Effect of Silver Nanoparticles on Fusarium Basal-Rot of Onion

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ABSTRACT---This study was conducted to use silver nanoparticles as a non-traditional method for managing plant diseases and to study their effect on the growth and incidence of Fusarium basal-rot of onion caused by *Fusarium oxysporum* f.sp. *cepae*. Dakahlia isolate of *F.o.* f.sp. *cepae* was the highest pathogenic isolate to onion plants, whereas Nobaria isolate was the lowest pathogenic isolate. The results of growing the treated onion transplants with silver nanoparticles and transplanting them in soil infested with the causal fungus showed that the tested concentrations resulted in significant reduction in the total count of fungi and *F.o.* f.sp. *cepae*, the infection by basal-rot compared with control treatment.. The reduction in the infection by basal-rot was reflected on crop parameters of the treated plants, which significant increase was recorded in plant height , root length, bulbs diameter weight compared with control treatment was recorded. The *In vitro* experiments indicated that all the tested nanoparticles concentrations, *i.e.* 25, 33, 50 and 100% caused different degrees of antifungal effect on the mycelial growth of *F. solani* and *F.o.* f.sp. *cepae*. Crude concentration (100%) of silver nanoparticles gave the highest effect on the mycelial growth of *F.o.*f.sp. *cepae* compared with the cheek treatment(90 mm). However, the fungicide Topsin-M70 was of the highest inhibitory effect. Also, the treatments with silver nanoparticles considerable increase in the activities of peroxidase, polyphenol oxidase and ß-1,3-glucanase enzymes compared with control plants. The concentrations of silver nanoparticles were detected with low residue in onion bulbs within the acceptable limits.

Keywords: Onion, silver nanoparticles, *Fusarium oxysporum* f. sp. *cepae*, crop parameters, polyphenol oxidase, β -1,3-glucanase, peroxidase.

1. INTRODUCTION

Onion (*Allium cepae* L.) is of great importance in A.R.E for local consumption and exportation. It is subjected to attack by several diseases, which causing massive losses in the field, during transportation and in storage. Fusarium basal-rot of onion caused by *Fusarium oxysporum* f. sp. *cepae* can effect on onion, garlic, and the other *Allium* spp. The fungus lives a long time in soil (Lager, 2011). Infection is often associated with mechanical or insect damage and some other important onion diseases (Cramer, 2000 and Ghanbarzadeh *et al.*, 2014). Foliar symptoms appear on one side or general wilt and a yellow to tan die-back of leaf tips during mid to late season. Death area may seem over several weeks. In onions, red to brown-rot appear, where roots are attached to the basal plate. Rot and discoloration usually effect on the base and up into the bulb scales. Affected tissues appear brown and watery within bulbs tissues. Sometimes, a white moldy growth evolves on the stem plate or between affected scales. Bulbs may appear normal at harvest, but rot may progress in storage (Kodama 1983; Pinto *et al*., 1995 ; Lager, 2011 and Taylor *et al.* ,2016).

Many studies have been conducted for controlling this disease chemically (El-Shehaby *et al*., 1997 and Behrani *et al*., 2015), biologically (Abd - Elbaky, 2005 and Ghanbarzadeh *et al.,* 2016), crop rotation and host plant resistance (Cramer, 2000).

The world is facing the problem of environmental pollution from pesticides. The intensive use of pesticide leads to increase risk of contamination of the environment and harmful effects on biodiversity, food security, water resources and groundwater. Residual pesticides may have effects on soil, water, turf, and other vegetation (Malaj *et al*., 2014 and Queyrel *et al*., 2016). Pesticides became more common in agricultural, public health, and nuisance applications throughout the first half of the 20th century, a myriad of problems were being discovered such the development of resistant races of the pathogens, harm effect on non-target species, environmental pollution, overall ecological degradation, and public health problems (Kogan, 1998 and Özkara *et al*., 2016). To overcome all these problems, there is a need to search for the development of methods to prevent the plants from the diseases. So, control increases the quantity and the quality of plant products available for use. Methods of control vary considerably from one disease to another, depending on the kind of pathogen, the host, the interaction of the two, and many other variables (Abbas, 2015).

The application of silver nanoparticles against onion white rot caused by *Sclerotium cepivorum* under lab.and greenhouse conditions resulted in encouraging results (Jung *et al*., 2010) .Thus, it has become an alternative approach to manage another plant diseases (Kim *et al*., 2009; Jo *et al*., 2009 ; Min *et al*., 2009; Singh *et al*., 2015 and Hassan *et al*., 2016).

Peroxidase (Po), polyphenol oxidase (PPO) and Pyrogallol peroxidase (POD) activity was increased by Zn-NPs treatment (Raigond *et al*., 2017 and Xihong *et al*., 2011). In addition, Azad *et al*. (2019) mentioned that increasing silver and other five nanoparticle materials concentrations increased (PO), (PPO) and total phenol activities.

The aim of this work was to study the effect of different concentrations of silver nanoparticles on the growth of the causal fungus and management of its infection to onion. Also, to determine the residual of silver nanoparticles in the bulbuls of the treated onion plants.

2 MATERIALS AND METHODS

Isolation, purification and identification of the isolated fungi to onion basal -rot:

Onion samples showing typical symptoms of basal-rot were collected from Kalubia, Gharbia, Dakahlia, Bani Sewif and Minia governorates (Giza 20 cv.) during April, 2019. The infected bulbs were washes thoroughly with tap water then the infected portions of basal-stem tissues were cut into small pieces (1.0 x 0.5 cm). Portions were sterilized with a 2% solution of sodium hypochlorite , washes twice in sterilized water , dried between fold of sterilized filter paper then transferred into Petri-plats containing 9 ml (PDA) medium. The plates were incubated at 28±2˚C and the isolated fungi were purified using single spore technique .The purified fungi were identified depending on their morphological features and the description of Domsch *et al.* (1980). The identification was verified by Mycol. Res. and Plant Dis. Sur. Dept., Plant Path. Res. Inst., Agric. Res. Center, Giza .

Preparation of silver nanoparticles.

Silver nanoparticles were prepared using microwave method in the Plant Pathol. Res. Inst., ARC according to Kildeby *et al.* (2005). The original concentration of silver nanoparticles used was 0.1g $AgNO_3$/100 ml ethanol. Polyvinyl

pyrrolidone (PVP) was used in the preparation of silver nanoparticles. The size was assessed by transmission electron microscope (TEM) 5-10 nm (at Fac. of Agric., Cairo University).

