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Protecting Tomato Seedlings from Infection with Pathogenic Fusarium Oxysporium Using Rosemary Oil and Nano-Chitosan

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Abstract

The investigation aimed to evaluate the effectiveness of rosemary oil (*Rosmarinus officinalis* L.) and regular and nano chitosan in reducing the severity of tomato seedling infection caused by *Fusarium oxysporium*, as well as evaluating some growth parameters and chemical properties. The results of the study showed that all treatments achieved a significant increase in germination rates and protection of tomato seeds and seedlings from infection by the disease before and after emergence, as well as reducing the severity of infection compared to the treatment with the pathogenic fungus. The treatment of extracted rosemary oil and nano and regular chitosan in the presence of the pathogen reduced the severity of infection to 9.38%, 6.75%, and 23.56%, respectively, compared to 70.34% in the pathogen treatment. The effect was also positively reflected on the fresh and dry weight of the shoot and root system. The used factors proved their efficiency in inducing systemic resistance in tomato plants by increasing the activity of the peroxidase enzyme and phenols in the plant leaves.

Keywords: Tomato, Pathogen, Rosemary oil, Chitosan, Fusarium oxysporium.

حماية بادرات الطماطة من الاصابة بالفطر الممرض Fusarium oxysporium باستعمال زيت أكليل الجبل والكايتوسان النانوي

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الخلاصة

هدفت الدراسة لتقيم فعالية زيت أكليل الجبل .*Rosmarinus officinalis* L والكايتوسان العادي والنانوي في خفض شدة اصابة بادرات الطماطة من مرض موت بادرات الطماطة الناجم عن المسبب *Fusarium oxysporium* وبعض معاير النمو والصفات الكيميائية. بينت نتائج الدراسة أن المعاملات جميعها حققت زيادة معنوية في نسب الإنبات وحماية بذور وبادرات الطماطة من الإصابة بالمرض قبل وبعد البزوغ واختزال شدة الإصابة مقارنة بمعاملة الفطر الممرض. وحققت معاملة زيت أكليل الجبل المستخلص والكايتوسان النانوي والعادي بوجود الممرض نسبة اختزل شدة الاصابة إلى 9.38، 6.75، 23.56% مقارنة مع 70.34% في معاملة الممرض، وإنعكس التأثير أيجابياً على الوزن الطري والجاف للمجموع الخضري والجذري، واثبتت العوامل المستعملة كفاءتها في استحثاث المقاومة الجهازية في نباتات الطماطة من خلال زيادة فعالية انزيم البيروكسيديز والفينولات في اوراق النبات.

كلمات مفتاحية: الطماطة، المسبب المرضي، زيت اكليل الجبل، الكايتوسان، Fusarium oxysporium.

Introduction

Tomato (*Solanum lycopersicum* L.), which belongs to the Solanaceae family, is one of the most economically important crops that contributes to the sustainability of food systems for the world's population (1). Tomato crops are affected by many agricultural pests, including seed rot and seedling death in nurseries and greenhouses, resulting in significant economic losses. Soil-borne fungi cause this disease, and its severity increases with climate change, such as high and low humidity and temperature. Infection with pathogens reduces the quality of seeds upon germination and their transition to other growth stages. Due to the increasing world population and its food needs, it has become necessary to use environmentally safe and human-friendly pest control methods to combat pathogens, in addition to stimulating them to increase productivity and improve plant quality (12). The use of extracted volatile plant oils has proven its effectiveness in reducing fungal diseases, as they have antifungal, antiviral, and antibacterial properties, and are non-toxic and biodegradable. This is due to their possession of a wide and effective range of chemical compounds, such as aldehydes, ketones, and phenols, which have proven highly effective in combating them (4 and 6).

