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Research Article

The Efficacy Test of Nano Chitosan and Phylex in Resistance Early Blight Disease in Tomato Caused by Alternaria Solani Fungus

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ABSTRACT
This study was conducted at the University of Baghdad / College of Agricultural Engineering / Department of Plant Protection / Mycotoxins Laboratory for the academic year 2019-2020.The study results of Chitosan and Nano Chitosan against the pathogenic fungus showed that all the concentrations led to an increase in the inhibition percentage of fungus compared to the comparison treatment, as the Nano Chitosan achieved high efficiency in inhibiting the pathogenic fungus. Also, it was given the highest inhibition percentage at a concentration of 6 mg/ml reached 93.88. Whereas the Chitosan gave an inhibition percentage at a concentration of 6 mg reached 58.33% 0. The results observed that the inhibition percentage increases with increasing concentration. Furthermore, The results also showed that the concentrations of Phylex 0.1, 0.2, 0.3 ml / 100 ml achieved an inhibition percentage of 82.96 and 100 and 100 respectively, compared with the comparison treatment, in inhibiting the growth of A. solani on a PDA (potato dextrose agar) culture medium. The results in the pots experiment showed a significant decrease in all treatments in the infection percentage and severity for the pathogenic fungus in the tomato plant. It protected the tomato plant and showed the lack of A. solani fungus effect, where the Nano Chitosan treatment was superior in reducing the infection percentage and severity, as the percentage reached 16.66 % and 12.497 %, respectively. However, the results of Chitosan showed an infection percentage and severity reached 33.33% and 16.887 respectively. As well as the Phylex treatment, achieved 16.66% and 10.413% respectively. The statistical results showed that there were significant differences in the biochemical indicators to stimulate the systemic resistance of tomato plants, as it was observed that the treatments used had a positive effect on the peroxidase enzyme (change in absorption/minute/gm fresh weight). The effectiveness of the biochemical indicators of the Nano Chitosan , Chitosan and phylex treatments reached the highest rate after 14 days from adding of the concentration, The results of the chlorophyll test showed the superiority of all treatments in increasing the chlorophyll content over the comparison treatment, The results showed that all of the treatments achieved a significant increase in the studied growth parameters represented by the length of root and shoot, wet and dry weight of root and shoot. Besides.

Keywords: Alternaria solani, Tomato, Inhibition percentage, Resistance, Chitosan, Phylex.

INTRODUCTION

Early blight disease (EB) is one of the most important diseases that affect tomatoes in many countries of the world such as the United States of America, Australia, the United Kingdom, India, and Iraq. Besides, it is one of the most important fungal diseases that affect the tomato crop and the most widespread in the world, as an economic disease that affects directly on the National Economy (Gomes et al., 2010). It affects that grown in protected and open fields, where the protected fields are more exposed to this type of disease due to the high humidity present in the greenhouses, where a significant decrease for crop produced was observed by 79% (Kumar et al., 2019). However, very difficult to isolate diseased plants from healthy ones during the active phase of the disease cycle. (Roy et al., 2019) observed that in severe cases, the disease leads to complete leaf defoliation and is more harmful to tomatoes in areas with heavy dew, rain, high humidity, and high temperatures. A wide range of temperatures 8-38 ° C characterizes the disease, where the symptoms of this disease can appear in all the plant parts. Furthermore, the tomato crop is susceptible to bacterial, viral, nematode, and fungal diseases, among the fungal diseases, is early blight, where the failure to control this disease can cause decreased production (Datar, 1981; Mayee, and Malik et al., 2014). Although there are many methods of biological control, they are very expensive and its method of work is often slow, and as a result, there is a need to use modern techniques that allow efficient control of the pathogens without environmental risk (Quiterio et al., 2019). Nano Chitosan, a biopolymer from the residue of Glucosamine and

N-acetyl, is an acetylated free product of chitin. It is available in abundance at an economical cost from the exoskeleton remains of lobster and the shrimp wall (Hadrami et al., 2010). Chitosan is a biological material that is well recognized in agriculture and is applied successfully in many crops (Zeng et al., 2012), and is known to enhance the defense response in plants and also possess antimicrobial properties (Thuesombat et al., 2014) as nanotechnology advances. Chitosan-based nanomaterials are largely adapted for exploration in plants along with other nanomaterials and increasing the number of the scientific paper of Chitosan nanoparticles in plant growth and protection adds the tremendous potential of Nano Chitosan over Chitosan because Nano Chitosan compared to Chitosan has superior Physico-chemical properties provides improved biological activities (Van et al., 2013). Hence, there is a need to explore the biopolymer of Chitosan not only for its new antimicrobial and plant defense property, but also for its role in plant growth and pest control (Saharan et al., 2014). Chitosan can be used to control these pathogens by inhibiting growth at various points in their life cycle, and Chitosan can inhibit the development of Physalospora piricola and Alternaria kikuchiana in pear fruits (Meng et al. 2010). Phylex is a product of transparent brown color with a density of 1.07, the stress of C35, a boiling degree of C104, a viscosity of 13.1, and a pH of 13.1 because it contains several acids: propionic acid, lactic acid, orthophosphoric acid, formic acid, formic acid, and sorbic acid and citric acid (Gosh and Haggblom, 1985). It is a product that has a high solubility in water and is not flammable as well as non-toxic, as Phylex has proven that it has high efficiency in inhibiting fungi as well as destroying the toxins produced by them, where some experiments have proven how to inhibit different types of fungi (Al-Qaisi 2010). Due to the severity of the disease and the lack of studies on its resistance by biological methods, this study aimed at using environmentally friendly materials (Chitosan and Nano Chitosan) and using Phylex as a chemical material that has proven successful in resisting many fungi.