Greenhouse experiments:

Identification the forma specialis of *F . oxysporum* and pathogenicity test of the isolated fungi belonging to *F.o.*f.sp. *cepae :*

Greenhouse experiments were done in 2019/2020 to prove the pathogenicity test of *F. oxysporum* and *F. solani* isolates (Tables 1 and 2) in addition to identify the forma specialis of *F. oxysporum* isolates. The inoculum of *F. oxysporum* and *F.solani* was prepared by growing pure culture of each isolates on sorghum - washed sand (1:1 v/v) and incubated at 28±2C˚. After15 days, the inoculum of each fungus isolates was added to sterilized soil at the rate of 2% (w/w). Five onion transplants (Giza 20 cv.) of 45 days age were transplanted in each infested soil pot. Another five onion transplants were transplanted in each uninfested soil pot. The infection by the isolates of both fungi was assessed two months after transplating.

Alo, onion (transplants of 45 days age), pea (Maser pea cv.), bean (Pronco cv.), broad bean (Giza 3 cv.), tomato (888 cv.), cotton (Giza 78 cv.) , lentil (Precose cv.) and cucumber (Prince cv.) were used to determine the ability of the fungus isolates to infect the tested plants.

Five onion transplanted (Giza 20 cv.) as well as five seeds from any of the tested plants were planted in each pot and three pots were used as replicates. The grown plants were examined for infection by the tested fungus isolates two months days after planting as infected plants.

Effect of nanoparticles on onion basal-rot infection :

Sliver nanoparticles were prepared at the concentrations of 25, 33, 50 and 100% and the fungicide Topsin-M70 was used as a comparison at a recommended dose (2g/l water). Formalin sterilized pots (25 cm in diameter) were filled with formalin sterilized clay soil, which was infested with *F.o.* f.sp. *cepae* grown on barley medium, at the rate of 3% . Fifteen seedlings (45 days old) were separately treated with each single treatment of the nanoparticles and the fungicide by dipping for 15 minutes just before transplanting. Transplant dipped in water only were used as control treatment. The pots were irrigated when it was necessary and fertilized as recommended doses. The incidence of infection by basal-rot was assessed 2 and for months as dead plants and at the end of experiment (5 months). The percentages of crop parameters of onion plants (plant height, root length, bulb diameter and weight of bulbs) were, also, estimated. In addition, the produced bulbs were used for determination of silver nanoparticles residue.

Laboratory experiments:

Inhibitory effect of silver nanoparticles and the fungicide Topsin-M70 on the growth of *F.solani* and three isolates of *F. o.* f.sp. *cepae*:

A laboratory experiment was carried to study the inhibitory effect of silver nanoparticles (1000 ppm) and the fungicide Topsin-M70 (thiophanate methyl) at 50 and 100 µl on the growth of the three isolates of *F. oxysporum* f.sp. *cepae* and one isolate of*F.solani*.by agar well diffusion method using sterile cork borer (6.0mm) according to Bobbaralal *et. al.* (2009). Each concentration was added to Petri-plate pores and inoculated with 0.5 cm. in diameter discs of 7-days- old cultures of any of *F.o.* f.sp. *cepae* and *F. solani*, then incubated at 28±2˚C for 5days. Wells free from any material were used as control. Three replicates were used for each

treatment. Linear growth was measured when the fungal growth covered the surface of any treatment.

Diseases assessment:

Onion plants were carefully examined and the percentages of dead plants resulted from the infection by the tested fungal isolates were recorded two and four months after planting and efficiency of the treatments were assessed as follows:

% Efficiency of treatment = ((A-B/A) ×100)

Where: A : Number of dead plants due to disease infection of the control.

B : Number of dead plants due to disease infection of the treatment.

Determination of microbial densities:

Plate count technique was followed to determine the microbial densities in the soil of the different treatments following according to the method of Johansen *et al*. (1960).

% Reduction of colony forming unit (CFU) = A-B/A×100

Where: A: Number of colony forming unit in control.

B: Number of colony forming unit in treatment.

Enzymes activity:

Instrument used in enzymes activity determination:

Determination of enzymes activity and phenolic compounds were carried out using spectrophotometer model UV-Vis spectronic 601.

Preparation of enzyme extracts and assay: One gram of onion plants was extracted in 3 ml of phosphate buffer pH 7 (0.1 M), then centrifuged at 3000 rpm for 15 min. at 4C°. The supernatant was filtered and collected as an enzyme extract. Enzyme extract was stored at 2-5° C and aliquots of these assayed for enzyme activity (Aluko and Ogbadu, 1986).

Peroxidase enzyme assay:

Peroxidase activity was determined using the method described by Allan and Hollis (1972) as follows: 0.1 ml extracted enzyme sample was added to 0.5 ml sodium phosphate buffer at pH7.1, 0.1ml H_2O_2 1% and 1.5 ml pyrogallol 0.05 mM. The mixture was completed to 3 ml using distilled water and the increase in absorbance was determined spectrophotometer at 430 nm every 30 second for 10 reads**.**

Polyphenol oxidase enzyme assay:

Polyphenol oxidase activity was measured using the method described by (Esterbaner *et al*., 1977). The enzyme activity was measured as the change in absorbance per minute at 495 nm immediately after the addition of catechol solution, which initiated the reaction.

ß-1, 3 glucanase enzyme assay:

ß-1,3 -glucanase activity was assayed using a reaction system containing 250 µL of a laminarin solution (1%) dissolved in sodium phosphate (pH 7) containing the substrate laminarin. buffer and 125 µL of enzyme solution. Reaction was allowed to proceed for 30 min at 37° C and stopped by addition of 1.5 mL of 3,5-dinitrosalicylic acid reagent. The reducing sugar formed was then determined spectrophotometrically at 550 nm according to Miller (1959). The enzyme activity was expressed as µg glucose released min-1 mg-1 of sample.

Determination of silver nanoparticles in the treated onion bulbs:

The produced bulbs from the experiment (4.2) were taken to determine the residue of silver nanoparticles concentrations in the bulbs.

a. **Extraction:** 1g dried onion bulbs was taken and placed in small beaker. 10ml of aqua regna (HNO_3 - HCL 1:3 v/v) was added. The sample was heated on a hot plate until digested. After cooling, a small amount (5ml) of H_2O_2 was added.

