Zagazig J. Agric. Res., Vol. 49 No. (1) 2022 29-39

Plant Protection and Pathology Research

Available online at http://zjar.journals.ekb.eg
http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

SAFE MANAGEMENT OF FABA BEAN CHOCOLATE SPOT USING CHITOSAN AND SILVER NANOPARTICLES SUBSTANCES

Omnia A.E. Ghareib[1*], A.Z. Aly[1], M.I. Abou Zaid[1] and K.I. Zaki[2]

1. Plant Pathol. Dept., Fac. Agric., Zagazig Univ., Egypt

2. Plant Prot. Dept., Desert Res. Cent., Mataria, Cairo, Egypt

Received: 04/08/2021 ; Accepted: 03/02/2022

ABSTRACT: Faba bean is amang the most strategic crops in Egypt and suffering from many destructive diseases especially chocolate spot caused by *Botrytis fabae* Sard. which can reduce yield production. Effect of chitosan nanoparticles (ChNPs) and silver nanoparticles (AgNPs) with average particle size of 7.54 and 8.043 nm, respectively at five concentrations (20, 40, 60, 80 and 100 ppm), as well as recommended dose of carbendazim (CZ) 100 g/100L (field rate) were studied *in vitro* against aggressive isolate of *Botrytis fabae* (B.f4). All concentrations significantly inhibited the radial growth of B.f4 compared with the control treatment. *In vitro* results showed that ChNPs followed by AgNPs were effective over conventional chemical fungicide CZ. Where the radial growth of B.f4 decreased by increasing concentration of the different materials especially ChNPs at 20-40 ppm recording 73.33% and 100% growth inhibition, respectively. While 100 ppm of AgNPs and recommended dose of CZ rate of 75.33% and 46.11% growth inhibition, respectively. In addition, scan electron microscope (SEM) showed that, the mycelium of B.f4 treated by AgNPs did not have detruation changes at 20 ppm. Contrary to B.f 4 mycelia treated with ChNPs 20 ppm exhibited seriously shrunk after 72 h post treatment in relative to the control. The antifungal effect of ChNPs, showed marked covered distorted mycelia. Most B.f4 spore morphology treated with AgNPs 20 ppm appeared to be shrunk and abnormal shape 72 h post treatment compared with the control. In addition, AgNPs lead to deforming *B. fabae* spores and explosion of others. On the other hand, spore production of *B. fabae* treated with ChNPs 20 ppm was completely inhibited 72 h post treatment. The obtained results offers a possibility of developing new manufacturing fungicides using chitosan and silver nanoparticles as substances for save control of faba bean chocolate spot disease and minimize the impact of conventional fungicides in the agri-ecosystem.

Key words: Nanoparticles, silver, chitosan, chocolate spot, *faba bean*.

INTRODUCTION

Faba bean (*Vicia faba*) is mainly grown in Egypt for its green pods and dried seeds, which are rich in protein (18.5 to 37.8%) that can substitute for animal protein in humans, as well as other compounds (**El Hendawy *et al*., 2010; Sahile *et al*., 2011**). Also, as the other legumes, faba bean plays a significant role in the restoration of soil fertility by fixing nitrogen and is used as suitable rotation crop for cereals and other crops (**Teshome and Tagegn, 2013**).

Faba bean is suffering from many destructive diseases in Egypt, especially chocolate spot caused by *Botrytis fabae*. It is one of the most economically important diseases that damage the foliage, limit photosynthesis activity, and reduce faba bean production (**El-Kholy, 2014**).

Several strategies can be employed to management chocolate spot disease in faba bean crop. Application of fungicides has been the most effective and widely used strategy and provides reliable effective disease control measure (**Teshome and Tagegn, 2013**). Previous

*** Corresponding author: Tel. :**+201007411687
E-mail address: Omniaghareeb16@gmail.com

studies showed that, carbendazim, gave good control of this disease in faba bean (**El-Kholy, 2014**). However, the widespread and uncontrolled use of conventional fungicides in plant health management has contributed significantly in deteriorating the quality of biotic and abiotic components of ecosystem (**Damalas and Eleftherohorinos**, **2011**). To overcome such difficulties, researchers focused attention to find economical and environmentally friendly alternative strategies against the disease by the applications of nanotechnology and bio-agents (**Cho *et al*., 2010; Esh *et al*., 2010; Ouda, 2014 and Massalimov *et al*., 2016**).

In recent years, great considerable attention is paid to antifungal effects of chitosan and silver nanoparticles due to their antifungal properties and their different properties compared with bulk (**Vaidyanathan *et al*., 2009; El-Hadrami *et al*., 2010; Bhattacharjee *et al*., 2017 and Kriti, 2020**). Chitosan based nanoparticles (ChNPs) are preferably used for various applications owing to their biodegradability, high permeability towards biological membranes, and a low toxicity for mammalian cells, cost effectiveness and broad antifungal activities. ChNPs imbued versatility in biological activities due to altered physico-chemical characteristics like size, surface area, cationic nature, active functional groups, higher encapsulation efficiency etc. (**Xu *et al*., 2007; Meng *et al*., 2010; Saharan *et al*., 2013; Youssef *et al*., 2019**). In this respect **Kheiri *et al*. (2017)** recorded that, ChNPs had high potential of antifungal activity on suppress of *Fusarium* sp. growth in wheat. ChNPs showed antifungal activites against all tested phytopathogenic fungi at different concentration compared with the control treatment (**Saharan *et al*., 2013; OH *et al*., 2019**). Silver in ionic or nanoparticle forms (AgNPs) is a metal had highly toxic effect to fungal pathogens, some of the *in vitro* assays showed strong inhibitory effects of AgNPs even at low concentration against various fungal plant pathogens **(Kim *et al*., 2012; Krishnaraj *et al*., 2012; Gopinath and Velusamy, 2013; Lee *et al*., 2013; Pulit *et al*., 2013; Mishra *et al*., 2014; Ouda, 2014**).

