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# Evaluation of the effectiveness of chitosan and Threonine in protecting tomato plants from root and stem rot disease caused by the fungus *Fusarium solani*

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**ABSTRACT**

The aim of the study was to protect tomato plants from root and stem rot disease caused by the fungus *Fusarium solani* under test using regular chitosan and threonine (Thr) at concentrations of 0.5, 1.0, 1.5, and 2.0 g L-1 for each. This was done by measuring the intensity of certain growth parameters and peroxidase activity. Compared with the control treatment, the results showed that all plant treatments, including chitosan and threonine, protected tomato plants by inducing systemic resistance to root and stem rot disease. The plant defense mechanisms were activated as evidenced by increased peroxidase activity, which typically results in growth parameters such as increased length and weight of both fresh and dry vegetable groups with statistically significant differences compared to the pathogenic fungus treatment.

## 1. Introduction

The tomato *Solanum lycopodium* L, a member of the Solanaceae family, is one of the most produced and economically significant crops in terms of tomato production, Iraq comes in sixth place among Arab nations with 744,000 tons produced in 2023, while Egypt leads the pack with 6.2 million tons (FAOSTAT, 2024). One of the most detrimental fungi to plants is *Fusarium* Spp., which results in large financial losses and a 30–40% drop in worldwide production, and in favorable conditions, up to 80% (Nikitin et al., 2023; St. Epien, 2023). According to lrulappan et al., (2022), it can infect a variety of plant components, including roots, stems, leaves, fruits, and seeds. One of the most damaging illnesses affecting tomatoes is rot in the roots and stems, which is brought on by a fungal infection. *F. solani* is a highly dangerous pathogen that causes wilt, root rot, crown rot, and seedling wilt in a variety of host plants (Saengchan et al., 2022; Li & Li, 2022). In tomatoes, it can result in notable output losses, particularly in favorable environmental conditions (Star, 2022; Jamali, 2024). Although chemical pesticides are one of the most popular ways to manage plant fungal diseases, they have several drawbacks, chief among them being environmental contamination and the emergence of pathogen resistance (Kumar et al., 2024). To combat tomato root and stem rot, scientists are now focusing on environmentally acceptable techniques. One such technique is the use of bio stimulants, which are substances that include microorganisms that, when applied to a plant or its rhizosphere, promote biological processes. These increase the plant's resilience to biotic stressors, the most significant of which are several plant diseases (Kumari et al., 2023; Husen, 2024). Based on their place of origin, plant bio stimulants can be categorized into two groups: naturally occurring ones, like

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chitosan, yeast extract, hormones, plant growth regulators, and amino acids, and manufactured ones, like synthetic hormones, phenolic compounds, and inorganic salts (Lau et al., 2022; Bartsch et al., 2023). After the acetyl group is removed from chitin, the amine group remains free, allowing chitin to dissolve in weak, diluted acids. This natural molecule is called chitosan (Bade & Wick, 1988). As a bio stimulant for plant growth, chitosan acts as an antifungal and anti-pathogen for a variety of plant diseases by activating several genes, proteins, and secondary metabolites of the plant (Pongrayoon et al., 2022). When a plant host is attacked with a pathogen, studies have demonstrated that chitosan triggers defense responses and systemic acquired resistance (Ali et al., 2011). St (2022) showed that the percentage and severity of plant infection with tomato root rot disease were significantly reduced by using regular and nano-chitosan against the fungi *F. solani* and *R. solani* and *M. phaseolina,* the causal agents of the illness. By examining how well chitosan and the bio fungicide *T. viride* work to create systemic resistance against *F. solani* and *M. phaseolina*, the disease-causing microorganisms of cowpea root rot in Babylon Governorate, Al-Jubouri (2024) provided additional clarification. The study showed that chitosan could inhibit the growth of harmful fungus, particularly when coupled, by analyzing the proportion of infections and the severity of infections. According to Takai et al. (2001) and Ghule et al. (2021), chitosan was utilized as a stimulant for plant defenses against infections by raising the defensive enzymes such Chitinase, all kinase, and phenol in infected plants.