Thereafter, it was heated on hot plate again and allows evaporation to a small volume. The sample was transferred into a 50ml-flask and diluted to final volume (30 ml) with distilled water and filtrated.

b. Determination: Silver concentration as a residual was determined in the Soil ,Water and Environ. Res. Inst. Determination was carried out according to Environmental Protection Agency (EPA) (1993) by using inductively coupled plasma (ICP) Spectrometry (model Ultima 2 JY Plasma) (Isaac and Kerber, 1971).

Statistical analysis:

Data were statistically analyzed using "F" test and treatments were compared by L.S.D. values according to Snedecor and Cochran (1998).

3. RESULTS AND DISCUSSION

Silver nanoparticles (AgNPs) are the most widely used nanomaterials worldwide in different areas such as the pharmaceutical, food, biomedical, textile and agricultural industries, due to their high capacity as antimicrobial and antiviral agents (Burdusel *et al* ., 2018). Due to the AgNPs diverse areas of application, it is fundamental to know, as much as possible, the toxicological profile of each nanoparticle formulation.

Isolation, purification and identification of the isolated fungi to onion basal –rot:

Isolation trials from onion bulbs showing basal-rot symptoms collected from Kalubia, Gharbia, Dakahlia, Bani Sewif and Minia governorates (Giza 20 cv.) during April, 2019 yielded many fungal isolates. In addition, isolates belonging to genus *Fusarium* were selected, purified and identified as *Fusarium solani* and *F. oxysporum.* Similar results were obtained by Somkuwar *et al.* (1996) ; Stadnik and Dhingra (1997); Boehnke *et al.* (2015) and Summerell (2019).

Greenhouse experiments:

Identification the forma specialis of *F . oxysporum* and pathogenicity test of the isolated fungi belonging to *F.o.*f.sp. *cepae :*

Data of growing the tested plants ,*i.e. onion*, pea, bean, broad bean, tomato, cotton, lentil and cucumber in soil infested with the different isolates of *F. oxysporum*r revealed that the isolates infected only onion plants and no apparent infection was not noticed on the other tested plants. Therefore, these isolates of *F. oxysporum* took the forma specialis *cepae* .

All isolates of the tested *F.o.* f.sp. *cepae* resulted in death of onion plants two and four months after transplanting (Table,1), with exception of Nobaria isolate, which no incidence of the disease was occurred four months after transplanting with the highest percentage of survived plants.(55%). Meanwhile, Dakahlia (isolate 1) was the most aggressive isolate (90.0%) followed by Kalubia isolate 1 (87.5%) then both Minia isolate 3 and Dakahlia isolate 2 (85.0 %) and 15.0% survived plants . Other isolates showed moderate figures of infection and survived plants. Control treatment recorded 7.5 % infection by basal-rot and 92.5% survived plants.

Pathogenicity test of *F. solani* isolates :

Data present in (Table, 2) clear that pathogenicity test of *F.solani* isolates significantly decrease to basal-rot incidence compared with control treatment .Minia isolate was the more aggressive one followed by Nobaria isolate. Meanwhile, Kalubia isolate caused the lowest percentages of basal-rot incidence (45.0%) and 55.0 % survived plants was recorded followed by Nobaria isolate, being 55.o and 45.o%, respectively.**.** Meanwhile, Minia isolated resulted in the highest percentage of the disease (67.5%) and the lowest percentage od survived

plants(32.5%).Control treatment recorded 7.5 % infection by basal-rot and 92.5% survived plants.

Effect silver nanoparticles on the total count of fungi and *F.o.* f.sp. *cepae* in the rhizospher of onion plants and the percentages of infected plants:

Transmission electron microscopy (TEM) characterization of silver nanoparticles gives the actual size and shape; the droplets in silver nanoparticles appear dark. The TEM micrograph showed that the silver nanoparticles were spherical in shape and moderately mono or di-dispersed and was in the range of 3.68 to 22.80 nm (Fig. 1).

Table 1. Pathogenicity test of *F. oxysporum* f.sp. *cepae* isolates on Giza 20 cv., 15 and 30 days after sowing, greenhouse experiment.

Source of isolates	% Basal-rot incidence after (months)		Total (%)	% Survived plants
	2	4		
Minia 1	57.5	22.5	80	20.0
2	65.0	10.0	75	25.0
3	82.5	2.5	85	15.0
Dakahlia 1	87.5	2.5	90	10.0
2	67.5	17.5	85	15.0
Nobaria 1	45.0	0.0	45	55.0
Bani Sewif 1	52.5	20.0	62.5	27.5
2	77.5	5.0	82.5	17.5
Kalubia 1	72.5	15.0	87.5	12.5
2	65.0	7.5	72.5	27.5
Non-infested pots (Control)	7.5	0.0	7.5	92.5
L.S.D. at 5%	5.5	4.7		3.9

Table 2. Pathogenicity test of *F. solani* isolates on Giza 20 cv.15 and 30 days after sowing , greenhouse experiment.

Source of isolates	% Basal-rot incidence after (months)		Total (%)	% Survived plants
	2	4		
Kalubia	30.0	15.0	45.0	55
Nobaria	45.0	10.0	55	45
Minia	47.5	20.0	67.5	32.5
Non-infested pots (control)	7.5	0.0	7.5	92.5
L.S.D. at 5%	4.0	3.1	3.9	3.8

Significant decrease in the total count of fungi and the percentages of the infected plants was observed with the concentrations of silver nanoparticles and the fungicide Topsin-M70 compared with positive control (Table, 3). In addition, this decrease was gradually increased with increasing the concentration of silver nanoparticles . Furthermore, the treatment with the fungicide Topsin-M70 was the superior treatment in reducing the total count of the total fungi and *F.o.* f.sp. *cepae* as well as the infected plants followed by the crude concentration(100%).

In agriculture, nanotechnology can be exploited by the use of natural resources in the conservation, production and protection of crops and livestock (Mujeebur and Tanveer, 2014). Recently, biosynthesis of nanoparticles (NPs) or green synthesis of NPs has received much attention due to the biocompatibility, low toxicity, and eco-friendly nature of the process and NP products (Mohammadlou *et al*., 2016).