Therefore, the objective of this study was to evaluate the sensitivity of *B. fabae* isolate (B.f 4) to AgNPs and ChNPs at different concentrations comparing with the commercial fungicide CZ, achieve the following objectives; 1) Effect on mycelia growth of *B. fabae*, 2) Involve mechanism or morphological change on the spores and mycelia under the direct observation of scanning electron microscope (SEM) to establish the efficacy mechanism on plant pathogen controls.

MATERIALS AND METHODS

The Used Isolate Pathogen

Isolate B.f4 has been previously isolated and identified as *B. fabae*. B.f4 which was isolated from Sakha at Kafr El Sheikh and was found to be the most aggressive isolate during two successive growing seasons (2017/2018 and 2018/2019) (**Ghareib *et al*., 2021**).

Growth Media for the Fungal Pathogen

In this study two growth media were used *i.e*. faba bean leaf agar (FBLA) medium was used for preparing spore suspention of *B. fabae* (**Leach and Moore, 1966**), and potato dextrose agar (PDA) medium was also used for *B. fabae* mycelial growth according to **Riker and Riker (1936)**.

Tested Materials

Silver nanoparticles (AgNPs) and Chitosan nanoparticles (ChNPs) as well as conventional fungicide carbendazim (CZ) were used in this study. Silver nanoparticles was obtained from Naqaa Nanotechnology Labe, Cairo, Egypt, while ChNPs was prepared according to **Tang *et al*.** (2007) in Nanotechnology lab. Desert Research Center (DRC), Cairo, Egypt. Carbendazim SC 50%. was used at 100 ml/100 L (field rates) as compared treatment.

Chitosan Nanoparticles

Chemicals and reagents

Chitosan (deacetylation 85%) was procured from STARCHEMIC sodium tripolyphosphate from SRL Company and acetic acid glacial were used without any further purification.

Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared based on ionic gelation method of chitosan with

tripolyphosphate (TPP) anions. TPP anions has often been used to prepare chitosan nanoparticles because TPP is nontoxic. The interaction can be controlled by the charge density of TPP and chitosan, which is dependent on the pH of the solution. Chitosan 20 gm was dissolved in 40 ml of 2% (v/v) acetic acid aqueous solution. Twenty milliliter of 0.75 mg/ml sodium tripolyphosphate. The TPP solution was prepared by double distilled water. Tripolyphosphate was dropped wisely under magnetic stirring at 1000 rpm, 1 hour under room temperature (**Tang *et al*., 2007**).

Concentrations used

Metal nanoparticles were diluted at different concentrations of 20, 40, 60, 80 and 100 ppm using distilled water at room temperature 24°C. The prepared solutions were stored at 4°C for experimental use. Sterilized distilled water was used as control treatments (**Ahmed**, 2017).

Characterization of NPs using transmission electron microscope (TEM)

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. There prepared grids were used to study. The morphological and particles size of AgNPs and ChNPs were demonstrated using TEM model JEM-1230, Japan, operated at 120 kV, with maximum magnification of 600 × 103 and a resolution until 0.2 nm.

***In vitro* screening of tested materials against pathogenic fungi**

Effect of different concentrations of ChNPs, AgNPs and CZ were tested against *B. fabae in vitro*. The concentrations of nanoparticles and recommended dose of CZ were prepared by adding the amounts of the stock solutions of nanoparticles to 100 ml of PDA media cooled to 45°C. Five replicates (Petri dishes 9 cm in diameter) were used for each concentration. Five mm in diameter agar plugs were obtained from the actively growing edge of fungal cultures (6 days old cultures) inoculated in the center of plates supplemented with different concentrations. The plates were incubated at 25°C and colony diameters were measured after 3 days when the control reached full growth. The percentages of growth inhibition were calculated relative to control growth treatment using the following formula:

$$\text{Inhibition rate (\%)} = (R - r) / R \times 100$$

Where R is radial growth of the fungi in control plates and r is the radial growth of fungi in treated plates (**Lamsal *et al*., 2011**).

Scanning electron microscopy

Mycelia of *B. fabae* grown on PDA medium treated with AgNPs or ChNPs were fixed in 2.5% glutaraldhyde at 4°C for 24 hr and post–fixed in 1.0% osmium tetraoxide for one hr at room temperature (**Harley and Fergusen, 1990**). The speciments were then dehydrated with ascending concentrations of acetone, critical point dried, and finally sputter coated with gold. The examination and photographing was done through Joel Scanning Electron Microscope (**Salem *et al*., 2019**).

Statistical Analysis

Variance analysis was calculated for assessed traits using M Static 10^{17} Program described by **Steel and Toori (1980)**. The comparison of means were calculated for traits based on least significant differences ($LSD_{0.05}$) of interaction between stated factors (pathogenic fungus and nanoparticles).

RESULTS AND DISCUSSION

Characterization of AgNPs and ChNPs Using Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) characterization represents the morphological structure of ChNPs that was found to be uniform and spherical with average particle size 7.54 nm while, AgNPs was more spherical with average particle size 8.043 nm (Fig. 1).