Among these volatile oils is rosemary oil *(Rosmarinus officinalis* L.), which has proven highly effective in combating pathogens. Yılar (20) indicated in a study that using rosemary oil against the fungus *Fusarium oxysporum* at a concentration of 8 μL resulted in inhibition rates of 71.97 and 61.32%. He also found that using rosemary oil against the fungus *F. oxysporum* at concentrations of 10, 20, 30, and 40 μL inhibited the growth of the fungus within 7 days under laboratory conditions (3). Research has also focused on the use of chitosan, which is considered a safe biological material for the environment and human health, in combating pathogens such as fungi, bacteria, and nematodes (7). The importance of chitosan in combating fungi lies in its effect on the permeability of the plasma membrane, changes in the composition of the cell wall, or its interaction with messenger RNA (mRNA) (16). Nanochitosan stimulates plant growth and germination to a large extent, in addition to being an induction factor for pathogen resistance. Given the importance of this disease, the study aimed to evaluate the efficiency of rosemary oil, nano-chitosan, and regular chitosan in reducing the incidence and severity of the disease and its positive impact on some growth parameters, and to detect biochemical reactions such as peroxidase enzyme and total phenols.

Materials and Methods

Rosemary (*Rosmarinus officinalis* L.) oil was extracted in the laboratories of the Plant Protection Department, College of Agriculture, University of Anbar, according to the Clevenger method (1928). Rosemary seedlings were collected from nurseries, and their leaves were separated and dried. 100 g of the sample to be extracted was placed in a flask, and 1000 ml of distilled water was added to the sample using a Clevenger distillation apparatus for 3 hours. A volume of 1-2 ml of oil was extracted from the leaves of the plant. The oil was separated from the water, then filled into tightly sealed plastic bottles and stored in a refrigerator at 4°C until use. The fungus *F. oxysporum* was isolated from the roots of infected tomato seedlings, which showed yellowing, wilting, and root rot. The isolate was morphologically identified and molecularly confirmed in the laboratories of the Wahj DNA Company (ASCO Learning Center) using the polymerase chain reaction (PCR) technique. It was deposited in the gene bank under bank number (OQ565149). The experiment was conducted in the greenhouse of the Plant Protection Department, College of Agriculture, University of Anbar, during the spring season of 2023. Aseel cultivar seeds produced by the Horticulture Department, National Program for the Propagation of Local Vegetable Crops Lines and Hybrids, were used for planting.

Plastic pots (25 cm in diameter) were sterilized with sodium hypochlorite and then filled with a mixture of soil and peat moss at a ratio of 2:3. The mixture was applied at a rate of 5 kg per pot. The soil and peat moss were sterilized with formalin at a rate of 3 liters per 1 m3, and the pots were covered with nylon. The containers were tightly sealed for 14 days, after which the nylon was removed and stirred continuously before use. The pots were then transferred to a greenhouse. The *F. oxysporum* inoculum grown on millet seeds was added to the potting soil at a rate of

25 g/pot. For the control treatment, millet seeds without the pathogenic fungus were added and mixed well with the soil. The potting soil was moistened with water and covered with a plastic sheet to maintain a moisture content suitable for pathogen growth. The containers were then left for three days. Tomato seeds were sown, 10 seeds per pot, surface-sterilized with a 1% sodium hypochlorite solution (free chlorine) for 2 min. They were then washed with sterile distilled water to remove traces of sterilization. The tomato seeds were then soaked for two hours before planting in the 2% rosemary oil, nano-chitosan, and regular chitosan treatments at rates of 3000 and 6000 mgL^{-1}, respectively, and the benzoyl perchlorate (Pentazole Combi) pesticide. Metalaxyl + Hymexazol) at a rate of 0.25 $mgL^{-1.}$ The results were recorded after all seeds germinated, and the germination rate and the percentage of infection before emergence were calculated 10 days after planting the tomato seeds using the following equation:

%of germination = (number of germinated seeds)/(number of total seeds) x 100

%of pre-emergence infection = (number of non-germinated seeds)/(number of total seeds) x 100

The percentage of post-emergence infection due to root rot was calculated 35 days after planting tomato seeds according to the following equation: % of post-emergence infection = (number of infected plants)/(number of total plants) x 100