The fundamental constituents of amino acids are nitrogen, carbon, oxygen, and hydrogen. Some amino acids also contain other elements that are a part of their side chains. It is the fundamental component and a significant source that plants employ to produce proteins and stimulate growth (Abbas, 2022; Almashhadany et al., 2022). Only twenty of the approximately 500 known naturally occurring amino acids are found in the genetic code, but they can be categorized in a variety of ways (Nelson & Michael, 2005). Moreover, amino acids can control the activity of auxin plant hormones, provide carbon and energy, and protect plants (Stasik, 2009; Ibrahem, 2016). A necessary amino acid for plants is threonine (Thr), also referred to as alpha-amino-beta-hydroxybutyric acid. It contributes significantly to the health of plants and increases their resistance to harmful fungus. It is applied as a fungicide and effectively suppresses plant fungus (Kassap, 1998). According to studies, threonine inhibits the growth of the common plant pathogen *F. solan.* To preserve tomato plants by creating systemic resistance to root and stem rot, the purpose of this study was to examine the possible applications of chitosan both by itself and in conjunction with the amino acid threonine.

## 2. Methodology

*2.1, Isolation and identification of associated fungi:*

Tomato seedlings exhibiting signs of stem and root rot were pulled out and properly cleaned with running water to get rid of any soil that had adhered. Each stem and root were chopped into tiny pieces that ranged in length from 0.5 to 1 cm. Sodium hypochlorite ($NaCIO_4$), which contains 1% free chlorine) was used to sterilize the surface for two minutes. The disinfectant was then rinsed off with sterile distilled water for another two minutes. A laminar flow hood was used to dry the components on sterile, clean filter paper. After that, they were moved in three duplicates, four pieces per dish, to 9 cm Petri dishes filled with water agar (WA). The plates were kept at 25 ± 2°C for incubation. After fungal growth started to form around the infected plant parts, a piece of the fungal colony's edge was removed using a sterile needle and moved, in triplicate for each isolate, to a different set of Petri dishes that contained potato dextrose agar (PDA). At 25 ± 2°C, these dishes were incubated. A light compound microscope (LCM) was then used to study the fungus under 4X, 10X, and 40X magnifications once they had been identified based on colony

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morphology. Using taxonomic keys and diagnostic traits outlined by Booth (1977) and Leslie & Summerell (2006), fungi were identified down to the genus and species level.

The following formula was used to determine each sample's frequency and percentage of fungal presence:

$$\text{The percentage of fungal recurrence in the sample} = \frac{\text{The number of plant pieces showing fungal growth}}{\text{The total number of pieces used}} \times 100 \quad \text{(eq.1)}$$

*2.2. Pathogen testing of fungi isolated on tomato seeds on Water Agar (WA) medium:*

The laboratory investigated the pathogens of the ten most common fungal isolates found in this investigation using Water Agar (WA) medium. The medium was injected with three duplicates of each isolate using a 0.5 cm disc that was cut from the edge of a fungal colony that was 5 days old. The plates were incubated at 25°C for three days. After being surface sterilized for two minutes in a 1% sodium hypochlorite solution (free chlorine), six tomato seeds were rinsed with sterile distilled water and allowed to dry on sterile filter paper in an isolation chamber. One centimeter separated the seeds from the fungal colony's developing edge. The control group consisted of three plates with just tomato seeds and no fungal inoculation. The plates were incubated at 25°C for seven days. The tomato seedlings were inspected once the seeds in the control treatment had completely sprouted. To get the germination percentage, the following formula was used:

$$\text{Percentage of seed germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad \text{(eq.2)}$$

*2.3. Preparation of the inoculum for the pathogenic fungus F. solani:*

They prepared fungal inoculants using the Dewan (1988) method using local millet seeds, Panicum milliaceum. To get rid of dust and other contaminants, the millet seeds were properly cleaned with water, steeped for six hours, and then placed on a piece of gauze for 30 minutes to drain any remaining water. In a 250 ml glass beaker, 50 g of the seeds were put. They autoclaved the beakers for 30 minutes. They were cleaned once more and allowed to cool after a day. Five discs of PDA with a diameter of 0.5 cm and containing *F. solani* fungus were then added to the beakers, one at a time. To guarantee aeration and the distribution of the fungal inoculum among the millet seeds, the flasks were incubated for 14 days at 25 + 1 °C, with stirring every three days.