MATERIALS AND METHODS OF WORK

- **Alternaria solani isolate**

A highly pathogenic isolate, diagnosed, and deposited in the NCBI Gen-Bank was obtained with an E code under the global accession number MT199154.1 (Al-Tamimi, 2020).

- **Preparation of the alternaria solani inoculum**

The fungal inoculum was prepared using Petri dishes with a diameter of 9 cm containing a PDA medium by culturing isolate E (Al-Tamimi, 2020). The dishes were incubated in the incubator at a temperature of 2+27 °C for 10 days, then 10 ml of sterilized and distilled water was added into the fungus colony, and the fungus colony was stirred using a sterile needle. The spore's suspension was collected, after that, sterile and distilled water was added to it, and the concentration was set at 2x 10^5 spore/ml.

- **The efficiency test of Chitosan and Nano Chitosan test in inhibiting the growth of Alternaria solani in vitro**

Chitosan and Nano Chitosan was obtained from the office of Dr. Zuhair Shafeeq Al-Taie / Al-Bashir Office / Baghdad and then tested at the College of Science / University of Baghdad / Department of Chemistry / Dr. Abdul-Karim Ali Al-Samarrai. Moreover, its size was measured and the accurate access to the nanoscale was achieved by atomic force microscopy SPM AA 3000 Angstrom Advanced INC., USA AFM. The Nano Chitosan has reached the nanoscale nm (69.55) and according to the examination forms and according to the specifications of the classified nanomaterial. Four concentrations (0.75, 1.5, 3, 6) g / ml of Chitosan and Nano Chitosan were selected both separately (Abboud et al., 2002) with some modification to prepare a solution with higher concentration. Accordingly, 1 g of Chitosan and Nano Chitosan were added separately in a plastic flask and adding 0.2 ml of glacial acetic acid to chitosan, and chitosan is stirred using a needle for dissolving it with the acid. Similarly, 40 ml of distilled water was added, and Chitosan is mixed with water by the magnetic stirrer for 5 minutes, and then the solution is left to the next day at room temperature. In addition, 1 N sodium hydroxide was added to the solution on the next day to reach the required concentration (6 Ph), where these concentrations were added to five 100 ml flasks containing 50 ml of water, and 2 g of the culture medium PDA was added. Then, they placed in the autoclave at 121 °C for 30 minutes, and after it was removed from the autoclave, the temperature reached approximately 40 °C, 25 mg of Amoxicillin was placed and then the medium was poured into sterile Petri dishes 9 cm in diameter each concentration of three dishes as well as three comparison dishes. All dishes are inoculated with the isolate of A. solani fungi of a 5 mm diameter disc taken from the edge of the growing colony on a culture medium. Then it was incubated at 2±27 °C temperature, and after the growth in comparison reached the inner edge of the dish, the growth rate was calculated in each treatment from calculating the growth rate in two perpendicular diameters for three replicates and the inhibition percentage of fungal growth according to the following equation: (Kim et al., 2012):

$$\text{Inhibition percentage} = \frac{Av.diamiter\ of\ comparision\ colony\ \ - Av\ diamiter\ of\ treatment\ colony}{Av.diamiter\ of\ comparision\ colony} \times 100$$

- **The efficiency test of Phylex in inhibiting the growth of Alternaria solani in vitro**

Three concentrations were taken from Phylex (0.1, 0.2, 0.3) ml per 100 ml of the PDA culture medium, as the Phylex was added to the culture medium after it was sterilized in the autoclave at a temperature of 121 °C for an hour. After the medium reached 40 °C, Phylex was added according to the concentrations and the PDA culture medium was poured into the Petri dishes, where three replicates of each concentration were made. As well as, the dishes were contaminated with A.solani fungus where a smear of fungus was taken and placed in the center of each dish and were incubated at a temperature of 2 ± 27 °C for 7 days, the dishes were read and the results recorded.

- **The efficiency test of Chitosan, Nano Chitosan, and Phylex in resisting early blight disease caused by Alternaria solani in pots plants under greenhouse conditions.**

This experiment was carried out under the shade of the Plant Diseases Laboratory of the Plant Protection Department - College of Agricultural Engineering Sciences - University of Baghdad, on 1/3/2020. A 2 kg pots with a diameter of 15 cm, where loamy soil was mixed with peat moss at a ratio of 1: 2, a mixture of soil and peat moss was sterilized with an autoclave under a temperature of 121 °C and a pressure of 1.5 kg/inch for 30 minutes. Then, the sterilization process was repeated the next day, and the mixture of soil and peat moss was distributed in the pots. The San variety of Tomato plants obtained from a nursery in Dujail district was used at the age of 30 days and planted four seedlings/pots with three replicates per treatment. The treatments included adding Chitosan and Nano Chitosan at a concentration of 6 mg/ml to the soil during cultivation, as well as adding Phylex extract at a concentration of 0.2 ml per 100 ml. The soil was contaminated with a fungal suspension that was prepared in the previous paragraph after two days of planting. As well as, the contaminating only three pots with fungus, while three pots were left without contamination for comparison, where the pots were transferred to the shade and distributed randomly according to a completely randomized design CRD. Finally, the pots were placed in polyethylene bags to provide adequate relative moisture for 5 days of contaminating the pots, and after 40 days of adding the fungal inoculum, the results were obtained, and the experiment included the following treatments:

Treatment of Nano- Chitosan N.CH at a concentration of 6mg / ml + A
Treatment of Chitosan CH at a concentration of 6mg / ml + A
Treatment of Phylex F at a concentration of 0.2 ml / 100 ml + A
Treatment of pathogenic fungus A
Treatment of N.CH at a concentration of 6 mg/ml
Treatment of CH at a concentration of 6 mg/ml
Treatment of F at a concentration of 0.2 ml / 100 ml
Where:
A = A.solani
N-ch = Nano chitosan, ch = chitosan, F = Phylex