The obtained results are in agreement with the obtained data by Min *et al.* (2009) and Tyagi and Malik (2010) who mentioned that such conditions

may be attributed to the disruption of plasma lemma and structural disorganization of the cytoplasm caused by deposition of SNPs. Also, Topsin-M70 preventing the roots from spores penetration. There is a possibility that the increase in the presence of other fungi cause inhibition of Fusarium effect, then reduced the infection percentage.

There are inversely proportional between the number of bulbs left in the end of the experiment and the rate of addition of sliver nanoparticles, where increasing the rate of sliver nanoparticles resulted in an increase in the number of bulbs. This results are in accordance with the obtained results by Jung *et al*. (2010).

The authors believe that, high concentration of silver nanoparticles induce general activation for vegetative growth and thus induced an increase in root growth. Wani and Shah (2012) tested zinc oxide (ZnO) and magnesium oxide nanoparticles against *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus*. They found that, the highest inhibition in the germination of all the tested fungi was observed at higher concentrations followed by lower concentrations of ZnO nanoparticles. In addition they reported that**,** the effect of nanoparticles of magnesium and zinc oxide on the inhibition of spore germination may be due to their fungicidal effect on the tested fungi.

Table 3. Effect of silver nanoparticles on existence of total count of the of fungi and *F.o.* f.sp. *cepae* in the rhizospher of onion plants and percentages of basal rot infection and survived plants .

Concentrations (%)	**Total count of total fungi (..X10^3) spores/g** soil	**Total count of *F.o.* f.sp. *cepae* (..X10^3) spores/g soil**	**% Infected plants** [a]	**% Survived Plants**
25	**5.49**	**2.15**	**66.67**	**33.33**
33	**4.57**	**2.88**	**40.00**	**60.00**
50	**3.65**	**1.43**	**33.33**	**66.67**
100	**2.72**	**1.32**	**13.33**	**86.67**
Topsin-M70	**0.41**	**1.23**	**10.00**	**90.00**
Non infested control	**0.63**	**3.30**	**0.0**	**100**
Infested control	**9.35**	**4.47**	**80.00**	**20.00**
LSD at 5%	**1.85**	**1.40**	**4.34**	**3.78**

[a] Mean of 3 replicates (pots).

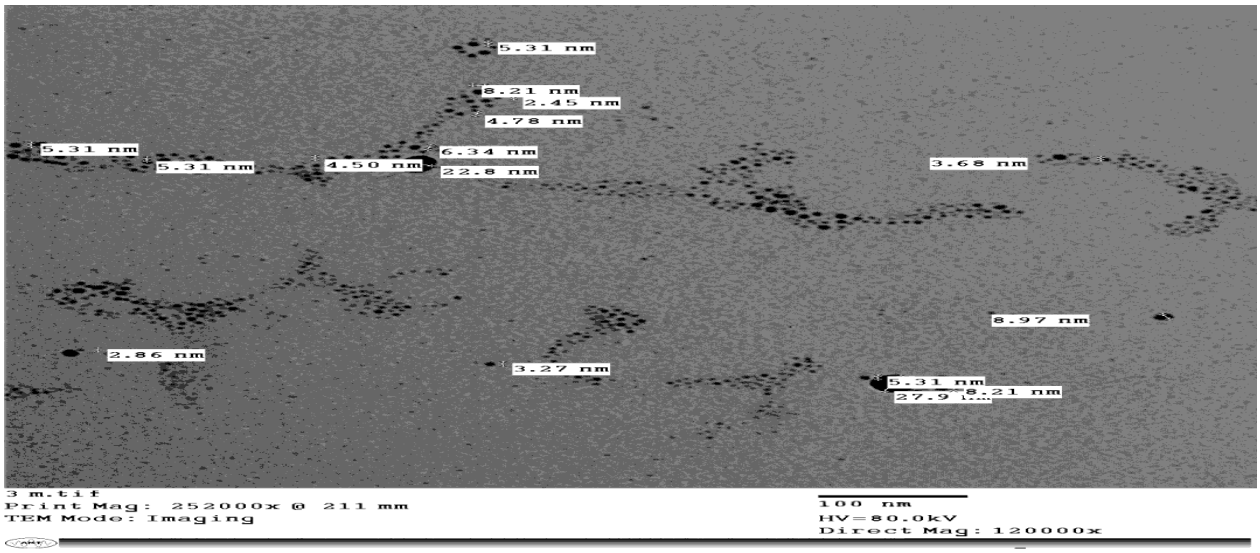

Fig. 1. TEM image of the size and distribution of silver nanoparticles. The particles are spherical in shape with approximately size 3.68- 22.80 nm (Scale bar = 100 nm).

Fig. 2.Effect of silver nanoparticles on the growth parameters of onion plants under greenhouse conditions.

Effect of silver nanoparticles and the fungicide Topsin-M70 on the crop parameters of onion plants grown in soil infected with *F. o.* f. sp. *cepae* under greenhouse conditions:

Table (4) reveals clearly that the different crop parameters of onion plants treated with silver nanoparticles and the fungicide Topsin-M70 were significantly greater than that of non-treated control. The lower effect was found on plant height at the crude concentration (100%) followed by Topsin-M70 and 50 % dilution, being 37.3, 35.7 and 35.00 cm, respectively. The same trend was found in case of the effect on root length and diameter and weight of onion bulbs. Un-infested control recorded the averages of 28.7 cm plant height, 13.0 cm root length, 7.8 cm bulb diameter and 170.8 g bulb weight, meanwhile infested control recorded 20.0 cm, 7.2 cm, 5.0 cm and 59.9 g, respectively.

Table 4. Effect of silver nanoparticles and the fungicide Topsin-M70 on the crop parameters of onion plants grown in soil infected with *F. oxysporum*f.sp*cepae* under greenhouse conditions.

Concentrations	Plant height (cm)	Roots length (cm)	Bulb diameter (cm)	Mean of bulbs weight (g)
25	33.0	13.5	6.5	171.3
33	33.7	13.6	7.3	174.0
50	35.0	14.3	7.5	180.5
100	37.3	16.0	8.2	183.7
Topsin-M70	35.7	14.0	8.5	192.6
Non infested control	28.7	13.0	7.8	170.8
Infested control	20.0	7.2	5.0	59.9
LSD at 5%	2.3	1.8	1.2	1.7

These results are in harmony with the results of Jung *et al.* (2010) which found that under greenhouse experiments the nano-silver liquids increased biomass and dry weights of green onion compared with control treatments .