Effect of ChNPs, AgNPs and carbendazim on radial growth of *B. fabae*

Data in Table 1 represented the antifungal effects of five concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm) of each AgNPs and ChNPs as well as recommended dose of conventional fungicide Carbendazim against *B. fabae* (B.f4) isolate. The nanoparticles inhibited the radial growth of *B. fabae* at different

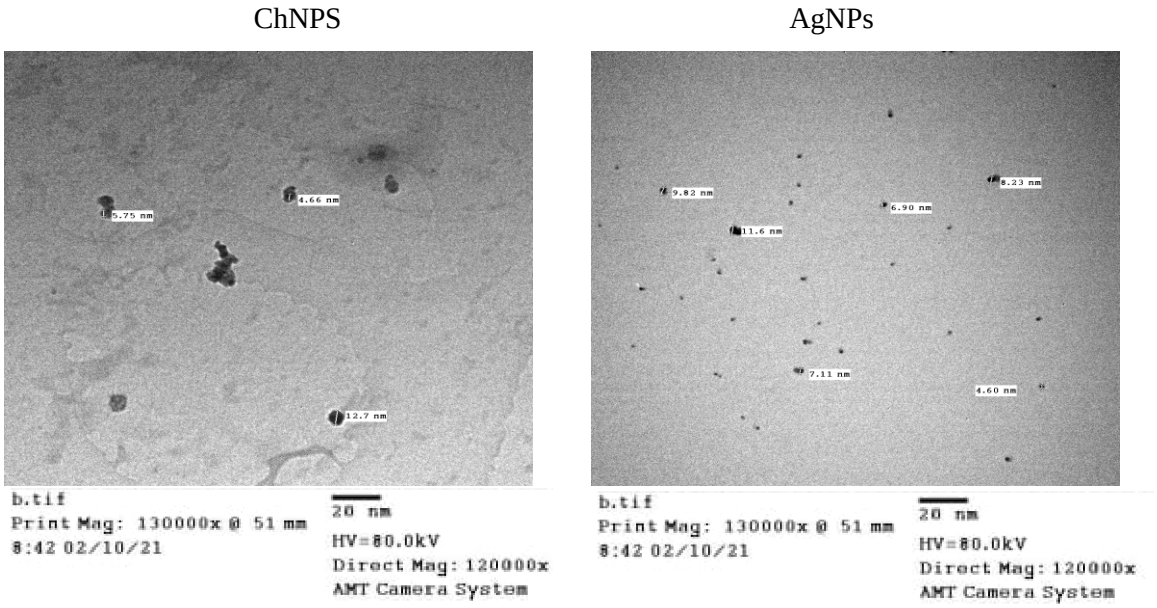

Fig. 1. Trasmission electron microscopy characterization size and morphological structure of nano chitosan (ChNPs), and nano silver (AgNPs)

Table 1. Effect of different concentrations of silver and chitosan nanoparticles on *B. fabae* isolate (B.f 4) mycelial growth after three days from inoculation.

Treatments	Means	Reduction (%)
AgNPs 20ppm	2.93	67.44
AgNPs 40ppm	2.60	71.11
AgNPs 60ppm	2.60	71.11
AgNPs 80ppm	2.33	74.11
AgNPs 100ppm	2.22	75.33
ChNPs 20ppm	2.40	73.33
ChNPs 40ppm	0.00	100.00
ChNPs 60ppm	0.00	100.00
ChNPs 80ppm	0.00	100.00
ChNPs 100ppm	0.00	100.00
Carbendazim	4.85	46.11
Control	9.00	0.00
LSD $_{0.05}$	0.137	-

concentration where all treatments showed significant effects compared with the control and conventional fungicide Carbendazim. The most effective treatments was 40 ppm of ChNPs where inhibited *B. fabae* growth by 100%. While, AgNPs at 100 ppm recorded inhibition rate by 75.33% followed by 80 ppm (74.11%) inhibition rate. On the other hand, chitosan nanoparticles were effective at 20 ppm and recorded 73.33% of inhibition (Table 1 and Fig. 2). The results of laboratory study showed that ChNPs followed by AgNPs were effective over conventional fungicide Carbendazim (Fig. 3). Results showed that, increase of effectiveness especially with increasing concentrations of ChNPs (20-40 ppm). These findings were in agreement with many investigators. In this respect **El-Mohamedy *et al.* (2019)** indicated that, there was a higher fungicidal effects as chitosan nanoparticles concentration increased (0.0125%- 0.1%), the radial growth, spors and sclerotia germination of all tested fungi were decreased.

Saharan *et al.* (2013) revealed that, chitosan nanoparticles showed antifungal activities against *Alternaria alternata*, *Macrophomina phaseolina* and *Rhizoctonia solani*. Where ChNPs exhibited maximum inhibitory growth effect 87.6% *in vitro* of *M. phaseolina* mycelial growth at 0.1% concentration. While, **Kheiri *et al.* (2017)** found that, maximum percentage of *Fusarium* sp. growth reduction was 68.18%, and 77.5% by ChNPs at concentrations of 1000 and 5000 ppm. Chitosan nanoparticles exhibit the inhibition activity on different growth stages namely mycelial growth, sporulation, spore viability germination, and the production of fungal virulence factors (**Xu *et al.*, 2007; Saharan *et al.*, 2013; Kheiri *et al.*, 2017**).

Also**, Derbalah *et al.* (2013)** evaluated the efficacy of some non-traditional methods, alone and in combination with recommended fungicides, for controlling *B. fabae* and showed that carbendazim and its mixtures was the lowest while, AgNPs alone showed significant inhibition of *B. fabae* radial growth relative to control. Also, **Bhattacharjee *et al.* (2018)** found that nano-formulations were effective over conventional chemicals, and among the most effective nano-formulations, AgNP followed by ChNP. **Ahmed (2017)** studied the antifungal effect of AgNPs and ChNPs on *B. fabae* and *Alternaria alternata*, and showed that the most effective treatments were 100 ppm of AgNPs that inhibited the fungal growth by 79% and 70% in *B. fabae* and *A. alternate*, respectively, followed by 80 ppm of AgNPs with inhibition rate 77% and 67% of radial growth of *B. fabae* and *A. alternata*. Also, ChNPs 80 ppm was effective, recording the inhibition rate 69% of *B. fabae* and 63% of *A. alternata*, respectively. While, 100 ppm recorded 72% and 68% inhibition rate of *B. fabae* and *A. alternata* radial growth, respectively. **Lamsal *et al.* (2011)** reported that AgNPs inhibited growth of *Colletotrichum* spp. with concentrations of 50 ppm and 100 ppm by 84.56% and 93.5%, respectively. These findings were contrast with previous studies which showed that, carbendazim, gave good control of this disease of faba bean (**Giltrap, 1991; Davidson and Kimber, 2007; El-Kholy, 2014**).

