Antifungal activity of some nanoparticles against kidney bean root rots pathogens

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ABSTRACT

The inhibitory effects of silver oxide nanoparticles (Ag NPs), Zinc oxide nanoparticles (Zn NPs) and Chitosan nanoparticles (Ch NPs) against the causal pathogens of kidney bean root rot comparing with fungicide Topsin-70® Wp were evaluated under greenhouse and laboratory during the summer season of 2023. In the pathogenicity test under greenhouse, based on infection of damping-off or root rot, the isolates No 5 of *Sclerotium* genus, No 4 of *Rhizoctonia*, No 1 of *Pythium* and No 2 of *Fusarium* were the most aggressive isolates, respectively. *In vitro* experiment, both fungicide and high level of any of nanoparticles used (30 ppm) entirely suppressed the linear spread of four examined fungal genera (*Fusarium solani*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium ultimum*). Zn NPs were the most effective followed by Ag NPs. In greenhouse experiment, under artificially contaminated soil with a diverse range of pathogenic fungi, Topsin-70® Wp and all tested nanoparticles as seed soaking treatments led to protecting bean plants against damping-off or root rot infection. The fungicide Topsin-70® Wp was more efficient followed by Zn NPs and Ag NPs. This study clearly demonstrated that there are no statistically important differences among the leverage of nanoparticles and fungicide against bean root rot pathogens.

Keywords: Nanoparticles, Silver nanoparticles, Zinc nanoparticles, Chitosan nanoparticles, Beans, Root rot pathogenic fungi.
Article type: Research Article.

INTRODUCTION

Kidney bean, *Phaseolus vulgaris* L. is one of the foremost vegetable legume crops for human exhaustion and a great figure export. It has also a beneficial effects of soil fertility, since supplying it with fixing atmospheric nitrogen by Rhizobium nodules (Anonymous 2005; Mahmoud *et al.* 2013). Multiple complex fungi like *Fusarium solani*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium* spp. cause damping-off and root rot diseases of kidney bean, affecting germination, seedlings growth and productivity (El-Shami 2008; Abd-El-Khair *et al.* 2011; Abd El-Hai & Ali 2018; EL-Saman *et al.* 2023). The control of these diseases is often complicated, since they have a vast host variety and create robust structures to last in the soil for a long time such as sclerotia or clamydospores in the absence of the host. The control of these pathogens depends only on applying chemical fungicides as a seed treatment before sowing. However, these compounds exhibit a harmful effect on beneficial micro-organisms, Rhizobia death and also negative effects on environmental balance and public health (Rauf 2000; Al-Kahal *et al.* 2009). Moreover, Chattopadhyay *et al.* (2017) added that the plant diseases control is the most difficult part of agricultural production, and thus the use of pesticides increased and become a serious problem threatening public health. To eliminate the damage of excessive use of fungicides, alternative strategies against plant pathogens are urgent. Therefore, the use of nanotechnology appears more alluring and favourable than chemical fungicide domination. Nanoparticle, which can vary from 1 to 100 nm, has engaged in a range of biological activities. It is actually more adequate than its molecular and macro-scale counterpart, since it has unique physical and chemical features such as the small size, extensive surface area to volume ratio, extreme reactivity, and alterations in molecular interactions (Wang *et al.* 2016). Many authors found that the application of nanoparticles has a fruitful

outcome against plant fungal pathogens such as silica silver (Park *et al.* 2006), Ag NPs (Jo *et al.* 2009), Zn O (Dimkpa *et al.* 2013; Al-Zaidi *et al.* 2023), silver-chitosan compost (Moussa *et al.* 2013) and chitosan (El-Mohamady *et al.* 2019). The current investigations goal was to evaluate antifungal properties of silver, zinc and chitosan nanoparticles against root rot pathogenic fungi of kidney bean and comparing them with Topsin-70® Wp fungicide under laboratory and greenhouse circumstances.

MATERIALS AND METHODS

Plant material

Kidney bean seeds cv. Giza 3 were bought from Department of Vegetables Crop Production Research, Horticultural Research Institute, Agriculture Research Centre, Giza, Egypt.

