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Original article

Silver nanoparticles synthesized by *Nigrospora oryzae* showed antifungal activity

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ABSTRACT

In this investigation, an alternate green-route based on myco-synthesised silver nanoparticles (Ag NPs) was evaluated to control plant disease to reduce the usage of synthetic chemicals. Here, we described biologically synthesised Ag NPs using the corn grain contaminant, *Nigrospora oryzae*, and were well-characterised by UV–visible spectrophotometer, X-ray powder diffraction (XRD), transmission electron microscopy (TEM), Energy Dispersive Spectroscopy (EDS) and particle size analyzer. The pathogenic behaviour of the *Fusarium* spp. were checked on Giza 86 and Giza 90 cultivars under greenhouse conditions. *F. moniliforme* and *F. oxysporum* exhibited high pathogenicity against Giza 90 and Giza 86 cultivars respectively. The antifungal activity of biosynthesised Ag NPs was evaluated against eight species of *Fusaria* causing damping-off of cotton seedlings. *In vitro* treatments with different concentrations of Ag NPs were achieved on Czapek Dox agar and Potato dextrose agar plates. Fungal growth was drastically retarded from 25 to 200 ppm of Ag NPs interaction. The antifungal activity of Ag NPs against the *Fusarium* spp. was clearly proven.

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1. Introduction

In recent years, in the field of nanoscience, there has been increasing interest in the fabrication of antimicrobial nanomaterials (Cheeseman et al., 2020). Metal nanoparticles, in particular silver, copper and zinc, have attracted great attention and have emerged as a novel class of nanomaterials (Ghodake et al., 2020; Pariona et al., 2019; Vishvanath et al., 2018). The conventional chemical synthesis processes are expensive, and both utilize and produce toxic chemicals that are hazardous to the environment. Researchers have therefore, examined the ambient biological process that usually occurs in nature. Microbial and plant extract-based processes have resulted from the exploration of natural systems and emerged as an alternative for nanosynthesis. The green

synthesis of nanomaterials such as silver nanoparticles (Ag NPs) using microorganisms has generated considerable attention. The biological and physicochemical properties of these NPs nanoparticles are valuable in various applications in many fields including biomedical and agricultural production (Li et al., 2014). Several microorganisms, including different genera of fungi, have successfully been employed for bio-fabrication of Ag NPs (Spagnoletti et al., 2019; Aygun et al., 2020; Schlüter et al., 2014). Moreover, different plant pathogenic fungi have successfully been tested for bio-synthesis of Ag NPs (Yassin et al., 2016). Of these, cereal contaminating fungi such as *Aspergillus clavatus*, *Curvularia pallescens*, *Penicillium citrinum*, and *Phoma leveillei* have also been used in the green fabrication of Ag NPs (Elgorban et al., 2016; Yassin et al., 2016; Yassin et al., 2017). Utilization of bio-synthesised Ag NPs in the control of other phytopathogenic fungi to eradicate them, or at least minimize their effects, is promising (Abdel-Hadi et al., 2014).

Fusarium species are one of the most serious plant pathogens. Their large host range can cause a variety of damages depending on the susceptibility of the host, the infected regions of the plant, and the virulence of the isolate. Diseases of cotton seedlings caused by *Fusarium* spp. result in seedling death and root rot of adulate plants in most cotton-producing areas (Palmateer et al., 2004,

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Costa, et al., 2005 El-Samawaty, et al., 2008; 2012). The extent of injuries and the amount of damage in the host plants depends on the virulence of the infecting *Fusarium* species and the susceptibility of the cotton cultivars (Aly et al 2000, Palmateer et al., 2004; El-Samawaty et al 2013). Eradicating or at least minimizing the Fusarial rot disease of cotton seedlings is vital (El-Samawaty et al., 2013).

Chemical fungicides are detrimental to the biological balance and can prompt the development of resistant plant pathogenic fungi (Calhelha et al., 2006). Thus, the identification and use of alternatives are required (Reddy et al., 2010; Yassin et al., 2013). This study investigated the green route for synthesis of Ag NPs using the corn grain contaminant, *Nigrospora oryzae*. The successful formation of Ag NPs was verified using various analytical techniques UV-vis, XRD, TEM, and EDS measurements. Mycosynthesis Ag NPs was tested for antifungal effect against eight species of *Fusaria* causing damping-off of cotton seedlings (Elgorban et al., 2017; Yassin et al., 2017a,b). The study aimed to eradicate pathogenic plant microorganisms without using synthetic chemical.

