Antifungal activity of chitosan nanoparticles against some plant pathogenic fungi *in vitro*.

Riad S. R. El-Mohamedy[1], Mahmoud E. Abd El-Aziz[2*], Samir Kamel[3]

(1. *Plant Pathology Department, National Research Centre, 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt*;
2. *Polymers and Pigments Department, National Research Centre, 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt*;
3. *Cellulose & Paper Department, National Research Centre, 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt*)

Abstract: The inhibitory effects of different concentrations of high and low molecular weight of chitosan (CH-HMW and CH-LMW, respectively) and chitosan nanoparticles (CH-HMW-NPs and CH-LMW-NPs, respectively) against linear growth and spores/sclerotia germination of the 18 phytopathogenic fungi of tomato, potato and green bean were evaluated *in vitro*. The studies showed that CH-HMW-NPs and CH-LMW-NPs were highly effective against all tested pathogenic fungi compared with CH-HMW and CH-LMW. Furthermore, CH-HMW-NPs and CH-LMW-NPs at concentrations 0.1% and 0.05% showed completely inhibited (100%) the mycelial growth of all tested pathogens. Meanwhile, chitosan HMW and LMW at 1.0% caused 100% reduction of all tested pathogenic fungi. *Rhizoctonia solani* and *Fusarium solani* followed by *F. oxysporum* and *Macrophomina phaseolina* were highly sensitive to all concentrations of chitosan than *Alternaria solani*, *Sclerotium rolfsii* and *Phytophthora infestance*. This study claimed that chitosan NPs could be a new development for the generation of chitosan based bio-nanopesticides against fungal diseases exploited for delivery of agrochemicals.

Keywords: chitosan nanoparticles, pathogenic fungi, tomato, potato, green bean

Citation: El-Mohamedya, R. S. R., M. E. Abd El-Aziz, and S. Kamel. 2019. Antifungal activity of chitosan nanoparticles against some plant pathogenic fungi *in vitro*. Agricultural Engineering International: CIGR Journal, 21(4): 201–209.

1 Introduction

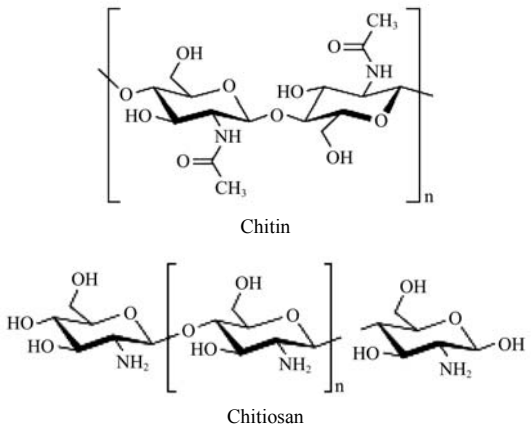
There is a growing interest in the development of alternative strategies in plant disease management to reduce dependency on synthetic chemicals. Plant pathogenic fungi are indubitably the most versatile agent for environmental adaptation and destruction of plant growth. Amongst the numerous strategies, nanotechnology assisted inventions have generated quantifiable data against plant fungal diseases mainly by the applications of nanoparticles (Rabea et al., 2003; Park et al., 2006; Cho et al., 2010). Chitosan is a natural linear biopolymer obtained by alkaline deacetylation of chitin (Scheme 1). Chitin is the mean component of protective cuticles of crustaceans such as crabs, shrimps, lobsters and prawns and it is a homopolymer consist of β-(1,4)-linked N-acetyl-glucosamine units. It has been widely applied in the environmental, pharmaceutical, biomedical and agricultural fields (Cho et al., 2010; Hanafi, 2012; Mahmoud et al., 2018; Abd El-Aziz et al., 2019). Chitosan has several advantages over other types of disinfectants in that it possesses a high-antimicrobial activity, a broad spectrum of activity, and a low toxicity for mammalian cells (Liu et al., 2001; Youssef et al., 2019).

The current situation galvanizes the search for natural antifungal compounds such as chitosan as a safe substitute to synthetic chemicals. Biodegradability, non-toxicity and antimicrobial property has made chitosan biopolymer most important material in agricultural nanotechnology. Studies have concluded that chitosan possesses antifungal activity via affinity of its cationic amino groups to cellular components (Badawy and Rabea, 2009; Meng et al., 2010). Nevertheless, the

Received date: 2018-09-17 **Accepted date:** 2018-11-01

***Corresponding author: Mahmoud E. Abd El-Aziz,** Researcher (author title, like professor), Polymers and Pigments Department, National Research Centre, 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt. Tel: 00233371718, Fax: 00233371718, Email: mahmoud_essam12@yahoo.com.

chitosan biopolymer has not been widely applied as antifungal agent mainly because of its insolubility in aqueous media and lower antifungal activity (Saharan et al., 2013; Youssef et al., 2018).



