Integrative
Plant Biotechnology
ISSN: 3007-2735 (Online), 3007-2727 (Print)

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Research Article

Application of Silver Nanoparticles and Fungicides for *in vitro* Suppression of *Pestalotiopsis psidii*, a Pathogen Associated with Guava Stem Cracking

Ameer Hamza Aslam[†1], Usman Ahmad[†1], Rabia Irshad[1], Muhammad Faheem Khan[2], Zia Ullah Ashraf[1], Muhammad Usman[1], Abdul Muhaimin Nadeem[1], Amjad Abbas[1*]

[1]*Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.*
[2]*National Seed Research and Training Center, University of Agriculture, Faisalabad, Pakistan.*
[†]*These authors have equal contributions.*

ABSTRACT

Guava (*Psidium guajava* L) is a widely cultivated fruit plant in tropical and subtropical countries, including Pakistan, where it is attacked by numerous pathogenic fungi. Guava stem cracking is caused by a destructive disease that significantly affects the stem and other parts of the plant. This study was conducted to isolate the causative pathogen of stem cracking and its management with chemicals and silver nanoparticles (AgNPs). Samples were obtained from seven different locations of Punjab, Pakistan, and the pathogen was isolated and purified on PDA using the hyphal tip technique. After the confirmation of pathogenicity, spore suspension was plated in a 96-well plate for chemical evaluation and a 9-day-old culture was used to find the AgNPs effect by the agar dilution method. Antifungal activity was measured in terms of optical density (OD) for chemicals and percent growth inhibition (PGI) for AgNPs. Four fungicides (Mancozeb, Iprodione, Chlorothalonil and Deconil) were evaluated at 7 different concentrations (50mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml, 1.56 mg/ml and 0.78 mg/ml) against *P. psidii* and the maximum antifungal effect was seen at the concentration of 50mg/ml. *In vitro* evaluation of fungicides showed Iprodione maximum spore growth inhibition against *P. psidii*. Silver nanoparticles were used to analyze their antifungal potential at different concentrations (1 ppm, 10 ppm, 100 ppm), whereas the maximum inhibition zone was seen at 100 ppm of AgNPs. Data was statistically analyzed by using factorial design under Complete Randomized Design (CRD). The outcomes of the present study revealed that using chemicals and silver nanoparticles can help to manage guava stem canker.

Keywords: Guava, Stem cracking, *Pestalotiopsis psidii,* Survey, Fungicides, Silver nanoparticles

OPEN ACCESS

***Correspondence**
Amjad Abbas
amjad.abbas@uaf.edu.pk

Article History
Received: April 29, 2025
Accepted: May 20, 2025
Published Online: May 23, 2025

Cite this article
Aslam, A. H., Ahmad, U., Irshad, R., Khan, M. F., Ashraf, Z. U., Usman, M., Nadeem, A. M., & Abbas, A. (2025). Application of silver nanoparticles and fungicides for in vitro suppression of Pestalotiopsis psidii, a pathogen associated with guava stem cracking. Integrative Plant Biotechnology, 3, 125–134.

INTRODUCTION

Guava (*Psidium guajava* L.), the “apple of the tropics” belonging to the Myrtaceae family, is one of the most cultivated fruit in tropical and subtropical areas across the globe (Radha and Mathew, 2007). It can grow naturally in regions with high annual rainfall and can also withstand drought in the temperature range from 20°C to 30°C. Guava is grown over a wide range of soil types, while the optimum growth conditions for the guava are well-drained soil with high organic matter and a pH range between 5 to 7 (Pereira *et al.,* 2017). *Psidium guajava* L. is known by different names all over the world, for example, Amrood (Pakistan), Goyavier (France), and Biyabas (Indonesia). Guava fruit contains 10-30 times more vitamin C than bananas, 10 times more than papaya and 6 times more than oranges (Sanda *et al*., 2011).

India and Pakistan are the two largest guava-producing countries in Asia with yearly production of 4.10 and 0.52 million tons, respectively (Singh *et al*., 2024). Guava is Pakistan's fourth-largest fruit in terms of area planted and production after citrus, mango, and apple. Pakistan contributes approximately 10% of guava's production worldwide (FAO, 2021). Approximately 80% of guavas are grown in Punjab because of their higher tolerance and adaptability to diverse agroclimatic conditions (Mehmood *et al*., 2014). Guava cultivation is concentrated in central Punjab, particularly in Lahore (Pattoki, Kasur and Paridhi), Sheikhupura (Nankana Sahib, Sharkpur and Paridhi), Faisalabad (Jaranwala, Shahkot, Sumundari, Toba Tek Singh and Paridhi), Pakpattan (Arifwala and Paridhi) and district of Sahiwal (Chichawatni and Paridhi). With 5000 hectares of guava, the Sheikhupura district is the primary guava-growing region in Punjab (Anonymous, 2011). Only the Kohat area in Khyber Pakhtunkhwa (KPK) is significant for the production of high-quality guava (Anonymous, 2015).

