Research Article

Antifungal Activity of Silver Nanoparticles with the Potential to Control Fungal Contamination in the Male Inflorescences of Palmyra Palm

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Abstract
Fungal contamination of the male inflorescences of palmyra palm (*Borassus flabellifer* Linn.) is a serious problem during their storage prior to being used as the raw material for making the local One-Tambon-One-Product, a cloth holder known as "Nguang Taan Hom" made by the Phutaan Entrepreneurship, Takua Thung District Phangnga Province, Thailand. Morphological and molecular techniques were used to identify the isolated fungi from male palmyra palm inflorescences. Three major fungal strains *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus sclerotiorum* were studied for antifungal activities and the minimum inhibitory concentration (MIC) of silver nanoparticles (AgNPs) was found to range from 13.5–27 mg mL^{-1}. Then, the efficiency of the AgNPs and silver nanoparticles stabilizing by Polyvinylpyrrolidone (AgNPs-PVP) on the male palmyra palm inflorescences was studied by constructing a chamber model over a period of 9 weeks. The results showed that the group inoculated with *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus sclerotiorum* spores developed fungal growth after 4 weeks whereas no fungal growth was observed in the group treated with AgNPs and AgNPs-PVP at 27 μg/mL until week 7, indicating that dipping with either AgNPs or AgNPs-PVP is able to inhibit the growth of the fungi which cause damage to raw materials and also threaten human health. Therefore, it can effectively extend the storage time of male palmyra inflorescences.

Keywords: Palmyra palm, Silver nanoparticles, Antifungal, Contamination, Biocontrol

1 Introduction

The palmyra palm (*Borassus flabellifer* Linn.) is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, and Malaysia. In Thailand, the male inflorescence of the palmyra palm is the part that is stimulated to produce a sap flow. After the sap is collected by cutting the outer end at the head of the inflorescences, the used inflorescences have been applied in the manufacture of the One Tambon One Product (OTOP) product as a cloth holder called "Nguang Taan Hom" by the Phutaan Entrepreneurship, Takua Thung District Phangnga Province, Thailand.

However, the main problem that the entrepreneurship suffers is the contamination by fungal growth on the Nguang Taan used as a raw material to produce the cloth holder. The damage caused by the fungi is a serious problem both in the product and also to human health.

Agricultural production is broadly affected by various kinds of diseases including natural disasters

caused by pathogenic organisms which lead to huge economic loss and a tendency to produce toxicity in the environment [1]–[6]. Over the past few decades, nanotechnology has been considered as an emerging technology that has become an interdisciplinary field of research that is growing rapidly with amalgamation into other areas of science and technology. The unique electronic, optical, mechanical, magnetic and chemical properties of nanoparticles are a wide range of applications in medicine, pharmacy, cosmetology, electronics and agriculture [7]–[9]. Among other things, many studies point to their antiviral, antibacterial and antifungal properties [10]–[14]. Recently, silver nanoparticles (AgNPs) have been reported in many antifungal applications as both a preservative and treatment in the area of agricultural research to be highly effective due to their unique properties [15]–[21]. The effective antimicrobial properties and low toxicity of AgNPs toward mammalian cells have made them to be easily utilized in many consumer-based products [14]. Therefore, this research work aimed to investigate AgNPs used as an antifungal agent for the growth inhibition of isolated fungus from the male inflorescences of palmyra palm.

The objectives of this research are to isolate, identify and quantify the fungal microorganisms on the male palmyra palm inflorescences (MPPIs). The application is to determine the effective concentration dose of AgNPs necessary to treat the most prevalent fungi isolated from MPPIs. In addition, preventive and post-infection treatments were performed *in situ* on MPPIs to control fungal contamination.

2 Materials and Methods

2.1 *Materials*

The MPPIs were provided by Phutaan, 40/1 Village No.5, Khok Kloi Sub-district, Takua Thung District, Phangnga Thailand. An ITS primer with sequences of ITSF 5' TCC GTA GGT GAA CCT GCG G 3' and ITSR 5' TCC TCC GCT TAT TGA TAT GC 3' was purchased from Integrated DNA Technologies, Singapore. Potato dextrose agar (PDA) medium was used to grow and maintain the cultures. Sodium borohydride ($NaBH_4$), Silver nitrate and Polyvinylpyrrolidone (PVP) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2 *Identification of fungi on the male inflorescences of palmyra palm*

The fungi on the MPPIs were inoculated and grown on Potato Dextrose Agar (PDA) with the addition of antibiotics. The incubation plates were maintained at room temperature for 2 weeks and observed every day. Pure cultures were isolated from the PDA plates by the hyphal tip isolation method under a stereo microscope in order to identify the morphology of the fungi. Further, the extraction of genomic DNA was conducted using the CTAB method and the genomic DNA was amplified using the ITS gene as a molecular marker to find homologues for identification. The purified PCR product was directly used for DNA sequencing and the sequence was run on the BLASTN program for comparison with the GenBank database (http://www.ncbi nlm.nih.gov). The sequences of the selected fungi and other sequences obtained from the GenBank database were aligned by ClustalW and MEGA6.