Fungicide and nanoparticles

A commercial fungicide product (Topsin-70® Wp) has the active ingredients. Thiophanate methyl was purchased from Sigma Company and used as a fungicide of root rot at the rate of 3 g L^{-1}. Silver nanoparticles (Ag O NPs) and Zinc nanoparticles (Zn O NPs) were obtained from King Aba Alla Institute for nanotechnology, College of Science, King Saud University, Saudi Arabia. Silver oxide nanoparticles size ranged from 20 to 30 nm while zinc oxide nanoparticles size from 10 to 30 nm. Moreover, chitosan nanoparticles (Ch NPs) was obtained from Advanced Research Centre Nanotechnology and Advanced Nano-materials Laboratory (NANML), Plant Pathology Research Institute, ARC, Giza, Egypt. The average size of Ch NPs ranged from 10 to 20 nm (a Malvern Instruments Nanosizer Nano ZS equipment). Different concentrations of the three nanoparticles i.e. 10, 20 and 30 ppm were prepared at room temperature by diluting the initial stock solution (1000 ppm) utilizing sterile distilled water. Afterward, chitosan nanoparticles was dispersed in acetic acid solution 1% (v/v) according to the method of Tang *et al.* (2007). For control treatment, sterile distilled water was used. All solutions were stored at 4 ℃ until use for further studies. Before adding nanoparticles to sterilized fungal growth media or directly to seeds as seed soaking, they were exposed to sonication for 30 min using Transonic Type 420, Elma, Germany Sonicator.

Kidney bean root rot fungal pathogens

Five isolates of each of *Fusarium solani*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium ultimum* were separated from naturally infested bean plants appearing typically signs of damping-off or root rot diseases. Bean infested roots cleaned completely with tap water, sliced into tiny sections in addition to surface- sterilized with 2% sodium hypochlorite, and re-washed using sanitized distilled water, then dried between two disinfected filter papers. The sanitized infected pieces put upon PDA medium in petri dishes accompanied by streptomycin-sulfate (100 μg mL^{-1}) to suppress the bacterial germination. Petri-dishes were incubated at 26 ℃ for five days. The growing fungi were recognized depending on their culture properties, morphological and microscopic characters according to Sime *et al.* (2002), Sneh *et al.* (1992) and Booth (1977).

Preparation of fungal inoculum

Individually fungal inoculum of every fungus isolate was made utilizing the media of sorghum: coarse sand: water (2: 1: 2 v/v). The components media were appropriately blended, bottled and autoclaved for 20 min at 1.5 air pressure. Sanitized medium was inoculated with agar discs acquired from the perimeter of five days old culture colony of every isolate from the isolated fungi. The inoculated media were incubated at 27 ℃ for 15 days to use for soil infestation to study the pathogenic ability of all fungal isolates in the pathogenicity test.

Pathogenicity test under greenhouse conditions

The previously prepared inoculum of every pathogen isolate was compiled with the autoclaved clay soil at the rate of 2% w/w then set in sanitized pots (25 cm in diameter). The infested pots were irrigated three times at 2days intervals before strewing to boost and insure the spread of fungal inoculum growth. Sanitized bean seeds cv Giza 3 were planted in pots at the rate of ten seeds per pot under greenhouse in the summer season of 2023. Three replicates were utilized in every specific treatment. Also, three pots contained autoclaved soil without fungal inoculum were prepared to serve as check. Pre- or post-emergence damping of hygienic survival plants were registered at 15, 30 and 45 days from strewing, respectively. The most aggressive isolate from each fungal genera was selected for further studies.

In vitro, antifungal effectiveness of nanoparticles towards pathogenic fungi

The antifungal impact of Ag NPs, Zn NPs and Ch NPs at 10, 20 and 30 ppm against the linear growth of the most aggressive isolate from each fungal genus comparing with Topsin-70® Wp was tested *in vitro*. The different nanoparticles concentration and Topsin-70® Wp (3 g L^{-1}) were added to 10 mL sanitized PDA (1:9 v/v, respectively) before solidification, then filled in sanitized petri-dishes (9 cm in diameter). After solidification, the plates were inoculated in the middle with fungal disc of the pathogenic fungal isolate then incubated at 26 ℃. Three plates were utilized for every treatment and three plates were made as a control for every fungal genus. Linear outgrowth was recorded when the full outgrowth of any examined pathogenic fungi was spotted in the control treatment and the inhibition percentage compared to control was calculated according to the equation shown below:

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

where C = average fungal outgrowth in check;

T = average outgrowth of pathogen with every treatment.