Scheme 1: structure of chitin and chitosan

Efforts have been commenced to amend the physico-chemical characteristics of chitosan for enhanced antifungal activity (Beaney et al., 2005; Meng et al., 2010). Chemically-modified chitosan viz. triethylene diamine dithiocarbamate chitosan and o-hydroxy phenylaldehyde thiosemicarbazone chitosan have shown higher antifungal activity as compared to chitosan (Meng et al., 2010). Chitosan based nanoparticles (NPs) are preferably used for various applications owing to their biodegradability, high permeability toward biological membranes, non-toxicity to human, cost effectiveness and broad antifungal activities. Chitosan NPs imbued versatility in biological activities due to altered physico-chemical characteristics like size, surface area, cationic nature, active functional groups, higher encapsulation efficiency etc. alone and/or through blending of other components (Saharan et al., 2013). Despite their potential applications in agriculture, few reports are available on the use of chitosan-NPs in plant disease management especially against fungal pathogens. So, this study focuses on the antifungal activity of low and high molecular weight chitosan and their nano-particles against major pathogenic fungi of tomato, potato and green bean in vitro.

2 Materials and methods

2.1 Pathogenic fungi isolates

Eighteen isolates of phytopathogenic fungi were used in this study as follow: seven fungal isolates of tomato i.e., *Fusarium oxysporium* f. sp. *lycopersici* (Fol 2), *F. oxysporium* f. *radicis*. *lycopersici* (Forl 5), *F. solani* (Fs 5), *Rhizoctonia solani* (Rs 5), *Sclerotium rolfsii* (Sr 5), *Alternariasolani* (As 3) and *Phytophthora infestance* (Ph 3), seven isolates of green bean *i.e. Fusarium oxysporium* (Fox 1), *Fusarium solani* (Fs 3), *Rhizoctonia solani* (Rs 1), *Macrophomina phaseolin* (Mph 3), *Sclerotium rolfsii* (Sr 3), *Botryties cienertea* (Bc 3), *Sclerotina. Sclerotiorum* (Sc 2) as well as four fungal isolates of potato *i.e*., *Fusarium sembaticum* (Fse 2), *Rhizoctonia solani* (Rs 2), *Phytophthora infestance* (Ph1) and *Alternariasolani* (As1). These isolated fungal were previously identified at Plant Pathology Department, National Research Centre, Giza, Egypt. The pathogenicity of each fungus was tested and recorded in previous studies by El-Mohamedy et al. (2013).

2.2 Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared based on the ionotropic gelation method between chitosan and sodium tripolyphosphate. Briefly, 2 g chitosan was dissolved in 1% (v/v) acetic acid solution was kept under magnetic stirring at room temperature for 24 h. Sodium tripolyphosphate was dissolved in double-distilled water to a concentration of 1%, under magnetic stirring at room temperature. Then 10 mL of tripolyphosphate solution was added dropwise to 100 mL of chitosan solution. The mixture was stirred at 3000 ppm for 30 min (Tang et al., 2007).

2.3 Characterization

2.3.1 Fourier transform **i**nfrared (FT-IR) spectral analysis

FT-IR spectra of CH and CH-NPs were recorded in the range of 400–4000 cm^{-1} on (Shimadzu 8400S) FT-IR Spectrophotometer.

2.3.2 Transmission electron microscope (TEM)

The morphological and particles size of chitosan and chitosan nanoparticles were demonstrated by using TEM model JEM-1230, Japan, operated at 120 kV, with maximum magnification of 600 × 10^3 and a resolution until 0.2 nm. A drop of an aqueous dispersion of the nanomaterial was placed on a carbon-coated copper grid and allowed to dry in air before characterization.

2.3.3 Zetasizer

Particle size and zeta potential were measured using a

Zetasizer Nano-ZS-90 (Malvern Instruments, UK).

2.4 Bioassay of chitosan and chitosan nano particles CH-NPs against pathogenic fungi

The inhibitory effect of four concentrations of CH-HMW and CH-LMW i.e., 0.125%, 0.25%, 0.5%, 1.0% (w/v) as well as CH-HMW-NPs and CH-LMW-NPs i.e., 0.0125%, 0.025%, 0.05%, 0.1% (w/v) on linear growth, spores and sclclerotia germination of the tested isolates of pathogenic fungi maintained above was evaluated. Poison food technique was used to measure the antifungal activity (Pochanavanich and Suntornsuk, 2002).