Nanoparticles may be more effective due to its higher adherence to the fungal cell surface, degrading its lipopolysaccharide molecules and increase the permeability of cell membrane (**Morones *et al.*, 2005**). Studies have concluded that chitosan possesses antifungal activity by affinity of its cationic amino groups to cellular components forming polyelectrolytic complexes, and affecting membrane permeability as well as causing leakage of intracellular electrolytes and proteinaceous constituents (**Badawy and Rabea, 2009; Meng *et al.*, 2010; Suhartono, 2015**). This decrease of protein could be due to the adherence of the chemicals with cell wall of the fungi and denaturation of proteins, inhibition of DNA replication (**Feng *et al.*, 2000**) and mRNA synthesis (**Sudarshan *et al.*, 1992**), inactivation of the expression of ribosomal subunit proteins, production of reactive oxygen species (ROS) and increasing the permeability of cell membrane causing leakage of cellular contents and accumulation of proteinase inhibitors etc. Similar effect was reported in *B. fabae* due to. ChNPs and AgNPs (**Hwang *et al.*, 2008; Kong *et al.*, 2010; Lamsal *et al.*, 2011; Kim *et al.*, 2012 and Ahmed, 2017**).

Morphological Observation of *B. fabae* Treated with AgNPs and ChNPs by Scanning Electron Microscope

The microscopic observation revealed that, the two types of nanoparticles clearly damaged

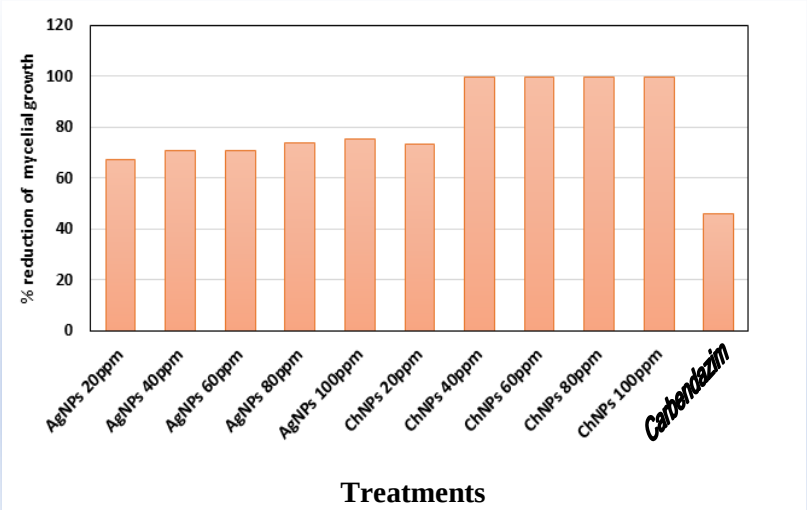

Fig. 2. Reduction percentage of *B. fabae* mycelial growth after three days from incubation with different concentrations of silver, chitosan nanoparticles and recommended dose of carbendazim