After 40 days of planting, four plants were uprooted from each treatment based on the pathological index and severity of infection, as described before (18). The severity of infection was calculated for each treatment according to equation (9). The fresh and dry weight of the shoot and root system, the number of leaves, the plant height, and the chlorophyll percentage were calculated (5). Some chemical characteristics, such as the peroxidase enzyme percentage and the total phenolic content in tomato leaves, were measured after 30 days of treatment. A completely randomized experimental design (CRD) was used with 12 treatments and four replicates for each treatment. The results were analyzed and compared statistically using the LSD test at a probability level of 0.05.

Results and Discussion

Effect of extracted rosemary oil and chitosan on germination percentage, total infection, and infection severity: The results of the tomato seed experiment, Table 1 showed that all treatments achieved a significant reduction in germination percentage, total infection percentage, and infection severity, as well as protection of tomato seeds from seed rot caused by the pathogenic fungus *F. oxysporum*. The germination percentage reached 37.5%, the total infection percentage reached 75%, and the infection severity reached 70.34%. The nano-chitosan treatment recorded the highest germination percentage of 95%, achieving high protection against the pathogen. The total infection percentage was 10.0% and the infection severity was 6.75%, which did not differ significantly from the extracted rosemary oil treatment, which achieved a germination percentage of 90.0%, a total infection percentage of 12.5%, and an infection severity of 9.38%. This was followed by the Pentazol Combi pesticide treatment, which gave a total germination, infection rate, and infection severity of 92.5%, 17.5%, and 12.38%, respectively. Then, the regular chitosan treatment gave

80.0%, 27.5%, and 23.56%, respectively. The germination rate reached 100% for each of the extracted oils, regular chitosan, and nano chitosan treatments, and the total infection rate and infection severity were 0.0% without the pathogenic fungus. This is attributed to the method of using the treatments and their ability to inhibit fungal growth, as it was shown that rosemary oil has a high effectiveness and biological activity in inhibiting the growth of fungal mycelium, in addition to interfering with the work of the enzymatic reactions that contribute to cell wall synthesis. This is due to the chemical properties of the basic substances in the oil, such as cineole and camphor, which work to break down the fungal cell wall, cause cytoplasmic leakage, inhibit it, and reduce the number of conidia formed by the fungus (17). Chitosan also plays a role in inhibiting fungi through its mechanism of stimulating the plant to acquire systemic resistance by stopping the activity of some enzymes and proteins necessary for fungal growth, or by increasing the permeability of the cell membrane as a result of the interaction of chitosan with the fungal membrane. It also works to inhibit the synthesis of proteins and enzymes necessary for fungi (21). The effect of the chemical pesticide in its ability to inhibit fungi may be attributed to the effect of the active ingredients Metalaxyl and Hymexazol, which work to disrupt the synthesis of ribosomal RNA and prevent fungal growth (13).

Table 1: Efficiency of rosemary oil and chitosan in germination rate, total infection rate, and infection severity of tomato.

Treatments	Germination rate %	Infection before emergence %	Infection after emergence %	Total Infection %	Overall injury severity %
Sterile soil	100	0.0	0.0	0.0	0.0
Soil contaminated with F.oxysporum	37.5	62.5	12.5	75.0	70.34
Local rosemary extract oil	100	0.0	0.0	0.0	0.0
Rosemary oil + F. oxysporum	90	12.5	0.0	12.5	9.38
Commercial oil + F. oxysporum	72.5	27.5	15.0	42.5	36.31
Regular chitosan	100	0.0	0.0	0.0	0.0
Chitosan normal + F.oxysporium	80.0	20.0	7.5	27.5	23.56
Nano chitosan	100	0.0	0.0	0.0	0.0
Nanochitoson + F. oxysporum	85.0	5.0	5.0	10.0	6.75
Pantazole Combi + F. oxysporum	92.5	7.5	10.0	17.5	12.38
Acetic acid + F. oxysporum	35.0	65.0	15.0	80.0	74.31
Tween 20 + F. oxysporum	42.5	60.0	20.0	80.0	74.25
LSD 0.05	12.4	11.9	10.2	11.7	4.4