Guava is significantly affected by many pathological issues causing a significant decline in the quantity and quality of fruit (Lin *et al*., 2003). Out of the biotic diseases of guava, stem canker is one of the most devastating diseases which is also known as scabby fruit canker and necrosis of guava. This disease affects not only fruit production in pre-harvest stages but also post-harvest storage. The reported disease, stem canker (also called stem cracking), is caused by *Pestalotiopsis psidii* (Kwee and Chong,1990). Earlier, the pathogen was reported as *Pestalotia psidii* (Chibber, 1911), but later on it was placed in the genus *Pestalotiopsis* and described by Nag Rag (1993). The disease has been reported to cause 20 to 50 % losses; however, under severe infection losses may be up to 100 % (Antu, 2013). The development of stem cracking disease is favored by temperatures ranging from 10°C to 35°C (Bhogal *et al*., 2022). Spore germination of *Pestalotiopsis psidii is* inhibited below 15°C and above 40°C, while a high relative humidity of approximately 98% is essential for sporulation (Naqvi, 2004).

The disease symptoms generally appear on green fruits while leaves are rarely affected (Moustafa *et al*., 2015). The first sign of infection is the appearance of small, circular, brown, or rust-colored necrotic areas on fruits. As the infection progresses, these lesions rupture the epidermis in a circular pattern. Infected fruits remain underdeveloped, becoming hard, malformed, and mummified, often dropping in large numbers. On leaves, small angular spots with a color of rusty brown may develop (Keith and Zee, 2010). Cankerous lesions are more common during winter while in the rainy season, tiny reddish specks form. The infected fruits exhibit stunted growth and give distorted shapes leading to the premature shedding of fruits (Misra, 2004).

Different methods have been used for the management of *P. psidii* such as the use of resistant varieties, biological control agents, cultural control approaches, nanotechnology and chemical control. Resistant varieties offer initial protection and are being widely used against stem cracking disease. The pathogen *Pestalotiopsis psidii* exhibits high genetic variability and but can overcome resistance over time (Rao *et al*., 2012). Additionally, environmental factors like drought, poor nutrition, and mechanical injuries predispose even resistant plants to infection. Therefore, relying solely on resistant varieties is inadequate (McDonald and Linde, 2002). The disease is complex and influenced by both biotic and abiotic factors. The use of biocontrol agents for disease management is eco-friendly and cost-effective than fungicide application but it has not been proven on a large scale due to the problem of the survival of microbes under adverse climatic conditions (Dwivedi *et al*., 2012). Therefore, management through chemicals is being widely used for quick and effective disease management for the prevention of economic losses. The current study aims to investigate the *in vitro* evolution of fungicides and nanoparticles to check their fungicidal effect against *Pestalotiopsis psidii.*

MATERIALS AND METHODS

Isolation, purification and morphological identification of the desired strain

Pestalotiopsis psidii was isolated by collecting diseased stems, leaves, and branches of three different varieties (Gola, Chuti Surahi, and Bari Surahi) from various guava-growing regions in Punjab province [UAF (PARS), UAF (Square-9), Khurrianwala, Chak No. 164 R.B, Sangla Hill, Shakar Pur, and Alawalkot], and brought to the Mycology and Biocontrol Laboratory at the University of Agriculture, Faisalabad (UAF). Infected parts were cut into small 0.5 cm bits, disinfected with 1% sodium hypochlorite (NaOCl) for 30 seconds, washed three times with distilled water, and placed on tissue paper to remove excess moisture. The dried samples were placed on Petri plates containing Potato Dextrose Agar (PDA) media and incubated at 28°C for 48 hours. Fungal isolates were obtained by picking a single hyphal tip under a stereoscope from an isolated culture and transferring it to separate plates containing PDA media. The inoculated Petri plates were incubated at 28°C for 5-6 days for sporulation. Temporary slides were done according to the protocol and viewed under a microscope to determine the morphological characteristics of the fungus.

Pathogenicity test

The isolated fungus culture was used for the confirmation of Koch's postulates. For this purpose, three healthy guava plants were selected, and the stem surface was scraped with the help of a sterile needle. After this, a spore suspension of fungal isolates was sprayed on the cut parts and covered with tape. Based on the disease symptoms that appeared after 6–7 days on the inoculated parts of the guava plants, the incubation period was recorded, and the associated pathogen was reisolated from the infected part and compared with the original culture.