*2.4. Evaluating the efficiency of chitosan and its interaction with threonine inhibiting the growth of Fusarium solani on Potato Dextrose Agar (PDA) medium.*

Concentrations are using (0.5, 1.0, 1.5, and 2.0) g $L^{-1}$ for threonine and chitosan, respectively. In a 100 ml glass flask, chitosan concentrations were added once by themselves and once with threonine. The volume was then increased to 100 ml of PDA culture medium while being shaken. Following the pouring of the mixture into 9 cm diameter Petri dishes, the mixture was inoculated with a 0.5 cm diameter disc of a continuous *F. solani* fungus isolate (Fs1) that was 5 days old. Three replicates were added for each concentration, and as a control treatment, three replicates were left without the addition of any culture medium concentration. The dishes were incubated for seven days at 25 +/- 1 °C. The proportion of fungal growth inhibition was determined by measuring the average diameter of two perpendicular columns from each colony. Each of the two induction factors' effective concentrations was selected for use in the tests that followed.

*2.5. Evaluating the efficiency of chitosan and its interaction with threonine in protecting tomato plants from infection by the pathogenic fungus Fusarium solani and some plant growth parameters under woody canopy conditions.*

The experiment was carried out during the 2024 growing season in the wooden canopy of the Plant Production Technology Department at Al-Musayyab Technical Institute-Al-Furat Al-Awsat Technical University. In all treatments that required the addition of the inoculum at a rate of 1%, 25 seeds per pot of 2 kg of sterilized sandy soil were treated by dipping sterile tomato seeds for two minutes in a concentration of 1 g/L of chitosan once and then interacting with threonine again. Peat moss was added to the soil at a 2:1 ratio, and the pathogenic fungus *F. solani* was added to the soil, loaded onto local millet seeds. The only additions made to the control treatment were sterilized millet seeds (Khader, 2007). Chitosan and threonine at a concentration of 1.5 g/L were sprayed on the green mass until it was wet; 45 days after the seedlings were planted. Three duplicates of each treatment were used in the completely randomized design (C.R.D.) experiment. Fourteen days following planting, the germination percentage was determined. Five tomato seedlings per pot were used for the thinning. Following the development of symptoms on the plant, the percentage and severity of infection were then determined using the pathological guide as described in Al-Ghazali (2022) and Al-Mashhadani (2022). This is as follows: 0 indicates healthy roots, 1 indicates secondary root discoloration (rot), 2 indicates secondary root discoloration and a portion of primary roots, 3 indicates main root discoloration without stem base rotting, 4 indicates main root discoloration and stem base decay and rotting, and 5 indicates plant death. As in the preceding paragraph, the percentage of infection was determined, and the McKinney (1923) equation cited in Al-Ghazali (2022) and Al-Ghanimi (2023) was used to determine the degree of infection:

$$\%\ \text{Infection Severity} = \frac{\text{Total (number of plants in class X class number)}}{\text{2Total number of plants X Highest score}a} X\ 100 \qquad \text{(eq.3)}$$

After 75 days of planting, the plants were trimmed to one plant per pot for assessment. This involved determining the tomato shoots' fresh and dried weights as well as their stem length.

*2.6. Measuring peroxidase (PO) activity in tomato plants:*

Samples were taken three times, seven days apart, at the Al-Musayyab Technical College Pathology Laboratory for Postgraduate Studies to assess peroxidase activity fourteen days after tomato seedlings were planted. Plants in each treatment had their leaves sampled using the Hammer Schmidt et al. (1982) procedure. With a spectrophotometer, absorbance was measured at 420 nm every 30 seconds for ten readings. The following equation was used to determine the change in absorbance:

Change in absorbance = $(\Delta A / \Delta T)$ / gram fresh weight (eq.4)

$\Delta A$ = change in light absorbance

$\Delta T$ = change in time / minute

## 3. Results and discussion

*3.1. Isolation and Identification:*

The presence of multiple fungal species at different frequency rates was discovered by the isolation and identification of the roots and stems of the tomato plants under study that displayed signs of root and stem rot disease. 25 isolates from various isolated fungal species were found when fungal growth from infected plant sections grown on PDA medium was examined under a microscope using the taxonomic keys that were specified (Table 1). *F. solani* was the most isolated fungus, occurring 40.25% of the time, followed by *R.*

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*solani* (22.50%). With a frequency of 3.00%, *Aspergillus niger* was the least common. These findings align with a number of research, such as Star (2022), which verified that *F. solani* was the most commonly isolated pathogenic fungus species from the roots of tomato plants infected with root rot disease in some regions of the Babil Governorate, Iraq, with a frequency of 55.9%.