The effect of rosemary oil and chitosan on some growth traits of tomato plants: The results (Table 2) showed that reducing the severity of infection led to a significant increase in some plant heights compared to the pathogenic fungus treatment. The rosemary oil and nano-chitosan treatments significantly outperformed the pathogenic fungus in soil contaminated with it, achieving plant heights of 9.77 and 9.57cm, respectively. The regular chitosan treatment resulted in 9.65 cm, compared to the pathogenic fungus treatment, which achieved 7.38 cm. Meanwhile, the combi-pentazol herbicide and commercial oil achieved plant heights of 11.70cm and 9.05cm. The results of Table 2 indicated that the used agents achieved a significant increase in the number of leaves 35 days after adding the pathogen inoculum, and an increase in the total chlorophyll content in the plant measured in SPAD compared to the pathogen treatment (without any addition). The treatment of rosemary oil and nano-chitosan recorded an average number of leaves of 22.75 and 19.10 leaves per plant^{-1}, and a chlorophyll content of 32.27 and 32.40 SPAD, respectively. However, it did not differ significantly from the regular chitosan treatment, which gave some leaves of 20.10 leaves plant^{-1} and a total chlorophyll content of 31.90 SPAD, respectively. At the same time, the combi-pentazole herbicide and the commercial oil gave some leaves of 24.0 and 17.25 leaves per plant^{-1} cm and a chlorophyll content of 34.65 and 27.80 SPAD, respectively. The results showed that natural treatments such as extracted rosemary oil, nano-chitosan, and regular chitosan were significantly superior in inhibiting the pathogen, which is consistent with what was mentioned by (4). Camphor, one of the main compounds of rosemary oil, inhibited the growth of the fungus *R.solani*, which was positively reflected on the vegetative growth parameters of pepper plants. Chitosan also has a role in improving plant growth, increasing crop productivity, increasing chlorophyll content in the plant, and reducing the level of salt stress in the plant (15).

Table 2: Efficiency of rosemary oil and chitosan on infection severity and some vegetative growth traits of tomato.

Treatments	Plant height (cm)	Number of Leaves (leaf/plant)	Chlorophyll percentage (SPAD)
Sterile soil	11.88	25.00	34.42
Soil contaminated with F.oxysporum	7.38	10.50	22.9
Local rosemary extract oil	11.15	24.00	38.42
Rosemary oil + F. oxysporum	9.77	22.75	32.27
Commercial oil + F. oxysporum	9.05	17.25	27.80
Regular chitosan	12.38	25.80	36.58
Chitosan normal + F.oxysporium	9.65	20.10	31.90
Nano chitosan	10.40	21.40	34.73
Nanochitoson + F. oxysporum	9.57	19.10	32.40
Pantazole Combi + F. oxysporum	11.70	24.00	34.65
Acetic acid + F. oxysporum	7.55	14.00	22.93
Tween 20 + F. oxysporum	7.65	11.23	21.43
LSD 0.05	1.40	3.21	3.68