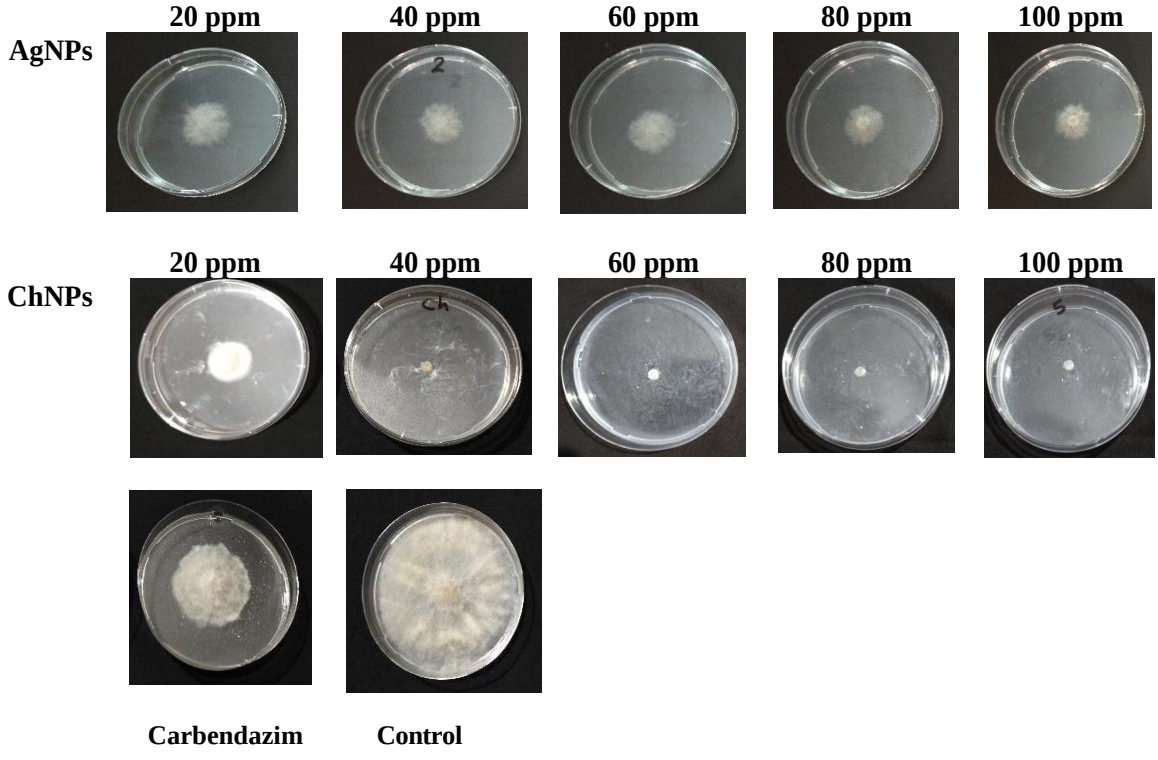

Fig. 3. Effect of different concentrations of nano silver (AgNPs), nano chitosan (ChNPs) and CZ on the radial growth of *B. fabae* isolate (B.f4) after three days from incubation and recommended dose of carbendazim

hyphae of *B. fabae* compared with control treatments (Fig. 4). Microscopic observation revealed, that nanoparticles caused detrimental effects not only on fungal hyphae but also on conidial germination. Moreover, the spore morphology of *B. fabae* treated with AgNPs indicated that most of spore surface appeared to be shrunk and abnormal shape 72 h post AgNPs 20 ppm treatment (Fig. 4. B2) in comparison with the control. Inaddition, AgNPs lead to deformation of *B. fabae* spores and explosion of others. On the other hand, spore production of *B. fabae* treated with 20 ppm of ChNPs was completely inhibited (Fig. 4 A1). Also, the mycelia of *B. fabae* treated with AgNPs did not have signifcantly changed at the concentration of 20 ppm. Contrary, *B. fabae* treated with ChNPs at 20 ppm caused serious mycelial shrunk 72 h post treatment (Fig.4 A2) in relative to the control. The antifungal effect of ChNPs, showed marked distorted mycelia and ChNPs covered fungal mycelia (Fig. 4 A3.).

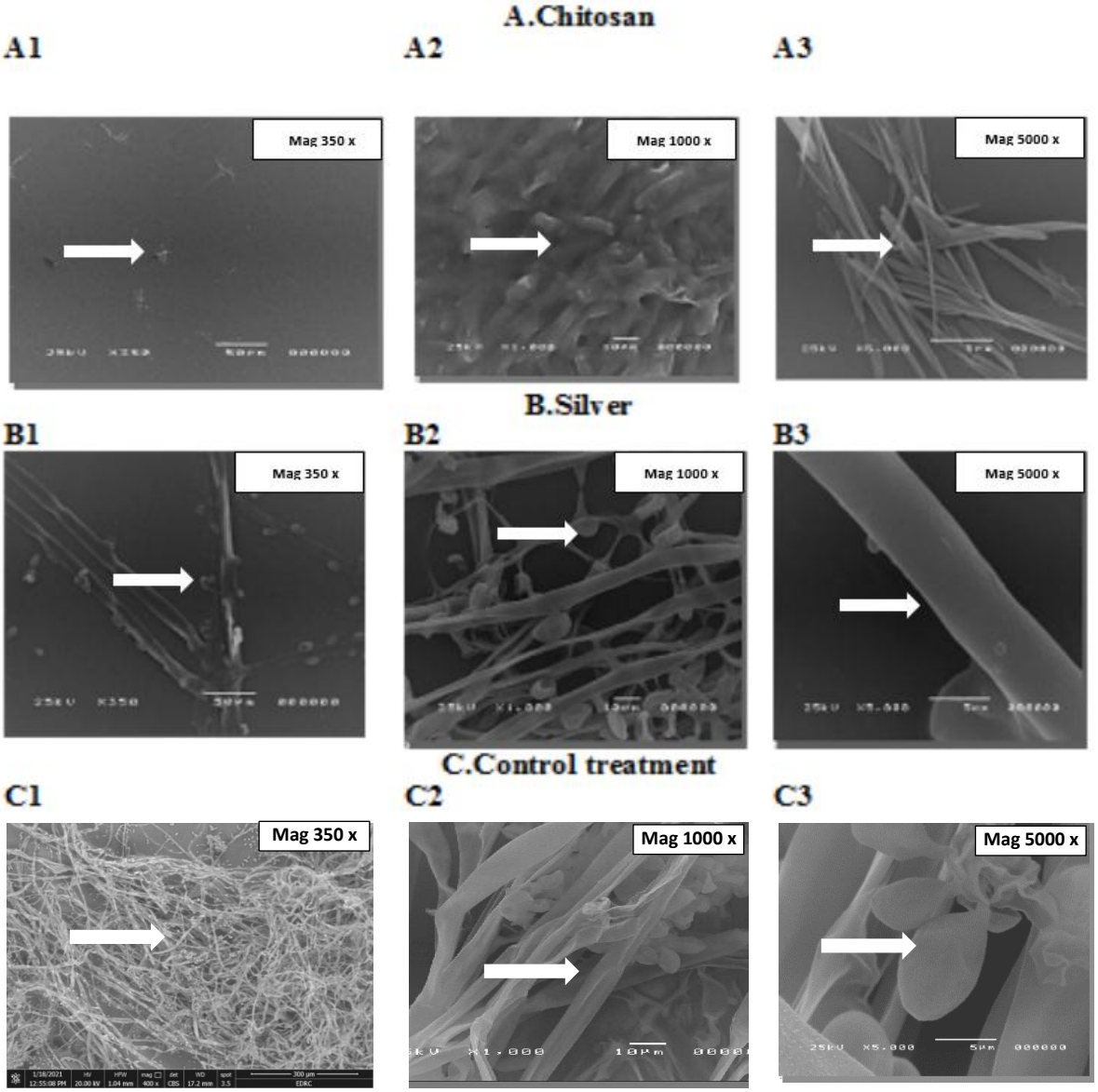

Fig. 4. Morphological observation of *B. fabae* treated with nano silver (AgNPs) and nano chitosan (ChNPs)s by scanning electron microscope at concentration 20 ppm and 72 h post treatment