Preparation of spore suspension

To prepare a spore suspension of the isolated pathogen, 10 mL of sterile water was added to a one-month-old fungal culture. The mycelial surface was gently scraped with a spatula and mixed thoroughly. The resulting spore suspension was stored in Falcon tubes at 4 °C.

***In vitro* evaluation of different fungicides**

Four fungicides (Mancozeb, Deconil, Iprodione and Chlorothalonil) were used at 7 concentrations (50ml, 25mg/ml, 12.5ml, 6.25ml, 3.12ml, 1.56ml along with control (distilled water). For this reason, 96-well plates were employed and in each well, measured amounts of spores, fungicides, and distilled water were poured. A microplate spectrophotometer (ELISA reader, BioTek ELx800) was used for recording data as it was able to detect optical density at 600 nm, which is optimal for measuring fungal biomass using the turbidity method. Moreover, the reason behind using this method is the ability to perform precise, non-destructive monitoring of *P. psidii* growth inhibition.

In vitro* evaluation of AgNPs against *Pestalotiopsis psidii

In vitro evaluation of AgNPs was done against *Pestalotiopsis psidii*. For this purpose, a 9-day-old culture of isolated fungus was treated with AgNPs at the concentrations of 1 ppm, 10 ppm, 100 ppm and 0 ppm (as the control). The agar dilution method was used for the pathogenic fungus to investigate the minimum inhibitory concentration required to inhibit fungal growth. The data were recorded in the diametric growth of the fungus (mm) while the growth inhibition in relation to control (untreated) was calculated by using the formula given by Begum *et al*. (2010) as follows:

$$\text{PGI\%} = \frac{C-T}{C} \times 100$$

Whereas,

PGI= Percent Growth Inhibition

T= Treatment (AgNPs) colony diameter (mm)

C= Control (0 ppm) colony diameter (mm)

Statistical analysis

Laboratory experiments were conducted to evaluate the fungicide and AgNPs against *P. psidii* and results were statistically analyzed by using Factorial design under Completely Randomized Design (CRD). The comparison of means was done using the least significant difference (LSD) as a post-hoc test.

RESULTS

Disease incidence on selected varieties

A survey was conducted in 2022 in different areas of Punjab province of Pakistan to record the disease prevalence of stem canker on guava. Based on the apparent symptoms, the recorded data in terms of disease incidence (%) is elaborated in Table 1. The data show that the disease prevailed in all observed locations of Punjab. The prevalence of disease ranged from 23% in Chuti surahi variety at Alawalkot to 54% in Bari surahi at Khurrinwala at all the locations surveyed in Punjab province. As far as the means of disease incidence in observed areas is concerned, it was found maximum in Shakar Pur (46%) followed by Khurrianwala (42%), Sangla Hill (40.33%), UAF-Square-9 (38.67%), Chak No.64 R. B (38.33%), Alawalkot (35.68) and UAF (PARS) (34.33). Whereas the mean of observed varieties showed that minimum disease prevailed in Chuti surahi variety (37%) and the most susceptible variety against *Pestalotiopsis psidii* was Gola variety (42.71%). Figure 1 depicts the visual symptoms of Stem cracking disease on guava plants caused by *Pestalotiopsis psidii*.

Isolation, purification and morphological Identification

The pathogen was isolated and purified on Potato Dextrose Agar (PDA) media and maintained at 4-5°C in the refrigerator (Figure 2 (A, B)). The mycelium of *P. psidii* gives whitish growth in color, having fluffy and cottony growth and a ring pattern growth observed in the petri plate (Figure 2 (C, D)). The pathogen produces dark black and round pycnidia on the culture media that contain conidia and conidiophores. Conidia are generally 5 cells, rod or oval, spindle-shaped, upright, with few narrow septal walls, 13-31 x 5-10 microns in size and 3 central cells were dark brown shown in Figure 2 (E, F). The thickest and most convex part of the conidium was its central part and the rest of the cells were

more transparent. The apical cells were conical or cylindrical, hyaline and thin-walled. Basal cells were dull, upright and stalked, growing into appendages. The seedling mycelium was aerial, serrated, septate, thin, cottony, and it was white to pink in color, unevenly branched and up to 3 µm in diameter. There were more mycelial thicknesses in aged cultures.

Table 1. Occurrence of stem canker disease of guava at different growing areas of Punjab of three varieties.