2.3 *Synthesis and characterization of silver nanoparticles*

AgNPs were prepared by the reduction of silver nitrate using $NaBH_4$ as a reducing agent. A 10 mL of 1.0 mM silver nitrate was gently added at about 1 drop/second into 30 mL of 2.0 mM sodium borohydride solution chilled in an ice bath. The reaction mixture was stirred vigorously on a magnetic stir plate with a stirring rate of 1,400 revolutions min^{-1}. The solution turned light yellow after the addition of 10 mL of silver nitrate and thereafter became bright yellow. Nanoparticles in colloidal solutions can be stabilized by adsorbed PVP and 4 mL of 0.3% (w/v) PVP was added to the yellow silver to prevent aggregation (AgNPs-PVP). The size of the AgNPs was measured using transmission electron microscopy (TEM).

2.4 *Antifungal test*

To examine the antifungal effect of the AgNPs, the isolated fungi from the MPPIs were cultured in PDA liquid medium at 37 °C for 3–5 days. Then 1×10^5 CFU mL^{-1} cells were plated on fresh PDA solid media containing 27 μg mL^{-1} of AgNPs and AgNPs-PVP and incubated for 7 days at 30 ± 2 °C. Fungal cultures treated with deionized water (silver-free PDA plates) and the commercial fungicide (Mancozeb) were cultured

under the same conditions and used as negative and positive controls. The assays were performed in triplicate and the mycelial radial growth was measured. The Percentage Inhibition of Diameter Growth (PIDG) was calculated using the following formula: PIDG = (DT-D)/DT × 100 where DT is the mean diameter of mycelial growth in the control and D is the mean diameter of mycelial growth in the treatment [9].

2.4.1 Measurement of minimum inhibitory concentrations

The antimicrobial activity of the AgNPs and AgNPs-PVP was determined using the microbroth dilution method described by the Clinical and Laboratory Standards Institute (CLSI) guidelines, document M27-S4. The minimum inhibitory concentration (MIC) of the AgNPs and AgNPs-PVP were determined by monitoring the growth of fungi in a microplate reader (Multiskan FC, Thermo Scientific) at 600 nm (OD600). Serial two-fold dilutions of AgNPs and AgNPs-PVP were prepared in sterile 96-well plates over a range of 0.42–27 μg mL^{-1}. The wells were then inoculated with 100 μL of the diluted broth culture to give a cell density of 10^5 CFU mL^{-1} and incubated at 37 °C for a period of 18 to 24 h. The experimental procedure involved treating samples inoculated with fungi with a cell density of 10^5 CFU mL^{-1} with the silver nanoparticle solutions prepared, and samples inoculated with fungi and with the commercial Mancozeb were used as positive and negative controls, respectively. All the experiments were conducted in triplicate and the results were expressed as the mean values from three replicates. In the experiments, differences in the effect of variables whose *p*-values were lower than 0.05 were considered to be significant. Bacterial growth was detected as an increase in absorbance at 600 nm and the comparative analysis of differences was conducted by one-way ANOVA (SPSS version 15). The MIC of the antimicrobial activity of the silver nanoparticles was evaluated according to the National Committee for Clinical Laboratory Standards with fungal growth being completely inhibited under the MIC.

2.4.2 Preservation assay for the male inflorescences of Palmyra palm

MPPIs were obtained from the target site in Phangnga and samples of moderate size, consistent maturity and without bruises were selected. The samples were chopped into 5 cm lengths for use in the experiment. They were sterilized using ethylene oxide gas at 55 °C for 16 h and stored at a constant temperature in a humidity chamber (20 × 25 × 5 cm) at room temperature with a relative humidity of 74.5 ± 0.8% (consistent with the real conditions used for storage at the target site).