The percentage of infection, wet and dry weight, length of shoot and root, the infection severity were calculated:

$$\text{Percentage of infection} = \frac{\text{infected plants number}}{\text{total number of infected plants}} \times 100$$

The infection severity was estimated on the shoot, and the pathological index described by (Souza et al. 2010) was used, with some modifications

Degree	The appearance of infection
0	There is no infection on the roots or the vegetative parts
1	1-25% of leaf area affected by the disease
2	26-50% of leaf area affected by the disease
3	51-75% of leaf area affected by the disease
4	76-100% of leaf area affected by the disease with the death of the plant

The percentage of infection severity was calculated using Mckinney's formula (1923)

$$\%\ \text{of infection severity} = \frac{(No.of\ plants\ in\ degree\ 0 \times 0) + \cdots + (No.of\ plants\ in\ degree\ 4 \times 4)}{(total\ number\ of\ tested\ plants \times degree\ 4)} \times 100$$

- **The effect of (Peroxidase PO) enzyme in tomato plants**

The activity of the Peroxidase enzyme in a plant was estimated according to (Song et al., 2011), and the solution absorption of light was recorded directly by a spectrophotometer every 30 seconds and the readings were recorded, and the amount of change in absorption was recorded according to the following equation: (Müftügil, 1985)

$$\text{The enzymatic activity} = \text{the device reading} / \frac{\text{Model Weight}}{\text{Extraction Volume}} * \text{The volume has taken for reading}$$

- **Measuring the chlorophyll content in tomato plants**

The total chlorophyll percentage of tomato leaves were estimated by the method of extraction by making three replicates of each treatment, where 1 g of wet leaves was taken randomly from each replicate of the treatments. Besides, the chlorophyll was extracted from the selected samples, as acetone was added to the sample at 80%, and the leaf tissue was crushed using a ceramic mill, and the solution was then filtered. Finally, the optical density was read at a wavelength of 660, 642 nm for total chlorophyll (Ranganna, 1977) with a Spectrophotometer, the total chlorophyll was calculated according to the following equation:

$$T.chl = \frac{[(7.12 \times 660) + (16.8 \times 642)] \times v}{w \times 1000}$$

V = volume of extraction solution (ml)
W = weight of sample (g)

RESULTS AND DISCUSSION

- The effect of different concentrations test of Chitosan and Nano Chitosan in inhibiting A.solani on the PDA culture medium.

The results of Table (1-2) showed the efficiency of Chitosan and Nano Chitosan in inhibiting the isolate of A.solani, which was the most pathogenic on the culture medium (PDA). It showed a high inhibitory efficiency, as the inhibition percentage reached (53.88, 73.14, 87.59, and 93.88%) respectively, for the concentrations (0.75, 1.5, 3, 6 mg/ml) used for Nano Chitosan. Also, the concentrations showed significant differences for the inhibition percentage of the A.solani growth on PDA compared with the comparison treatment of 00%, where the highest inhibition percentage at a concentration of 6 mg/ml were 93.88%, while the inhibition percentage at a concentration of 0.75 mg/ml was 53.88%. Moreover, the Chitosan treatments showed a significant difference in the inhibition percentage reached 20.74, 26.29, 43.33, 58.9%, respectively, for the same concentrations, where the highest inhibition percentage at a concentration of 6 mg/ml amounted to 58.9, while the inhibition percentage at a concentration of 0.75 mg/ml was 20.74%. The results concluded that there was a direct correlation between the fungus inhibition percentage and the tested concentrations, and it was observed from this experiment that Nano Chitosan gave higher differences in the inhibition percentage from Chitosan in all concentrations as shown in Figure (1-2). This action may be due to that the nanoparticles of Nano Chitosan are effective in the inhibiting of fungus due to its high surface area and small size that can easily penetrate the cell wall. Thus, it leads to the deformation and decomposition of the fungal hypha, which is followed by the flowing cytoplasm out of the cell and the fungus death (Pal and Saharan, 2016).

Table 1: The efficiency test of different concentrations of Nano Chitosan in inhibiting A.solani in vitro

Seq.	Concentration	Colony diameter	Inhibition percentage %
1	0.0	9.0000	00
2	0.75	4.1500	53.88
3	1.5	2.4167	73.14
4	3	1.1167	87.59
5	6	0.5500	93.88
	LSD	1.1696	

