JJBS

Volume 18, Number 2,June 2025
ISSN 1995-6673
Pages 221 – 230
https://doi.org/10.54319/jjbs/180203

Jordan Journal of Biological Sciences

# Application of Chitosan/Hydroquinone Nanaoemulsions for Management Root Rot Disease on Cucumber Plants in Plastic Houses

El-Sayed H. E. Ziedan[1,*] and Abdel-Tawab H. Mossa[2]

[1]Plant Pathology Department and [2]Pesticide Chemistry Department, National Research Centre NRC), 33 El Bohouth Street (former El Tahrir St.)، P.O. 12622, Dokki, Giza, Egypt.

Received: May 9, 2024; Revised: September 10, 2024; Accepted: October 15, 2024

**Abstract**

Root rot is an epidemic fungal disease in cucumber caused by soil borne fungi of *Fusarium* spp., significantly reducing numbers of plants, vegetative growth and fruit yield during growing in plastic houses. Several nanoemulsions of chitosan alone and chitosan loaded with hydroquinone, sorbic acid, or propionic acid were prepared, characterized and *tested in vitro* against mycelial linear growth, condial sporulation and pathogenic potential of the fungal isolates, i.e. *Fusarium oxysporum* (MG018778) and *Fusarium solani* (MG018781) on cucumber. These nanoemulsions were also evaluated in greenhouse as soil drench for their effect on root rot incidence in cucumber plants, vegetative growth and fruit yield. Results *in vitro* indicated that nanaoemulsion of chitosan/hydroquinone was more effective than the other tested nanaoemulsions, with high efficacy against morphological characters tested of fungal isolates. Moreover, application of the chitosan/hydroquinone nanoemulsion, with 122.8 nm drop size, by the rate 0. 5 % as soil drench on transplanting's before cultivation and in combination with soil drench 30 days after plants cultivation in plastic house*s* was significantly suppressive root rot incidence on cucumber plants, increased survival plant, plant height, fresh weight and fruits yield of cucumber plants. Therefore, the nanoemulsion of chitosan/hydroquinone is an effective eco-friendly agent alternative fungicide for controlling root rot on cucumber cultivation.

**Keywords:** Cucumber, root rot, fungi, Fusarium, nanoemulsion, chitosan, hydroquinone.

## 1. Introduction

In order to provide fresh food for human consumption, industrial food, and medicine purposes, cucumber plants (*Cucumis sativus* L.) are among the most important economically fresh vegetables due to their rapid vegetative growth and extended fruiting period. They are widely distributed in open fields and under various soil and geographic conditions in protected houses (Essa *et al*., 2017). Cucumber is planted in open fields and protected houses with worldwide cultivation in 2,231,402 hectares, producing approximately 87.805 tons of fresh fruits (FOASTAT 2021). In Egypt, cucumber agriculture is expanding in newly reclaimed lands in open fields during the summer and in greenhouses throughout the autumn, winter, and summer seasons (Mossa *et al.,* 2021; Ziedan *et al.,* 2022; Ziedan, 2024a), and the global cultivation area reached, 19702 hectares which produced 433440.85 tons (FAOSTAT 2023).

Cucumber is one of the most important economic fresh vegetables due to its rapid growth and extended fruiting period. It is widely distributed in open fields and in various soil and geographic conditions in protected houses (Essa *et al.*, 2017).

Cucumber fruits have great economic and health benefits for humans due to their rich content of the high nutritional values. They are very important as fresh vegetables, medicinal plants, and weight loss remedy by reducing fat and including a high percentage of fibre. Cucumber fruit is rich in minerals, sugar, protein, thiamin, riboflavin, vitamin C, niacin; and they have antimicrobial properties and anticancer ones and many other bone disease remedies due to their content of secoisolariciresinol, lignans, lariciresinol, and pinoresinol (Pal *et al.,* 2020; Sambou *et al.,* 2023).

Many genera of soil-borne pathogenic fungi, *F. solani*, *F. oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Sclerotinia* spp. attack cucumber plants and cause wilt and root rot diseases (Elwakil *et al*., 2015; Farrag *et al*., 2013; Ziedan and Saad 2016; Ziedan *et al.,* 2022; Ziedan 2024 a). Synthetic fungicides were regularly used to treat fungal diseases and have a number of negative side effects, including environmental contamination, residues in edible plant portions, and resistant strains. For avoiding ricks of fungicides, alternatives of organic acids of sorbic and salicylic acids were used for controlling grapevine root rot (Ziedan *et al.,* 2020), polymer of chitosan and essential oils as the natural agents were used for management crown and anthracnose on banana fruits after harvest

* Corresponding author. e-mail: ziedanehe@yahoo.com.

(Hossain and Iqbal 2016; Zoeir *et al*., 2017; El-Zahaby *et al.*, 2018; Ziedan 2024 b). Chitosan and hydroquinone as a promising natural organic extraction are non-toxic, biodegradable, with direct harmful antifungal effect on growth, morphology and populations of plant pathogens, as well, enhancing systemic induce resistance in plant against invasion of root rot and wilt pathogens , in addition increasing productivity in cucumber (Elwakil 2003; Hanafi 2004), on fenugreekm (Ghule *el al.,* 2021), on chickpea (Hemeda 2006; Zian *et al.,* 2023). Chitosan directly significantly reduced mycelial growth, sporulation, spore germination and induce malformation, swellings and lysis of mycelial hyphal of *F. solani* the causal of root rot disease on fenugreek. It also enhances plant seed germination, increasing defense resistance enzymes, of chitinase, β-1, 3-glucanase (Ghule *el al.,* 2021). Chitosan and hydroquinone were controlling wilt disease of chickpea caused by fungi of *F. oxysporum* f. sp. *ciceris* and *Rhizoctonia solani* by enhancing the activation of enzymes related plant resistant, such as peroxidase, polyphenol oxidase and increase content of phenol and photosynthetic pigments ( Zian *et al.,* 2023). As a result, the use of nanoparticles in managing plant diseases has become crucial to integrated pest management since various chemical, physical, and biological processes may be used to create eco-friendly agents (Mossa *et al*., 2021; Ziedan *et al.,* 2022). Nanoparticles are an efficient and convenient way to defend plants from diseases, such as bacteria, fungi, viruses, and nematodes, which are significant limiting factors in the production of food material (Khan *et al*., 2012). Recent studies have evaluated the effectiveness of nanoparticles and nano formulations in controlling fungi, and the results show that they are substantially more effective than conventional fungicides (Hossain and Iqbal 2016; Ziedan and Saad 2016; Mossa *et al.,* 2021; Ziedan *et al.,* 2022). In this manner, chitosan nanoemulsion were found to be more effective than chitosan, nanoemulsion of chitosan at 1.0% with droplet size (200 nm and 600 nm) respectively, its highly significances reduced mycelial growth of *Colletotrichum musae* (Berk. & Curt) Arx., the causal of anthracnose on banana fruits and *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc., the causal of anthracnose on papaya and dragon fruits. In addition, *in vivo* was significantly suppressive incidence of anthracnose on fruits and maintaining their quality to 28 days under cold storage (Zahid *et al.,* 2012). Different application treatments of chitosan nanoparticles formulation were enhancing plant growth and yield components such as composite nano-size silver/chitosan nanoformulations are more efficient in combating a number of seed-borne fungi, of *Aspergillus flavus*, *Alternaria alterneta* and *R. solani* of chickpea (Kaur *et al.,* 2012). Cu-chitosan nanoparticles signifcantly enhanced growth of maize plants, chlorophyll content dry weight and enhanced defense responses against *Curvularia* leaf spot disease incidence by increasing enzymes activities of superoxide dismutase, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase (Choudhary *et al*., 2017).