2.4.1 Effect on linear growth

Potato dextrose agar (PDA) plates were amended with different concentrations of CH-HMw, CH-LMW as well as CH-HMW-NPs and CH-LMW-NPs according to Laflamme et al. (1999). Un-amended PDA plates with 0.05% final concentration of acetic acid (pH 5.6) served as negative controls. Mycelial bit from peripheral end of uniform size (diameter, 5.0 mm) was taken from 7 days old culture of test pathogens and placed in the center of test Petri dishes. All the Petri dishes were incubated at 28°C±1°C for 7 days and the observation of radial mycelial growth was recorded when control Petri dish cover full growth (90 mm). All the treatments consisted of three replications and experiment was repeated twice. The inoculated plates were compared with control (without nanoparticles) to calculate the % inhibition rate of mycelia of the pathogen.

2.4.2 Effect on spores and sclerotia germination

Sclerotia germination of *R. solani*, *S. rolfsii*, *M. phaseolina*, *Botryties cienertea* and *Sclerotina. Sclerotiorum* (Sc 2) produced on PDA in each treatment as maintained above were determined according to Manning et al. (2003). Meanwhile, spore germination of *F. oxysporum*, *F. solani*, *Fsembaticum*, *A. solani* and Phytophthora *infestance* were evaluated using microscope slide technique. Slides were covered with 1 mL of spore's suspension of each tested pathogen in aqueous solution of the desired concentrations of CH-HMW, CH-LMW as well as CH-HMW-NPs and CH-LMW-NPs in Petri dishes, and then incubated at 27°C±1°C for 8 h in complete darkness. The percentage of germination was assessed according to El-Abyad and Saleh (1971) and El-Abyad et al. (1983). Five plates were prepared for each treatment and the means were compared. Antifungal activity was calculated and expressed as percentage of reduction in both linear growths (LG) as well as on spores and sclerotia germination (SG and SCG) of each pathogenic fungus under investigations.

3 Results and discussion

3.1 FT-IR analysis

Figure 1 represents the FT-IR spectrum of chitosan and chitosan nanoparticles. The characteristic FTIR pattern of chitosan exhibited the absorption band at band 3450 cm^{-1} corresponding to the contribution of -OH and -NH stretching vibration Figure 1a. Also the absorption bands at 1650 and 1380 cm^{-1} corresponded to the C=O and C–O stretching of amide group, respectively. In addition, the absorption band at 1596 cm^{-1} was due to the N–H deformation of amino groups, while the absorption band at wave numbers 1152 and 1075 cm^{-1} corresponded to the symmetric stretching of the C–O–C and concerned skeletal vibration of the C–O stretching, respectively.

Figure 1b shown a shift of absorption band at 3450 to 3427 cm^{-1} and two new sorption bands appear at 1632 cm^{-1}, due to the ammonium groups of chitosan are crosslinked with tripolyphosphate. Thus it is postulated that polyphosphoric groups of sodium polyphosphate interact with the ammonium groups of chitosan, which serves to enhance both inter- and intramolecular interaction in chitosan nanoparticles (El-Abyad et al., 1983).

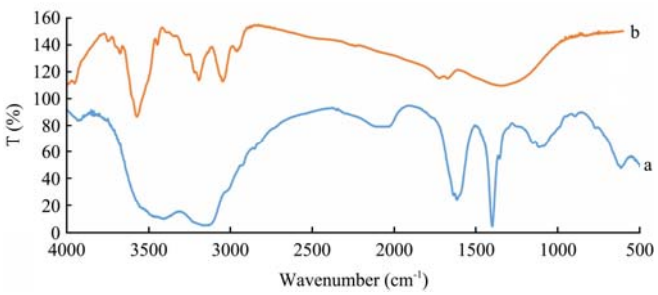


Figure 1 FT-IR spectra of chitosan (a) and chitosan nanoparticles (b)

3.2 Transmission electron microscope (TEM)

Figure 2 represents the morphological structure of chitosan and chitosan nanoparticles. The particles of chitosan were disproportionate as shown in Figure 2a while chitosan nanoparticles were more uniform and

spherical with average particle size 50 nm.

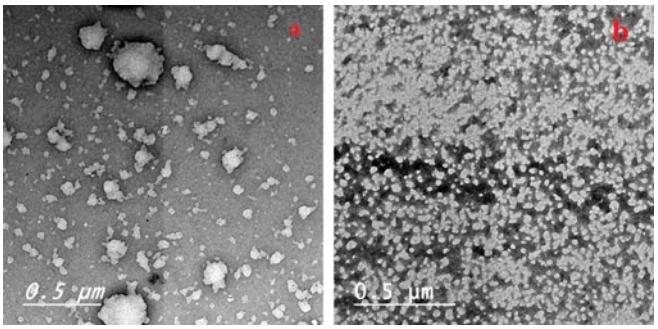


Figure 2 TEM of chitosan (a) and chitosan nanoparticles (b)

3.3 Particle size and zeta potential

Figure 3 showed the zeta potential and the particles size distribution of chitosan nanoparticles. The average particle size was 50 nm with size distribution from 40 to 70 nm (Figure 3a). In addition, the zeta potential of chitosan nanoparticles solution was 48 mV which supports the stability of the formed solution Figure 3b.