**Table 1:** The percentage of recurrence or appearance of fungi associated with tomato plant roots.

| ID | Isolated Fungi | Number of isolates | Frequency % |
|---|---|---|---|
| 1 | *Fusarium solani* | 14 | 40.25 |
| 2 | *Rhizoctonia solani* | 6 | 22.50 |
| 3 | *Fusarium oxysporum* | 2 | 18.33 |
| 4 | *Macrophomina phaseolina* | 1 | 10.33 |
| 5 | *Penicillium* Spp. | 1 | 5.51 |
| 6 | *Aspergillus niger* | 1 | 3.00 |

*3.2. Evaluating the pathogenicity of isolated fungi on tomato seed germination in Water Agar:*

Table (2) showed that all of the investigated fungal isolates were responsible for the decrease in tomato seed germination percentage as compared to the healthy control treatment, where 100% of the seeds germinated. With a seed germination rate of 0.0%, the isolates Fs1 and Fs10 experienced the largest drop in germination percentage. These isolates were followed by Fs2, Rs1, and Fo1, which recorded 5.50% with an average number of seed germinations of 0.33. The remaining isolates' germination percentages ranged from 27.67 to 83.38%, with a germination rate of 1.66 to 5 seeds. The variance in the rates of seed germination could be explained by variations in the pathogenicity and genetic composition of the fungal isolates, as well as variations in the amounts of fungal secretions. The production of enzymes that break down cellulose and pectin, which causes root rot and inhibits germination, was linked to toxic secondary metabolites that killed embryos. According to the findings, (Mollan, 2006 and Star, 2022). Based on the test results, isolates that most inhibited tomato seed germination was chosen for further research. One such isolation was Fs1, which had the highest frequency in the prior trial.

**Table 2:** Pathogenicity of the isolated fungi on tomato seed germination in Water Agar

| ID | Isolate | Average seed germination | % Seed germination | ID | Isolate | Average seed germination | % Seed germination |
|---|---|---|---|---|---|---|---|
| 1 | control | 6.00 * | 100.00 | 7 | Fs 13 | 4.66 | 77.67 |
| 2 | Fs 1 | 0.00 | 0.00 | 8 | Rs 1 | 0.33 | 5.50 |
| 3 | Fs 2 | 0.33 | 5.50 | 9 | Rs 3 | 1.66 | 27.67 |
| 4 | Fs 4 | 1.66 | 27.67 | 10 | Rs 5 | 5 | 83.33 |
| 5 | Fs 7 | 2.33 | 38.83 | 11 | Fo 1 | 0.33 | 5.50 |
| 6 | Fs 10 | 0.00 | 0.00 | L.S.D = 0.05 | | 0.225 | 2.027 |

Each No, = three repetitions - *Fs=F. solani* - *Rs = R. solani* - FO=*F. oxysporum*

*3.3. Evaluating the efficiency of chitosan and its interaction with threonine in inhibiting fungal growth on Fusarium solani on Potato Dextrose Agar (PDA) medium.*

The results (Table 3) demonstrated that the *F. solani* isolate (Fs1) was inhibited from growing when plant bio stimulants (chitosan and threonine) were added to the culture medium at all concentrations employed in the experiment. While doses of 0.5 and 1.0 g L-1 slowed the fungal colony's (Fs1) growth rate, reaching 3.08 and 1.92 cm, chitosan alone demonstrated the maximum growth inhibition rates of 100% at concentrations of 1.5 and 2.0 g L-1. The percentage of inhibition was 65.73 and 78.70%, respectively, in contrast to the 0.00% inhibition rate obtained with the fungal treatment alone. Regarding incorporating the synergistic therapy (threonine and chitosan) into the culture medium, the addition of 1.0, 1.5, and 2 g of concentration. After 0.5 g of

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concentration therapy, L-1 demonstrated a considerable drop in the pathogenic fungus' inhibition rate, which reached 100%. When compared to the control treatment, L-1 demonstrated an efficient effect in inhibiting the growth of the pathogenic fungal colony, reaching 1.58 cm with an inhibition rate of 82.40%. Along with the ability of the amino acid threonine to fend off plant diseases by producing antifungal compounds like phenols and flavonoids, chitosan's antifungal effect may be due to its effect on the fungal DNA, which inhibits the synthesis of vital enzymes and proteins (Timofeeve et al., 2022 and Ibrahim, 2016). These findings align with those of (Star, 2022, Abbas, 2022 and Al-Jabouri, 2024).