Clay/chitosan nanocomposite at 20 µg mL -1 directly completely inhibited growth of *Penicillium digitatum* and incidence of green mold disease of orange cv. Valencia after harvest, inducted resistance of orange fruits and caused morphological alternations in mycelial hyphae of the fungal pathogen (Youssef and Hashim 2020). Chitosan nanoparticles (CNPs) as antifungal of *F. equiseti* was controlling tomato wilt and increased the efficacy of biocontrol agents (El-Morsy *et al.,* 2023). In addition, chitosan- potassium nanoparticles formulation increased plant vegetative growth, fruit yield and high and total soluble solids as well vitamin C, acidity, sugar strawberries fruits (Abd-Elrahman *et al*., 2023).

This investigation, aimed at preparation, characterization of antifungal nanoemulsions from safety organic material to suppress growth of fungi causing root rot disease on cucumber *in vitro* and their application *in vivo* for protecting cucumber plant during growth in plastic houses.

## 2. Materials and Methods

### *2.1. Chemicals and reagents*

Chitosan (M.Wt. = 100.000-300.000), Polysorbate 80 (Tween 80), Sodium tripolyphosphate (TPP) and hydroquinone were obtained from VWR International 201, Rue Carnot F-94126 Fontenay/Bois, France, while systemic fungicide named Topsin M- 70% was provided from El-Deeb of Developmental Agriculture Company, Egypt.

### *2.2. Preparation of chitosan nanoemulsion*

For preparation chitosan nanoemulsion, native chitosan and TPP were used for preparation an ionic gelation at room temperature. (Calvo *et al*., 1997; Rampino *et al.,* 2013; Waly *et al.,* 2015). Nanoparticles are created by inter- and intra-molecular linkages between amino groups of chitosan and charged phosphate groups of (TPP). Chitosan solution was prepared by swirling a 3 mg/ml chitosan into a 1% (v/v), pH 5, acetic acid solution. Afterward, 120 ml of TPP solution (0.5 mg/ml, w/v) was added under stirring to 100 ml of chitosan solution. Chitosan nanoparticles (NPs) were collected, cleaned by suspending in deionized water two to three times, centrifuged for 30 minutes at 10,000 rpm, then re-suspended in deionized water.

### *2.3. Preparation of hydroquinone-loaded chitosan nanoemulsion*

Hydroquinone-loaded chitosan nanoemulsion according ionic cross-linking method (Rampino et al., 2013; Ding *et al.,* 2017). Chitosan (1000 mg) after dissolved in 100 ml of 1.0 % acetic acid was mixed with 1 mg/ml of hydroquinone then TPP solution (1 mg/mL) was dropped dropwise to chitosan suspension while being stirred (4000 rpm) for two hours. The resulting nanoparticles (NPs) were freeze-drying after being collected by centrifugation for 30 min then being re-suspended in deionized water.

### *2.4. Preparation organic acids and hydroquinone loaded chitosan nanoemulsion*

Sorbic or propionic acids were loaded with chitosan nanoparticles according method described by (Ding et al., 2017). 1000 mg of chitosan was dissolved in 1.0 % acetic acid, pH 5) or 0.1%. of propionic acid, and sorbic acid. TPP was prepared in deionized water. Then, TPP solution was added drop wise into the chitosan solution at room temperature under magnetic stirring for 2 hrs; then

nanoparticles were obtained by after centrifugation at 13 000 rpm for 30 min at 4 °C then re-suspended in deionized water.

*2.4.1. Nanoemulsion characterizations*

Distribution of particle size of various nanoparticles was determined by dynamic light scattering PSS instrument (Santa Barbara, CA, USA) , at 23 °C with 632 nm helium-neon (HeNe) laser as the light source. The scattering angle was set to 90°.

*2.4.2. Transmission electron microscopy (TEM)*

Various prepared chitosan nanoemulsions were observed their dimentions size of nanoparticles by TEM (model JEM-1230, Jeol, Tokyo, Japan). The drops of chitosan nanoparticles only and its loaded either one of sorbic acid and propionic acid, and hydroquinone in deionized water, were diluted then transferred in grid of carbon-coated copper then dried at 27ºC then images were taken at 80 KV voltages at Centeral laboratory of scientific services in National Research Centre unit, Egypt.

*2.5. Effect of nanoemulsion on fungi growth and pathological activity Fungal isolates tested*

Two highly pathogenic isolates of fungi caused root rot on cucumber were identified according to morphological, cultural and molecular biology with accession numbers, i.e. *Fusarium oxysporum* (MG018778) and *Fusarium solani* (MG018781), in previous work (Attallah *et al*., 2019). These isolates were tested under stress of nanoemulsions of chitosan.

*2.5.1. Mycelial linear growth*

Different chitosan, nanoemulsion singly and in combinations with hydroquinone and organic acids of propionic and sorbic were tested on mycelial linear growth of fungi tested on PDA agar medium. Three Petri dishes plates (9 cm in diameter) were used as a replicates for each formulation, and tree plates free treatment were served as a control. Plates were incubated at 27±2 C for 7 days. The average diameters of mycelial linear growth of each plate was determined and calculated as the reduction % of fungal growth according to the following formulation (Duarte *et al*., 2014).

$$GI\ (\%) = [\ (Gc - Gt)/Gc] \times 100$$

Where: GI = Percentage of mycelial growth inhibition; Gc = linear mycelial in negative control; Gt = linear mycelial of the treatment

*2.5.2. Conidia sporulation count*

Conidiospores count was determined using haemacytometer slide. The average number of spores was calculated per cm² of fungal growth.