*Each number represents an average of three replicates

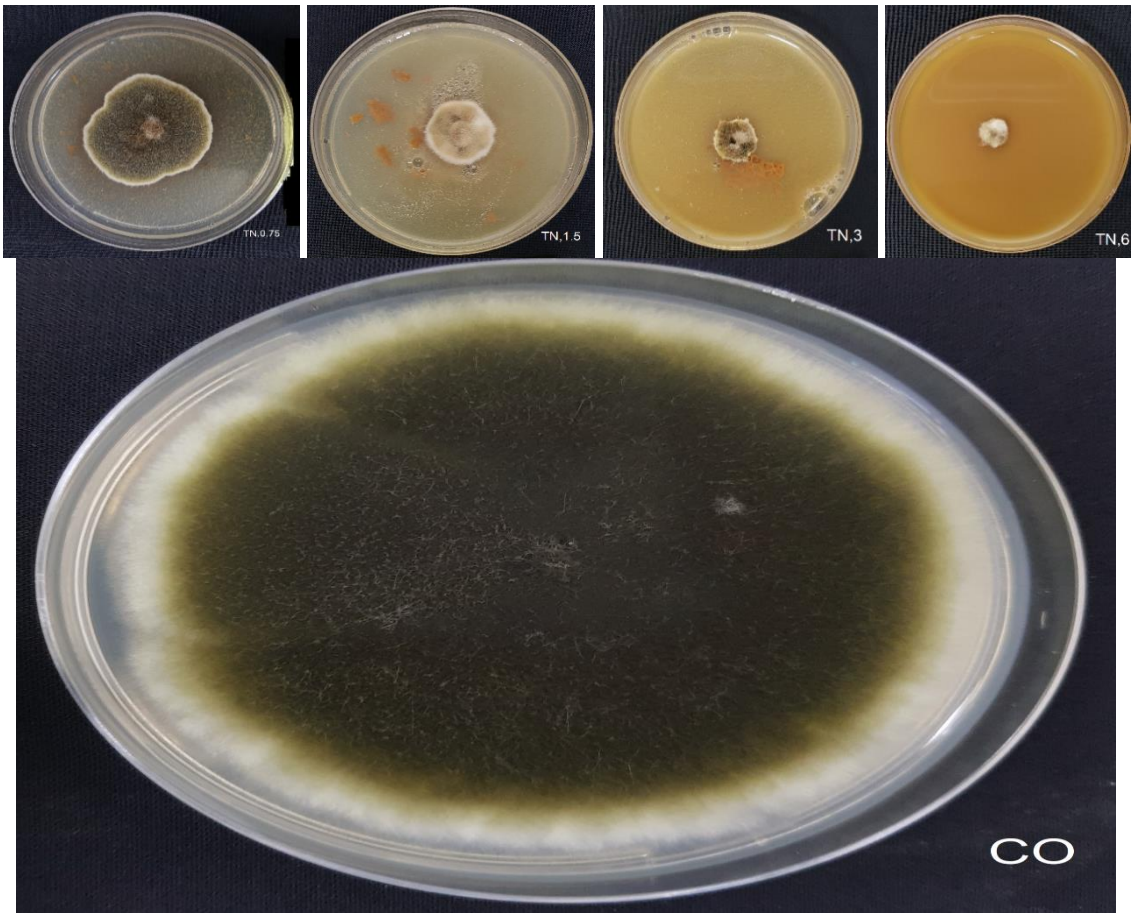
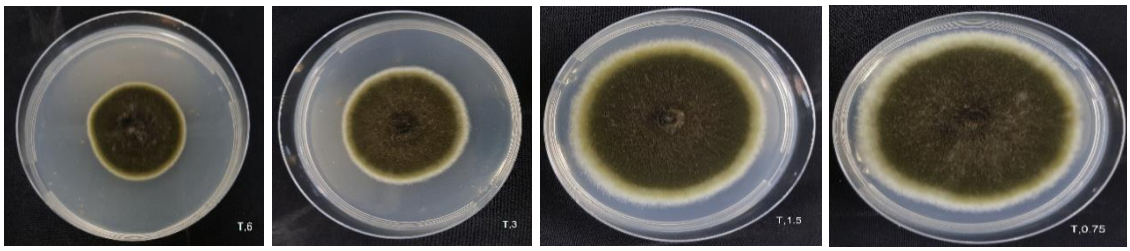


Fig.1: Inhibition ability of nano chitosan against the pathogenic fungus after 7 days for four concentrations of 0.75, 1.5, 3, and 6 mg / ml and comparison 0

Table 2: The efficiency test of different concentrations of Chitosan in inhibiting A.solani in vitro

Seq.	Concentration	Average of two diameter	Inhibition percentage %
1	0.0	9.0000	00
2	0.75	7.1333	20.74
3	1.5	6.6333	26.29
4	3	5.1000	43.33
5	6	3.7500	58.33
	LSD	0.6043	

*Each number represents an average of three replicates



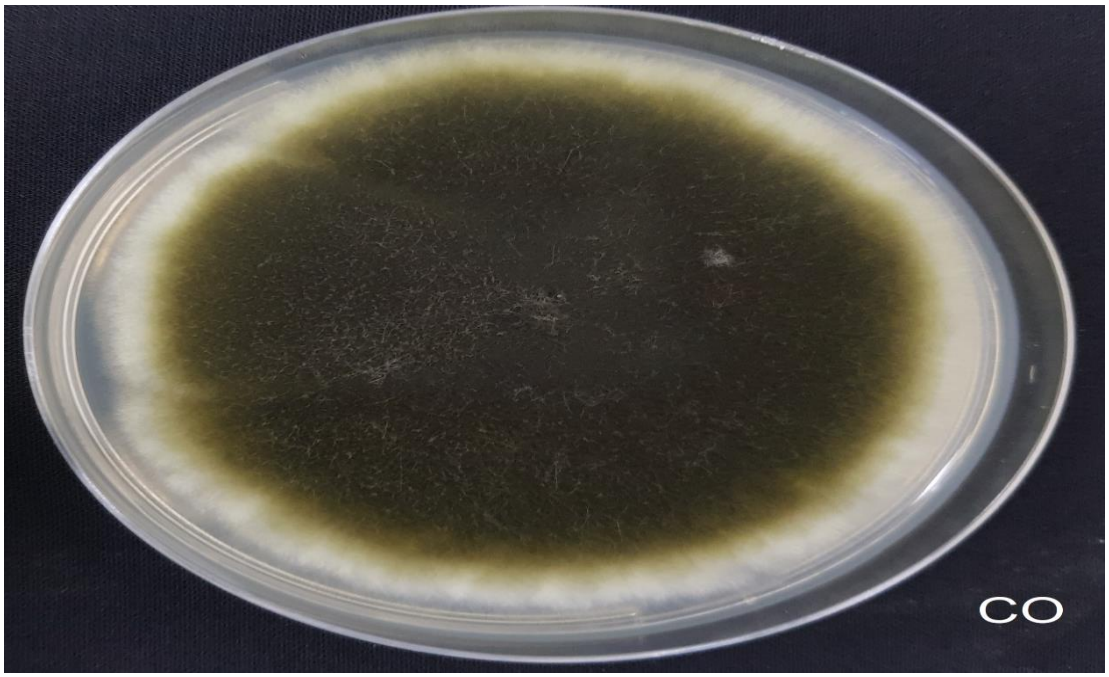


Fig.2: Inhibition ability of Chitosan against the pathogenic fungus after 7 days for four concentrations of 0.75, 1.5, 3, and 6 mg/ml and comparison 0

