | 65.48 |
| $T_3$ | Mancozeb @ 0.2 % | 7.48 | 10.8 | 13.4 | 16.10 | 19.50 | 26.25 | 68.97 |
| $T_4$ | Propiconazole @ 0.2 % | 8.15 | 9.5 | 13.4 | 16.70 | 20.10 | 28.65 | 66.13 |
| $T_5$ | Agritecnanofungicide @ 100 ppm | 9.43 | 12.52 | 16.35 | 19.61 | 24.70 | 30.60 | 63.82 |
| $T_6$ | Selenium nanofungicide @ 100 ppm | 10.82 | 14.27 | 19.50 | 22.48 | 25.30 | 32.50 | 61.58 |
| $T_7$ | Nickel nanofungicide @ 100 ppm | 14.97 | 17.13 | 20.86 | 24.26 | 28.86 | 35.50 | 58.03 |
| $T_8$ | Silver nanofungicide @ 100 ppm | 6.32 | 9.2 | 12.4 | 15.80 | 19.70 | 23.47 | 72.25 |
| $T_9$ | Control | 28.1 | 42.1 | 58.9 | 67.5 | 79.10 | 84.60 | - |
| **CD** | | 1..385 | 1.946 | 2.681 | 3.118 | 3.685 | 2.650 | - |
| **SE (m)** | | 0.463 | 0.650 | 0.895 | 1.041 | 1.231 | 0.885 | - |

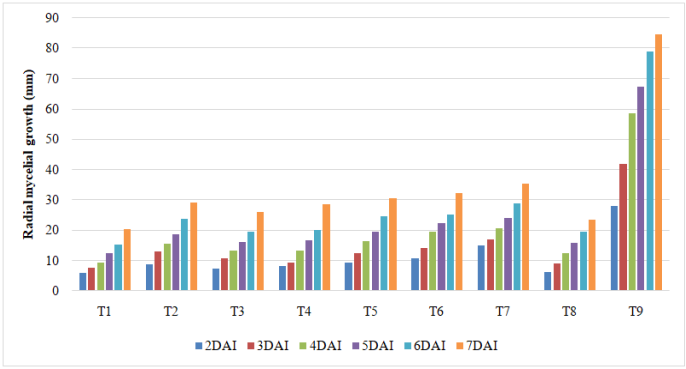


**Fig. 2.** Comparative effect of different nano-fungicides and fungicides on radial mycelial growth (mm) of *Phytophthora infestans* during 2022-23.

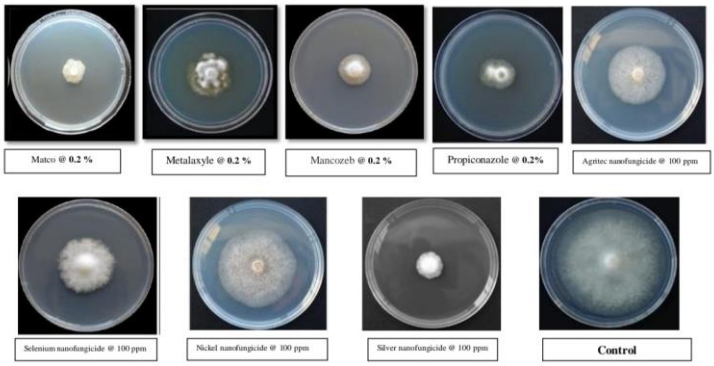


**Plate 1.** Comparative effect of nano-fungicides and fungicides on radial mycelial growth (mm) of *Phytophthora infestans.*

## CONCLUSIONS

The data presented in Tables 1 and 2 indicated that both conventional fungicides and nano-fungicides significantly inhibited the radial growth of the *P. infestans* pathogen compared to the control. Among the conventional fungicides, Matco at 0.2% demonstrated the most effective inhibition, with 80.25% and 75.95% reductions in mycelial growth during 2021-22 and 2022-23, respectively. Among the tested nano-fungicides, Silver at 100 ppm was the most effective, achieving significant mycelial growth inhibition of 75.70% and 72.25% during the both years, respectively. All the nano fungicides and fungicides evaluated significantly control the disease. So, they can be used to manage the disease under natural field condition. Nanofungicides may be applied widely and safely instead of using the commercially available synthetic fungicides, which show higher toxicity to humans.

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**Conflicts of Interest**. None.

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