Inhibitory effect of silver nanoparticles andthe fungicide Topsin-M70 on the linear growthof *F.solani* and three isolates of *F. o.* f.sp. *cepae*:

Data presented in Table (5) show that isolate 1 of *F. o.* f.sp. *cepae* was the most affected one by the tested silver nanoparticles and the two concentrations of the fungicide Topsin-M70, being 28.11, 16.33 and 31.11% reduction in the linear growth, respectively. Meanwhile, the lowest affected isolates by silver nanoparticles and the fungicide Topsin-M70was isolate 3 of *F.o.* f.sp. *cepae* followed by isolate 2 of the same fungus. Moderate effect of the tested materials appeared on *F. solani*.

Table 5. Effect of silver nanoparticles and the fungicide Topsin-M70 on the growth of *F. solani* and thre*e* *F.o.* f.sp. *cepae* isolates.

Tested isolates*	Silver nanoparticles (1000 ppm)		Topsin-M70 (50µl)		Topsin-M70 (100 µl)	
	Linear growth (mm) [a]	Reduction (%)[b]	Linear growth (mm) [a]	Reduction (%)[b]	Linear growth (cm) [a]	Reduction (%)[b]
***F.solani* (M1)**	**63.3**	**29.44**	**75.3**	**16.33**	**60.3**	**33.00**
***F.o.* f.sp. *cepae* (F1)**	**64.7**	**28.11**	**75.3**	**16.33**	**62.0**	**31.11**
***F.o.* f.sp. *cepae* (F2)**	**58.3**	**35.22**	**74.0**	**17.78**	**59.0**	**34.44**
***F.o.* f.sp. *cepae* (F3)**	**56.7**	**36.88**	**70.0**	**22.22**	**59.3**	**34.11**
Control	**90.0**	**0.00**	**90.0**	**0.00**	**90.0**	**0.00**
LSD at 5%	**4.96**	**--**	**0.7**	**--**	**1.1**	**--**

[a] Mean of 3 replicates (plates),[b] Inhibition % = (Control-treatment /Control) x100, (F1) Dakahlia isolate(1) , (F2) Dakahlia isolate (2), (F3) Minia isolate (3) and (M1) Minia isolate (1).

Table (6) shows that all the tested silver naoparticles concentrations, *i.e.*25, 33 ,50 and 100% caused different degrees of fungistatic effect to the mycelial growth of the tested fungi. This reduction was gradually increased by increasing the concentration In addition, Topsin-M70 was the most efficient in this regard, which recorded 86.67 %reduction to the fungal growth followed by the crud concentration (100%) 25.89%. Meanwhile, low effect was obtained by the concentrations of 33%, being3.33 % compared with check treatment.

Enzymes activity:

Fig.(3) shows that the treatment with silver nanoparticles increased the activity of oxidative enzymes , *i.e.* peroxidase (POD) , polyphenol oxidase (PPO) and ß- 1,3 glucanase. The highest activity of peroxidase and polyphenol oxidase were obtained at the concentration of 50%. Meanwhile, ß-1, 3 glucanase showed the highest activity at the concentration of 33%. Many researchers have found the instability of both POD and PPO activities by using different concentrations of silver nanoparticles (Raigond *et al*., 2017 and Xihong *et al*., 2011). These results are in agreement with the obtained results by Omid *et al*. (2013) who stated that nanoparticle iron chelate at 1, 3 and 5 g obtained different effect on peroxidase activity, being 0.26 ,0.31 and 0.18 OD./min-1. Meanwhile, the treatment with the three previous concentrations plus foliar spraying of nano iron chelate resulted in 0.26, 0.21 and 0.21 OD./min-1 peroxidase activity, respectively. Our results also confirmed by Azad *et al*. (2019) who observed that including six nanoparticles examined at 25, 50 and 100 µgml-1 to control chili die-back disease caused by *Alternaria tenuissima*, silver significantly increased peroxidase, polyphenol oxidase activity and total phenol content followed by aluminum nanoparticles. Meanwhile, zinc dioxide recorded low activity at the same concentrations compared with check treatments.

Table 6. Effect of silver nanoparticles and fungicide Topsin-M70 on the linear growth of *F. O.* f.sp. *cepae* (isolate 1 of Dakahlia governorate), on PDA medium, 5 days after incubation at 28 ±2ºC.

Concentrations(%)	Average linear growth (mm)[a]	%Reduction [b]
25	**87.0**	**3.33**
33	**84.7**	**5.89**
50	**76.0**	**15.56**
100	**66.7**	**25.89**
Topsin-M70	**12.0**	**86.67**
Control	**90.0**	**0.00**
LSD at 5%	**1.5**	**--**

[a] Mean of 3 replicates, [b] Inhibition % = (control-treatment /control) x100.,

The increase in the concentration of AgNPs could be causes a reduction in the growth of different plants depending to AgNP's penetration and transport to the plant tissues (Thuesombat *et al*., 2014; Vannini *et al*., 2014; Nair and Chung, 2014). However, in a previous report by Sharma *et*

al. (2012), declared that AgNPs could be cause an enhance in growth and antioxidant status growth of *Brassica juncea*. Therefore, the effects of nanoparticles must be well evaluated before their widespread application.

2.6. Residue of silver nanoparticles in onion bulbs:

Data presented in Table (7) show that the residual amount of silver nanoparticles in onion bulbs was increased with the increase of silver nanoparticle concentration. However, the concentrations of 33 and 25 % are may be the best and safe concentrations for human due to their low residue in onion bulbs. Mathematical modeling of silver nanoparticles administered to the gastrointestinal tract bioaccumulation and bio-distribution was performed by Gmoshinski *et al.* (2013) using kinetic equations of 1-st order for describing of interior an exchange of this NPs. In this way it was shown that potentially dangerous levels of Ag NPs can be achieved in critical organs (liver, spleen) after acute or sub-acute oral administration of these NPs in a daily dose of at least 5-10mg / kg body weight.