Sr. No.	Locations	Disease incidence (%)			
		Gola	Bari surahi	Chuti surahi	Mean
1	UAF (PARS)	40	20	43	34.33
2	UAF (Square-9)	38	38	40	38.67
3	Khurrianwala	38	54	34	42.00
4	Chak No.64 R. B	32	47	36	38.33
5	Sangla Hill	53	28	40	40.33
6	Sharkpur	50	45	43	46.00
7	Alawalkot	48	36	23	35.66
	Mean	42.71	38.29	37	39.33

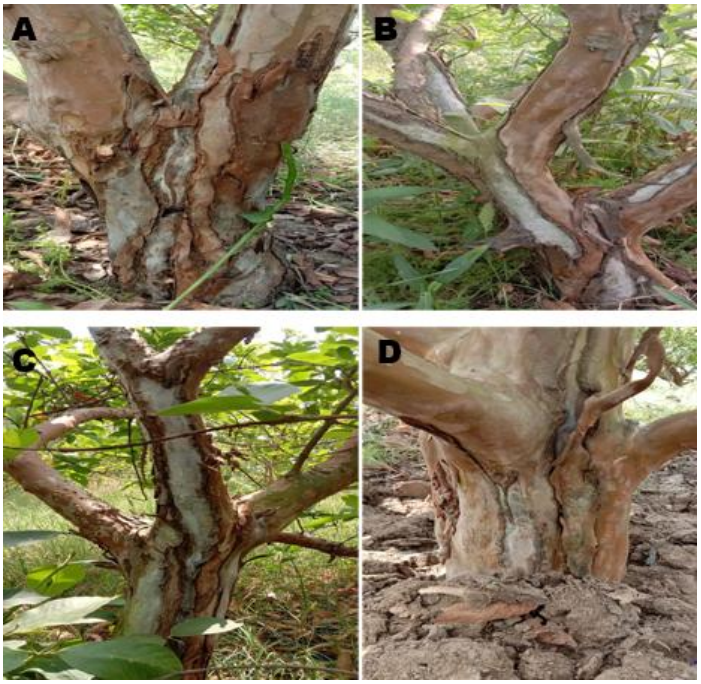


Figure 1. Visual symptoms of Stem cracking disease on guava plants caused by *Pestalotiopsis psidii* A) Longitudinal symptom with brown necrotic lesion and peeling bark B) Sunken and corky lesions at the branching zone of the main stem C) Deep vertical cracks with coalescing lesions D) Cracking and decay symptoms at the stem base extending into the soil surface.

Effect of temperature on colony growth and acervulus production of *Pestalotiopsis psidii*

Four temperatures, 20, 25, 30 and 35°C, were evaluated for the determination of the optimum temperature for the growth of *Pestalotiopsis psidii* (Figure 3 (A)). The results demonstrated that out of four temperatures, maximum growth (90.00mm) of the fungus was recorded at 30°C, indicating that 30°C is the most favorable temperature for the growth of the pathogen. Colony growth reduced to 84.25 mm at 35 °C, 74.50 mm at 25 °C, and was lowest at 53.75 mm at 20 °C. ANOVA analysis (Figure 3 (C)) revealed that temperature treatments were significantly different in their influence on colony growth (F value significant). Figure 3 (B) shows that acervulus production varies with temperature. As far as the total number of acervulus production is concerned, maximum acervulus production (13.25) was observed at 30°C, making it the most favorable temperature for reproductive growth of *P. psidii* (Figure 3 (B)). The acervulus production reduced to (9.50) when the temperature raised to 35°C. Moreover, minimum production of acervuli (4.00) has been observed at the temperature of 20°C which is not significantly different from the acervulus production (6.5) at 4°C.

Figure 3(D) depicts that the results were statistically significant, as the temperature significantly affects the colony growth of *P. psdii*.