**Table3:** Effect of chitosan and interaction with threonine on inhibiting the growth of *Fusarium solani* on PDA culture medium.

| Treatment | Concentration g.L-1 | Fungus colony diameter (cm) | Inhibiting fungal growth (%) |
|---|---|---|---|
| Control | 0 | 9 * | 0.00 |
| Ch. + Fs1 | 0.5 | 3.08 | 65.73 |
| | 1.0 | 1.92 | 78.70 |
| | 1.5 | 0.00 | 100.00 |
| | 2.0 | 0.00 | 100.00 |
| Ch. + Thr + Fs1 | 0.5 | 1.58 | 82.40 |
| | 1.0 | 0.00 | 100.00 |
| | 1.5 | 0.00 | 100.00 |
| | 2.0 | 0.00 | 100.00 |
| LSD: 0.05 | | 0.122 | 1.670 |

*3.4. Testing the efficiency of chitosan and its interaction with threonine in protecting tomato plants from infection by the pathogenic fungus Fusarium solani and some plant growth parameters under woody canopy conditions.*

The percentage and intensity of infection of tomato plants with the pathogenic fungus F.s1 were decreased by treating them with a concentration of 1.5 g L-1 of the bio stimulant chitosan and then spraying the vegetative system until wet after planting. This was done in combination with threonine treatment. All treatments, including chitosan and the interaction with threonine, significantly reduced the percentage and severity of infection, according to the results (Table 4). The interaction treatment achieved the highest rate of both the percentage and severity of infection, reaching 0.00%, in contrast to the treatment of the pathogenic fungus alone, which achieved a percentage of infection of 100.00%. And a 93.33% infection severity, whereas the chitosan therapy produced 30.66% and 28.00% infection percentage and severity, respectively. The capacity of the interaction treatment (chitosan with threonine) to shield tomato roots from infection accounts for its great disease-controlling effectiveness. By altering fundamental development mechanisms, it affects harmful fungus. Chitosan is characterized as a plant defines stimulant that promotes the production of defines chemicals, such as phenols, and growth regulators in tomato root exudates (Suarez-Fernandez, 2020). It also inhibits a number of enzymes that are involved in fundamental biological processes by altering the DNA of pathogenic fungus. Amino acids also contribute to protein production, which strengthens the plant's defences against disease and works in tandem with chitosan (Younes and Rinaudo, 2015; Star, 2022; Su et al., 2022; Hazem, 2025).

Table 4 also demonstrated that, in comparison to the control treatment of the pathogenic fungus F.s1, treating tomato plants with the bio stimulant chitosan and the interaction treatment with threonine at a concentration of 1.5 g L-1 improved growth parameters, such as stem length and fresh and dry weight of the vegetative mass. The average stem length ranged from 142 cm in the chitosan treatment to 170 cm in the interaction treatment, while the average stem length in the pathogenic fungus treatment was 77 cm. In comparison to the pathogenic fungal treatment, the two study treatments also showed a considerable improvement in the average fresh and dry weight of the tomato plant's vegetative mass, as the interaction treatment attained. The highest growth parameter averages, with fresh and dried weights of the vegetative mass of 1860 and 315 g, respectively, in contrast to 820 and 135 g for the pathogenic fungal treatment. This is explained by the function of chitosan and how it interacts with threonine to protect tomato plants from stress. According to Aboelmagd (2021), chitosan is more successful in lowering the prevalence of root rot disease and improving the vegetative growth characteristics of tomato plants. In addition to improving the availability of nutrients for simple absorption, chitosan is the most appropriate compound for use in biocompatibility with macro and micronutrients and growth promoters, as it increases the efficiency of roots in absorbing vital nutrients for growth and development (Habte-Tsion et al., 2015 and Palac-Marquez et al., 2022). These results are in line with numerous studies that found that using multiple control agents together has a greater impact on lowering the rate and severity of infection with numerous pathogens. This is due to the synergistic action of these factors, which have an inhibitory effect on pathogens, as well as their role in promoting systemic resistance in plants against pathogens through the production of phytoalexins and pathogenesis-related proteins. Additionally, the production of compounds and substances, such as growth regulators, facilitates plant growth (Al-Asadi, 2020; Al-Taie, 2021; Al-Ghazali, 2022; & Al-Ghanimi, 2023.