The experiment was conducted on four groups as follows: the first group consisted of negative control in which the samples, were soaked in deionized water. The second group, in which the sterilized MPPI samples with no treatment were used as controls. In the third group, the sterilized samples after treatment with AgNPs and AgNPs-PVP solutions at the determined MIC (27 and 24.5 μg mL^{-1}) were challenged by inoculation with a fungal concentration of 5×10^4 spores mL^{-1}. In the last group (positive control), sterilized samples with no treatment were inoculated with a fungal concentration of 5×10^4 spores mL^{-1}. The treatments were administered to the MPPIs by soaking for 15 min following, which the samples were dried at room temperature for 15–20 min. The inhibition of fungal growth by the various treatments was then determined.

The samples were examined by Electron Microscope (EM) and images were collected every day. The experiment was performed in triplicate, and the data were statistically analyzed by the mean ± standard deviation (SD).

3 Results

3.1 *Identification of fungi on the male Palmyra palm inflorescences*

Preliminary identification of isolated fungi from the MPPIs was conducted using the morphological characteristics of the mycelium and spores. There were 224 isolates, which were divided into nine groups as shown in Figure 1. Molecular identification was conducted using the ITS gene, which is a standard molecular marker for 500–800 bp sequences used to identify species from the fungal kingdom using primers that are applicable for the broadest possible taxonomic group.

A 544 bp ITS-containing region was amplified from each of nine pure cultured isolates (sp1–sp9).

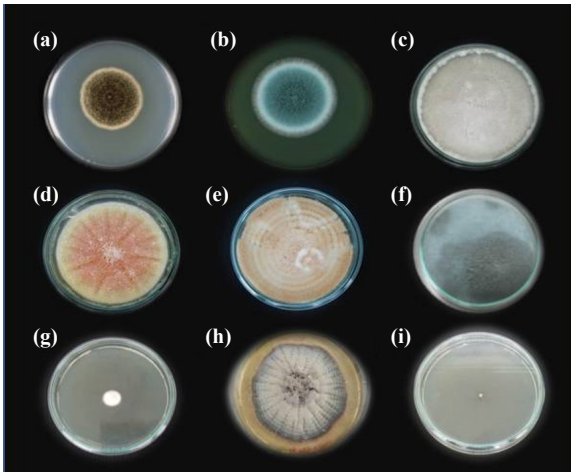

Figure 1: Identification of fungi on Ngang Taan showing *Aspergillus niger* (a), *Aspergillus fumigatus* (b), *Sebipora aquosa* (c), sp4: unknown (d), *Aspergillus sclerotiorum* (e), *Penicillium funiculosum* (f), *Talaromyces* sp. (g), *Gelatoporia subvermispora* (h) and *Sympoventuriaceae* sp. (i).

All nine amplicons were sequenced and all were found to contain a complete 495 bp ITS region (ITS1/5.8SrRNA/ITS-2) flanked by short 18S and 28S rRNA sequences. NCBI BLAST searches of the ITS regions for the fungal isolates showed that they were 99–100% identical to nine species of fungi (Table 1).

Table 1: Comparison of Nucleotide sequences of fungi isolated from the MPPIs from the NCBI database

Code	Identified Species	Number of Isolated Fungi	GenBank Accession No.	% Identity
sp1	*Aspergillus niger*	73	MW413309	100%
sp2	*Aspergillus fumigatus*	75	MW413310	100%
sp3	*Sebipora aquosa*	7	MW413311	99%
sp4	unknown	3	-	-
sp5	*Aspergillus sclerotiorum*	44	MW413312	100%
sp6	*Penicillium funiculosum*	10	MW413313	100%
sp7	*Talaromyces* sp.	6	MW413314	100%
sp8	*Gelatoporia subvermispora*	4	MW413315	99%
sp9	*Sympoventuriaceae* sp.	2	MW413316	100%

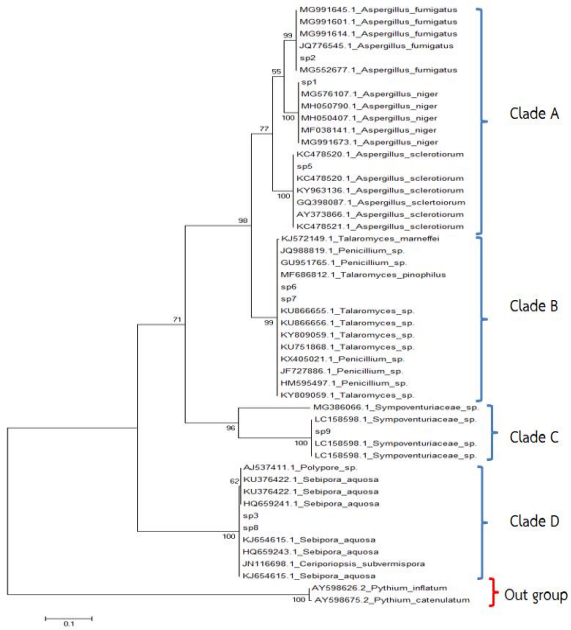

Figure 2: Neighbour-joining phylogenetic tree of fungi 9 isolates.