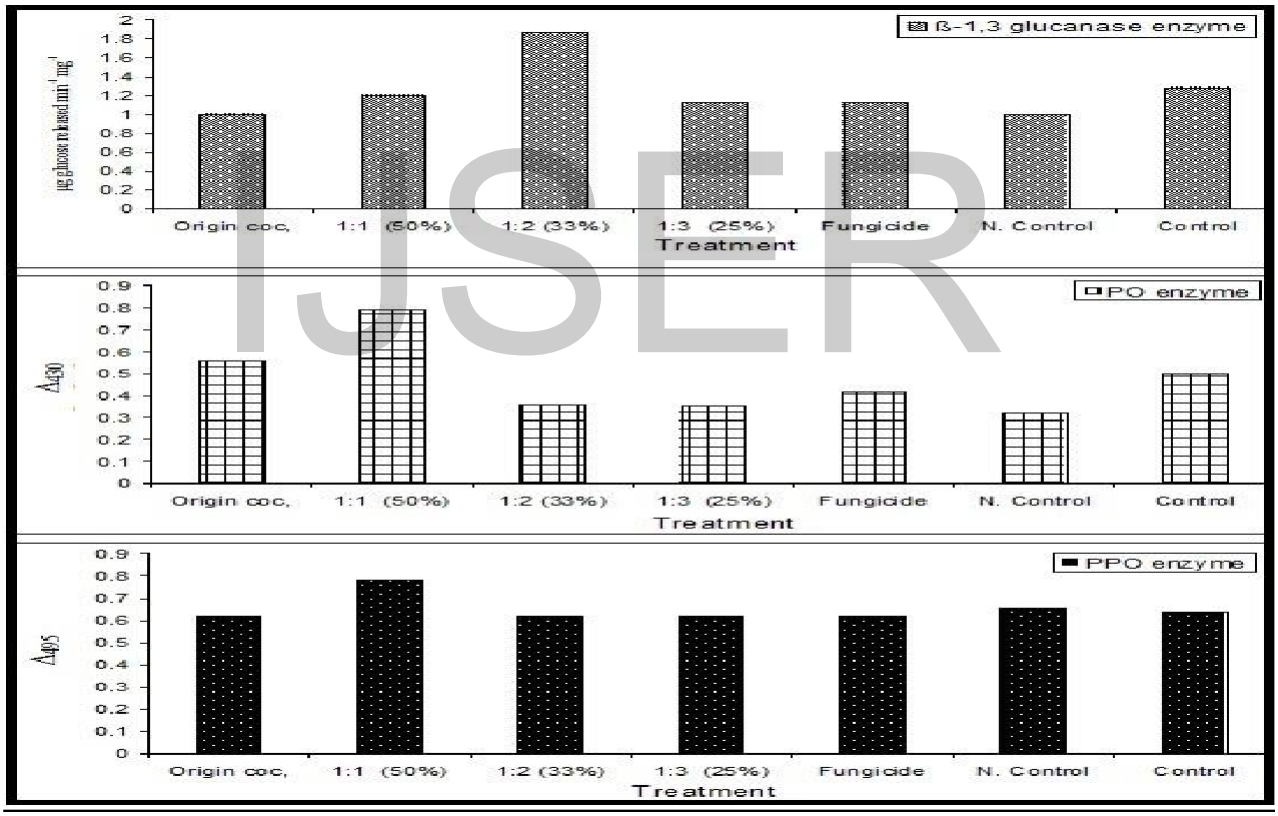

Fig.3. Effect of silver nanoparticles on the activity of peroxidase, polyphenol oxidase and ß- 1,3glucanase.

Recently, silver nanoparticles are widely used as powerful antimicrobial agents in agriculture. On the other hand, only the limited health effects of silver nanoparticles on humans have been documented to date and less known about their environmental effects (Lowry *et al* ., 2010 and Kuhnel and Nickel, 2014). Therefore, more experiments are needed to determine the actual concentration of nano-silver needed to control different microorganisms and the movement of silver nanoparticles in the food chain

and their environmental impacts. Also, the analysis of nanotechnology products assortment presented in the turnover in the Russian Federation indicates that various forms of nano-sized colloidal silver are the most widely used. Also, Korani *et al.* (2015) indicated that currently nano scale silver is one of the most widely studied nanomaterials from the point of view of its toxic effect on biological objects including laboratory animals *in vivo*. However, the available results of toxicological studies are largely inconsistent, which may be due either to differences in the samples of used NPs (with different particle size and shape, functionalization of the surface), and lack of a unified methodology in the planning the biological experiment. Thus, reliable epidemiological and biological toxicological studies are required. The toxicity of silver nanoparticles is associated with many physical and chemical properties such as size, chemical nature, surface area, reactivity, charge, compositions and ease of assembly. Small molecules are thought to be more toxic than larger ones, but particle size is not the only factor determining the toxicity of silver nanoparticles (Brown *et al*., 2001; Duffin *et al.*, 2007; Xiong *et al*., 2013 and Wang *et al*., 2014).

4. CONCLUSION

Based on the our results in the present study, it was concluded that nanotechnology can play as a catalyst for enhancing agricultural growth rate. Therefore, Silver nanoparticles applications can be used to reduce the infection by onion basal rot through an integrated control program. Moreover, this work needs further research to clear the mode of action of silver particles on the phytopathogenic fungi.

Table 7.The residue of silver nanoparticles in treated bulbs of onion after harvesting..

% Conc.(s)	Residue of silver nanoparticles in onion bulbs (mg\kg) during
25	0.13
33	0.23
50	1.20
100	1.95
Control	0.0

5. REFERENCES

[1] Abbas, A. (2015). Management of plant diseases. M.Sc. (Hons). The University of Agriculture, Peshawar Pakistan, 41pp.

[2] Abd-Elbaky, A.A. (2005).Control of some onion rots in North Africa by field and storage treatments. Ph.D. Thesis, Afr. Res.and Studies Inst., Cairo Univ.,pp.l09.

[3] Allan, A. I. and Hollis J. P. (1972). Sulffide inhibition of oxidase in rice roots. Phytopathology, 62: 634-639.

[4] Aluko, R and Ogbadu, G. H. (1986). Analysis of eggplant varieties for enzymes related to their organoleptic properties. Trop. Sci., 26: 163-171.

[5] Azad, C.S; Kumar A.; Chand, G. and Ranjan, R.D. (2019) . Evaluation of host defense inducing nanoparticles against *Alternaria tenuissima* (Kunze ex pers.)Wiltshire causing die-back disease of chilli. J. Pharmacog. and Phytoch., 8(3): 222-226.