2.5.3. *Pathogenic potential of fungal isolates*

Mycelial colonies 10 days old of two fungal isolates tested as mentioned before being grown under stress of nanoparticles formulations were used for testing effect of nanoemulsion prepared on pathogenicity test of fungi using germinated seeds (Golden) of cucumber on wetted filter paper for two days then cultured on fungal mycelial growth. Ten germinated seeds were used of each plate; three plates were used as replicates for each treatment and ten plates without nanoparticles were served as a control. Plates were incubated at 27±2 C for 7 days. Root rot syndromes of developing cucumber seedlings were visually examined according to (Ziedan and Saad 2016).

*2.5.4. Scanning electron microscopy (SEM) observations*

For studying morphological changes of fungal isolates, pieces 4x4mm of each fungal isolates 5 days old growth, on potato dextrose agar medium (PDA) supplemented nanoemulsion of chitosan/hydroquinone 4000 ppm and fungi free stress (control). Each fungal isolate was fixed in buffer it in osmium tetroxide, dehydration in a graded ethanol series of ethanol solutions (25%, 50%, 75%, and two 100%) for 10 minutes, then coat with gold and view in scanning electron microscope quanta FEG250 field emission (Scan EM) at National Research Centre unit, Egypt.

*2.6. Nanoemulsion for controlling root rot disease on cucumber*

Different nanoemulsion of chitosan/hydroquinone at 0.5 and 1.0 % concentration as soil drench during transplanting's growth 2 days before cultivation in green house and /or soil drench by the rate 200 ml/plant, 30 days after cultivation of transplantings in open field (greenhouse). Treatments were distributed in randomized block design during growing season 2018/2019 in protective plastic green houses, Dokki, Agriculture Research Centre, Minsitry of Agriculture, Egypt as follows:

0- Control (plants free treatment)

Soil drench of transplanting's by chitosan/ hydroquinone nanoemulsion (0.5 %)

Soil drench of transplanting's by chitosan/ hydroquinone nanoemulsion (1.0%)

3-Soil drench of transplanting's and in field with chitosan/ hydroquinone nanoemulsion ( 0.5 % )

4- Soil drench of transplanting's and in field with chitosan/ hydroquinone nanoemulsion (1.0%)

5- Soil drench of transplanting's with (fungicide) Topsin M-70 (0.5%).

6-Soil drench of transplanting's and in field with (fungicide) Topsin M-70 (0.5%)

2.6.1. *Root rot assessment*

Root rot disease incidence on hundred cucumber plants, disease severity was determined according to (Downes and Ito 2001).

$$\text{Disease severity} = \Sigma\ (n \times r)/N \times 100$$

Where: n = number of plants in each numerical disease grade;r = number of the disease grade; $N$ = total number of plants multiplied by the maximum numerical disease grade.

Disease severity was determined on the cucumber shoot by the linear scale (0-4) as grade of wilt syndromes on leaves, 3 months after cultivation according to (Carver et al., 1996) where:

0 = healthy plant
1 = intial sing of wilt on plant
2 = wilt more 25% of plant
3 = wilt more 50% of plant
4 = wilting more 75% to plant.

*2.6.2. Morphological characters and yield components of cucumber plants*

Plant height of cucumber plants, fresh weight of shoot, number of buds, and total number and yield of cucumber fruits (Kg) were calculated 5 months after cultivation.

*2.7. Statistical analysis*

Data were statistically analyzed using analysis of variance (ANOVA) comparing among means using least significant differences (L.S.D ) *P*= 0.05 according to procedures outlined by (Snedecor and Cochran 1980).

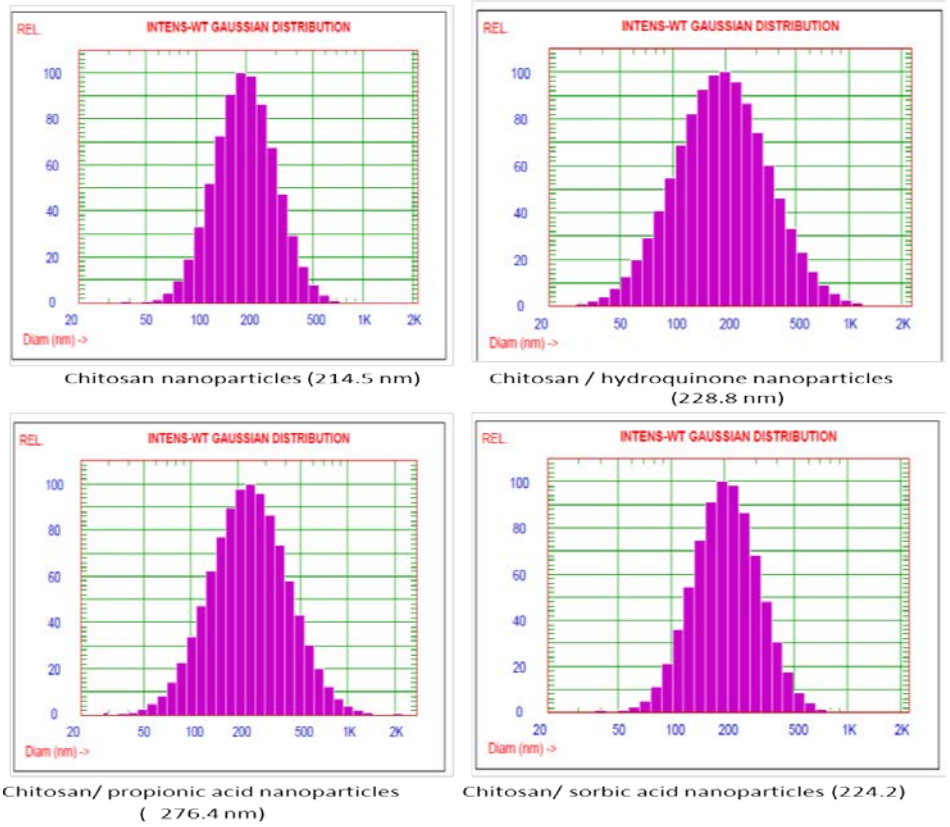
## 3. Results

*3.1. Preparation of nanoparticles of chitosan, hydroqinone and organic acids*

Chitosan nanoemulsions and its loaded with each one of hydroquinone, sorbic acid and propionic acid were prepared based on chitosan high molecular weight, polysobate (tween- 80) and tripolyphosphate (TPP) by ionic gelation at room temperature as simple ionic cross-linking at 50% in distilled water. Stable nanoemulsions were suspended for assay droplet size and their distributions, as shown in (Fig. 1) nanoparticles of chitosan (214.5 nm), nanoparticles of chitosan/hydroquinone (228.8 nm), nanoparticles of chitosan/sorbic acid (224.2) and nanoparticles of chitosan/propionic acid (276.4).