Nucleotide sequences obtained from the NCBI database and the MEGA 6 program were studied to create a phylogenetic tree calculated with neighbor-joining and bootstrap values calculated over 1,000 replicates using *Pythium catenulatum* (AY598675.2) with *Pythium inflatum* (AY598626.2) as the out-group. Nine isolates in this study were classified into 4 Clade (A-D) (Figure 2). Clade A consisted of the *Aspergillus* genus including sp1, sp2 and sp5 isolates closely related to *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus sclerotiorum*, respectively. However, Clade D including sp3 and sp8 showed the farthest relationship with other clades. For sp3 is grouped together with genus *Sebipora* and sp8.

3.2 *Sampling and conditions for in situ experiment*

The MPPIs were cut into pieces about 10 in length and kept at the storage house at the Phu Tan Palm Processing Group, Village No. 5, Khok Kloi Sub-district, Takua Thung District, Phangnga Province, which is 12 × 15 × 3.5 m in size. Since the storage house is open, so the growth of fungi is aided by ready access to nutrients, oxygen and water and without any control of the temperature, humidity or light conditions.

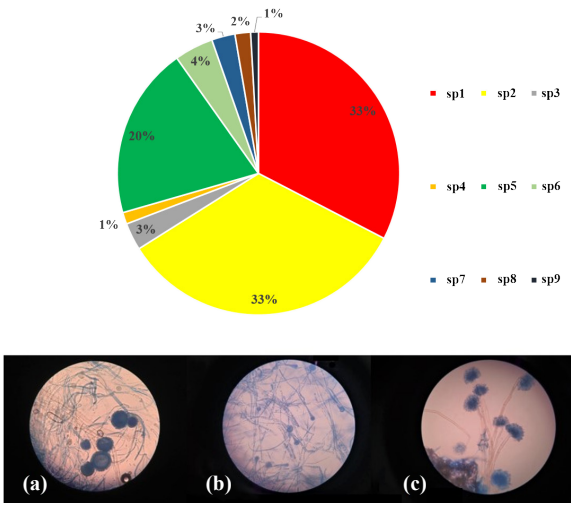

Figure 3: Distribution of fungi (224 isolates) isolated from contaminated MPPIs and microscopic observation of the most frequently found fungi in male inflorescences, *Aspergillus niger* (a), *Aspergillus fumigatus* (b) and *Aspergillus sclerotiorum* (c).

Water content is one of the factors that affect the growth of fungi. The amount of water in a sample can be measured by the moisture content and water activity. Fungi usually require less water for growth than other microorganisms, yeast, and bacteria, and can grow in a sample with both low moisture and water activity, such as paper, wood, etc. The relative humidity at the storage house was measured during March and April and it was found that the mean relative humidity was in the range of 74.53 ± 0.80%, which would be sufficient to promote the growth of fungi on the MPPIs. This was in agreement with previous work indicating the amount of water that is suitable for the growth of the group of fungi that causes contamination in agricultural products and it has been found that most of these fungi tend to thrive with a water activity of below 0.85 [22]. The average temperature in the storage house was 30.9 ± 2.2 °C and such a temperature can also promote the growth of fungi.

Based on the identification of the fungal species from their morphology and molecular characteristics, it was found that sp1 (*Aspergillus niger*), sp2 (*Aspergillus fumigatus*) and sp5 (*Aspergillus sclerotiorum*) were the most common and they are the major cause of the contamination shown in Figure 3. Therefore, the researcher chose to use these three types of fungi to study the effects of AgNPs and AgNPs-PVP on the preliminary growth of fungi and their effectiveness in inhibiting those fungi on MPPIs.

3.3 *Synthesis and characterization of AgNPs*

The AgNPs were synthesized according to the method described in the previous section, and their characteristic size was investigated using TEM (JEOL JEM-2010). The average particle sizes of AgNPs and the AgNPs-PVP are 20 ± 2.8 nm and 36.7 ± 1.3 nm, respectively.