2. Materials and methods

2.1. Biosynthesis of Ag NPs

Ag NPs were biosynthesized by *N. oryzae* isolate. Synthetic autoclaved broth was used for the biomass production that was described previously by Yassin et al. (Yassin et al., 2016). After obtaining biomass, the fungal cells were incubated in deionized water for 72 h at 140 rpm in an orbital shaker. Then the cell-free filtrate was collected by washing (Elgorban et al., 2016). The filtrate and 1.0 mM silver nitrate suspension was mixed in 1:1 ratio. The suspension was incubated at 200 rpm in a rotary shaker at 28 ± 2 °C in the dark to allow complete reaction and development of the yellowish colour for 2 days. The solution was then centrifuged at 10,000 rpm for 14 min. The pellet was dried at 60 °C for 24 h.

2.2. Characterisation of Ag NPs

Dried pellet was used to analyze energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) patterns. XRD was applied to evaluate metallic nature of biosynthesized Ag-NPs. X-pert pro diffractometer was used for XRD analysis with Cu-K α radiation at 40 KV and 40 mA. The dried powder was dispersed in 1 ml double distilled water prior to measurement using an ultraviolet-visible (UV-VIS) spectrophotometer and was sonicated for 1 h prior to transmission electron microscopy (TEM) using 740 and 740X Ultrasonics Sonicator TEM and the Ag NPs. EDS is clubbed with TEM; used to enumerate the compositional analysis of NP.

2.3. Source and behaviour of *Fusarium* spp.

Fusarium spp. isolates used in this study were provided by Cotton and Fiber Disease Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt (Table 1). To determine the pathogenicity of these isolates the soil infestation technique was used. Briefly large amounts of mycelia was obtained by transferring a small piece of PDA containing the growing fungus to 250 ml Erlenmeyer flasks containing 100 ml yeast broth and held at 25 °C for 10–12 days in the dark. After this period, the mycelial mat was taken from the Erlenmeyer flasks, washed with tap water, and 0.3 g was blended for 1 min with 100 ml tap water. In general, 100 ml of the blended mycelia was used to infest 3 kg of soil, After adding the fungal suspension, the

soil was hand mixed to obtain uniform distribution of the propagules. The experiment was performed under greenhouse conditions. The seedling damping-off was monitored on Giza-86 and Giza-90 cotton cultivars. Seedling damping-off was recorded 15–45 days after planting (Fig. 1). Table 2. Table 3.

2.4. Antifungal activity of Ag NPs

Czapek Dox and PDA media containing 50, 100, 150, and 200 ppm Ag NP were used. Four-millimeter diameter plugs of each *Fusarium* spp. isolate was cultured in 90 mm Petri plates containing the Ag NP supplemented media. Four replicate plates per treatment along with untreated control plates were used, and all of these plates were incubated at 28 ± 2 °C. Colony diameters were measured daily and the collected data were used for efficacy calculations.

3. Results

3.1. Ag NP biosynthesis

The reaction of silver nitrate with the culture supernatant and the gradual colour change to the yellowish colour was visually observed after 2 days of incubation.

The preliminarily characterization of prepared biosynthesized Ag NPs was done with UV-vis spectrophotometer and it gave an absorbance peak at 420 nm (Fig. 2). The excitation of NPs was due to strong localized SPR property. The yellow colour appearance and the absorbance peak around 400 nm represents the Ag NPs formation.

3.2. Ag NP characterisation

Ag presence were confirmed by EDS analysis (Fig. 3). The presence of other elements in the EDS spectra were from biomolecules in the reaction mixture.

The crystalline nature of the obtained Ag NPs was determined by XRD analysis, which revealed the presence of pure Ag metal with a polycrystalline nature (Fig. 4). An average grain size (D), dislocation density (δ), and strain (ϵ) for Ag NPs were 46.4 nm, $4.64 \times 10^{-4} \text{ nm}^{-2}$, and 7.3×10^{-3} , respectively, as calculated by TEM revealed mostly spherical nanoparticles with a size range from 3 to 13 nm size, with good Gaussian variation (Fig. 5).