Nanoparticles *vis* root rot pathogenic fungi under greenhouse situations

Pot experiment was done under greenhouse to study the effects of the three tested nanoparticles on disease assessment of bean plants under synthetically infested with root rot pathogenic fungi. A same quantity of every fungal inoculum was mixed well to form a mixture of fungal inoculum, then blended with clay soil in pots (25 cm in diameter) at the rate of 2% w/w. Infested pots were irrigated and kept for seven days before strewing to promote the spread of mixed fungal inoculum. Bean seeds were soaked in Ag NPs, Zn NPs and Ch NPs at 10, 20 and 30 ppm and Topsin-70® Wp fungicide at 3 g L^{-1} for 2 h then dispersed in infested pots at the rate of 10 seeds per pot. The rate (%) of pre- or post- emergence damping-off and root rot were assessed at fifteen, thirty and sixty days from strewing, respectively by subsequent equations:

$$\text{Pre-emergence damping-off rate (\%)} = \frac{\text{Number of non-germinated seeds after fifteen days}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Post-emergence damping-off rate (\%)} = \frac{\text{Number of dead seedlings after thirty days}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Root rot rate (\%)} = \frac{\text{Number of plants infected with root rot after 60 days}}{\text{Total number of sown seeds}} \times 100$$

$$\text{Survived plants rate (\%)} = \frac{\text{Number of survival plants after 60 days}}{\text{Total number of sown seeds}} \times 100$$

Statistical analysis

The information acquired were statistically evaluated utilizing the analysis of variance (ANOVA) approach for fully randomized layout utilizing CoStat software (4.6 version) for comparing among means at 1% in laboratory experiment and at 5% in greenhouse experiment.

RESULTS

Fungal isolation and their pathogenicity

Five isolates of four fungal genera (Numberd from 1 to 5) were isolated from normally infected bean plants appearing tipically root rot signs. The isolated fungi recognized as *Fusarium solani*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium ultimum*.These fungal isolates were examined for their pathogenic capability on kidney bean cv Giza 3.

Table 2. Pathogenicity test of root rot isolated fungi on bean damping-off.

Fungus	Isolate Number	Pre-emergence	Post-emergence	Survival
Check	………………..	0.00 [h]	1.00 [g]	99.00 [a]
	1	23.67 [g]	39.67 [b]	36.33 [cd]
	2	25.67 [g]	42.33 [a]	32.00 [e]
F. solani	3	24.00 [g]	40.33 [b]	35.67 [c]
	4	24.33 [g]	41.33 [ab]	34.34 [d]
	5	25.00 [g]	41.67 [ab]	33.33 [de]
Mean	………..	24.534	41.066	34.334
	1	42.67 [b]	36.00 [c]	21.33 [h]
	2	41.00 [b]	33.33 [d]	25.67 [g]
S. rolfsii	3	43.00 [ab]	36.33 [c]	20.67 [h]
	4	41.33 [b]	34.67 [cd]	24.00 [g]
	5	45.33 [a]	38.67 [b]	16.00 [i]
Mean	………….	42.666	35.868	21.534
	1	39.67 [c]	29.00 [ef]	31.33 [e]
	2	39.00 [c]	27.67 [f]	33.33 de
R. solani	3	37.33 [cd]	26.00 [f]	36.67 [c]
	4	41.67 [bc]	29.67 [ef]	28.66 [f]
	5	38.00 [c]	26.33 [f]	35.67 [cd]
Mean	………..	39.134	27.734	33.126
	1	36.00 [d]	32.33 [d]	31.67 [e]
	2	34.33 [de]	30.00 [e]	35.67 [cd]
P. ultimum	3	30.67 [f]	29.00 [ef]	40.33 [b]
	4	35.00 [d]	31.00 [e]	34.00 [d]
	5	33.67 [e]	29.00 [ef]	37.33 [c]
Mean	……….	33.394	30.266	35.800

Note: Various characters in a column show significant variations at p ≤ 0.05.

Information shown in Table 1 demonstrate that, all examined isolates of four fungal genera were pathogenic in addition to reasoned pre and post-emergence damping-off diseases on bean seedlings. The greatest rate of pre-emergence damping-off occurred under infested soil with *S. rolfsii* with an average of 42.666% followed by *R. solani* (39.134%) then *P. ultimum* (33.934%), while *F. solani* came in the last order (24.534%). On the other side, *F. solani* followed by *S. rolfsii* exhibited the greatest rate (%) of post-emergence damping-off with an average of 41.066% and 35.868%, respectively. While *P. ultimum* came in the third order (30.266%) followed by *R. solani* (27.734%). Concerning survival plants, *S. rolfsii* was the most aggressive fungus leading to 21.534% healthy survival followed by *R. solani* (33.126%) then *F. solani* (34.334%), while *P. ultimum* was less effective (35.800%). Generally, the most aggressive isolates were No. 5 of *S. rolfsii*, No. 4 of *R. solani*, No. 1 of *P. ultimum* and No. 2 of *F. solani*, therefore they were selected for further studies.

Nanoparticles *vis* root rot pathogens linear growth
The antifungal activity of three tested concentrations of three nanoparticles and Topsin-70® Wp against the linear spread of the most hostile isolate from each the four fungal pathogens were estimated in *in vitro*.