3.4 *Preliminary antimicrobial assay*

The antimicrobial effects of the synthesized AgNPs and AgNPs-PVP were estimated by the agar dilution technique against three fungi: *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus sclerotiorum* isolated from the MPPIs. A fresh culture of isolated fungi was suspended in 1 mL of sterile distilled water and 100 µL of the suspension was spread-plated on solidified PDA. Five millimeter diameter plugs of actively growing mycelia from the fungi isolates were placed at equal distances on the inoculated PDA plates. All the fungi isolates were analyzed in triplicate. The plates were incubated aerobically at 30 °C for 24–48 h with AgNPs and AgNPs-PVP at 27 and 24.5 µg mL^{-1}. The effect of the AgNPs and AgNPs-PVP on *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus sclerotiorum* are shown separately in Figure 4. It was found that the PIDGs for the AgNPs were 52, 74, and 71%, respectively, while for the AgNPs-PVP, the PIDGs were 40, 59, and 58%, respectively.

3.5 *MIC determination*

The antifungal effect of both the AgNPs and AgNPs-PVP against various fungi isolated from the MPPIs were estimated with Mancozeb 80% WP as a positive control based on the agar dilution technique. The inhibition in the growth of the fungi was determined based on the MIC, that is, the lowest concentration that completely inhibited growth. The results are shown in Table 2. The concentrations of AgNPs and AgNPs-PVP required to achieve the MIC were observed to be in the range of 12.25–27 µg mL^{-1}. At concentrations as low as 13.5 and 12.25 µg $mL^{-1}$for both the AgNPs and the AgNPs-PVP, substantial inhibition of fungal

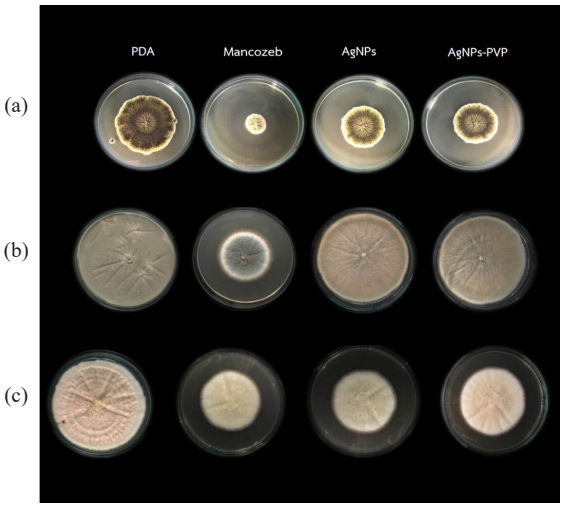

Figure 4: Effects of antifungal agents on the growth of *Aspergillus niger* (a), *Aspergillus fumigatus* (b) and *Aspergillus sclerotiorum* (c) on PDA only (control), PDA with Mancozeb 200 µg mL^{-1}, AgNPs 27 µg mL^{-1} and AgNPs-PVP 24.5 µg mL^{-1}, respectively.

growth was noted. These results also suggest different forms of silver nanoparticles have an equivalent antifungal effect.

Table 2: The Minimum inhibitory concentration (MIC) of agents against various fungi

Fungi Species	MIC (µg mL^{-1})		
	Mancozeb	AgNPs	AgNPs-PVP
Aspergillus niger	3.13	27.0	24.5
Aspergillus fumigatus	1.56	13.5	12.25
Sebipora aquosa	0.78	13.5	12.25
Aspergillus sclerotiorum	1.56	13.5	12.25
Penicillium funiculosum	1.56	13.5	12.25
Talaromyces sp.	0.78	13.5	12.25
Gelatoporia subvermispora	0.78	13.5	12.25
Sympoventuriaceae sp.	0.78	13.5	12.25

3.6 *The antifungal activity of AgNPs and AgNPs-PVP in male palmyra palm inflorescences: the model system*

The sterilized 5 cm-long pieces of MPPIs were treated in AgNP solution at the MIC (µg mL^{-1}) for 15 min, then dried at room temperature for 15–20 min and transferred to a constant temperature and humidity