The effect of rosemary oil and chitosan on the fresh and dry weight of the shoot and root system of tomato plants: The results of Table 3 showed that the treatments used led to a significant increase in growth parameters, including the fresh and dry weight of the shoot and root system, compared to the pathogenic fungus treatment. The rosemary oil and nano-chitosan treatments in soil contaminated with the pathogenic fungus outperformed the fresh and dry weight of the shoot and root system, giving them a fresh weight of 1.90 and 0.11 g, respectively, and a dry weight of 0.32 and 0.2 g, respectively. This was followed by the regular chitosan treatment at 1.52, 0.9 g, 0.30, and 0.2 g, respectively, compared to the pathogenic fungus treatment, which gave 1.00, 0.11, 0.12, and 0.1 g, respectively. At the same time, the Pentazol Combi pesticide and the commercial oil gave a fresh weight of 1.90 g, 0.11 g, and a dry weight of 0.27 g, 0.2 g, 1.32 g, 0.17 g, 0.27 g, 0.1 g, respectively. The results of Table 2 indicate that the effect of treatments such as volatile rosemary oil on other growth parameters such as fresh and dry weight of the vegetative and root system is due to the active compounds that the oils contain that stimulate growth, by activating the plant's defense mechanisms, making it more resistant to the disease, and may stimulate the plant to produce phytoalexins that inhibit the fungus and lignin, which accumulates in the infected area to prevent the fungus from entering the plant tissues (19), and this is consistent with what was found by (8). The used volatile oils increased the weight of roots and stems by 0.12 and 0.22 grams for tomato plants after treatment with oil. Chitosan also played a role in increasing the wet and dry weight of tomato plants in contaminated treatments. This was a result of inhibiting the fungus and stimulating the plant to induce resistance, which was reflected in growth characteristics (14).

Table 3: Efficiency of rosemary oil and chitosan in the wet and dry weight of the green and root system of tomatoes.

Treatments	Fresh weight (gm plant^{-1})		Dry weight (gm plant^{-1})	
	vegetative	root	vegetative	root
Sterile soil	2.20	0.21	0.45	0.07
Soil contaminated with F.oxysporum	1.0	0.11	0.12	0.01
Local rosemary extract oil	1.97	0.24	0.47	0.03
Rosemary oil + F. oxysporum	1.90	0.11	0.37	0.02
Commercial oil + F. oxysporum	1.32	0.17	0.27	0.01
Regular chitosan	3.40	0.12	0.4	0.03
Chitosan normal + F.oxysporium	1.52	0.09	0.3	0.02
Nano chitosan	2.00	0.13	0.42	0.03
Nanochitoson + F. oxysporum	1.55	0.1	0.32	0.02
Pantazole Combi + F. oxysporum	1.90	0.11	0.27	0.02
Acetic acid + F. oxysporum	0.82	0.07	0.15	0.01
Tween 20 + F. oxysporum	0.9	0.06	0.15	0.01
LSD 0.05	0.61	0.04	0.15	0.01

The effect of rosemary oil and chitosan on the activity of peroxidase enzymes and phenols in tomato leaves: The results in Figure 1 indicate that the activity of the peroxidase enzyme, estimated based on the rate of change in optical absorbance per minute g^{-1} fresh weight in tomato seeds, did not differ significantly between the oil, chitosan, and chemical pesticide treatments in peroxidase activity, compared to the soil treatment without the pathogenic fungus, which recorded 26.40 min g^{-1} fresh weight. However, it differed significantly from the pathogenic fungus treatment (19.60 min g^{-1} fresh weight). The rosemary oil treatment outperformed the treatment (27.90 min g^{-1} fresh weight), which did not differ significantly from the regular and nano-chitosan treatments (25.35 and 26.30 min g^{-1} fresh weight, respectively). The chemical pesticide and commercial oil treatments gave enzyme activity percentages of 25.80 and 24.60 min g^{-1} fresh weight, respectively. The treatments of extracted oil, regular chitosan, and nano chitosan without pathogenic fungus recorded 28.30, 30.45, and 29.20 min g^{-1} fresh weight, respectively. Treating tomato seeds with the materials used in the experiment (oil and chitosan) led to an increase in the activity of the peroxidase enzyme, which plays an important role in plant defense by preventing the pathogen from penetrating the plant cell walls. This is consistent with what was mentioned by (2). The volatile oil has a positive effect in increasing the concentration of peroxidase content in tomatoes after 5 days of treatment. The use of nanoparticles also stimulates the induction of systemic resistance in the plant, thus increasing the activity of the peroxidase enzyme in the tomato plant, in addition to enhancing the biological activity of the plant (11).