Morphology and dimensions of chitosan nanoparticles were viewed by transmission electron microscopy (TEM) as shown in **(Fig. 2).** The droplets of nanoemulsion were spherical shape in a good dispersion. This visualization confirmed the distribution of droplet diameter sizes in the chitosan nanoemulsions**.**

**Figure 1** . Particle size distribution of chitosan nanoparticles alone and those loaded with hydroquinone, sorbic acid, and propionic acid.



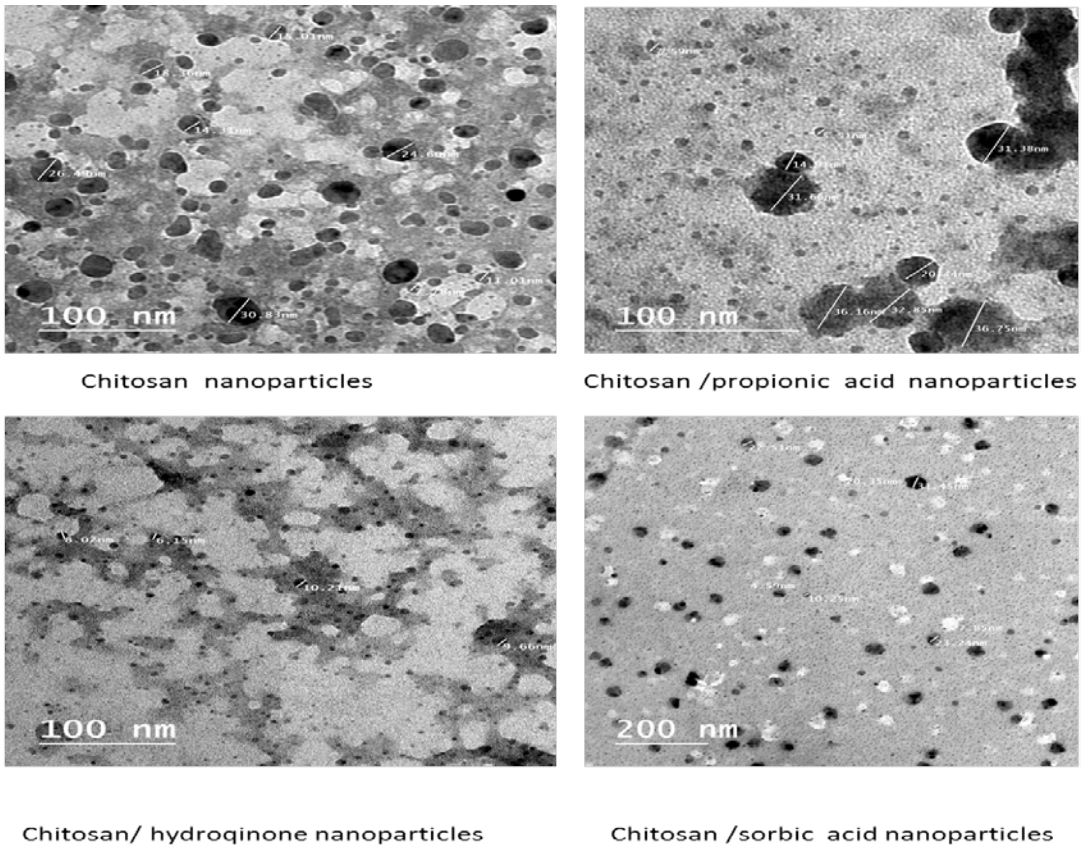



**Figure 2** . TEM of exhibiting chitosan nanoparticles size

*3.2. Effect of chitosan nanoemulsions on growth and pathogenic activity of fungi*

Data in Table (1) indicated that testing 1000 and 2000 ppm of nanoemulsions of chitosan only or chitosan / propionic acid, chitosan / sorbic acid and chitosan / hydroquinone were reduced mycelial linear growth of the tested fungus of *Fusarium solani* and *F. oxysporum* than in untreated (fungi free nanoemulions test) which increased with higher concentrations. In this manner, the chitosan/hydroquinone nanoemulsion at 2000 ppm was the most effective against the mycelial linear of fungal growth which reduced *F. oxysporum* by 60% followed by chitosan / sorbic acid nanoemulsion (33%) then the chitosan alone nanoemulsion (34%), meanwhile the least effective was nanoemulsion of chitosan / propionic acid (24%). Meanwhile, nanoemulsion of chitosan significantly reduced mycelial growth of *Fusarium solani* by (41%), meanwhile nanoemulsions of chitosan/hydroquinone, chitosan / sorbic acid chitosan / propionic acid, which reduced mycelial linear growth by (33%).

In addition, data in Table (2) shows that all nanoemulsions significantly reduced conidia count production on both tested fungal isolates compared to the fungi free treatments (control). Nanoemulsion of chitosan/hydroquinone and chitosan nanoemulsion alone, respectively, were the best treatments that reduced conidia count production of *Fusarium solani* (81.3) and *F. oxysporum* (47.4%). In this respect, nanoemulsions of chitosan/sorbic acid and chitosan/propionic acid were the least effective in reducing the conidia count of *F. oxysporum* (28.4%) and *Fusarium solani* (62.5%) , respectively. Furthermore, as shown in Fig (3) , all chitosan nanoemulsions in this study reduced the rotting of the germinated seed of cucumber and mortality of developing cucumber seedlings than in the untreated (control). Nanoemulsion of chitosan/hydroquinone was the best treatment that reduced seed rotten and seedling death of cucumber caused by *Fusarium solani* followed by nanoemulsion of chitosan/ propionic acid, nanoemulsion of chitosan, respectively. Meanwhile, nanoemulsion of chitosan/sorbic acid showed the least effect.

**Table 1** . Effect of chitosan nanoemulsions on growth of fungi pathogens

| Treatment | | % Reduction of mycelial linear growth | |
|---|---|---|---|
| Nanoemulsion | Con. ppm | *F. oxysporum* | *F. solani* |
| Control | 000.0 | 00.0 g | 00.0 e |
| Chitosan / propionic acid | 1000 | 02.0 f | 00.0 e |
| | 2000 | 24.0 e | 33.3 b |
| Chitosan / sorbic acid | 1000 | 50.0 b | 33.0 b |
| | 2000 | 50.0 b | 33.0 b |
| Chitosan /hydroquinone | 1000 | 50.0 b | 16.7 d |
| | 2000 | 60.0 a | 33.0 b |
| Chitosan | 1000 | 24.0 e | 25.1 c |
| | 2000 | 34.0 d | 41.3 a |

The same letter in each colum are not significantly differences at $P \leq 0.05$.