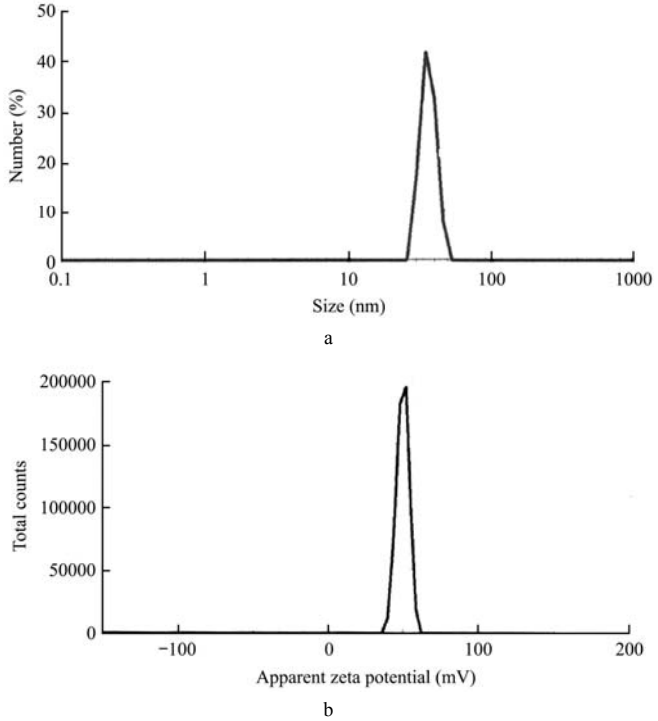


Figure 3 Particle size distribution (a) and zeta potential (b) of chitosan nanoparticles

3.4 Antifungal of chitosan and their nanoparticles against tested fungi

The inhibitory effect of different concentrations of chitosan and chitosan nano particles with high and low molecular weight (CH-HMW, CH-HMW, CH-HMW-NPs and CH-HMW-NPs) against pathogenic fungi of tomato (*Fusarium ox* f. sp. *lycopersici* Fol 2), *Fusarium ox*. f. *radicis*. *lycopersici* Forl 5, *F. solani* Fs 5, *Rhizoctonia solani* Rs 5, *Sclerotiumrolfsii* Sr 5, *Alternaria`solani* As 3 *and Phytophthora infestance* Ph 3), green bean (*Fusarium oxysporium, F. solani* Fs 3, *Rhizoctoniasolani* Rs 5, *Macrophominaphaseolin*, *Sclerotiumrolfsii* Sr 5, *Botryties cienertea*, *Sclerotina. Sclerotiorum* Sc 2 and *Potato* (*Fusarium sembaticum, Rhizoctoniasolani* Rs 5, *Phytophthorainfestance and Alternariasolani*) was investigated and the results are in Table 1, 2, 3 and 4.

3.4.1 Effect on linear growth

Results in Table 1 show that all tested concentrations of chitosan with high and low molecular weight (CH-HMW and CH-LMW) had capability effect in inhibiting linear growth of all tested pathogenic fungi, the inhibition consistently increased with concentration. CH-HMW-NPs and CH-LMW-NPs displayed strong inhibition against all tested pathogens if compared with chitosan. CH-HMW and CH-LMW at 1.0% caused complete (100%) reduction of all tested pathogenic fungi Table 1, wherever, CH-HMW-NPs and CH-LMW-NPs at 0.1% and 0.05% concentrations caused complete reduction (100%) the mycelial growth of the same fungi Table 2. Highest records of reduction (80.0%, 100% and 90.2%) were obtained at 0.5% of CH-HMw with Rhizoctoniasolani Rs5, Rs1 and Rs2 followed by 75.5%, 92.2% and 90.0% with *Fusarium solani* isolates Fs5, Fs3 and *Alternaria solani* isolate As1, but the least reduction records (65.5%, 52.2% and 57.8%) were with *Sclerotium rolfsii* (Sr 5), *Botrytis* ceneriea (Bc 3) and *Phytophthora infestance* (Ph 1). CH-HMW NPs at 0.025% caused reduction in linear growth of *Rhizoctonia solani* Rs3, *Fusarium solani* Fs2, *Rhizoctonia solani* Rs1 and Rs1 by 80.0%, 87.8%, 65.5%. However, CH-HMw at 0.125% and 0.25% concentrations showed fairly growth reduction of all tested pathogenic fungi, especially with *Phytophthora infestance* and *Sclerotium scleotorium*, as the reduction rate reach 27.8% to 11.1% .CH-HMW-NPs at 0.0125% showed considerable rate of reduction (62.2%-83.3%).