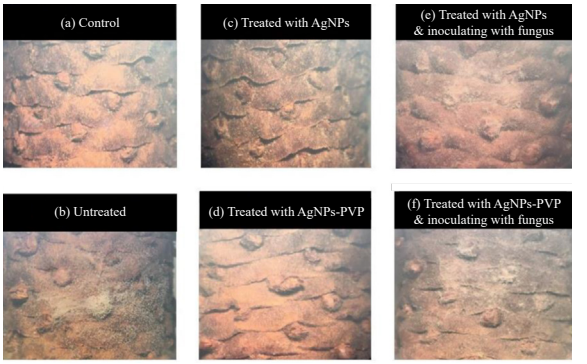

Figure 5: The growths of fungi on MPPIs observed by stereo microscope after incubation for 60 days. Control (a), The sterilized MPPIs inoculated with fungal spores (b), MPPIs treated with AgNPs (c), MPPIs treated with AgNPs-PVP (d), MPPIs treated with AgNPs then inoculated with fungal spores (e) and MPPIs treated with AgNPs-PVP then inoculated with fungal spores (f).

chamber (20 × 25 × 5 cm). Sterilized MPPI samples with no treatment were used as controls. A spore suspension of *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus sclerotiorum*, the three most common species of fungi traced in the MPPIs were prepared (5 × 10^4 spores mL^{-1}) and approximately 10 µL of the spore suspension was spread on the samples. The inoculated samples were incubated at room temperature in 74.5 ± 0.8% RH (The conditions used in previous studies) and the fungal growth were examined every day for 7 weeks. White mycelia were observed in the untreated samples 4 weeks after inoculation [Figure 5(b)] and after 7 weeks for the treated samples as shown in Figure 5(e) and (f). The growth of only *Aspergillus niger* species was observed in the experiment. Furthermore, *Aspergillus fumigatus* and *Aspergillus sclerotiorum* did not germinate in the samples treated at the MIC concentration of AgNPs and AgNPs-PVP before 7 weeks. Therefore the storage time which AgNPs and AgNPs-PVP are able to prevent the growth of fungi is around 7 weeks compared with the control sample without treatment in which germination occurred after only 4 weeks. Moreover, a concentration of 13.5 µg mL^{-1} of the silver-nano solution was able to inhibit the germination of *Aspergillus fumigatus*, *Sebipora aquosa*, *Aspergillus sclerotiorum*, *Penicillium funiculosum*, *Talaromyces* sp., *Gelatoporia*

subvermispora and *Sympoventuriaceae* sp. whereas the higher concentration of 27 μg mL^{-1} was necessary only to inhibit the development of *Aspergillus niger*.

4 Discussion and Conclusions

According to morphological and molecular techniques, *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus sclerotiorum* were identified from MPPIs. All three fungi are closely related and belong to the same genus, which causes the spoilage of food and agricultural products and is also found in the environment [23]. Clade B consisted of sp6 and sp7 (*Penicillium funiculosum* and *Talaromyces* sp.) which are also closely related according to a previous study [24]. Clade C contained the fungus sample sp9, *Sympoventuriaceae* sp., which is a filamentous fungus from the Ochroconics genus which is found in the soil [25]. The last group, Clade D, consisted of sp3 and sp8 (*Sympoventuriaceae* sp. and *Gelatoporia subvermispora*), which are closely related and are Polyporals found in rotten wood [26]. Those 3 major strains of fungi were studied for antifungal activities using two antifungal agents; Silver nanoparticles (AgNPs) and Silver nanoparticles stabilizing by Polyvinylpyrrolidone (AgNPs-PVP). From these results it can be seen that, the AgNPs were smaller than the AgNPs-PVP.

Moreover the AgNPs-PVP solution was more stable and could be kept for 12 months, while the AgNPs solution could only be kept for 3 months. PVP is a polymer that can be used to maintain the stability of the AgNPs. In general, polymers or organic materials with long chains or functional clusters can be used to attach to the surface of particles, which helps to prevent them from making direct contact with each other or aggregating when solution conditions change [27]. Unlike the work of other authors, our studies point to selective antifungal activity in both AgNPs and AgNPs-PVP where similar concentrations (MIC of 3 fungi species; Table 2) were concerned. These MIC results were found to be consistent with other studies [18], [20], [28] which is in the range of 1–100 μg mL^{-1}. Further study, we investigated the efficiency of antifungal effect on MPPIs in a real situation. The conditions of a moist chamber are closed to the storehouse in Phangnga Province. The treatment was then conducted and challenged with AgNPs whereas the Mancozeb was used as an agricultural fungicide. When compared with Mancozeb, both types of nano-silver solution showed effective in inhibiting the growth of fungi. Therefore, it is suggested that treating with a silver-nano solution can be used to alternatively prevent fungal growth for storage periods of up to 7 weeks. However, the storage periods exceeding 7 weeks, a repeated coating is necessary.