**Table 2.** Effect of chitosan nanoemulsions on conidia count of fungal pathogens

| Nanoemulsion (2000 ppm) | Conidia (x $10^5$)/ $cm^2$ | | | |
|---|---|---|---|---|
| | *F. oxysporum* | | *F. solani* | |
| | count | % reduction | count | % reduction |
| Control | 9.5 a | 00.0 | 4.0 a | 00.0 |
| Chitosan / propionic acid | 6.0 c | 36.8 | 1.5 b | 62.5 |
| Chitosan / sorbic acid | 6.8 b | 28.4 | 1.3 c | 67.5 |
| Chitosan /hydroquinone | 6.0 bc | 36.8 | 0.5 e | 87.5 |
| Chitosan | 5.0 d | 47.4 | 0.8 d | 81.3 |

The same letter in each colum are not significantly differences at $P \leq 0.05$.

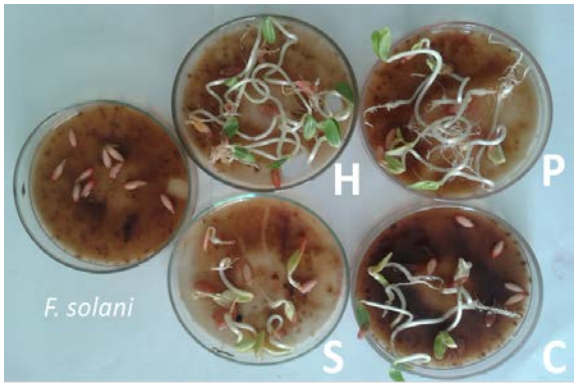



**Fig ure 3.** Effect of chitosan nanoemulsions on pathogenic activity of *F. solani* free treatment, H= chitosan/hydroquinone, P = chitosan/propionic acid, S = chitosan/sorbic acid and C = nano chitosan alone)

### *3.3.* Application chitosan nanoemulsions on root rot disease incidence

Promising nanoemulsion of chitosan / hydroquinone as the a promising antifungal against pathogenic fungi of cucumber plants by the rate ( 0.5 and 1%) compared the systemic fungicide (Topsin-M-70) at (10g/L) were application were by suspended as the oil drench 2 days before cultivation the cucumber transplanting's or / and soil drenching 30 days after cultivation in greenhouse. Various applications of chitosan / hydroquinone nanoemulsion as in combination soil drench by 0.5% followed by 1.0% respectively significantly reduced root rot incidence, disease severity of cucumber plants and increased number of survival plants as well morphological characters and yield fruits of cucumber plants compared in untreated plants and the same treatments by fungicide (Tables 3 and 4 ), as shown in Table (3) and Fig (4). Applications of nanoemulsions of chitosan / hydroquinone by the rate (0. 5%) as soil drench of transplanting's alone or in combination with soil drench were the best treatments that reduced root rot incidence on cucumber plants 5 months after cultivation in greenhouse and recorded the highest survival % of cucumber plants, significantly enhancing morphological characters of cucumber plants than in the treatments by fungicide and in the control (no treatment), *i.e* shoot length, fresh weight of shoot, number of buds , number of cucumber fruits / plant and total yield of cucumber fruits, in Table ( 4 ) and Fig (4). In this respect, the significant of plant height of cucumber (230 cm) in combined treatment of transplanting and soil drench by (0.5%) of chitosan / hydroquinone nanoemulsion compared (110 cm) of plant height in untreated and fungicide application (120 cm). Moreover, the combined treatment of transplanting and soil drench after cultivation by nanoemulsion of chitosan / hydroquinone recorded, the highest values of the fresh weight of cucumber plant shoot (453.0 g /plant), yield components was (10) buds and yield was (9.2 kg of cucumber fruit / plant followed by the same the application by at (1.0 %) of nanoemulsion of chitosan / hydroquinone the fresh weight of shoot (226 g /plant), (6) buds and (5) kg of cucumber fruit / plant. Meanwhile, the lowest value was recorded with fungicide and plant free treatments (control) which recorded fresh weight/plant ( 74.8 and 40 g/plant), 1.6 and 1.0 kg/ fruit/plant respectively.

**Table 3 .** Effect of nanoemulsion of chitosan / hydroquinone on root rot disease incidence on cucumber plants under natural infestation in plastic houses

| Soil drench | | | Survival plant % | Root rot incidence | |
|---|---|---|---|---|---|
| Materials | Con % | method | | % Disease | D. severity |
| Nanoemulsion (chitosan/hydroquinone) | 0.5 | T | 90 a* | 40.0 c | 1.0 d |
| | | T + S | 90 a | 10.0 f | 1.0 d |
| | 1.0 | T | 85 b | 35.0 e | 1.8 c |
| | | T + S | 82.5 c | 40.0 c | 2.0 b |
| Topsin M-70% | 0.5 | T | 75 e | 46.0 b | 2.0 b |
| | | T + S | 80 d | 30.0 d | 1.0 d |
| Control | | 0 | 65 f | 70.0 a | 3.7 a |

**T**= transplanting's soil drench 2 days before cultivation

**S** = soil amendement 30 days after cultivation in greenhouse

The same letter in each colum are not significantly differences at $P \leq 0.05$.*

**Table 4.** Effect of nanoemulsion of chitosan / hydroquinone on morphological characters of cucumber plant grown under natural infestation by root rot causal pathogen in plastic houses

| Soil drench | | | Morphological characters of cucumber plant | | | | |
|---|---|---|---|---|---|---|---|
| Materials | Con | Method | length | bud/ | fresh weight | fruit/plant | |
| | % | | shoot (cm) | plant | shoot (g) | No. | Kg |
| Nanoemulsion (chitosan/hydroquinone) | 0.5 | T | 200.0 c* | 7 | 249.3 c | 47 c | 3.9 c |
| | | T + S | 230.0 a | 10 | 453.0 a | 110 a | 9.2 a |
| | 1.0 | T | 190.0 d | 2 | 177.8 d | 43 d | 3.6 c |
| | | T + S | 220.0 b | 6 | 266.4 b | 60 b | 5.0 b |
| Topsin M-70% | 0.5 | T | 120.0 e | 0 | 51.6 f | 15 f | 1.3 e |
| | | T + S | 120.0 e | 0 | 74.8 e | 20 e | 1.6 d |
| Control | | ------ | 110.0 f | 0 | 40.0 g | 14 f | 1.1 f |

**T**= transplanting's soil drench 2 days before cultivation

**S** = soil drench 30 days after cultivation in greenhouse

* The same letter in each colum are not significantly differences at $P \leq 0.05$.