Table 2. Impact of nanoparticles on fungal linear outgrowth (cm).

tments	*F. solani* N0. 2	*S. rolfsii* No. 5	*R. solani* No. 4	*P. ultimum* No. 1
k				
IPs 10 ppm	5	1	2	3
IPs 20 ppm	4	4	7	2
IPs30 ppm				
IPs 10 ppm	5	3	7	3
IPs 20 ppm	1	3	)	2
IPs 30ppm				
IPs 10 ppm	2	2	5	1
IPs 20 ppm	)	3	)	3
IPs 30 ppm				
in-70® Wp				

Note: Various characters in a column show significant variations at p ≤ 0.01; LG= Linear Growth and IP = Inhibition Percentage.

As shown in Table 2, the linear growth of both of chick treatment of *S. rolfsii* and *R. solani* completed its growth and filled the petri-dishes after 6 days from inoculation, so at 6 days the linear growth was estimated. Generally,

P. ultimum was more sensitive to nanoparticles accompanied by *F. solani* then *R. solani* while, *S. rolfsii* was more tolerant it. Both fungicide and the high concentration of any of nanoparticles prohibited the germination of all examined pathogens. Moreover, Zn NPs was the most effective in minimizing the fungal outgrowth, Ag NPs came second while, Ch NPs came late. Also, it was found oppositely correlation between increasing nanoparticles concentration as well as growth of the examined pathogenic fungi. Regardless of the fungicidal treatment, the lower outgrowth and inhibition percentage of all pathogens happened under the administration of Zn NPs at 20 ppm accompanied by Ag NPs at 20 ppm.

Greenhouse experiment

In this experiment, the efficacy of nanoparticles in protecting bean plants from infected with root rot fungi, compared to Topsin-70® Wp was estimated in infested soil with a combination of equal amount of each fungal genus.

Table 3. Disease assessment of damping-off in addition to root rot as response to nanoparticles.

tments	emergence	-emergence	: rot	ival rate (%)
k (Mixture fungi)	3 a	7 a	3 a	7 j
IPs 10 ppm	7 d	) c	7 c	5 f
IPs 20 ppm	f	d	e	) d
IPs30 ppm	gh	ef	fg	) b
Ps 10 ppm	3 e	cd	3 d	5 e
Ps 20 ppm	g	de	ef	) c
Ps 30ppm	gh	ef	fg	) b
IPs 10 ppm	3 b	3 b	7 b	7 i
IPs 20 ppm	7 c	) b	7 b	5 h
IPs 30 ppm	) d	7 bc	7 c	) g
in-70® Wp	h	f	g	) a

Note; Various characters in a column show significant variations at $p \le 0.05$.

Table 3 reveals that, all examined nanoparticles at any dose then fungicide lowered significantly the rate (%) of damping-off (pre or post), subsequently increased survival rate. In this respect, Zn NPs was more effective compared to Ag NPs while, Ch NPs came at the end. Topsin-70® Wp recorded the greatest lowering of pre, post-emergence damping-off or root rot, where exhibited the least values of these parameters. With regard to nanoparticles effect, the maximum lowering in both damping-off parameters and root rot was recorded by Zn NPs (30 ppm) followed by Ag NPs (30 ppm) without any significant differences between them. In general, noteworthy, pre- emergence damping-off was registered the highest values followed by root rot the post-emergence damping-off. Also, it can be easily observed the negative correlation increase in nanoparticles concentration and disease occurring of damping-off and root rot of bean plants.

DISCUSSION

Kidney bean, *Phsealus valgaris* L. from one of the foremost vegetables for local exhaustion and export. Damping-off and root rot diseases are considered as limiting factors of bean cultivation, outgrowth and productivity. Using chemical fungicides to dominate soil-borne pathogens is a common practice to protect the plants from infection of seeds and seedlings rot, damping-off and root rot diseases. However, it leads to reduction in the beneficial micro-organisms and also is responsible for environmental pollution, which negatively affects human health (Al-Kahal *et al.* 2009). Moreover, Sharma et al. (2019) added that the primary issue associated with soil and environmental health and also comprises alternation of soil physical and chemical properties, pesticide impedance in pathogens, as well as the raised pesticide residual in the soil and water. Nanotechnology can overcome most of these problems where it has wonderful utility as antimicrobial, therapeutic compounds and high sensitivity disease detection (Elmer *et al.* 2018). Therefore, the currently research was planning to search about the evaluation of fungicidal effects of Ag NPs, Zn NPs and Ch NPs at 10, 20 and 30 ppm against the causative pathogens of root rot disease in bean plants. In the present study, based on infection of damping-off and root rot, *S. rolfsii* was the most hostile fungus followed by *R. solani* then *F. solani*, while *P. ultimum* came at the end. The differences in the pathogenicity of fungal genera may be due to variances in infection genes and their tolerance to environmental situations through producing it resistant structures such as sclerotia (Adhikari *et al.* 2022). In addition, fungal enzymes causes cotyledons rot, which leads to seed rot consequently seedlings damping-off. In addition, *Fusarium* spp. secretes fusaric acid which inhibits seed germination (Mahmoud *et al.* 2013.). The obtained results showed