These results are consistent with (Saharan et al., 2013) findings that the nanoparticles of Chitosan showed pathogenic antifungal activity to the plant such as Alternaria alternata, Macrophomina phaseolina, and aseolina in vitro at a concentration of 0.1%. (Coqueiro and Di Piero 2011) reported that chitosan affected the growth of A. solani, as it showed an inhibitory effect on fungal cell growth and conidia germination, at concentrations from 2 to 5 mg/ml Rhizoctonia solani, and the maximum inhibitory effect (87.6%) on the growth of M. ph.

- **The efficiency test of different concentrations of Phylex in inhibiting the growth of Alternaria solani on PDA in vitro**.

The results in Table 3 showed a high inhibitory efficiency of the Phylex in its different concentrations against A. solani. It was observed a significant difference in the inhibition percentages of the concentrations 0.1, 0.2 and 0.3% reached 82.96, 100, 100 respectively, compared to the comparison treatment, and the concentration 0.2 was significantly superior over the concentration 0.1 as shown in Figure 3. Possibly the high efficiency of Phylex in inhibiting the growth of pathogenic fungi is due to the presence of strong acids such as formic acid, citric, lactic, orthophosphoric, probiotics, ammonia, and water. Besides, some previous studies have shown that the leprosy product that contains 99% of probiotic acid affects the growth of fungus that may reach 100% through the use of concentrations 20000-11000 ppm (Al-Haiti 1977), where these results are consistent with (Salome, 2007) on the Phylex activity in inhibiting the fungus.

Table 3: The different concentrations effect of Phylex on the growth of A.solani on a PDA in vitro

Seq.	Treatment name	Concentration	Colony diameter	Inhibition percentage %
1	Fungus	0.0	9.0000	00
2	Phylex	0.1	1.5333	%82.96
3	Phylex	0.2	0.0000	%100
4	Phylex	0.3	0.0000	%100
5	LSD		0.9922	

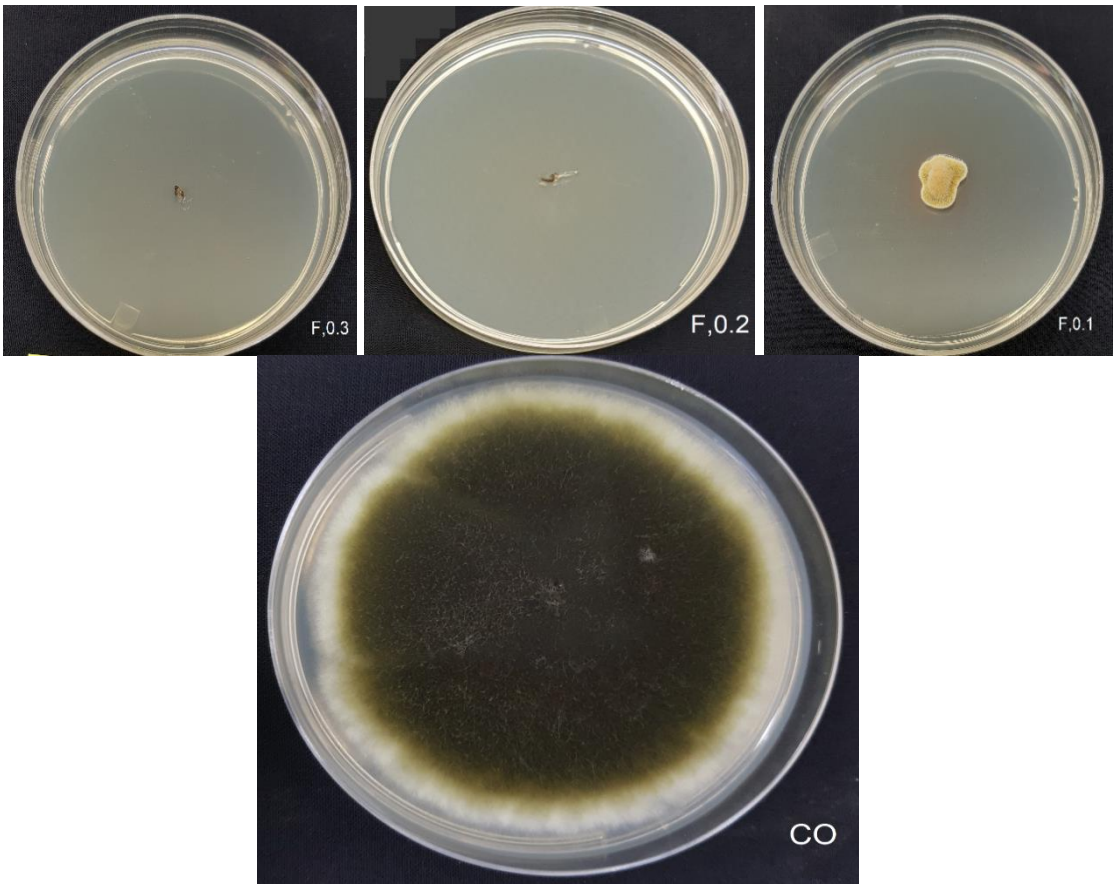


Fig.3: Inhibition ability of Phylex against pathogenic fungi after 7 days for three concentrations of 0.1, 0.2, and 0.3 ml per 100 ml and CO comparison