In this respect with **Salem *et al*. (2019)** tested the antifungal effect of AgNPs against the growth of *B. cinerea* by scanning electron microscope, the study showed marked distorted mycelia and AgNPs were covered on mycelia. In addition, deforming of *B. cinerea* spores and explosion of others. **Lamsal *et al.* (2011)** reveald that, AgNPs clearly damaged the hyphae of *Alternaria alternate* and *Botrytis cinerea* compared with control, and caused detrimental effects not only on fungal hyphae but also on conidial germination. Silver nanoparticle upon accumulation in fungal hyphae of *Bipolaris sorokiniana*, *Magnaporthe oryzae* and *Colletotrichum gloeosporioides* can provide greater protection to plants due to cellular disruption of fungi in addition to interrupting cellular processes such as respiration and metabolism due to reacting with molecules (**Kriti**, **2020**). Also, SEM showed that ChNPs caused inhibition of *B. cinerea* hyphal growth and alteration of hyphal morphology such as cell wall disruption, withering, and excessive septation (**Ayat *et al.*, 2019**). Chitosan nanoparticles causes fungal mat accumulation, and structural changes, such as excessive branching, cell wall swelling, and reduced hyphal size, which have been observed in *Penicillium expansum* and *Rhizopus stolonifer* (**Oliveira *et al.*, 2012**).

All the examined nanoparticle concentrations of AgNPs and ChNPs were found to be effective in controlling hyphal growth and spore germination of *B. fabae*. AgNPs and ChNPs at 20 ppm concentration while it was found less effective for inhibition of mycelial growth and spore germination.

Acknowledgement

All gratitude is due to Allah who guided and helped me to achieve this work. I wish to express my appreciation to Dr. Ahmed the directory of Nanotechnology Applications Laboratory and all the work team of the at the Plant Protection Department, Desert Research Center for their advice, technical and material support. Also, deep thanks to Mister, Eslam Mamdouh (Master student in Plant Pathology Dept. Faculty of Agriculture, Benha University.) for his assistance in preparation of chitosan nanoparticles.

REFERENCES

Ahmed, I.S.A. (2017). Chitosan and silver nanoparticles as control agents of some Faba bean spot diseases. J. Plant Pathol. Microbiol., 8:1-7.

Ayat, F.H., K. Youssef and K.A. Abd-Elsalam, (2019). Eco-friendly nanomaterials for controlling gray mold of table grapes and maintaining post harvest quality. Europ. J. Plant Pathol., 154: 377-388.

Badawy, M.E.I. and E.I. Rabea (2009). Potential of the bio-polymer chitosan with different molecular weights to control post harvest gray mold of tomato fruits. Postharvest J. Biol. and Technol., 51(1): 110–117.

Bhattacharjee, J., A. Sahoo, D. Bhattacharjee and A. Basu (2017). Nano-formulations and inducer chemicals having antimicrobial property against Alternaria leaf blight of potato. Int. J. Curr. Microbiol. Appl. Sci., 6 (12): 1-9.

Bhattacharjee, J., D. Bhattacharjee, A. Basu and C. Debnath (2018). Effect of different chemical compounds and nano-formulations on the cell wall components of *Alternaria solani* and *A. alternate*. Indian J. Hill Farming, 31 (2):323-328.

Cho, Y., R. Shi and R.B. Borgens (2010). Chitosan produces potent neuro protection and physiological recovery following traumatic spinal cord injury. J. Exp. Biol., 213 (9): 1513–1520.

Damalas, C.A. and I.G. Eleftherohorinos (2011). Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public Health., 8: 1402–1419.

Davidson, J.A. and R.B.E. Kimber (2007). Integrated disease management of Ascochyta blight in pulse crops. Europ. J. Pathol., 119: 99-110.

Derbalah, A.S., G.A. El-Kot, Y.M. Hafezand and A.F. Omar (2013). Non-traditional methods to control chocolate spot of faba bean caused by *Botrytis fabae* Sadr under greenhouse conditions. Egypt J. Biol. Pest. Control, 23: 137-144.

El-Hadrami, A., L.R. Adam, I. El Hadrami and F. Daayf (2010) Chitosan in plant protection. J. Mar. Drugs, 8: 968-987.

El-Kholy, M.A.R. (2014) Chemical and biological control of chocolate spot disease in faba bean under field conditions. Middle East J. Agric. Res., 3(2): 368-377.

El-Hendawy, S., W. Shaban and J.I. Sakagami (2010). Does treating faba bean seeds with chemical inducers simultaneously increase chocolate spot disease resistance and yield under field conditions. Turk. J. Agric., 34: 475-485.

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 J., 21 (4): 201–209.

Esh, A.M.H., M.M.M. Atia and S. Taghian (2010). Phyllosphere microflora of sugar beet and their interaction with *Corcospora beticaoly* the cousal of agent of cercospora leaf spot disease. Egypt. J. Appl. Sci., 25 (8B): 340-361.

Feng, Q.L., F. Cui and G. Chen (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus.* J Biomed. Mater. Res., 52 (4): 662-668.