3.4.2 Effect on spores and sclerotia germination

Results in Table 3 clearly show that chitosan with HMW and LMW at 1.0% concentration caused complete reduction (100%) of spores and sclerotia germination of all tested pathogenic fungi of tomato, green bean and potato. But at 0.5% it reduced spores and sclerotia germination of tomato pathogenic fungi up to 100% with

Fusarium solani, *Rhizoctonia solani*, 90.0% with *Fusarium oxysporum f. sp. Lycopersici*, 63.4% with *Sclerotiumrolfsii* (Sr 5). The same trend of results recorded with the pathogenic fungi of green bean by 100% except *Sclerotiumrolfsii* Sr 3 (80.0%), *Sclerotium scleotorium* Sc 2 (94.4%), *Fusarium solani* Fs 2 (72.2%) and *Phytophthora infestance* Ph1 (65.6%). CH-LMW NPs at 0.05% and 0.1% concentrations reduced the linear growth of all tested fungi of tomato, green bean and tomato by 100% meanwhile at 0.025 concentration it reduced the growth of *Rhizoctonia solani* (Rs 5), *Sclerotiumrolfsii* (Sr 5), *Rhizoctonia solani* (Rs 1), *Sclerotiumrolfsii* (Sr 3), Rhizoctonia solani (Rs 2) and *Phytophthora infestance* (Ph 1) by 78.9%, 52.2%, 77.8%, 48.9%, 85.5% and 70.0%, respectively. Chitosan at all concentrations were found less effective for inhibition of mycelial growth of all tested pathogenic fungi as compared to synthesized chitosan nano particles CH-NPs.

Table 1 Reduction % on linear growth of Pathogenic fungi on tomato, green bean and potato as affected by different concentration of CH-HMW and CH-LMW on PDA medium

Pathogenic fungi	Host plant	Chitosan concentration %							
		CH-HMW				CH-LMW			
		0.125	0.25	0.5	1.0	0.125	0.25	0.5	1.0
Fusarium ox f. sp. *lycopersici* (Fol 2)	Tomato	20.8	38.4	70.0	100	25.6	52.8	82.2	100
F. ox f. *radicis*. *lycopersici* (Forl 5)	Tomato	21.1	40.0	70.0	100	25.2	55.0	86.0	100
Fusarium oxysporium (Fox 1)	green bean	23.3	38.8	90.0	100	36.7	58.4	100	100
F. solani (Fs 5)	Tomato	28.2	42.0	85.5	100	41.8	62.2	100	100
F. solani (Fs 3)	Green bean	24.0	40.2	82.0	100	35.2	60.2	100	100
Fusarium sembaticum (Fse 2)	Potato	25.2	38.2	77.2	100	34.6	58.0	78.2	100
Alternariasolani (As 3)	Tomato	22.0	33.2	87.8	100	28.0	42.2	76.0	100
Alternariasolani (As 1)	Potato	24.0	38.4	70.0	100	40.0	62.2	84.4	100
Phytophthora infestance (Ph 3)	Tomato	24.4	36.0	68.0	100	30.0	50.2	71.0	100
Phytophthora infestance (Ph 1)	Potato	21.2	28.2	60.4	100	24.2	48.2	66.0	100
Rhizoctonia solani (Rs 5)	Tomato	27.8	57.3	80.0	100	46.8	66.4	100	100
Rhizoctonia solani (Rs 1)	Green bean	25.8	52.2	74.8	100	38.2	62.2	88.0	100
Rhizoctonia solani (Rs 2)	Potato	27.2	55.8	71.4	100	42.0	60.0	76.4	100
Sclerotium rolfsii (Sr 5)	Tomato	14.4	35.5	60.5	100	25.0	40.8	66.4	100
Sclerotium rolfsii (Sr 3)	Green bean	18.2	44.8	75.8	100	25.5	48.2	76.6	100
Sclerotina. sclerotiorum (Sc 2)	Green bean	22.0	52.2	80.0	100	30.0	42.0	70.0	100
Botryties cienertea (Bc 3)	Green bean	15.8	44.4	68.2	100	20.0	44.4	72.2	100
Macrophomina phaseolina (Mph 3)	Green bean	20.1	38.4	78.8	100	30.2	52.0	75.8	100