[6] Behrani R. N. ;Abro, S. M. A. Abro ; Jiskani, M. M. and Khanzada, M. A. (2015). Pathogenicity and chemical control of basal rot of nion caused by *F.oxysporum* f.sp. *cepae*. G.Q.Pak. J.Agric., Agic. Eng., Vet. Sci., 31 (1): 60-70.

[7] Bobbarala1, V; Katikala, P. K.; Naidu, K. C. and Penumaj, S. (2009). Antifungal activity of selected

plant extracts against phytopathogenic fungi *Aspergillus niger* f2723. Ind. J. of Sci. and Technol.,2: 87-90.

[8] Boehnke B.; Karlovsky P.; Pfohl K.; Gamliel A.; Isack Y. and Dehne H. (2015). Identification of different *Fusarium* spp. in *Allium* spp. in Germany. Comm. Agric. Appl. Biol. Sci.,80:453–463.

[9] Brown, D.M.; Wilson, M.R.; MacNee, W.; Stone, V.and Donaldson, K. (2001). Size-dependent pro inflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol. App. Pharmacol; 175:191–199.

[10] Burdusel, A.C.; Gherasim, O.; Grumezescu, A.M.; Mogoanta, L.; Ficai, A. and Andronescu, E. (2018). Biomedical

Applications of Silver Nanoparticles: An Up-to-Date Overview. Nanomaterials, 8: 681.

[11] Cramer, C.S.(2000). Breeding and genetics of Fusarium basal rot resistance in onion.Euphytica,115:159–166.

[12] Domsch, K. H.; Gams, W. and Anderson, T. H. (1980). Compendium of Soil Fungi, Vol. 1 & 2, Academic Press, London.

[13] Duffin, R.; Tran, L.; Brown, D.; Stone, V. and Donaldson, K . (2007). Proinflammogenic effects of low-toxicity and metal nanoparticles *in vivo* and *in vitro*: highlighting the role of particle surface area and surface reactivity. Inhal. Toxicol.,19:849–856.

[14] El-Shehaby, A.I; El-Ganieny, R.M.A.;Tadrous, M.I. and Osman, Nagwa A. (1997). Control of post-harvest bulb rots of onion.8th Cong. of the Egypt. Phytopathol. Soc.,Cairo,353-363.

[15] Environmental Protection Agency (EPA) (1993). Reregistration Eligibility Document (RED) : Silver. OPP ,Washington ,USA.115 pp.

39–45.

[16] Esterbaner, H.; Schwarzl, E.andHayn, M. (1977). A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid.Anal.Biochem.,77: 486-494.

[17] Ghanbarzadeh, B.; Ebrahim, M. G. and Naser, S.(2014). Identification of *Fusarium* species causing basal rot of onion in East Azarbaijan province, Iran and evaluation of their virulence on onion bulbs and seedlings. Arch. of Phytopathol. and Plant Protec., 47(9): 1050–1062.

[18] Ghanbarzadeh, B.; Safaie, N.; Goltapeh, E.R.; Danesh, Y. and Khelghatibana, F. (2016). Biological control of Fusaium basal rot of onion using *Trichoderma harzianum* and *Glomus mosseae*. J. Crop Protec., 5 (3): 359-368.

[19] Gmoshinski, I.V.; Khotimchenko, S.A.; Popov, V.O. ;Dzantiev, B.B.; Zherdev, A.V.; Demin, V.F. and Buzulukov, Y.P.(2013). Russ. Chem. Rev., 82(1): 48.

[20] Hassan, Maggie E.M.; ZawamHanaa S.; El-Nahas, Shereen, E.M. and Desoukey, Abeer F.(2016) . Comparison study between silver nanoparticles and two nematicides against *Meloidogyne incognita* on tomato seedlings. Plant Path. J., 15: 144- 151.

[21] Jo, Y.K; B.H. Kim, and G. Jung (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis., vol. 93, pp.1037-43.

[22] Johansen,L.E.;Curi, E.A. ; Bonf, J.H. and Fribourg, H.A. (1960). Method for studying soil microflora–plant disease relationship. Second Printing Burges publishing Co. pp77.

[23] Jung, J.H., Sang-Woo, K.;Ji-Seon,M.;Young-Jae, K.;Kabir, L., Kyoung, S. K. and Youn, S. L. (2010) . The Effect of nano-silver liquid against the white rot of the green onion caused by *Sclerotium cepivorum*. Mycobiology, 38(1):

[24] Isaac, R. A. and Kerber, J.D.(1971). Atomic absorption

and flame photometry: Techniques and uses in soil, plant and water analysis. In L.
M. Walsh (ed.) Instrumental methods for analysis of soils and plant tissue. Soil Sci. Soc. Am., Madison, WI. , 18-37.

[25]Kildeby ,N.L., Andersen, O.Z., Roge, R.E., Larsen, T., Petersen, R.and Riis ,J.F.. 2005. Silver Nanoparticle (Research project). Aalborg University .Faculty of Physics and Nanothechnology, 80 pages.

[26] Kim, K.J.; Sung, W.S.; Suh, B.K.; Moon, S.K.; Choi, J.S. and Kim, J.G. (2009) .Antifungal activity and mode of action of silver nanoparticles on *Candida albicans*. Biometals,22:235–242.

[27] Kodama, F. (1983). Studies on basal rot of onion caused by *F. oxysporum* f.sp. c*epae* and its control
.Hokkaido Prefectural Agricultural Exper. Stats., 39-65.

[28] Kogan, M. (1998). Integrated pest management: Historical perspectives and contemporary developments. Annu. Rev. of Entomol., 43:243–270.

[29] Korani, M., Ghazizadeh, E., Korani, S., *et al*. (2015). Effects of silver nanoparticles on human health. Eur. J. of Nanomedi.., 7(1): 51-62.

[30] Kuhnel, D. and Nickel, C. (2014).The OECD expert meeting on ecotoxicology and environmental fate – towards the development of improved OECD guidelines for the testing of nanomaterials. Sci. Total Environ., 472:347–53.

[31] Lager ,Sara (2011). Survey of *Fusarium* species on yellow onion (*Allium cepa*e) on Öland. Master thesis, biology and agronomy program, Swedish University of Agricultural Science. 34pp.