Ghareib, O.A.E., A.Z. Aly, M.I. Abou Zaid and K.I. Zaki (2021). Isolation, identification and frequency occurrence of *faba bean* chocolate spot disease and its associated fungi in different governorates of Egypt. J. Plant Archives. (In press).

Giltrap, N.J. (1991). Comparison of fungicides for chocolate spot control in winter *faba bean*. Aspects of Appl. Biol., 27:123-128.

Gopinath, V. and P. Velusamy (2013). Extracellular biosynthesis of silver nanoparticles using *Bacillus* sp. GP-23 and evaluation of their antifungal activity towards *Fusarium oxysporum*. Spectrochim Acta. A. Mol. Biomol. Spectrosc., 106:170–174.

Harley, M.M. and I.K. Fergusen (1990). The Role of SEM in Pollen Morphology and Plant Systematic. Association special, Clarendon press, oxford, 41: 45-68.

Hwang, E.T., J.H. Lee, Y.J. Chae, Y.S. Kim and B.C. Kim (2008). Analysis of the toxic mode of action of silver nanoparticles using stress-specifc bioluminescent bacteria. Small J., 4: 746-750.

Kheiri, A., S.A. Moosawi, A. Jorf, S.H. Malihipour and M. Nikkhah (2017). Synthesis and characterization of chitosan nanoparticles and their effect on Fusarium head blight and oxidative activity in wheat. Int. J. Biol. Macromol., 102: 526–538.

Kim, S.W., J.H. Jung, K. Lamsal, Y.S. Kim and J.S. Min (2012). Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiol., 40: 53-58.

Kong, M., X.G. Chen, K. Xing and H.J. Park (2010). Antimicrobial Properties of Chitosan and Mode of Action: A State of the Art Review. Int. J. Food Microbiol., 144: 51-63.

Krishnaraj, C., R. Ramachandran, K. Mohan and P.T. Kalaichelvan (2012). Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochim Acta. A. J., 93:95–9.

Kriti, A. (2020). Nano phytopathology-exploring future opportunities in the era of climate change. Int. J. Current Microbiol. and Appl. Sci., 9 (2): 1984-1989.

Lamsal, K., S.W. Kim, J.H. Jung, Y.S. Kim, K.S. Kim and Y.S. Lee (2011). Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. Mycobiol., 39: 194-199.

Leach, R. and K.G. Moore (1966). Sporulation of *Botrytis fabae* on sugar cultures. Translation Br. Mycol. Soc., 49:593-601.

Lee, K.J., S.H. Park, M. Govarthanan, P.H. Hwang, Y.S. Seo, M. Cho, W.H. Lee, J.Y. Lee, K.S. Kamala and B.T. Oh (2013). Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens. Materials Letters J., 105: 128 – 131.

Massalimov, I., Y. Medvedev, F. Urakaev, A.I.S. Ahmed and B. Muhambetkali (2016). Antifungal activity of inorganic micro-and nanoparticles against pathogenic fungi compared with some traditional organic drugs. Ame. Eurasian J. Agric. Environ. Sci., 16: 255-265.

Meng, X., L. Yang, J.F. Kennedy and S. Tian (2010). Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit. Carbohydrate Polymers, 81(1): 70–75.

Mishra, S., B.R. Singh, A. Singh, C. Keswani, A.H. Naqvi and H.B. Singh (2014). Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. PLOS One, 9 (5): 78-81.

Morones, J.R., J.L. Elechiguerra, A.K. Caacho, J.B. Kouri and J.T. Ramirez (2005). The bactericidal effect of silver nanoparticles. Nanobiotechnol., 16: 2346-2353.

OH, J.W., S.C. Chun and M. Chandrasekaran (2019). Preparation and *in vitro* characterization of chitosan nanoparticles and their broad-spectrum antifungal action compared to antibacterial activities against phytopathogens of tomato. J. Agron,, 9(21): 1-12.

Oliveira, J.E.N.D., I.S.D. Melo and T.T. Franco (2012). Changes in hyphal morphology due to chitosan treatment in some fungal species. Brazil Archiv. Biol. Technol., 55: 637–646.

Ouda, S.M. (2014). Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternate* and *Botrytis cinerea*. Res. J. Microbiol., 9 (1): 34–42.

Pulit, J., M. Banach, O.R. Szczyg and M. Bryk (2013). Nanosilver against fungi. silver nanoparticles as an effective biocidal factor. Acta. Biochim. Pol., 60: 795-798.

Riker, A.J. and R.S. Riker (1936). Introduction to Research on Plant Diseases. In: A Guide to the Principles and Practices for Studying for Various Plant Diseases Problems. Univ. Winconsin, USA, 117.

Saharan, V., A. Mehrotra, R. Khatik, P. Rawal, S.S. Sharma and A. Pal (2013). Synthesis of chitosan based nanoparticles and their *in vitro* evaluation against phytopathogenic fungi. Int. J. Biol. Macromol., 62: 677–683.

Sahile, S., P.K. Sakhuja, C. Finisa and S. Ahmed (2011). Potential antagonistic fungal species from Ethiopia for biological control of chocolate spot disease of faba bean. Afr. Crop Sci. J., 19 (3): 213-225.

Salem, E.A., M.A.S. Nawito and A.A. Ahmed (2019). Effect of silver nano-particles on gray mold of tomato fruits. J. Nanotechnol. Res., 1 (4): 108-118.

Steel, R.G.D. and J.H. Torrie (1980). Principles and Procedures of Statistics. A Biometrical Approach. 2nd Ed. McGraw-Hill, New York, USA, 20-90.

Sudarshan, N.R., D.G. Hoover and D. Knorr (1992). Antibacterial action of chitosan. Food Biotechnol., 6: 257-272.

Suhartono, D. (2015) Preparation of chitosan material and its antifungal activity for bamboo. Int. J. Sci. Res., 6: 1586-1590.