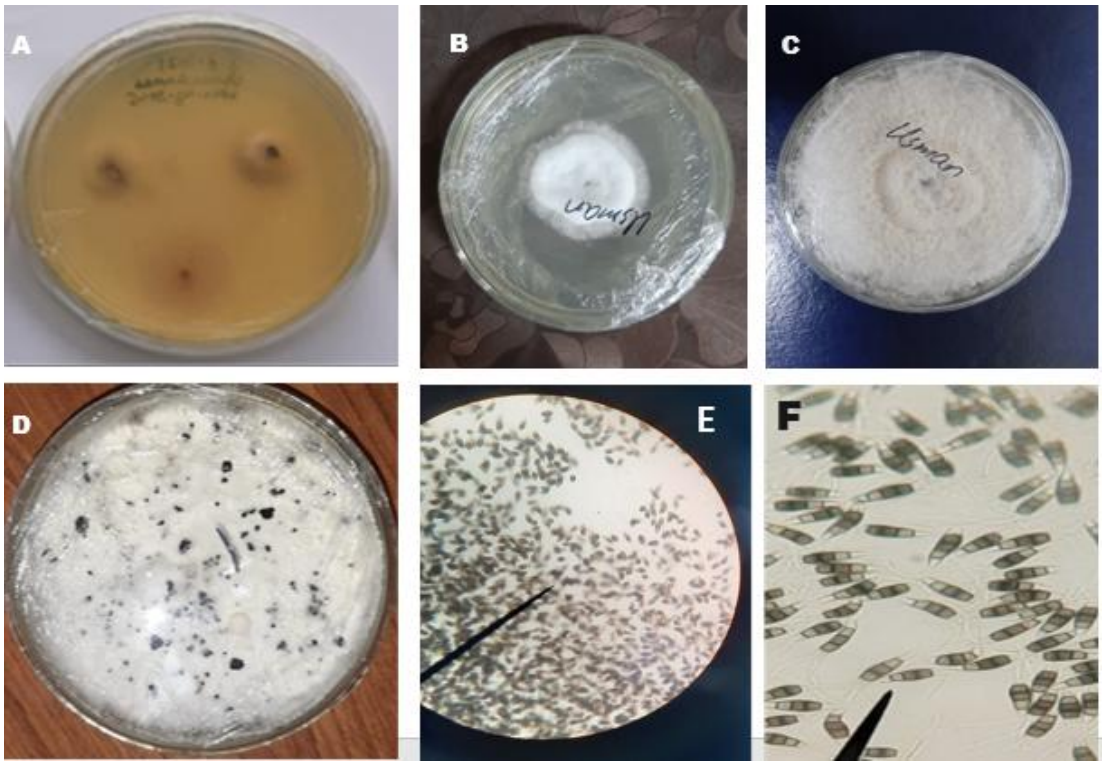
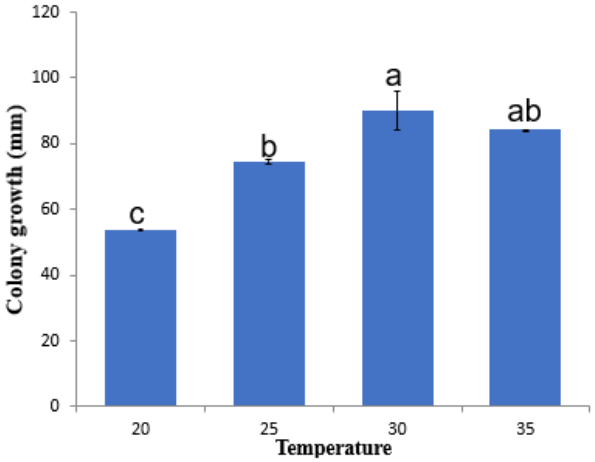
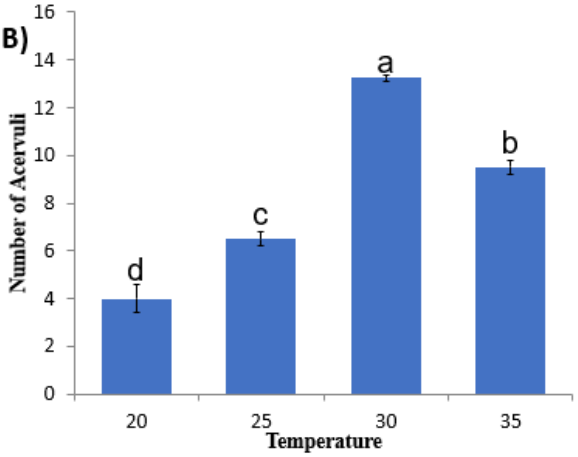


Figure 2. A) Initial isolation of *P. psidii* on Potato Dextrose Agar (PDA) B) Sub-culture of fungal isolate for purification C) Purified culture of *P. psidii* on PDA, exhibiting cottony and white colony D) Sporulation culture of *P. psidii* with black conidial masses E) Microscopic view of *P. psidii* conidia F) Detailed microscopic image of *P. psidii* showing multicellular conidia with apical and basal appendages

A)



B)



C)

Sov	Df	SS	MS	F-value
Between	4	3681.700	920.425	86.696**
Within	15	159.250	10.617	
Total	19	3840.950		

**highly significant(p<0.01)

D)

SOV	Df	SS	MS	F-value
Between	4	367.700	91.925	102.139**
Within	15	13.500	0.900	
Total	19	381.200		

**highly significant (P<0.01)

Figure 3. A) Effect of temperature on colony growth of *P. psidii.* B) Effect of temperature on acervulus production of *P. psidii.* C) Analysis of variance for the effect of temperature on colony growth of *P. psidii.* D) Analysis of variance for the effect of temperature on colony growth of *P. psidii*.