[32] Lowry, G.V.;Hotze, E.M.; Bernhardt, E.S.; Dionysiou, D.D.; Pedersen, J.A. and Wiesner, M.R.(2010). Environmental occurrences, behavior, fate, and ecological effects of nanomaterials: an introduction to the special series. J. Environ. Qual.,39:1867–74.

[33] Malaj, E.; von der Ohe, P.C.; Grote, M.;Kühne.R.; Mondy, C.P.; Usseglio-Polatera, P.; Brack, W. and Schäfer, R.B. (2014). Organic chemicals jeopardizethe health of freshwater ecosystems on the continental scale. Proc. of the Nat.Acad. of Sci.,111 (26): 9549–9554.

[34] Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugar.Anal. Chem., 31, 426-428.

[35] Min, J.S; K.S. Kim; S.W. Kim; J.H. Jung ; K. Lamsal; S.B.Kim ;M. Jung, and Y.S. Le.(2009).Effects of colloidal silver nanoparticles on sclerotium-forming phytopathogenic fungi. Plant Pathol. J., 25: 376-80.

[36] Mohammadlou, M.; Maghsoudi, H. and Jafarizadeh-Malmiri, H.(2016). A review on green silver nanoparticles based on plants: Synthesis, potential applications and eco-friendly approach.
Int. Food Res. J., 232: 446–463

[37] Mujeebur, R.K. and Tanveer, F.R. (2014). Nanotechnology: Scope and Application in Plant Disease Management. Plant Pathol. J., 2014, 13, 214–231.

[38] Nair, P.M.G. and Chung, I.M.(2014). Assessment of silver nanoparticle-induced physiological and molecular changes in *Arabidopsis thaliana*. Environ. Sci.Pollut.Res. ,21 (14): 8858- 8869.

[39] Omid Salarpour ; Soheil, P.; Hassan, S.M. ; Majid, A.J. (2013). Effect of nano-iron chelates on growth, peroxidase enzyme activity and oil essence of Cress *(Lepidium sativum*L.). Int. J. of Agro. and P. Prod. , 4 (S): 3583-3589 .

[40] Özkara,A.; Akyıl, D. and Konuk,M. (2016). Environmental Health Risk.Chapter 1, Pesticides Environmental Pollution, and Health, 3-27pp.

[41] Pinto, C. M.F.; De Paula Junior, T. J. and Zambolim,V (1995). Diseases caused by fungi on onion. Informe Agropecuario (Belo Horizonte) , 17 (183): 82-36.

[42] Queyrel, W.W; Habets, F.; Blanchoud, H.; Ripoche, D. and Launay, M. (2016). Pesticide fate modeling in soils with the crop model STICS: Feasibility for assessment of agricultural practices. Sci. of the Total Environ., 542: 787–802.

[43] Raigond, P., Raigond, B.; Kaundal. B. ; Singh, B. ; Joshi, A. and Dutt, S. (2017).Effect of zinc nanoparticles on antioxidative system of potato plants.(38) No 3 : 435-439 .

[44] Sharma, P.; Bhatt, D.; Zaidi, M. G. H. ; Pardha-Saradhi, P.; Khanna, P. K. and Arora, S. (2012). Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl. Biochem. Biotechnol ., 167:2225–2233.

[45] Singh, S.; Singh, B. K.; Yadav S.M. and Gupta, A.K. (2015). Applications of nanotechnology in agricultural and their role in disease management. Res. J. of Nanosci. and Nanotech., 5: 1-5.

[46] Snedecor, G.W. and Cochran, W.G. (1998).Statistical Methods 7th Ed. Iowa State Univ.,Press, Ames, Iowa, U.S.A.

[47] Somkuwar, R.G.;VeereGowda,R.; Singh, T.H. and Pathak, C.S. (1996). Screening of onion for resistance to onion basal rot. Madras Agric. J., 83: 273-275.

[48] Stadnik, M.J.and Dhingra, O.D. (1997). Root infection by *Fusarium oxysporum* f.sp. *cepae* at different growth stages and its relation to the development of onion basal rot. Phytopath Medit., 36(1): 8-11.

[49] Summerell, B.A. (2019). Resolving Fusarium: Current status of the genus. Annu. Rev. Phytopathol. ,57:323–339. doi: 10.1146/annurev-phyto-082718-100204.

[50] Thuesombat, P.; Hannongbua, S.; Akasit, S. and Chadchawan, S. (2014). Effect of silvernanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. Ecotoxicol. Environ. Saf.,104: 302-309.

[51] Tyagi ,A.K and Malik, A. (2010). In situ SEM, TEM and AFM studies of the antimicrobial activity of lemon grass oil in liquid and vapourphase against *Candida albicans*. Micron.;41(7):797-805.

[52] Taylor A.; Vágány V.; Jackson A.C.; Harrison R.J.; Rainoni A. and Clarkson J.P. (2016). Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *cepae*. Mol. Plant Pathol. ,17:1032–1047. doi: 10.1111/mpp.12346.

[53] Vannini, C.; Domingo.G.; Onelli, E.; Mattia, F.D.; Bruni, I.;Marsoni, M. andBracale, M. (2014).Phytotoxic and genotoxic effects of silver nanoparticles exposure on germinating wheat seedlings. J. Plant Physiol., 171: 1142- 1148.

[54] Wang, X.; Ji, Z.; Chang, C.H. ; Zhang, H.; Wang, M.; Wang, M. *et.al*.(2014).Use of coated silver nanoparticles to understand the relationship of particle dissolution andbioavailability to cell and lung toxicological potential. Nano-Micro Small, 10:385–98.

[55] Wani, A. H. and Shah, M.A.. (2012) .A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. J.of App. Pharmaceu. Sci.,2 (3): 40-44.

[56] Xihong, L.i.; Weili, L.I.; Jiang; Y.; Ding, Y. ; Yun, J.; Tang, Y. and Zhang, P. (2011). Effectof nano-ZnO-coated active packaging on qualityof fresh-cut „Fuji" apple .Int. J. of Food Sci. andTechnol., 46: 1947–1955.

[57] Xiong, Y.; Brunson, M.; Huh, J.; Huang, A.;Coster, A.; Wendt, K.; Fay ,J. and Qin, D.(2013).The role of surface chemistry on the toxicity of Ag nanoparticles. Nano-Micro Small,9:2628–2638.Wileyonlinelibrary. com.