Further, storage under vacuum conditions would improve the preservation time. The AgNPs-PVP is recommended as the optimum treatment agent for the MPPIs because they have a longer shelf life (12 months). Since the experiments for the anti-fungal effect of both AgNPs and AgNPs-PVP were studied after 12 months and its results still remained the same antifungal activities (unpublished data). Moreover, their abilities to prevent the growth of fungi on stored products would be of major significance to the health of humans and animals, and to the agricultural economy.

Acknowledgments

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References

[1] S. Savary, A. Ficke, J.-N. Aubertot, and C. Hollier, "Crop losses due to diseases and their implications for global food production losses and food security," *Food Security*, vol. 4, no. 4, pp. 519–537, Dec. 2012.

[2] M. Aleksandrowicz-Trzcinska, A. Szaniawski, J. Olchowik, and S. Drozdowski, "Effects of copper and silver nanoparticles on growth of selected species of pathogenic and wood-decay fungi *in vitro*," *Forestry Chronicle*, vol. 94, no. 2, pp. 109–116, Apr. 2018.

[3] A. Singh and S. M. Prasad, "Nanotechnology and its role in agro-ecosystem: A strategic perspective," *International Journal of Environmental Science and Technology*, vol. 14, no. 10, pp. 2277–2300, Oct. 2017.

[4] H. Ashraf, T. Anjum, S. Riaz, and S. Naseem,

"Microwave-assisted green synthesis and characterization of silver nanoparticles using melia azedarach for the management of fusarium wilt in tomato," *Frontiers in Microbiology*, vol. 11, no. 10, Mar. 2020, Art. no. 238.

[5] N. F. Ali and I. S. Abd-Elsalam, "Antimicrobial characteristics of wool fibers treated with chitosan-propolis nano composite and dyed with natural dye extracted from red prickly pear," *International Journal of Agricultural Technology*, vol. 16, no. 2, pp. 223–236, 2020.

[6] W. Pongprayoon, T. Siringam, A. Panya, and S. Roytrakul, "Application of chitosan in plant defense responses to biotic and abiotic stresses," *Applied Science and Engineering Progress*, 2020, Art. no. 3865, doi: 10.14416/j.asep.2020.12.007.

[7] M. Pogribna and G. Hammons, "Epigenetic effects of nanomaterials and nanoparticles," *Journal of Nanobiotechnology*, vol. 19, no. 1, Dec. 2021.

[8] S. AmirAshraf, A. J. Siddiqui, A. E. O. Elkhalifa, M. I. Khan, M. Patel, M. Alreshidi, A. Moin, R. Singh, Mejdi Snoussi, and M. Adnan, "Innovations in nanoscience for the sustainable development of food and agriculture with implications on health and environment," *Science of the Total Environment*, vol. 768, no. 10, May 2021, Art. no. 144990.

[9] D. Dutta and B. M. Das, "Scope of green nanotechnology towards amalgamation of green chemistry for cleaner environment: A review on synthesis and applications of green nanoparticles," *Environmental Nanotechnology, Monitoring and Management*, vol. 15, May 2021, Art. no. 100418.

[10] M. Vargas-Hernandez, I. Macias-Bobadilla, R. G. Guevara-Gonzalez, E. Rico-Garcia, R. V. Ocampo-Velazquez, L. Avila-Juarez, and I. Torres-Pacheco, "Nanoparticles as potential antivirals in agriculture," *Agriculture (Switzerland)*, vol. 10, no. 444, pp. 1–18, Oct. 2020.

[11] J. Zhou, N. Krishnan, Y. Jiang, R. H. Fang, and L. Zhang, "Nanotechnology for virus treatment," *Nano Today*, vol. 36, Feb. 2021, Art. no. 101031.

[12] L. d. C. Silva, A. A. de Oliveira, D. R. de Souza, K. L. B. Barbosa, K. S. F. E. Silva, M. A. B. C. Júnior, O. B. Rocha, R. M. Lima, T. G. Santos, C. M. de A. Soares, and M. Pereira, "Overview of antifungal drugs against paracoccidioidomycosis: How do we start, where are we, and where are we going?," *Journal of Fungi*, vol. 6, no. 4, pp. 1–21, Dec. 2020.

[13] P. Golinska, D. Rathod, M. Wypij, I. Gupta, M. Skladanowski, P. Paralikar, H. Dahm, and M. Rai, "Mycoendophytes as efficient synthesizers of bionanoparticles: Nanoantimicrobials, mechanism, and cytotoxicity," *Critical Reviews in Biotechnology*, vol. 37, no. 6, pp. 765–778, Aug. 2017.