Table 2. Mean comparison of concentration and chemicals for spore inhibition of *Pestalotiopsis psidii* based on optical density (OD) readings.

Chemicals	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.12mg/ml	1.56mg/ml	0.78mg/ml	Means
Mancozeb	1.3820B	0.5327C	0.2503 C	0.2747C	0.4117C	0.4427C	0.5023C	0.5423ab
Chlorothalonil	0.2657C	0.2747C	0.2983C	0.2877C	0.3063C	0.5190C	0.4537C	0.3436c
Iprodione	2.3287A	0.2660C	0.2457C	0.2123C	0.2723C	0.4640C	0.4423C	0.6045a
Deconil	0.2497C	0.4407C	0.3590C	0.4860C	0.4707C	0.5183C	0.4453C	0.4242bc
Conc. means	1.0565	0.3785	0.2883	0.3152	0.3652	0.486	0.4609	
Hom. groups	a	bcd	d	cd	bcd	b	bc	

Table 3. Growth of *Pestalotiopsis psidii* (colony diameter in mm) treated with different concentrations of the Ag NPs for 9 days

Concentration (ppm)	After 6 days	After 9 days	Growth Inhibition (%)
Control	3.5	46.2	0%
1	1.5	43.2	6.5 %
10	1.5	41.6	10 %
100	0.5	16	65.36%

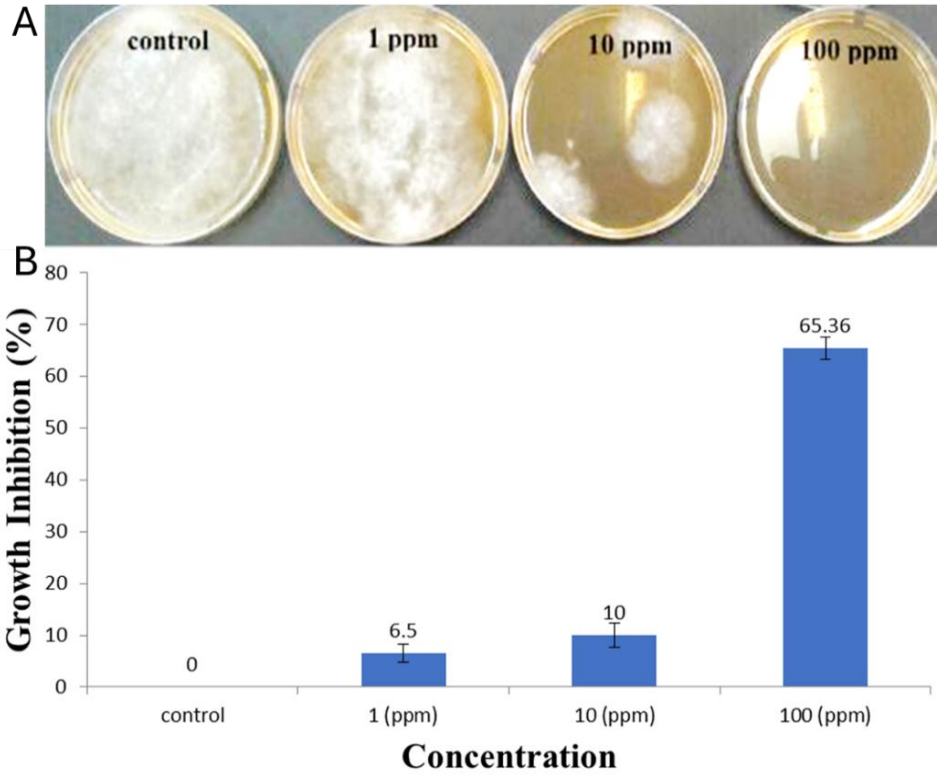


Figure 4. A) Growth of *P. psidii* at different concentrations of the AgNPs for 9 days, B) Growth inhibition percentage of P. psidii treated with different concentrations of the Ag NPs for 9 days

In vitro* efficacy of fungicides against *Pestalotiopsis pisdii

The *P. psidii* isolates exhibited varying sensitivities to the evaluated fungicide types and their concentrations (Table 2). The mean comparison between chemicals and concentrations shows that the maximum spore inhibition (2.3287) was observed with Iprodione at a concentration of 50 mg/ml, while the minimum inhibition (0.2123) was recorded with Iprodione at 6.25 mg/ml. Mancozeb proved effective against *P. psidii* at concentrations of 50 mg/ml and 25 mg/ml, showing a percent growth inhibition (PGI) of 80.35% and 73%, respectively. While its third and fourth concentrations, diluted to 12.5 mg/ml and 6.25 mg/ml, also remained effective, with PGI values of 75% and 62%, respectively. However, chlorothalonil was effective at all concentrations except 0.78 mg/ml, where PGI was 0%. Table 2 indicates that the interaction between fungicide type and concentration has a significant effect on spore inhibition.