Table 2 Reduction % on linear growth of Pathogenic fungi on tomato, green bean and potato as affected by different concentration of CH-HMW-NPs and CH-LMW-NPs on PDA medium

Pathogenic fungi	Host plant	linear growth Reduction %							
		CH-HMW-NPs				CH-LMW-NPs			
		0.0125	0.025	0.05	0.1	0.0125	0.025	0.05	0.1
Fusarium ox f. sp. *lycopersici* (Fol 2)	Tomato	57.8	67.8	100	100	48.2	55.8	100	100
F. ox f. *radicis*. *lycopersici* (Forl 5)	Tomato	55.2	72.2	100	100	50.0	66.2	100	100
Fusarium oxysporium (Fox 1)	green bean	55.5	75.5	100	100	50.2	70.1	100	100
F. solani (Fs 5)	Tomato	60.0	82.0	100	100	54.4	77.2	100	100
F. solani (Fs 3)	Green bean	58.2	80.0	100	100	51.1	72.4	100	100
Fusarium sembaticum (Fse 2)	Potato	52.2	74.2	100	100	45.6	68.4	100	100
Alternariasolani (As 3)	Tomato	52.2	74.4	100	100	46.0	65.2	100	100
Alternariasolani (As 1)	Potato	55.0	76.4	100	100	51.8	70.4	100	100
Phytophthora infestance (Ph 3)	Tomato	50.0	65.5	100	100	42.2	60.8	100	100
Phytophthora infestance (Ph 1)	Potato	53.0	64..2	100	100	40.2	58.0	100	100
Rhizoctonia solani (Rs 5)	Tomato	55.2	80.0	100	100	50.8	72.2	100	100
Rhizoctonia solani (Rs 1)	Green bean	57.4	84.4	100	100	53.0	77.8	100	100
Rhizoctonia solani (Rs 2)	Potato	54.5	82.0	100	100	50.2	75.0	100	100
Sclerotium rolfsii (Sr 5)	Tomato	38.8	67.2	100	100	38.0	54.2	100	100
Sclerotium rolfsii (Sr 3)	Green bean	41.2	60.4	100	100	32.0	51.4	100	100
Sclerotina. sclerotiorum (Sc 2)	Green bean	45.8	70.2	100	100	34.4	62.4	100	100
Botryties cienertea (Bc 3)	Green bean	42.8	72.4	100	100	38.8	66.4	100	100
Macrophomina phaseolina (Mph 3)	Green bean	53.2	74.2	100	100	42.8	70.2	100	100

Table 3 Reduction % on spores and sclerotia germination of Pathogenic fungi on tomato, green bean and potato as affected by different concentrations CH-HMW and CH-LMW on PDA medium

Pathogenic fungi	Host Plant	Chitosan concentration %							
		CH-HMW				CH-LMW			
		0.125	0.25	0.5	1.0	0.125	0.25	0.5	1.0
Spores germination Reduction %									
Fusarium ox f. sp. *lycopersici* (Fol 2)	Tomato	21.4	41.6	86.0	100	37.7	54.2	90.0	100
F. ox f. *radicis.lycopersici* (Forl 5)	Tomato	25.2	45.2	90.2	100	40.0	618	90.0	100
Fusarium oxysporium (Fox 1)	Green bean	22.8	40.8	88.0	100	38.8	58.6	100	100
F. solani (Fs 5)	Tomato	28.6	55.2	90.0	100	48.8	70.8	100	100
F. solani (Fs 3)	Green bean	25.0	42.2	82.0	100	40.0	62.2	100	100
Fusarium sembaticum (Fse 2)	Potato	21.4	43.8	92.0	100	36.4	66.2	92.0	100
Alternaria solani (As 3)	Tomato	20.8	40.6	80.2	100	38.2	50.8	88.8	100
Alternaria solani (As 1)	Potato	22.6	42.2	80.8	100	38.8	50.2	90.0	100
Phytophthora infestance (Ph 3)	Tomato	20.2	35.2	77.2	100	32.2	55.8	82.0	100
Phytophthora infestance (Ph 1)	Potato	22.6	38.8	80.0	100	35.8	60.4	84.4	100
Sclerotia germination Reduction %									
Rhizoctonia solani (Rs 5)	Tomato	24.2	52.2	82.0	100	32.4	70.0	100	100
Rhizoctonia solani (Rs 1)	Green bean	20.8	48.2	76.0	100	44.2	72.0	100	100
Rhizoctonia solani (Rs 2)	Potato	22.8	50.0	80.2	100	44.8	70.0	88.2	100
Sclerotium rolfsii (Sr 5)	Tomato	18.2	31.8	70.0	100	28.6	46.0	77.0	100
Sclerotium rolfsii (Sr 3)	Green bean	20.2	36.4	76.0	100	30.2	60.0	100	100
Sclerotina. sclerotiorum (Sc 2)	Green bean	22.8	44.4	72.2	100	32.8	60.8	100	100
Botryties cienertea (Bc 3)	Green bean	20.1	38.4	78.8	100	30.2	52.0	80.8	100
Macrophomina phaseolina (Mph 3)	Green bean	25.8	52.2	84.8	100	46.2	62.2	88.0	100