3.3. Antifungal evaluation of Ag NPs

Antifungal activity of Ag NPs against *Fusarium* spp. was clearly proven in this study. The analysis of variance (ANOVA) showed that *Fusaria*, concentration of Ag NPs, and their interaction were highly significant sources of variation in the fungal growth on PDA and Czapek Dox media. Relative contribution indicated that the concentration of Ag NPs was the most important source of variation, while the (F \times C) interaction was the least important topic (Fig. 6). Regardless of the culture media, all Ag NP concentrations were capable of inhibiting the fungal growth. All tested species were variably inhibited when grown on PDA and Czapek Dox media. Inhibition generally increased as the Ag NP concentration increase. Fungal growth was drastically retarded at a concentration of 200 ppm.

Table 1
Geographic origin, host plant, and previous crop of *Fusarium* isolates.

	<i>Fusarium</i> species	Geographic origin	Host	Previous Crop
F1	<i>F. sambucinum</i>	Minya	Giza 90	Onions
F2	<i>F. semitectum</i>	Sohag	Giza 90	Egyptian clover
F3	<i>F. sporotrichioides</i>	Sohag	Giza 83	Egyptian clover
F4	<i>F. anthropophilum</i>	Assuit	Giza 90	Egyptian clover
F5	<i>F. oxysporum</i>	Sohag	Giza 90	Egyptian clover
F6	<i>F. moniliforme</i>	Sohag	Giza 90	Egyptian clover
F7	<i>F. fusarioids</i>	Sohag	Giza 90	Egyptian clover
F8	<i>F. solani</i>	Assuit	Giza 90	Wheat

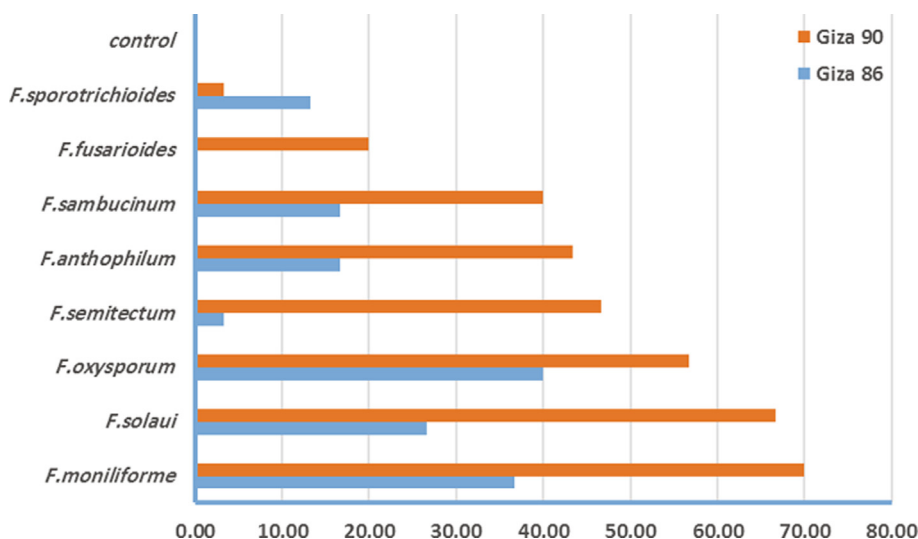


Fig. 1. Pathogenicity of *Fusarium* species on Giza-86 and Giza-90 cotton cultivars under greenhouse conditions.

Table 2
Effect of Ag NPs on the growth of *Fusarium* spp. on Czapek Dox medium.