- **Evaluation of the efficiency of Chitosan, nano Chitosan, and Phylex extract in resisting early blight disease caused by Alternaria solani in pots under greenhouse conditions**

The results Table 4 showed a significant decrease in all treatments in the infection percentage and severity for early blight disease in tomato plants, as it provided protection for tomato plants and showed the lack of A. solani effect. The treatment of Nano Chitosan was superior in reducing the infection percentage and severity under greenhouse conditions (pots) and reached 16.66% and 12.497%, respectively, while the treatment of Nano Chitosan with the pathogenic fungus achieved a significant reduction in the infection percentage. As well as, the severity amounted to 25% and 20.83% compared to the treatment of the pathogenic fungus that achieved 100% and 79.163%, respectively, while the infection percentage and severity results of Chitosan reached 33.33% and 16.887, respectively. Furthermore, the Chitosan treatment with the pathogenic fungus reached 41.66% and 24.997% respectively, as well as the Phylex treatment achieved 16.66% and 10.413% respectively. The treatment of Phylex with the pathogenic fungus amounted to 25% and 18.747 as shown in Figure 4, thus the treatment of Nano chitosan exceeded all treatments.

Table 4: The effect of Chitosan, Nano Chitosan, and Phylex in reducing the infection percentage and severity of early blight disease on tomato plants in pots under greenhouse conditions.

Seq.	Treatment	Infection percentage %	Infection Severity
1	A	100	79.163
2	Control	8.33	6.247
3	N ch	16.66	12.497
4	Ch	33.33	16.887
5	F	16.66	10.413
6	N ch +A	25	20.83
7	Ch+A	41.660	24.997
8	F+A	25	18.747
	LSD	10.631	4.2943

Each number represents an average of three replicates
N-ch = nano chitosan, ch = chitosan, A = A.solani, F = phylex

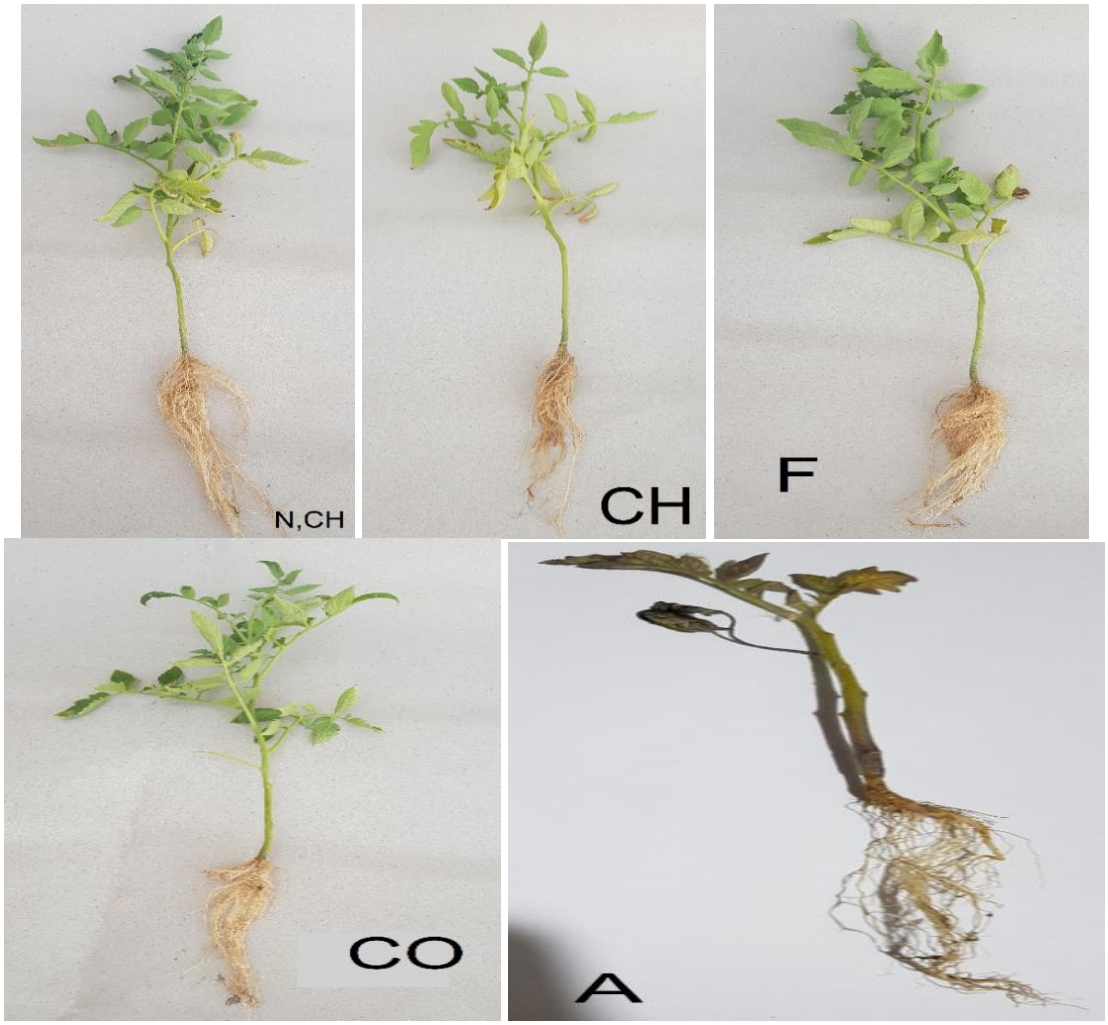


Fig.4: The ability of treatments N.CH, CH, F in reducing the infection percentage and severity of early blight disease on tomato plants in pots under greenhouse conditions
N-ch = nano chitosan, ch = chitosan, A = A.solani, F = phylex, CO=Control