Table 4 Reduction % on spores and sclerotia germination of Pathogenic fungi on tomato, green bean and potato as affected by different concentration of CH-HMW and CH-LMW NPs on PDA medium

Pathogenic fungi	Host Plant	Chitosan concentration %							
		CH-HMW NPs				CH-LMW NPs			
		0.0125	0.025	0.05	0.1	0.0125	0.025	0.05	0.1
Spores germination Reduction %									
Fusarium ox f. sp. *lycopersici* (Fol 2)	Tomato	55.8	80.0	100	100	50.2	77.0	100	100
F. ox f. *radicis. lycopersici* (Forl 5)	Tomato	59.4	84.6	100	100	52.2	82.2	100	100
Fusarium oxysporium (Fox 1)	Green bean	60.2	88.0	100	100	57.8	82.2	100	100
F. solani (Fs 5)	Tomato	65.4	94.0	100	100	55.8	90.0	100	100
F. solani (Fs 3)	Green bean	63.2	90.4	100	100	52.4	88.2	100	100
Fusarium sembaticum (Fse 2)	Potato	55.8	80.4	100	100	50.8	88.8	100	100
Alternaria solani (As 3)	Tomato	56.2	82.8	100	100	52.4	83.0	100	100
Alternaria solani (As 1)	Potato	55.0	86.6	100	100	51.0	80.2	100	100
Phytophthora infestance (Ph 3)	Tomato	42.0	68.2	100	100	51.8	74.0	100	100
Phytophthora infestance (Ph 1)	Potato	48.2	72.0	100	100	51.8	80.0	100	100
Sclerotia germination Reduction %									
Rhizoctonia solani (Rs 5)	Tomato	56.0	78.8	100	100	51.4	70.4	100	100
Rhizoctonia solani (Rs 1)	Green bean	52.2	70.0	100	100	60.4	82.8	100	100
Rhizoctonia solani (Rs 2)	Potato	54.4	74.0	100	100	42.2	90.0	100	100
Sclerotium rolfsii (Sr 5)	Tomato	38.2	60.0	100	100	44.6	60.0	100	100
Sclerotium rolfsii (Sr 3)	Green bean	40.4	66.2	100	100	41.4	66.4	100	100
Sclerotina. sclerotiorum (Sc 2)	Green bean	50.0	72.2	100	100	47.4	66.4	100	100
Botryties cienertea (Bc 3)	Green bean	45.6	74.6	100	100	52.8	77.6	100	100
Macrophomina phaseolina (Mph 3)	Green bean	52..2	72.6	100	100	52.0	75.0	100	100

Nano-materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and their ability to permeate cells. Many literatures reported that chitosan and its derivatives have antimicrobial and plant-defense elicit

function (Rabea et al., 2003; Kenawy et al., 2005), therefore, these compounds are considered as useful pesticides in the control of plant diseases. In our results, we found that by increasing concentration of both chitosan and chitosan nano particles (0.125%-1.0% and –0.0125%-0.1%), the radial growth, spors and sclerotia germination of all tested fungi of were decreased especially with CH-LMW. Many investigators recorded the same finding. Early, El Ghaouth et al. (1992) found that a chitosan at concentrations ranged from 750 to 6000 mg L^{-1} was very effective in inhibiting spore germination and germ tube elongation of *B. cinerea*and *R. stolonifer*. Furthermore, this biopolymer at a concentration greater than 1500 mg L^{-1} induced morphological changes in *R. stolonifer*. Elmer and Lamondia (1994) showed a linear decrease of growth of *R. solani*as the chitosan concentration gradually increased from 0.5 to 6.0 mg mL^{-1}, also Cheah and Page (1997) reported that the growth of Sclerotinia *sclerotiorum was* complete inhabited *when* chitosan concentrations increased from 1% to 4%. However, a complete growth inhibition was recorded against *F. oxysporum*, *R. stolonifer*, *P. digitatum*, and *C. gloeosporioides*at concentrations of 3% (Bautista-Baños et al., 2003; Bautista-Baños et al., 2004). Meng et al. (2010) noted that both chitosan and oligo chitosan strongly inhibited spore germination and mycelial growth of *Alternaria kikuchiana* Tanaka and *Physalospora piricola* Nose. Relatively, chitosan and oligo chitosan showed more obvious inhibitory effect on mycelial growth than spore germination.