<i>Fusarium</i> spp.	Concentration (ppm)								Mean
	Control	25	50	75	100	150	200		
<i>F. sambucinum</i>	90.00	90.00	84.00	72.25	70.25	64.75	54.67	75.13	
<i>F. semitectum</i>	90.00	76.50	58.50	42.50	37.00	35.00	33.38	53.27	
<i>F. sporotrichioides</i>	90.00	83.50	58.25	43.75	38.25	31.75	27.57	53.29	
<i>F. anthropophilum</i>	90.00	79.25	64.50	49.50	43.00	41.75	40.13	58.30	
<i>F. oxysporum</i>	90.00	80.25	57.75	46.00	36.50	32.25	30.17	53.27	
<i>F. moniliforme</i>	90.00	84.75	76.50	64.50	57.00	51.25	50.63	67.80	
<i>F. fusarioids</i>	90.00	73.75	62.00	55.50	40.75	30.00	25.07	53.86	
<i>F. solani</i>	90.00	85.50	79.75	67.25	52.75	52.00	42.38	67.09	
Mean	90.00	81.69	67.65	55.15	46.94	42.34	38		

LSD ($P \leq 0.05$) for fusarium (F) = 1.10.
LSD ($P \leq 0.05$) for concentration (C) = 0.52.
LSD ($P \leq 0.05$) for interaction F × C = 2.91.

4. Discussion

4.1. Ag NP biosynthesis and characterisation

The development of a yellowish colour was visually observed after 2 days. This colour change is considered the first indicator of the biological synthesis of Ag NPs (Sadowski et al., 2008). The reduction of silver ions that occurs during the formation of Ag NPs is responsible for the colour changes that might occur due to the activity of extracellular fungal enzymes such as nitrate reductase (Naveen et al., 2010).

XRD analysis revealed pure polycrystalline Ag metal. A similar crystalline and metallic nature with face centered-cubic structures of Ag NPs has been described (Sadowski et al., 2008; Gade et al.,

2014; Nanda et al., 2015). EDS analysis results suggested that Ag was the major element based on the very intense signal observed at 3 K eV in the EDS profile (Gade et al., 2014; Mallikarjuna et al., 2014). TEM indicated diverse morphology that nevertheless mostly consisted of spherical nanoparticles 3 to 13 nm in size with good Gaussian profile (Elgorban et al., 2016; Goswami et al., 2013).

UV–VIS near infrared spectroscopy indicated the extreme permeability of the reactive solution at 420 nm, suggesting the formation of Ag NPs. The results were similar to the previous description (Gade et al., 2014; Birla et al., 2009) that the absorbance peak of bio-synthesised Ag NPs was approximately 420 to 440 nm and the dependent particle size was 2.237 nm (Shivraj et al., 2014).

The antifungal activity of Ag NPs was investigated using *Fusarium* spp. All the species growth was suppressed when exposing to

Table 3
Effect of Ag NPs on the growth of *Fusarium* spp. on Czapek Dox medium.

<i>Fusarium</i> spp.	Concentration (ppm)							
	Control	25	50	75	100	150	200	Mean
<i>F. sambucinum</i>	90.00	90.00	75.50	60.50	51.00	40.75	34.61	63.19
<i>F. semitectum</i>	90.00	78.00	53.50	45.00	40.50	38.75	28.36	53.44
<i>F. sporotrichioides</i>	90.00	87.50	69.75	58.25	45.25	31.50	30.28	58.93
<i>F. anthophilium</i>	90.00	79.00	64.25	46.00	41.25	33.50	27.24	54.53
<i>F. oxysporum</i>	90.00	89.00	75.75	66.00	53.50	43.75	31.11	64.16
<i>F. moniliforme</i>	90.00	77.50	61.75	56.00	44.75	31.75	26.36	55.44
<i>F. fusarioids</i>	90.00	90.00	84.25	60.75	50.00	37.75	32.28	63.58
<i>F. solani</i>	90.00	72.25	65.25	51.50	42.50	37.25	31.74	55.78
Mean	90.00	82.97	68.75	55.50	46.09	36.87	30.25	

LSD ($P \leq 0.05$) for fusarium (F) = 1.02.
 LSD ($P \leq 0.05$) for concentration(C) = 0.48.
 LSD ($P \leq 0.05$) for interaction F × C = 2.68.