[14] M. Rai, A. Yadav, and A. Gade, "Silver nanoparticles as a new generation of antimicrobials," *Biotechnology Advances*, vol. 27, no. 1, pp. 76–83, Jan. 2009.

[15] J. Liang, J. Wang, S. Li, L. Xu, R. Wang, R. Chen, and Y. Sun, "The size-controllable preparation of chitosan/silver nanoparticle composite microsphere and its antimicrobial performance," *Carbohydrate Polymers*, vol. 220, no. 15, pp. 22–29, Sep. 2019.

[16] S. Rahman, L. Rahman, A. T. Khalil, N. Ali, D. Zia, M. Ali, and Z. K. Shinwari, "Endophyte-mediated synthesis of silver nanoparticles and their biological applications," *Applied Microbiology and Biotechnology*, vol. 103, no. 6, pp. 2551–2569, Mar. 2019.

[17] X. Zhao, L. Zhou, M. S. R. Rajoka, L. Yan, C. Jiang, D. Shao, J. Zhu, J. Shi, Q. Huang, H. Yang, and M. Jin, "Fungal silver nanoparticles: Synthesis, application and challenges," *Critical Reviews in Biotechnology*, vol. 38, no. 6, pp. 817–835, Aug. 2018.

[18] A. Ogar, G. Tylko, and K. Turnau, "Antifungal properties of silver nanoparticles against indoor mould growth," *Science of the Total Environment*, vol. 521–522, pp. 305–314, Jul. 2015.

[19] M. D. C. Sales, H. B. Costa, P. M. B. Fernandes, J. A. Ventura, and D. D. Meira, "Antifungal activity of plant extracts with potential to control plant pathogens in pineapple," *Asian Pacific Journal of Tropical Biomedicine*, vol. 6, no. 1, pp. 26–31, Jan. 2016.

[20] S. W. Kim, J. H. Jung, K. Lamsal, Y. S. Kim, J. S. Min, and Y. S. Lee, "Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi," *Mycobiology*, vol. 40, no. 1, pp. 53–58, Mar. 2012.

[21] Z.-K. Xia, Q.-H. Ma, S.-Y. Li, D.-Q. Zhang, L. Cong, Y.-L. Tian, and R.-Y. Yang, "The antifungal

effect of silver nanoparticles on *Trichosporon asahii*," *Journal of Microbiology, Immunology and Infection*, vol. 49, no. 2, pp. 182–188, Apr. 2016.

[22] D. Richard-Molard, L. Lesage, and B. Cahagnier, "Effect of water activity on mold growth and mycotoxin production," in *Properties of Water in Foods: in Relation to Quality and Stability*, Dordrecht, Netherlands: Springer, 1985, pp. 273–292.

[23] S. Mailafia, G. R. Okoh, H. O. K. Olabode, and R. Osanupin, "Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria," *Veterinary World*, vol. 10, no. 4, pp. 393–397, Apr. 2017.

[24] R. A. Samson, N. Yilmaz, J. Houbraken, H. Spierenburg, K. A. Seifert, S. W. Peterson, J. Varga, and J. C.Frisvad, "Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*," *Studies in Mycology*, vol. 70, pp. 159–183, Sep. 2011.

[25] K. Samerpitak, A. P. M. Duarte, D. Attili-Angelis, F. C. Pagnocca, G. Heinrichs, A. J. M. M. Rijs, A. Alfjorden, A. H. G. Gerrits van den Ende, S. B. J. Menken, and G. S. de Hoog, "A new species of the oligotrophic genus *Ochroconis* (Sympoventuriaceae)," *Mycological Progress*, vol. 14, no. 2, Feb. 2015.

[26] O. Miettinen and M. Rajchenberg, "*Obba* and *Sebipora*, new polypore genera related to *Cinereomyces* and *Gelatoporia* (Polyporales, Basidiomycota)," *Mycological Progress*, vol. 11, no. 1, pp. 131–147, Feb. 2012.

[27] A. Pongpeerapat, K. Itoh, Y. Tozuka, K. Moribe, T. Oguchi, and K. Yamamoto, "Formation and stability of drug nanoparticles obtained from drug/PVP/SDS ternary ground mixture," *Journal of Drug Delivery Science and Technology*, vol. 14, no. 6, pp. 441–447, Jan. 2004.

[28] J. Pulit, M. Banach, R. Szczygłowska, and M. Bryk, "Nanosilver against fungi. Silver nanoparticles as an effective biocidal factor," *Acta Biochimica Polonica*, vol. 60, no. 4, pp. 795–798, 2013.