**Figure 4** Application of nanoemulsion of chitosan /hydroquinone ( left) and control ( right) on root rot disease incidence and growth of cucumber plants

*3.4.* SEM observation chitosan/ hydroquinone nanoemulsion on morphology of mycelial growth of pathogenic fungi

The effect chitosan/ hydroquinone nanoemulsion (4000 ppm) on the morphological characteristics of the tested fungi i.e., *Fusarium oxysporum* and *Fusarium solani* were observed using Scanning electron microscopy (SEM). The mycelial growth of the fungal isolates was examined 5 days after treatment as shown in Fig (5); Malformation, reduced density of mycelial growth, branches of mycelial and conidia sporulation as well the absence of the conidiophores and swelling of mycelial cells were observed compared to the control ( fungus free treatment). Also in Fig (5) absence the conidia and chlamydospores which clearly observation with *Fusarium solani* free treatment.

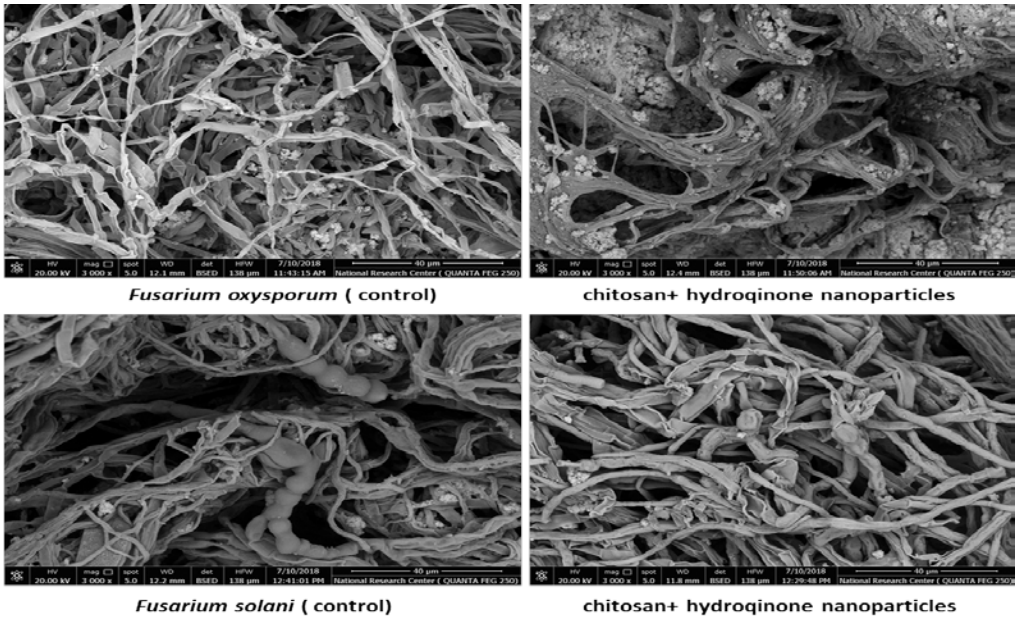



**Figure 5** . SEM observation of nanoemulsion of chitosan/hydroquinone on fungal morphology

## 4. Discussion

Cucumber plants (*Cucumis sativus* L). plants are attaked by several soil and seed borne pathogenic fungi of *Fusarium* genera causing high losses of total plants, growth and productivity. (Farrag *et al.,* 2013 and Elwakil *et al.,* 2015; Ziedan and Saad 2026; Ziedan 2024 a).

To avoid human health problems when eating cucumber fruits, highly dangerous chemical pesticides are used in addition to the contamination of environmental elements with residues of these pesticides, which persist for long periods without decomposition (Ziedan 2024 b). The trend to use the safety organic natural materials and chemicals is quick to decompose and is efficient in resisting the form of nanoparticles in small quantities and has become important in order to produce healthy food free of pollutants (Mossa *et al.,* 2021; Ziedan *et al.,* 2022).

Chitosan nanoemulsion particles prepared by simple ionic cross-linking at room temperature and loaded with each one of hydroquinone, sorbic and propionic acids were spherical shape with droplets size distribution of stable nanoparticles were, 214.5, 228.8, 224.2 and 276.4 nm respectively; the same findings were reported by other studies which reported that droplets of nanoemulsion ranging between 20-200 nm (Diaz *et al.,* 2005; Wang *et al.,* 2009; Rampino *et al.* , 2013; Gokce *et al.* , 2014). Moreover, also the droplet sizes obtained were 82.6, 95.9, 131.9, and 117.4 nm, respectively of nanoemulsions of clove, black seed, lemon and orange essential oils (Mossa *et al.,* 2021)

*In vitro*, several nanoemulsions of chitosan as individual or it loaded with each one of hydroquinone, sorbic acid and propionic acid were evaluated on the common criteria of pathogenic fungal isolates, i.e. mycelial linear growth, condia sporulation production, and their pathological ability on germinated seeds and growing seedlings of cucumber. All nanoemulsions prepared in this study were significantly antifungal effect against *Fusarium* isolates causing root rot disease on cucumber in greenhouse, chitosan / hydroquinone at 2000 ppm was the most effective against mycelial linear growth of *F. oxysporum* by (60% followed by nanoemulsion of chitosan significantly reduced mycelial linear growth of *Fusarium solani* by (41%). In addition, nanoemulsions of chitosan was the best treatment reduced conidia count production of *Fusarium solani* by (81.3%), while nanoemulsions of chitosan/hydroquinone was the mot treatments reduced conidia count of *F. oxysporum* (47.4%). In addition, nanoemulsion of chitosan/hydroquinone highly reduced seed rot and seedling death of cucumber caused by *Fusarium solani* followed by nanoemulsion of chitosan/ propionic acid, nanoemulsion of chitosan respectively. Meanwhile, nanoemulsion of chitosan/sorbic acid had the lowest effect. These results are agreements with data reported that, chitosan nanoemulsion at the rate 1.0% with droplet size (200 nm), was suppress mycelial growth of *Colletotrichum musae* the causal of anthracnose disease on banana fruits and *Colletotrichum gloeosporioides* with droplet size (600 nm), the causal of anthracnose on papaya and dragon fruits (Zahid *et al.,* 2012).