The interpretation of the results of the decrease in the infection percentage and severity of early blight disease due to the efficiency of the biological materials used to resist the pathogenic fungus. Besides, the reason may be due to the effect of nanoparticles in inhibiting the fungus growth, preventing the germination of spores, and reducing the production of toxins with which the fungus attacks the plant. In addition to the high surface area of nanoparticles and their small size, which can easily penetrate the cell wall, thus leading to deformation and decomposition of the fungal hypha that leads to flowing cytoplasm out of the cell and the fungus death. Also, the ability of bacteria to produce antibiotics and enzymes that can decompose the fungal cell walls such as amylase and lipase, and protease (Pal and Saharan, 2016). Phylex has proven that it can inhibit fungi by destroying the toxins produced by it due to the acids present in it, especially propionic acid, which works to inhibit most fungi. These results are consistent with several studies that have shown that nanoparticles have a role in controlling many plant diseases that lead to a decrease in the infection percentage and severity (Ahmed et al., 2016). (Ahmadizadehesfahani et al., 2019) stated that Nano Chitosan at a concentration of 7 g / liter has led to a significant reduction in the severity of A. solani in tomato plants. (Sathiyabama et al., 2014) stated that the use of Chitosan at a concentration of 5 g / l has led to a decrease of approximately 75% in infection severity, and Chitosan could protect tomato leaves from early blight disease caused by A. solani under greenhouse conditions. The test results of the materials used to inhibit the fungi in the Phylex treatment showed a significant increase over the rest of other treatments by 100% (Al-Hamiri, 2007). (Hussain, 2008) confirmed the efficacy of Phylex in inhibiting F. moniliforme.

- **The effect of Chitosan, Nano Chitosan, and Phylex solution on the enzyme peroxidase in inducing systemic resistance in tomato plants in pots under greenhouse conditions**

- Peroxidase enzyme measurement

The experiment results showed that there were significant differences between the treatments for their effect on the activity of peroxidase enzyme and the stimulating the systemic resistance after 7 days of adding the pathogen. As there was an increase in the biochemical indicators, and all the treatments exceeded after 14 days compared with the treatment of fungus alone. However, the Nano chitosan treatment was superior with the presence of fungus in increasing the Peroxidase enzyme and resistance induction efficiency estimated based on the average change in optical absorption/min / g fresh weight in the tomato plant, which reached 18.76, followed by the Phylex treatment with the presence of fungus, which reached 17.31. Then, followed by the Chitosan treatment with the presence of the fungus reached 14.37 compared to the treatment of pathogenic fungi only, that reached 11.76, and on day 21 it gradually decreased, but a significant activity of enzyme remained higher than day 7 of adding the pathogenic fungus compared to the comparison treatment. The biomaterials used have the ability to induce plant resistance by activating defence enzymes such as Peroxidase, this enzyme has a role in preventing a pathogen from penetrating the plant cell wall, by increasing the wall strength of plant cells through converting the extensins that secreted into apoplast from monomeric from the soluble to insoluble, depending on H_2O_2, which in turn increases plant defense, making the cell wall more resistant to mechanical stress and the difficulty of the fungus penetration through the adhesion organ (Thakker et al., 2013). As it evident that the use of nanoparticles leads to the induction of systemic resistance and works to increase the activity of Peroxidase enzyme in tomato plants, this study agreed with the study of (Nadendla et al., 2018). That the nanoparticles of Chitosan have led to increased biological activity in plants, which also led to an improved defense response against R. solani in tomato plants. (Weake Workman 2008) stated that chitosan induces acquired systemic resistance and rapidly induces disease-related and variable enzymes such as the salts of phenylalanine, Ammonia, peroxidase, polyphenol oxidase, catalase, superoxide dismutase, glucanase, and chitinase. (Al-Jubouri, 2016) also indicated the efficiency of Phylex in inducing systemic resistance in the field pistachio plant and increasing the activity of peroxidase enzyme, as it reached after 7 days of 0.272 compared to the fungus treatment, which amounted to 0.073.

Table 5: The effect of adding chitosan and Nano chitosan and Phylex to control early blight on the activity of peroxidase enzyme

Seq.	Treatment name	7 days	14 days	21 days
1	control	2.30	10.43	7.26
2	A	3.83	11.44	8.07
3	N CH+A	7.12	18.76	16.37
4	CH+A	4.86	14.37	10.71
5	Ph	6.173	17.31	14.95
	LSD	0.1965	0.5389	0.2137

*Each number has three replicates

N-ch = nano chitosan, ch = chitosan, A = A.solani, F = phylex

- **Measuring the amount of chlorophyll in the leaves of tomato plants**

The results of the chlorophyll test showed the superiority of all treatments in increasing the chlorophyll content over the comparison treatment, as the Nano Chitosan treatment was superior with the presence of fungus and reached 2.47, while the chitosan treatment with the presence of fungus in the chlorophyll content reached 1.68. As for the Phylex treatment with fungus, its chlorophyll content was 2.17 compared to the treatment of fungus that reached 1.11.