Tang, Z.X., L.E. Shi and J.Q. Qian (2007) Neutral lipase from aqueous solutions on chitosan nanoparticles. Biochem. Eng. J., 34: 217-223.

Teshome, E. and A. Tagegn (2013). Integrated management of chocolate spot (*Botiytis fabae* Sard.) of faba bean (*Vicia faba* L.) at highlands of Bale, south eastern Ethiopia. Res. J. Agric. and Environ. Manag., 2 (1): 11- 14.

Vaidyanathan, R., K. Kalishwaralal, S. Gopalram and S. Gurunathan (2009). Nanosilver-the burgeoning therapeutic molecule and its green synthesis. Biotechnol. Adv., 27: 924-937.

Xu, J., X. Zhao, X. Han and Y. Du (2007). Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. Pest Biochem. Physiol., 87: 220–228.

Youssef, A.M., M.E. Abd El-Aziz, E.S. Abd El-Sayed, M.A. Moussa, G. Turky and S. Kamel (2019). Rational design and electrical study of conducting bionanocomposites hydrogel based on chitosan and silver nanoparticles. Int. J. Biol. Macromolecules, 140: 886–894.

الإدارة الآمنة للتبقع البني الشيكولاتي في الفول البلدي باستخدام المركبات النانوية للشيتوزان والفضة

أمنية عبدالمحسن السيد غريب[1] – أحمد زكي علي[1] – محمد إبراهيم أبو زيد[1] – خالد إسماعيل زكي[2]

1- قسم أمراض النبات – كلية الزراعة – جامعة الزقازيق – مصر

2- قسم وقاية النبات – معهد بحوث الصحراء – المطرية – مصر

يعتبر الفول البلدى من المحاصيل الإستراتيجية في مصر، ويتعرض محصول الفول إلى من العديد من الأمراض المدمرة وخاصة مرض التبقع البنى الشوكولاتى المتسبب عن الفطر *Botrytis fabae* Sard. والذى يؤدى إلى نقص إنتاج المحصول تم إختبار تأثير خمسة تركيزات من جسيمات الشيتوزان و جسيمات الفضة النانوية بتركيزات 20 ، 40 ، 60 ، 80 و 100. جزء في المليون بالإضافة إلى المبيد الفطري للكربندازيم بالجرعة الموصى بها فى الاستخدام الحقلى 100مللي/100 لتر ضد عزلة من الفطر *Botrytis fabe*a (B.f4) تحت ظروف المعمل، أوضح توصيف الميكروسكوب النافذ TEM التركيب المورفولوجي لجسيمات الشيتوزان النانومترية انها وحيدة وكروية بمتوسط حجم جسيمي 7.54 نانومتر وكانت جسيمات الفضة النانوميترية أكثر كرويَّة بمتوسط حجم جسيمي 8.043 نانومتر. أظهرت النتائج تثبيط الجسيمات النانونية من الشيتوزان والفضه للنمو الميسليومى للفطر *B. fabae* بمستويات تركيز مختلفة حيث أظهرت جميع المعاملات تأثيراً معنوياً مقارنة بمعاملة الكنترول والمبيد الفطري كربندازيم. وقد كانت أكثر المعاملات فاعلية هي جسيمات الشيتوزان النانونية 40 جزء في المليون من حيث خفض نمو الفطر *B. fabae* بنسبة 100%. بينما أعطت جسيمات الفضة النانونية عند 100 جزء في المليون معدل تثبيط بنسبة 75.6% تليها 80 جزء في المليون بنسبة 74.4%. و من ناحية أخرى ، كانت جسيمات الشيتوزان النانوية فعالة عند 20 جزء في المليون وقد سجلت 73.3% كنسبة للتثبيط. كما أظهرت النتائج أن ChNPs متبوعًا بـ AgNPs كان أكثر فعالية عن المبيد الفطري التقليدي. كما أظهرت نتائج الفحص بالميكروسكوب الالكتروني الماسح أن الجسيمات النانوية تسببت فى تأثيرات ضارة على الميسليوم الفطري و أيضًا على إنبات الجراثيم الكونيدية للفطر *B. fabae*. علاوة على ذلك، فإن النتائج تشير إلى أن معظم سطح الجراثيم يبدو منكمشًا و غير طبيعي 72 ساعة بعد المعامله بـ AgNPs 20جزء في المليون مقارنةً بالكنترول. أدت المعاملة بـ AgNPs 20جزء في المليون إلى تشوه جراثيم *B. fabae* وإنفجار الأخرى. كما تم تثبيط إنتاج الجراثيم تمامًا من الفطر *B. fabae* المعامل بـ ChNPs 20 جزء في المليون. ولم يتغير بشكل ملحوظ الميسليوم المعامل بـ AgNPs عند تركيز 20 جزء في المليون. وعلى العكس، أدت المعاملة بـ ChNPs بتركيز 20 جزء في المليون إلى انكماش ميسليوم الفطر بشكل كبير بعد 72 ساعة نتيجة المعاملة مقارنة بالكنترول كما أوضحت النتائج التي تم الحصول عليها إمكانية تطوير إستخدام الجسيمات النانوية من الشيتوزان والفضة كمبيدات فطرية لمقاومة مرض التبقع الشيكولاتى فى الفول البلدى وبالتالى التقليل من تأثير مبيدات الفطريات التقليدية في النظام البيئي الزراعي.

المحكمـــــون:

1- أ.د. أحمد أحمد موسى أستاذ أمراض النبات المتفرغ – كلية الزراعة – جامعة عين شمس.

2- أ.د. محمد رضا أحمد تهامي أستاذ أمراض النبات المتفرغ – كلية الزراعة – جامعة الزقازيق.