The results also indicated that there is Variation in sensitivity between the tested pathogenic fungi, as the highest recodes of reduction in linear growth, spores & sclerotia germination were observed with *Fusarium solani*, *F. oxysporum*, *A. solani*and *R. solani*, *Fusarium sembaticum*, *Phytophthora infestance*, as they were highly sensitive to chitosan than *Macrophomina phaseolina*. *S. rolsfsii*, *B. ceneriea* and *S. sclerotiorum*. In this respect, Chien and Chou (2006) noted that the antifungal activity of chitosan depends on the type, concentration and test organism. For example, at 0.1%, chitosan of 92.1 kDa showed a higher growth inhibition of 76.2% on *P. italicum* than did chitosan of 357.3 kDa (71.4%), while at 0.2%, the antifungal activity exerted by chitosan of 357.3 kDa was higher than chitosan of 92.1 kDa against *P. italicum*. Benhamou et al. (1994) showed that chitosan derived from crab-shell at concentrations of 500 and 1000 mg L^{-1} was effective in reducing disease incidence caused by *F. oxysporum*f. sp. *radicis-lycopersici*. At the same time El Ghaouth et al. (1994) revealed that chitosan was effective in inhibiting mycelial growth of *P. aphanidermatum* completely at a concentration of 400 mg L^{-1}. Also, our results agreement with Stössel and Leuba (1984), they noted that chitosan has high-antifungal activity, but it is less effective against fungi with a chitin or chitosan component in their cell walls.

The results of our laboratory study showed that chitosan LMW was more effective than chitosan HMW in decreasing linear growth, spores and sclerotia germination of all tested fungi .These finding agreement with many investigators. In this respect Kheiri et al. (2017) recorded that low molecular weight (LMW) CS and its NPs had high potential of antifungal activity on suppress of fungus growth. The maximum percentage of growth reduction was 68.18%, and 77.5% by CS and its NPs at concentrations of 1000 and 5000 ppm. Saharan et al. (2013) found that Chitosan nanoparticles showed antfungal activites against phytopathogenic fungi namely *Alternaria alternata, Macrophomina phaseolina* and *Rhizoctonia solani* .The maximum growth inhibitory effects (87.6%) on *in vitro* mycelial growth of *M. phaseolina* at 0.1% concentration. Hirano and Nagao (1989) testing high- and low-molecular weight chitosan on different fungal species and they found that the best fungicidal activity on mycelia occurred in media supplemented with low-molecular-weight chitosan. However, Bautista-Baños et al. (2005) indicated that no differences in the fungicidal pattern among the three different types of chitosan, whereas there was a higher fungicidal effect as chitosan concentration increased (0.5%-2.0%).

The antimicrobial activity of different MWs chitosan and chitosan oligomers (DP 2-8) against several plant pathogens were examined by Hirano and Nagao (1989). It was observed that the increases in MW increased the number of inhibited fungi. The strongest growth inhibition was observed with LMW and the weakest was observed with HMW chitosan (Badawy and Rabea, 2009). Kendra

and Hadwiger (1984) examined the antifungal effect of chitosan oligomers on *F. solani*f. sp. *pisi*and *F. solani*f. sp. *phaseoli*. The antifungal activity was found to increase as the polymer size increased. Monomer and dimer units did not show any antifungal activity at 1000 μg mL^{-1}. However, heptamer (DP=7) showed maximal antifungal activity and the minimum concentrations were in an *in vitro* experiment, the result demonstrated that the antifungal activity increased as the chitosan MW decreased. In an *in vivo* study, chitosan with MW of 5.7 × 104 Da was the most effective among those tested. It is difficult to find a clear correlation between MW and antimicrobial activity, generally the antimicrobial activity increases as the MW of chitosan increases. However, the activity decreases over a certain high MW. The discrepancies between data may result from the different degree of deacetylation (DDA) and MW distributions of chitosan. The evaluation of only the MW dependence of the antimicrobial activity requires a wide MW range of chitosan samples with the same DDA. It is almost impossible to obtain this because chitosan is a natural polymer. From the existing data, it is difficult to determine what the most optimal MW for the maximal antimicrobial activity is. The selection of MW of chitosan could be thought to be more dependent on its application (Badawy and Rabea, 2009).

4 Conclusions

Chitosan nanoparticles could be used as bio-nanopesticides against fungal diseases exploited for delivery of agrochemicals.

Acknowledgement

This work was funded by a grant, code number 10120604 of In-House report from the National Research Center, Giza, Egypt.

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