Table 6: The effect of adding Chitosan and Nano Chitosan and Phylex and bacteria in the chlorophyll content in tomato plant under greenhouse conditions after 21 of adding it

Seq.	Treatment name	Chlorophyll content
1	control	1.4
2	A	1.11
3	N+CH+A	2.47
4	CH+A	1.68
5	Ph+A	2.17
	LSD	0.3182

Each number has three replicates

N-ch = nano chitosan, ch = chitosan, A = A.solani, F = phylex

These results are consistent with the (Nicholson and Vermerris 2006) study, that Chitosan increases the chlorophyll in leaves, as well as increases the phenols and enzymes that are important compounds for defense, like chlorophyll and total carbohydrates increased when using Chitosan at 250 mg / l (Farouk S, Amany, 2012). (Dzung et al., 2011) indicated that chitosan has significantly increased the concentration of chlorophyll, which indicates that chitosan can enhance the performance of photosynthesis in plants. (Al-Jabouri, 2016) indicated that the Phylex treatment achieved an increase in the chlorophyll content of 210.36 mg/gm fresh weight in the field pistachio plant.

- **The effect of Chitosan, Nano Chitosan, bacteria, and Phylex on some growth parameters of tomato plants in pots under greenhouse conditions**

The results in Table 7 showed that all of the treatments achieved a significant increase for the studied growth parameters represented by the length of shoot and root, wet and dry weight of root and shoot. As the Nano Chitosan treatment exceeded the rest of the treatments in increasing the length of the root and vegetative total, as it reached 21,333 cm and 25.167 cm respectively, while the wet and dry weight of the root reached 4.866 g and 1.553 g, and the shoot reached 18.323 g and 4.203 g, respectively. The Nano Chitosan treatment with fungus also achieved an increase in the length of root, shoot reached 17 cm and 17.667 cm respectively, and the wet and dry weight of the root, and shoot reached 1.613 g, 0.853 g, 6.967 g, and 2.263 g, respectively, compared to the treatment of fungus only. Accordingly, the length of root and shoot was 7.75 cm and 9.5 cm respectively, and the wet and dry weight of root and shoot was 0.626 g, 0.366 g, 3.493 g, and 1.053 g respectively. The Chitosan treatment achieved an increase in the length of 18.167 cm and 24.5 cm respectively, while the wet and dry weight of both root and shoot was 3.35 g, 1.16 g, 13.31 g, and 3.07 g, respectively. The chitosan treatment with fungus also achieved a length of the root and shoot of 11.167 cm and 14.167 cm respectively, and the wet and dry weight of each of the root and shoot reached 0.686 g, 0.40 g, 3.693 g, 1.11 g respectively, while the length of the root and shoot in the Phylex treatment reached 16 cm, 25.167 cm, and the wet and dry weight of root and shoot was 3.233 g, 1.09 g, 13.35 g, and 3.19 g, respectively, while the Phylex treatment with fungus achieved an increase in length compared with the treatment of fungus, where the length of root and shoot reached 11 cm, 14 cm and the wet and dry weight for root and shoot amounted to 0.873 g, 0.423 g, 4.557 g, and 1.29 g, respectively, compared to the fungus treatment only, as the length of the root and shoot was 7.75 cm and 9.50 cm, and the wet and dry weight of root and shoot was 0.626 g, 0.366 g, 3.493 g, and 1.053 g respectively.

Table 7: The efficiency test of Chitosan, Nano Chitosan, bacteria, and Phylex in its effect on some growth parameters

Seq.	Treatment	Root length	Shoot length	Root		Shoot	
				Wet weight	Dry weight	Wet weight	Dry weight
1	A	7.75	9.5	0.626	0.366	3.493	1.053
2	control	20	24.417	3.6467	1.3200	17.317	3.7533
3	N ch	21.333	25.167	4.866	1.553	18.323	4.203
4	ch	18.167	24.5	3.35	1.16	13.31	3.07
5	F	16	25.167	3.233	1.09	13.35	3.19
6	N ch +A	17	17.667	1.613	0.853	6.967	2.263
7	Ch+A	11.167	14.167	0.686	0.40	3.693	1.11
8	F+A	11	14	0.873	0.423	4.557	1.29
9	LSD	3.0291	2.9613	0.6905	0.2734	2.6807	0.6895

N-ch = nano chitosan, ch = chitosan, A = A.solani, F = phylex
Each number has three replicates

The results are consistent with (Quiterio-Gutiérrez et al., 2019), that the use of nanoparticles in many studies has an effective and successful role in controlling many pathogens compared to conventional methods. The nanoparticles were shown to increase the length of the root and shoot, the wet and dry weight of the root and shoot, and an increase in production and flowering characteristics. (El Amerany et al., 2020) reported that the use of Chitosan nanoparticles resulted in an increase in the length as well as an increase in the wet weight of the tomato plant contaminated with fungi and a decrease in the infection percentage. (ElAmerany et al., 2020 and Hidangmayum et al., 2019) showed a similar result in tomato plants grown in soil treated with Chitosan

and compost, as the positive effects of Chitosan on tomato growth and reproduction may be due to its properties to stimulate chlorophyll and to stimulate the growth of xylem vessels. Moreover, it is known that using Chitosan to the leaves increases the rate of photosynthesis, which leads to improved growth and plant development in general (Rendina et al., 2019). (Al-Jubouri, 2016) reported that the Phylex treatment with Aspergillase flavas increased the wet and dry weight of the field pistachios of 96.66 and 84.23 g, respectively. These results are consistent with the results of (Salome, 2007; Al-Hamiri, 2007; Hussain, 2008A; Al-Qaisi, 2010) stated that the Phylex gives a high efficiency in inhibiting the studied fungi, and this may be reflected positively on some growth parameters.

CONFLICT OF INTEREST

None

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