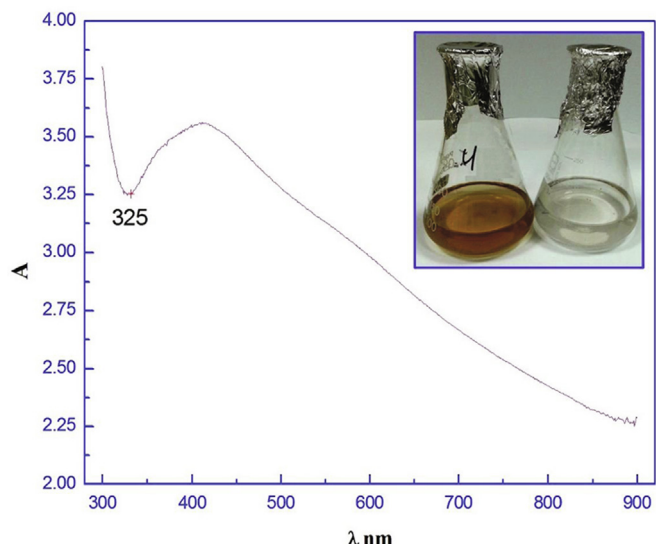


Fig. 2. Reaction solution showing the colour change in the culture filtrate with silver ions (b) compared with the control (a).

25 to 200 ppm of biosynthesized Ag NPs. The growth inhibition varied to great extents when grown on the two different media. In general, growth inhibition increased as the Ag NP concentration increased. Fungal growth was drastically retarded at a concentration of 200 ppm. Higher concentrations of Ag NPs in solution might be capable of saturating and adhering to fungal hyphae and disrupting the fungal cells. Such an inhibitory effect can be attributed to Ag⁺ that primarily affects the function of membrane-associated enzymes such as those found in the respiratory chain. Ag⁺ may also affect the expression of some microbial proteins and enzymes. Disruption of DNA replication has also been documented. Further examination for field applications is needed to ensure the antifungal activity of Ag NPs.

5. Conclusion

Ag NPs were bioengineered using the corn grain contaminant *Nigrospora oryzae*. These NPs displayed strong antifungal activity against *Fusarium* spp. plant pathogenic fungi. It is expected that the application of biosynthesised Ag NPs nanoparticles at low concentrations will be eco-friendly and decrease farm management

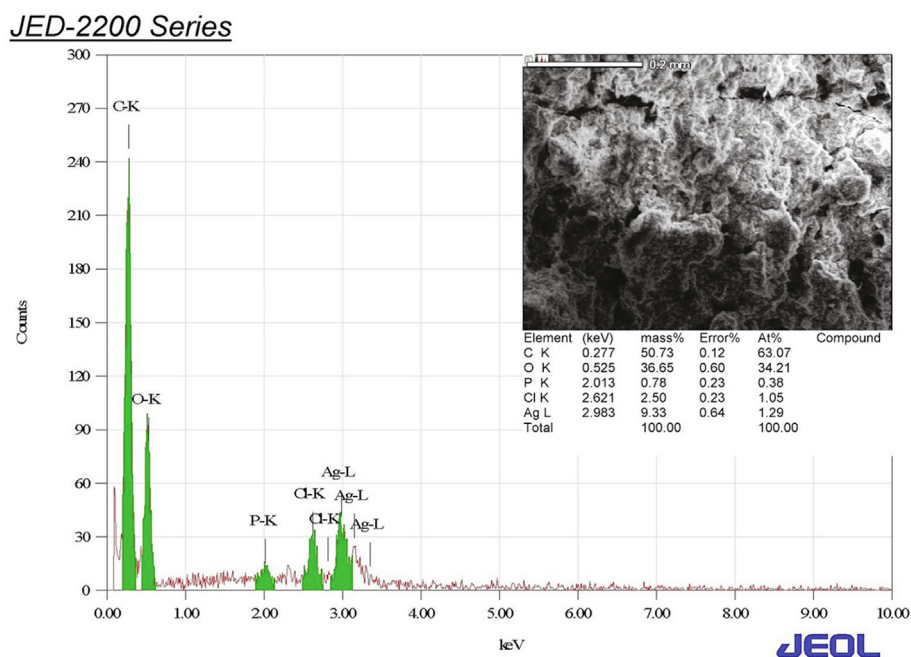


Fig. 3. EDS micrograph of biosynthesised Ag NPs.