Application of nanoemulsion of chitosan/hydroquinone by the rate (0.5%) as soil drench in plastic houses on the soil cucumber plant 2 days before cultivation followed by soil drench at 30 days after cultivation in greenhouses was highly reduced root rot incidence at 10% on cucumber plants and disease severity in addition, it highly significances increased plant shoot length, fresh weight of shoot and cucumber yield fruits compare the application with higher dose at (1.0%) and the systemic fungicide used Topsin - M70. These results are in agreements with results reported that, application of chitosan nanoemulsions *in vivo*, which significantly reduced incidence of anthracnose on fresh fruits of banana, papaya and maintaining their quality for 28 days in cold conditions. chitosan nanoemulsions could be used as a biofungicide for controlling anthracnose of fresh fruits in storage (Zahid *et al.,* 2012). Furthermore, composite of silver/chitosan nanoparticles was effective than single nanoparticles of silver or chitosan on various against fungi of seed borne plant pathogens on chickpea plants, i.e. *Aspergillus flavus, Alternaria alterneta, Rhizoctonia solani,* (Kaur *et al.,* 2012). Application of nanocomposite of clay/chitosan was completely application nanocomposite of clay/chitosan at 20 µg mL -1 was completely of was complete (100%) suppress green mold on orange cv. caused by fungal of *P. Penicillium digitatum*, illumination mycelial growth and sporulation of *Penicillium digitatum*, the pathogen, induce systemic resistant in orange fruits tissue and caused several malformations on the shape of mycelial hyphae (Youssef and Hashim 2020). In this respect, chitosan /hydroquinone as the main components of nanoemulsion formulation directly reduced fungal growth, sporulation and their germination, changed morphology of mycelial hyphae, reduced conidia spores and the propagules count of plant pathogens, and enhanced induce systemic resistance in plant against invasion of pathogens , by increasing defense resistance enzymes, of chitinase, β-1, 3-glucanase, peroxidase and polyphenol oxidase (Elwakil, 2003; Hanafi 2004; Choudhary *et al.,* 2017; Ghule *et al.,* 2021; Zian *et al.,* 2023).

Application nanoemulsion of chitosan / hydroquinone at (0.5%) as soil drench of transplanting's in combination with soil drench of plants in greenhouse significantly increased plant height, fresh weight of cucumber plant shoot, and fruit yield. In this manner, silver nanoparticles treatment during seed germination of spinach had positive effect on morphological and physiological parameters, water content, stomatal conductance, chlorophyll content, dry weight and leaf area of spinach plant grown free salt stress after seed germination and negatively effects under saline conditions (Bsoul *et al.,* 2023). In addition, chitosan nanoparticles was controlling wilt disease on tomato and enhanced antifungal of biocontrol agents (El-Morsy *et al.,* 2023). Furthermore, application of nano-sized chitosan loaded 1000 mg L−1 with nano K as spray and soil treatment of strawberries plants in sandy soil enhanced plant growth, increased fruit yield and maintained high of the marketable characters of strawberry fruits as well as increased the total soluble solids, vitamin C, acidity, sugar and anthocyanin (Abd-Elrahman *et al.,* 2023), chitosan nanoparticles was controlled wilt disease on tomato and enhanced antifungal of biocontrol agents (El-Morsy *et al.,* 2023).

## 5. Conclusions

*In vitro*, nanoemulsion of chitosan/hydroquinone was significantly reduced mycelial linear growth, condial spores production, pathogenic activity of the fungi of *Fusarium* spp. the causal of root rot disease of cucumber, Its application *in vivo* at (0.5%) was highly suppressive root rot incidence, disease severity and significantly enhancing the morphological characters of plant growth and fruit yield on cucumber plants in high quality. So, nanoemulsion of chitosan/hydroquinone as a promising alternative fungicides could be used as a biofungicide as eco-friendly agent, with cheap cost to control plant diseases and enhance quality and quantity.

## Acknowledgment

The authors extend their appreciation to the National Research Centre, Egypt, for funding this work through research project No. (P11030135) during the period 2016–2019.

## Author's contributions

EZ designed the research, conducted experiments, analyzed the data and submitted the manuscript for publication. AM prepared and characterized the nano formulation and contributed to writing and editing the manuscript.

## References

Abd-Elrahman, S.H., Y.A. El-Gabry, F.A. Hashem, AF Ibrahim E.I. El-Hallous, Z.K. Abbas, D.E. Darwish, N.A. Al-Harbi, S.M. Al-Qahtani, N.M.Taha. 2023. Influence of nano-chitosan loaded with potassium on potassium fractionation in sandy soil and strawberry productivity and quality. *Agron* **13**: 1126.

Attallah, A.G., E.H. Ziedan, A.F. Sahab. 2019. Molecular characterization and pathogenicity of *Fusarium* species causing root rot disease of cucumber in greenhouse in Egypt. *Plant Arch* **19** : 1301-1307.

Bsoul, E.Y., S.A. Othman, A.A. Al-Ghzawi, M.I. Massadeh. 2023. Effect of silver nanoparticles on growth and physiological responses of spinach *(Spinacia oleracea* L.) under salt stress. *Jordan J Biol Sci* **16 ( 1) :** 1 – 6.

Calvo, P., C. Remunan-Lopez,, J.L. Vila-Jato, M.J. Alonso. .1997. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *Journal Appl Polym Sci* **63 (1)** :125-132.

Carver, C.E., D. Pitt, D.J. Rhodes. 1996. Aetiology and biological control of Fusarium wilt of Pinks (*Dianthus caryophyllus*) using *Trichoderma aureoviride*. *Plant Pathol* **45**: 618-630.

Choudhary, R.C., R.V. Kumaraswamy, S. Kumari, S.S. Sharma A. Pal, R. Raliya, P. Biswas, V. Saharan. 2017. Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). *Scientific Rep* 7: 9754

Diaz, S., S. Espinosa, E.A. Brignole. 2005. Citrus peel oil deterpenation with supercritical fluids optimal process and solvent cycle design. *The J Super Flui* **35** :49–61

Ding, R.L., F. Xie, Y. Hu, S.Z. Fu, J.B. Wu, J. Fan, W.F. He, Y. He, L.L.Yang, S. Lin, Q.L.Wen. 2017. Preparation of endostatin-loaded chitosan nanoparticles and evaluation of the antitumor effect of such nanoparticles on the Lewis lung cancer model. *Drug del* **24** :300-308.

Downes, F., K. Ito. 2001. **Compendium of Methods for The Microbiological Examination of Foods** Washington, D.C.. American Public Health Association (APHA).

Duarte, Y., O. Pino, B. Martínez. 2014. Effect of four essential oils on phytopathogenic fungi associated with spotted rains in rice. *Rev Prot Veg* **29 (1):**62–65.

El Zahaby, H.M., H.F. Maswada, E.H. Ziedan, H.A. Zoeir. 2018. Safe integrated control of postharvest rot diseases on banana fruit. *Plant Arch* **18 ( 2)**: 1345-1351

El-Morsy, E.S.M., Y.S. Elmalahy, M.M.A. Mousa. 2023. Biocontrol of *Fusarium equiseti* using chitosan nanoparticles combined with *Trichoderma longibrachiatum* and *Penicillium polonicum*. *Fungal Biol. Biot* **10**: 5.

Elwakil, M.A. 2003. Use of antioxidant hydroquinone in the control of seed-borne fungi of peanut with special reference to the production of good quality seed. *Pakistan J Plant Pathol* **2 (2)**: 75-79.