**Table4:** Chitosan and its interaction with threonine in protecting tomato plants from infection by the pathogenic fungus *Fusarium solani* and some plant growth parameters under woody canopy conditions.

| Treatment | Rate Infection % | Severity Infection % | Plant height Cm | Fresh shoot weight gm | Dry shoot weight gm |
|---|---|---|---|---|---|
| Control (Fs1) | 100.00 * | 93.33 | 25.66 | 82.00 | 13.55 |
| Ch. + Fs1 | 30.66 | 28.00 | 48.00 | 122.67 | 25.33 |
| Ch. + Thr + Fs1 | 0.00 | 0.00 | 62.67 | 186.35 | 31.50 |
| L.s.D = 0.05 | 6.556 | 2.660 | 5.360 | 4.125 | 5-240 |

### *3.5. Testing the effectiveness of chitosan and interaction with threonine on the activity of the peroxidase enzyme in tomato plants infected with root and stem rot disease under woody canopy conditions.*

The outcomes (Figure 1) showed how the bio stimulant chitosan and its combination with threonine could effectively increase the activity of the peroxidase enzyme in tomato plants, hence causing systemic resistance against root rot disease. By raising the rate of change in light absorbance/minute/g of plant fresh weight, this was accomplished. When the peroxidase enzyme was measured as the rate of change in light absorbance per minute per gram of fresh weight, the interaction treatment had the highest levels, reaching 110.67 min $g^{-1}$ fresh weight. This contrasted with the control treatment, which recorded 40.55 min $g^{-1}$ fresh weight, and the chitosan treatment, which recorded 95.45 min $g^{-1}$ fresh weight. The high concentrations of peroxide in chitosan treatments and their interaction with threonine when F.s1 is present could be because peroxide helps plants develop resistance to a variety of diseases. The most significant process is the production of pathogenesis-related proteins, such as the peroxidase enzyme, which is found in plant tissues and plays a critical role in determining the degree of host resistance by stimulating exposure to pathogens or inducers.

It is an essential enzyme to produce lignin, sobering deposition, and cell wall biosynthesis, which increases structural defences (Jogaiah et al., 2013; Thakker et al., 2013; Ghuie et al., 2021).

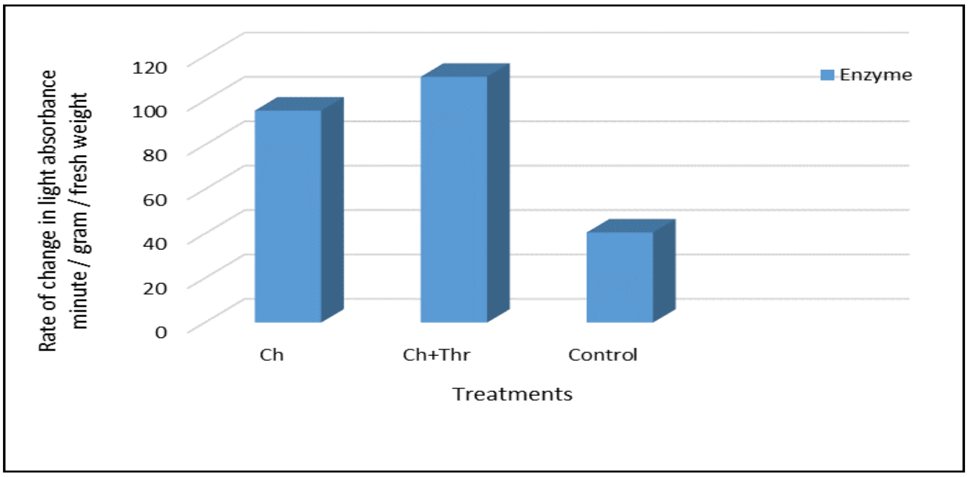


**Figure 1.** Effect of chitosan and threonine treatments on peroxidase activity (rate of change in absorbance/min/g fresh weight) in tomato plants

## 4. Conclusions

Tomato root rot has been found to be mostly caused by *Fusarium solani.* According to the findings, both chitosan and threonine treatments were better at lowering the fungus-induced infection's severity and producing the highest values for vegetative growth markers. Furthermore, due to their economic effectiveness and environmental safety, chitosan and threonine can both be employed as bio stimulants to fight illnesses

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