The results in Figure 1 in the tomato seeds experiment indicated that all treatments used (oil and chitosan) achieved a significant increase in the average plant content of phenols compared to the control treatment with pathogenic fungi, which gave 30.50 mg $g^{-1.}$ The nano-chitosan treatment showed a significant increase in phenol content, reaching 50.30 mg g^{-1}, compared to the extracted oil and regular chitosan treatments, which were 42.55 and 45.45 mg g^{-1}, respectively. At the same time, the chemical pesticide and commercial oil gave phenol amounts of 40.90 and 38.20 mg g^{-1}, respectively. As for the treatments of extracted oil, regular chitosan and nano-chitosan without pathogenic fungi, they recorded 49.20, 54.25, and 52.40 mg g^{-1}, respectively, compared to sterilized soil 41.40 mg g^{-1}. The reason for the superiority of plants treated with oil and chitosan in increasing the accumulated phenols in tomato leaves may be because volatile oils such as rosemary oil, which contains active compounds when treated with plants, increase the accumulation of phenols in the plant, and thus lead to enhancing the acquired resistance induced by activating gene expression, which enhances the accumulation of phenylpropane and amino acids (2). Chitosan also increases the activity and production of phenols in the plant, which also plays a role in defending the plant from pathogens (10).

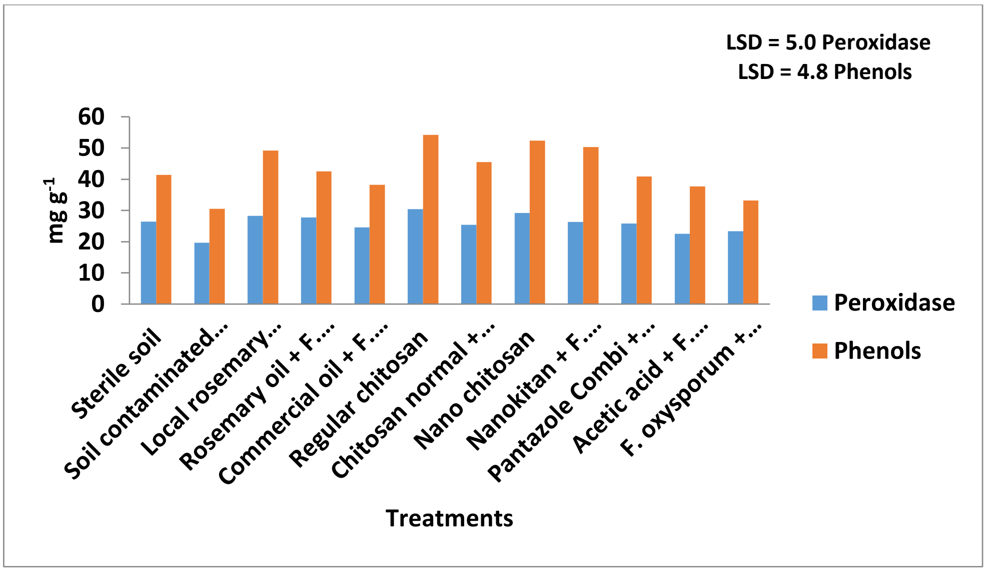

Figure 1: The efficiency of rosemary oil and chitosan in the activity of the peroxidase enzyme and phenols in tomato plant leaves.

Conclusions

Rosemary oil extract and both regular and nano-chitosan can protect tomato seeds from seed rot caused by the pathogenic fungus *F. oxysporum* under plastic conditions, with a positive impact on the studied growth parameters. This, in turn, may be an important factor and an alternative to chemical pesticides, or reduce their use, which has positive economic and environmental impacts.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author H. N. Jadoa; writing original draft preparation, Kh. W. Ibade; methodology, Lab. Analysis, check all figures, draw figure, read and rewrite some figures then agreed to the published version of the manuscript.

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Data available upon request.

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The authors declare no conflict of interest.

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