Elwakil, M.A., M.A. El-Metwally, A.Elsherbiny, K.N.M. Eisa 2015. Enhancing systemic acquired resistance in cucumber to control root rot and wilt diseases with reference to yield and quality. *Plant Pathol J* **14 (4)**: 223-233.

Essa, TA, A.H.M. El-Gamal, M.M.I. Afifi. 2017. Control of cucumber downy mildew by some plant growth promoting Rhizobacteria under greenhouse conditions. Middle East J Agric Res **6**: 395–408.

Farrag, E.S.H., M.H.A. Moharam, E.H.Ziedan. 2013. Effect of plant extracts on morphological and pathological potential of seed-borne fungi on cucumber seeds. *Inter J Agric Tech* **9 (1)** : 141- 149.

FOASTAT. 2021. **Food and Agriculture Organization of the United Nations**. Available online: https://www.fao.org/faostat/en/#search/Cucumbers %20 and %20 gherkins (accessed on 14 November 2021).

FAOSTAT. 2023. **Food and Agriculture Organization of the United Nations**, Rome, Italy. Retrieved January, 2023 on the world Wide Web: http://www. fao.org/faostat/en/#data/QC

Ghule, M.R., P.K. Ramteke, S.D. Ramteke, et al .2021. Impact of chitosan seed treatment of fenugreek for management of root rot disease caused by *Fusarium solani* under *in vitro* and *in vivo* conditions. *Biotech*. **11:** 290.

Gokce, Y., B. Cengiz, N. Yildiz, A. Calimli, Z. Aktas. 2014. Ultrasonication of chitosan nanoparticle suspension: Influence on particle size. Colloids and Surfaces A: *Physicochemical and Eng Aspects* **462**:75-81.

Hanafi,. AA. 2004. Effect of some antifungal compounds and Rizolex-Ton damping-off, root and pod rots diseases and pod yield of peanut. *Egypt J Appl Sci* **19** :52-61.

Hemeda, A.H. 2006. Induced resistance in chickpea against some seed-borne pathogenic fungi using the antioxidant, hydroquinone. *Egypt J Exp Biol (Bot)* **2**: 27 – 36 .

Hossain, M.S., A. Iqbal. 2016. Effect of shrimp chitosan coating on postharvest quality of banana (*Musa sapientum* L.) fruits. *Int. Food Res J* **23 (1)**: 277-283.

Kaur, P., R.Thakur, A.Choudhary. 2012. An in vitro study of the antifungal activity of silver/chitosan nanoformulations against important seed borne pathogens. *Inter J Sci & Tech Res* **1 ( 6) :** 83-86.

Khan, M.M.,M.R. Khan,F.A. Mohiddin. 2012. The relative performance of different inoculation methods with *Alternaria brassicae* and *A. brassicicola* on Indian mustard. *Plant Pathol J* **11**: 93-98.

Mossa, A.A., S.M.M. Mohafrash, E.H.E. Ziedan, I.S. Abdelsalam , A.F. Sahab. 2021. Development of eco- friendly nanoemulsions of some natural oils and evaluating of its efficiency against postharvest fruit rot fungi of cucumber. *Indusl Crops Prod* **159:**113049–113051

Pal, A., R. Adhikary, T. Shankar, A.K. Sahu, S. Maitra 2020. Cultivation of Cucumber in Greenhouse. In book: **Protected Cultivation and Smart Agriculture,** Smart Agriculture; Sagar Maitra, Djgats (ed), New Delhi Publishers: New Delhi, India, pp. 139–145. doi:10.30954/NDP-PCSA.2020.14.

Rampino, A., M. Borgogna, P. Blasi, B. Bellich, A. Cesàro A. 2013. Chitosan nanoparticles: preparation, size evolution and stability. *Inter J Pharma* **455 (1-2):** 219-228.

Sambou, A., N.W.Samb, N. Ayessou. 2023. Nutritional values of green and white cucumber (*Cucumis sativus* L.) and African horned cucumber (*Cucumis metuliferus* E.) J Hort and Post Res **6 (3):** 221-234

Snedecor, G.W., W.G. Cochran 1980. **Statistical Methods.** 7th Edn. Iowa State Univ. Press, Ames

Waly, A.I., A.F. Sahab, M.M. Marie, W.M. Elzoeerey, R. Abo Elela. 2015. Preparation of quaternized chitosan nanoparticle and its antimicrobial activity on polyester, cotton and polyester/cotton blend treated fabrics . *American-EurJ. Sust Agric* **9 (5) :** 52-59.

Wang, L., J. Dong, J. Chen, J. Eastoe, X. Li . 2009. Design and optimization of a new self-nanoemulsifying drug delivery system. *J Coll Interf Sci* **330 (2**) :443–448.

Youssef, K., A.F. Hashim .2020. Inhibitory Effect of Clay/Chitosan Nanocomposite against *Penicillium digitatum* on Citrus and Its Possible Mode of Action. *Jordan J Biol Sci* **13 ( 3**): 349 – 355.

Zahid, N., A. Ali, S. Manickam, Y. Siddiqui, M. Maqbool. 2012. Potential of chitosan-loaded nanoemulsions to control different *Colletotrichum* spp. and maintain quality of tropical fruits during cold storage. *J Appl Micr* **13**: 925–939, doi:10.1111/j.1365-2672.2012.05398.x

Zian, A.H., S.A. El-Blasy, H.M. El-Gendy, S.A. El-Sayed. 2023. Plant defense activation and down-regulation root rot and wilt in chickpea diseases by some abiotic substances. *In E3S Web of Conferences* 462, p. 02014). EDP Sciences.

Ziedan, E.H. 2024a. Distribution of epidemic diseases of cucumber under protective plastic houses in Egypt. *Analele Univ Orad Fasci Biol* **1:** 15-23

Ziedan, E.H., M.S. Saad .2016. Efficacy of nanoparticles on seed borne fungi and their pathological potential of cucumber . *Inter J Pharma Tech Res* **9 (10)** :16-24.

Ziedan, E.H.E. 2024 b. Application of essential oils and their volatile components as a green bio-fumigations pesticides eco-friendly on controlling various plant diseases. A review. *AFRIMED AJ –Al Awamia* **142:** 33-72.

Ziedan, E.H.E., M.M. Saad, A.A. El-Kafrawy, A.F. Sahab, A.T. Mossa. 2022. Evaluation of essential oils nanoemulsions formulations on *Botrytis cinerea* growth, pathology and gray mould incidence on cucumber fruits. *Bull Nati Rese Cent* 46:88.

Zoeir, H.A., H.M. El Zahaby, E.H. Ziedan, H.F. Maswada. 2017. Efficacy of antifungal activity of essential oils, salts and antioxidants acids on pathogenic fungi and their application methods for controlling postharvest diseases on banana fruits. *African J Micr Res* **11 (29)** : 1162-1170.