the antifungal activity against all tested pathogenic fungi of all tested concentration nanoparticles *in vitro* study. The high concentrate (30 ppm) of any nanoparticles used prevented all pathogenic fungal growth. Also, under greenhouse conditions, all nanoparticles and Topsin-70® Wp led to protecting bean plants from damping-off and root rot infections under artificially infested with root rot pathogenic fungi. The fungicidal treatment (Topsin-70® Wp) is required during germination stage for protecting against soil-borne fungal pathogens (Abd El-Hai & Ali 2018). Agricultural nanotechnology has appeared to be cultivating for disease management in addition to crop safeguarding (Gogos *et al.* 2012). In-organic nanoparticles such as Ag NPs and Zn NPs are non-toxic, hydrophilic biocompatible and highly stable (Kurtjak *et al.* 2017). Hanly *et al.* (2008) stated that ZnO has more effectively biosafety and fewer cytotoxicity indexes for mammalian cells which have been demonstrated via various cell line tests. Moreover, Premanathan *et al.* (2011) discovered that ZnO NPs led to damaging human cancer cells as opposed to normal cells. In addition, the microbial impact spectrum of Zn nanoparticles involves antibacterial, antifungal and antiviral characteristics (Reddy *et al.* 2007). Moreover, Wani & Shah (2012) stated that greater concentrations of NPs with magnesium iron in addition to zinc *in vitro* suppressed spores cultivation of *Penicillium notatum*, *Aspergillus niger* and *Nigrospora oryzae*. In the same line, Yehia *et al.* (2013) discovered that ZnO NPs prevented spore cultivation and linear outgrowth of *Fusarium oxysporum* and *P. expansum.* Also, ZnO nanoparticles suppress plant diseases due to impact towards various fungal and bacterial pathogens, i.e., *Penicillium expansum*, *Mucor plumbens*, *Aspergillus flavus*, and *Pseudomonas aeruginosa* (Servin *et al.* 2015). Application of nano ZnO progresses stress tolerance in plants since it is connected with raised cumulating of proline and other amino acids, progresses water shortage, stimulation of antioxidants enzymes as superoxide dismutase (SOD), nitrate reductase, catalase, and peroxidase; all of these activities protect plants from pathogen infections (Malandrakis *et al.* 2019). Zn is a co-factor of super oxide dismutase which is an enzymatic antioxidant and lessen the adverse consequences of free radicals (Reactive Oxygen Species "ROS") attributed to pathogen infections. On the other hand, Ag NPs is a prospective tool for management of crop diseases as it enhances a variety of plant defence approaches, involving raised lignin sedimentation in the vascular bundles (Moussa *et al.* 2013). Bholay *et al.* (2013) explained that the mix of Ag NP with fungicide fluconazole exhibited enhanced fungicidal activity against the main fungal pathogen for diverse commercially significant crops such as *Cladosporium berbarum, Alternaria alternata* and *F. oxysporum.* Ahmed (2017) found that the number of spores and mycelial growth were significantly decreased by the application of chitosan and silver nanoparticles (20 ppm, 40 ppm, 60 ppm, 80 ppm, or 100 ppm) on the development of aggressive isolates of *B. fabae* and *Alternaria alternata*. Moreover, various concentrations of chitosan nanoparticles exhibited antifungal activity against the mycelium outgrowth of some pathogenic fungi *in vitro* (El-Mohamedya *et al.* 2019). Chitosan NPs were elucidated as dominate products and plant safety towards *R. solani* and *Fusarium* spp. since they suppressed fungal cultivation and development, in addition to the cationic nature of chitosan due to the permanence of various amine signs that is the essential of their adequacy against (Berger *et al.* 2016). Chitosan nanoparticles caused inhibition of mycelia outgrowth and spore cultivation in *Alternaria solani* or *F. oxysporum* (Saharan *et al.* 2015). Data obtained from this study suggest the utilizing of various nanoparticles as a substitute safe way for controlling bean root rot disease.

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