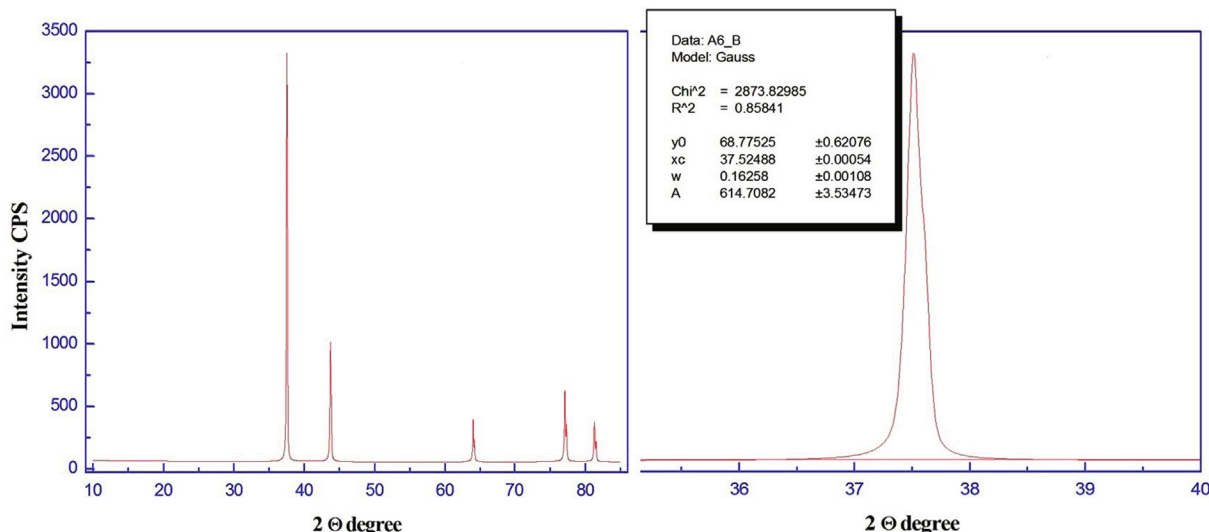


Fig. 4. XRD micrograph of Ag NPs.

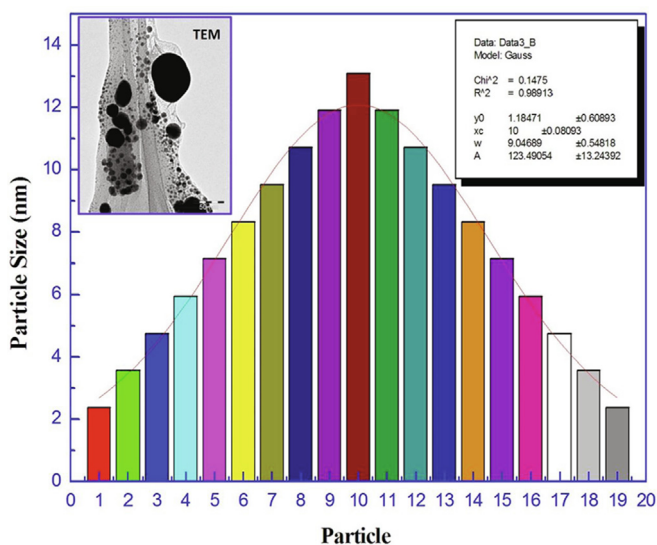


Fig. 5. TEM micrograph and Gaussian distribution of biosynthesised Ag NPs.

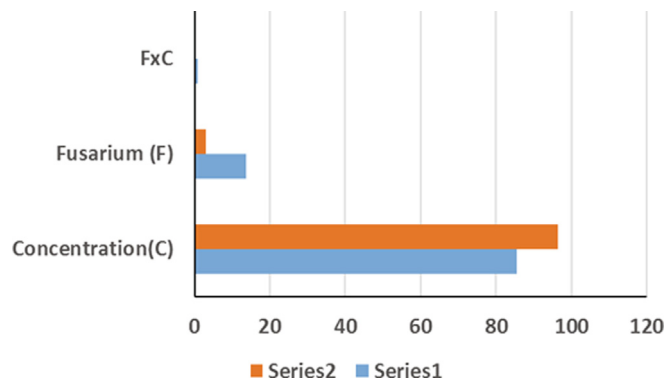


Fig. 6. Relative contribution of Ag NP concentration, *Fusarium* spp., and their interaction on the growth of *Fusarium* spp.

costs. It will be used as a alternate solution for controlling fungal pathogens affecting plants growth instead of using synthetic chemical.

Declaration of Competing 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.

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