In vitro* efficacy of AgNPs against *Pestalotiopsis psidii

The antifungal activity of silver nanoparticles (AgNPs) was evaluated by measuring colony diameter at 6- and 9-day post inoculation of Pestalotiopsis psidii at different concentrations: 1 ppm, 10 ppm, 100 ppm and 0 ppm (as the control). Among all the concentrations of AgNPs, the maximum inhibition zone (65.36%) was observed at 100 ppm, with a colony diameter of 0.5 mm after 6 days and 41.6 mm after 9 days (Table 3). It can be seen in Figure 4 that a strong antifungal effect is observed at the highest concentration of AgNPs. The control group (which received no treatment) shows that the pathogen grew extensively (46.2 mm) after 9 days of post inoculation, while no inhibition zone was observed without the application of AgNPs. The graph (Figure 4) indicates that the inhibition zone is dose dependent. For instance, slight inhibition (6.5%) was recorded at the concentration of 1 ppm, which increased to 10% at 10 ppm.

DISCUSSION

Guava fruit canker has already been reported in different countries of the world including Egypt, India and Hawaii. In Egypt and India, disease severity caused by *Pestalotiopsis* spp*.* on guava trees has been reported to range between 40-70% under favorable conditions (El-Argawy, 2016; Singh *et al*., 2017), whereas in our experimental conditions, severity was observed up to 54%, which falls within the same range but highlights the potential threat if not managed early in Pakistan. The signs of disease are observed as necrotic corky lesions. Similar symptoms were also noted on all the infected plants (cracks on stem, small moles on the stem). These observations of the development of the symptoms have maximum similarity to symptoms described by Keith and Zee (2008). Figure 1 illustrates the symptoms on the stem part of the plants. These results were supported by the findings of Keith *et al*. (2006) who reported the corky and brown lesions resembling symptoms that occurred in the field. Quantitatively, the current study recorded an incidence rate of 54% in the most affected location, which is comparable to the 45% incidence rate reported in Egypt (El-Argawy, 2016).

Studies have explained that *Pestalotiopsis* species were historically named according to the host from which they were first observed. They also added that many of the *Pestalotiopsis* species are not host-specific and are found on many substrates and hosts (Jeewon *et al*., 2004; Lee *et al*., 2006). However, species of *Pestalotiopsis* displayed a considerable diversity in the phenotype and grouped based on the similarities in morphology of conidia (Jeewon *et al.,* 2003; Maharachchikumbura *et al.,* 2011). Conidial characteristics such as conidial length, median cell length, median cell width, and length of the apical appendages appear to be stable characters within *Pestalotiopsis* (Jeewon *et al*., 2004; Hu *et al.,* 2007). Results of the experiment showed that the mycelium of *P. psidii* gives pure whitish growth while acervuli develop as black and shiny in cultures. The conidia are dark colored, spindle shaped and 5-celled. These findings were also supported by Keith *et al*. (2006) who identified the structures of conidia of *P. psidii*, the causal agent of stem cracking of guava.

Temperature of incubation has a great effect on the growth rate, growth of mycelium, production of acervuli and growth pattern of *P. psidii*. The fungus grows well at temperatures between 25 to 30 while 28 being most suited for the optimal growth of pathogen. These results were supported by Keith *et al*. (2006) who reported that reported pathogen grows maximally at 28. Moreover, findings were further supported by Younis *et al*. (2004) who concluded that optimal temperature for the growth of pathogen and acervuli production was recorded at 30 °C, followed by 25 °C and 20. These findings were confirmed by Rahman *et al*. (2003), who reported that optimum growth temperature of the fungus in culture is close to 30°C. Rayanathoola *et al*. (2025) reported that spores of *Pestalotiopsis* spp*.* germinate maximally at a temperature of 30 °C while spores do not germinate below 15°C or above 40°C.

Many scientists have described that *Pestalotiopsis* spp*.* are opportunistic pathogens that may cause infection in stressed plants (Maharachchikumbura *et al*., 2011). These pathogens harshly affect the yield at both pre and post-harvest stages in guava (Bhogal, 2020; Kwee and Chong, 1990). It confirms that the results that show it as a pathogen are reliable data for further research by considering it as pathogen of guava fruit canker in Pakistan. It is supported by literature such as (Gurunathan *et al.,* 2009; Pourali, 2015; Singh *et al.,* 2017). Zeta potential, size and distribution (obtained from dynamic light scattering) were powerful characterizations of the metallic nanoparticles as they provide further characterizations i.e., physical stability, saturation solubility, and even biocidal activity.

Many scientists have explained that modern techniques as nanotechnology have wide application in agriculture for controlling plant diseases in integration with other approaches (Bhattacharyya *et al.,* 2011; Rai and Ingle, 2012). This modern technology is gaining popularity for its effective use in plant pathology. For example, Tomey *et al.* (2007) described that the insertion of resistant genes in crop plants with suitable nanotechnology techniques increases the tolerance level of the plant, which will lead to a reduction in the use of chemicals in these methods. Nowadays, we have a lot of scientific evidence of the effectiveness of metallic nanoparticles having antimicrobial potential. As Oh *et*

al. (2006) assessed antimicrobial effect of nanoparticles against *Botrytis cineria*, Fateixa *et al.* (2009) tested against *Aspergillus niger*, Min *et al.* (2009) tested against secirotium, Kasprowicz *et al.* (2010) and Musarrat *et al.* (2010) tested against the *Fusarium*. Krishnaraj *et al.* (2012) tested against many of the fungal pathogens, including *Alternaria alternata, Macrophomina phaseolina, Sclerotinia sclerotium, Botrytis cinerea* and *Curvularia*. Most of these studies support our results, where silver nanoparticles have demonstrated antifungal potential against the *P. psidii* causing stem cracking in guava. Despite their *in vitro* effectiveness, AgNPs require further testing in field conditions. The environmental stability, degradation, and fate of silver nanoparticles in soil ecosystems are not well understood (Rai and Ingle, 2012), which raises concerns about their potential toxicity to beneficial microbes and long-term effects. Additionally, although the cost-effectiveness of the synthesis method used in this study was evident at a laboratory scale, costs could potentially increase for larger field applications (Thakkar *et al*., 2010).

We have adopted this nanotechnology for the management of emerging diseases in Pakistan. In this study, the Ag nanoparticles were used which is an economical and environment-friendly method and it was also supported by (Thakkar *et al.,* 2010). He said that other methods like chemical methods are costly because in those methods, more steps are involved. Many scientists have evaluated different nanoparticles like Sulphur and phosphorus nanoparticles (Gurunathan *et al.,* 2009 and 2014), Copper and silver-based nanoparticles (Cioffi *et al.,* 2004), but silver nanoparticles have shown the most effective results in reduction of the growth of different soil-borne pathogens, stimulating plant growth through improved microbial activity and uptake of nutrients (Prasad *et al*., 2017; Kim *et al*.,2012). However, excessive and repeated application may have a negative effect on soil health by disturbing microbial diversity and can lead to phytotoxicity (McGee, 2020).

Although AgNPs have been widely investigated for their antifungal properties but there is limited investigation into the combined use of AgNPs with fungicides, particularly for the management of *P. psidii*. This study suggests, for the first time, that the combined use of AgNPs with fungicides may provide a new and promising approach for the control of guava stem canker caused by *P. psidii*. Moreover, the use of fungicides in combination with AgNPs may result in a synergetic effect which can reduce the dose of both agents. Studies have been conducted on other pathosystems but no study has been conducted against *P. psidii* (Huang *et al*., 2018). Investigating AgNPs in combination with traditional fungicides such as carbendazim and mancozeb may result in more sustainable management strategies (McGee, 2020).

In general, the current study suggests the use of AgNPs for managing the guava stem cracking disease as one component of an integrated management strategy. The synergistic action of conventional fungicides with AgNPs has not been studied for the pathogen *Pestalotiopsis psidii* and future studies should be conducted to investigate their stability, compatibility and efficacy in formulated products under field conditions.

CONCLUSION

The present study found *Pestalotiopsis psidii* as the causal fungus of guava stem cracking in Punjab, Pakistan. Amongst test fungicides, Iprodine at 50mg/ml proved to be the most effective in inhibiting the growth of the fungus under laboratory conditions. Silver nanoparticles (AgNPs) showed strong antifungal activity, particularly at 100 ppm. The combined application of fungicides and AgNPs provides a promising effective control strategy for the stem cracking disease of guava. These findings help in disease control and may improve guava production and export value.

AUTHOR CONTRIBUTIONS

AHA conducted the experiments and wrote the original draft, UA conducted the experiments and wrote the original draft. RI helped with data collection, MFK wrote the methodology and reviewed the editing. ZUA wrote the results and helped with data analysis. MU reviewed the draft and improved the discussion part, AMN helped with the data analysis, AA reviewed the article and provided all lab facilities to conduct experiments.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

The authors are thankful to the Institute of Microbiology, GC University, Faisalabad, for the provision of AgNPs.

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