

## Evaluation the Effectiveness of Some Amino Acids in Resisting the Fungus *F.Oxysporum*, Causing Root Rot and Wilt of Tomato Plants

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**Abstract:** This study assessed the effectiveness of glutamic acid (G), proline (P), and tryptophan (T) against *Fusarium oxysporum*, the causal agent of root rot and wilting disease in tomato plants. Treating tomato plants with P+G and P+G+T mixtures prior to *Fusarium* inoculation significantly reduced fungal development. Disease incidence was 0.00% in both treatments, compared to 83.33% and 75.45% in untreated plants inoculated with *Fusarium*. The treatments also notably increased fresh and dry weights, chlorophyll content, and total protein content. Additionally, the treatments induced higher polyphenol oxidase activity (measured as the change in absorbance per minute per gram of fresh weight) compared to the control. These findings suggest that amino acid treatments can promote self-resistance in tomato plants against *Fusarium* infection.

**Key Words:** *Amino acids, induce resistance, tomato, F.oxysporum* .

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### INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a widely cultivated vegetable belonging to the Solanaceae. It can be grown and consumed extensively worldwide due to its high nutritional value; Tomatoes are consumed both fresh and as processed products and are considered the second most popular crop after potatoes globally (Brookie et al., 2018; Siddiqe et al., 2020).

Global tomato production faces numerous challenges, particularly from biotic factors such as plant pathogens including bacteria, fungi, nematodes, and viruses, as well as abiotic factors such as heat, water, and salinity stress, which reduce tomato growth and productivity (Singh et al., 2018; Huang, 2020). Among the most significant diseases affecting the crop and compromising its health is *Fusarium* wilt, one of the most serious and widespread diseases caused by *Fusarium oxysporum lycopersici* (FOL) (Borisade et al., 2017; Sidharthan et al., 2018). *Fusarium* wilt is a major economic disease that affects tomato plants wherever they are cultivated, whether in fields or greenhouses. The disease affects fruit quality and reduces yield by 50-60%, and can sometimes reach 100% in susceptible cultivars when soil conditions and temperatures are favorable for the pathogen throughout most of its life cycle (Bawa, 2016; Lopez-Zapata et al., 2021). Given the negative impacts of excessive use of chemical pesticides on human health and the environment, as confirmed by numerous studies, researchers have focused on developing alternative inputs to control diseases and reduce environmental pollution, such as the use of amino acids as an alternative strategy or as part of integrated pest management (Seo et al., 2016; Przemieniecki et al., 2021). Interest in amino acids for controlling plant pathogens is not recent, and the use of amino acids against fungal pathogens has shown significant and widespread progress (Nivanjan-Raj et al., 2004; Parthasarathy et al., 2021; Zhao et al., 2022).

Several studies have demonstrated the successful use of commercially produced amino acids in controlling plant pathogens. For instance, Nivanjan-Raj et al. (2004) showed that applying proline at a concentration of 50 mM effectively controlled *Sclerospora graminicola*, the causal agent of downy mildew in pearl millet. Similarly, Golo et al. (2020) found that external application of glutamate induced systemic resistance in *Arabidopsis* against pathogens. Kim et al. (2021) further demonstrated that glutamic acid can stimulate plant disease resistance. Deng et al. (2023) reported that applying amino acids at a concentration of 5 mg/ml inhibited the growth of the pathogenic fungus *Fusarium* sp. Moreover, Li et al. (2024) suggested that amino acids can enhance plant resistance to fungal diseases. Given the significance of *Fusarium* wilt in

tomato crops and the need for alternatives to chemical pesticides, this study aimed to evaluate the efficacy of certain amino acids in protecting tomato plants from the pathogen.

#### **MATERIALS AND METHODS:**

##### **Isolation and Diagnosis of the Pathogenic Fungus *Fusarium oxysporum***

The fungus *F. oxysporum* was isolated from the roots of yellowed and wilted tomato plants collected from several greenhouses in Baghdad Governorate. Infected roots were cut into small fragment (1 cm long), then surface-sterilized in 1% sodium hypochlorite solution for 2 minutes, rinsed with sterile water, and dried on sterile filter paper. Segments were then plated onto 9 cm Petri dishes containing sterilized potato dextrose agar (PDA) medium supplemented with 100 mg/L tetracycline to inhibit bacterial growth. Four segments were plated per dish. Plates were incubated at  $25 \pm 2^\circ\text{C}$  for three days. After incubation, the plates were examined for fungal growth. Pure cultures were obtained by transferring a small piece of the fungal hyphae to the center of a new PDA plate. Isolates were identified after 4 days based on colony morphology, hypha characteristics, spore types, and structures formed, using standard taxonomic keys (Booth, 1977; Rezaee et al., 2018).

##### **Pathogenicity Test of *Fusarium oxysporum* Isolates in Pots**

A study was conducted to evaluate the pathogenicity of nine *Fusarium oxysporum* isolates using a completely randomized design (CRD) in a greenhouse at the Plant Protection Department, College of Agricultural Engineering, University of Baghdad. A fungal inoculum, prepared by coating millet seeds with the fungus, was added to autoclaved soil mixture for 20 minutes. The soil was autoclaved twice with a 24-hour interval. The inoculated soil was then filled into 2 kg plastic pots at a rate of 1% (w/w). The pots were watered, covered with perforated polyethylene bags, and left for 3 days. Subsequently, seeds of a local tomato cultivar, surface-sterilized with 1% sodium hypochlorite solution, were sown at a rate of 10 seeds per pot. The control treatment involved sowing seeds in sterilized soil mixed with sterilized millet at a rate of 1% (w/w), following the same procedure. Germination percentage was calculated after 8 days, and continued until the germination of the control treatment was complete. The germination percentage was calculated using the following formula:

$$\% \text{ Germination} = (\text{No. germinated seed} / \text{No. All seeds}) * 100$$

Due to the high virulence of isolate FOL4, it was selected for all subsequent experiments.

##### **Investigation of the Inhibitory Effect of Certain Amino Acids on the Growth of *Fusarium oxysporum* In Vitro**

The effect of the amino acids glutamic acid, proline, and tryptophan on the growth of the pathogenic fungus *Fusarium oxysporum* was investigated. Three concentrations of each amino acid (500, 1000, and 1500 mg/L) were sterilized by filtration through a  $0.45\mu\text{m}$  Millipore membrane filter. These concentrations were then added to Potato Dextrose Agar (PDA) medium using a sterile pipette and mixed thoroughly. Sterile Petri dishes (9 cm diameter) containing the amended PDA medium were inoculated at the center with a 0.5 cm disc taken from the edge of a 5-day-old *F. oxysporum* (FOL4) colony grown on PDA. Each treatment was repeated 3 times. A control treatment containing PDA without amino acids was also included. The petri dishes were incubated at  $25 \pm 2^\circ\text{C}$ . The inhibition of fungal growth was calculated using the following formula

$$\text{Inhibition} = \frac{\text{mean of control diameter} - \text{mean of treatment diameter}}{\text{mean of control diameter}} * 100$$

### Evaluation of the Toxicity of Beltanol Fungicide against *Fusarium oxysporum* on PDA Medium

The toxicity of the commercial fungicide Beltanol, produced by Probelte (Spain) and containing 50% chinisol as the active ingredient, was evaluated against the pathogenic fungus *Fusarium oxysporum*. Different concentrations of the fungicide (500, 1000, 1500, and 2000 mg/L, based on the active ingredient) were added to Potato Dextrose Agar (PDA) medium. The treated PDA was poured into sterile Petri dishes (9 cm diameter). A 0.5 cm disc from a 5-day-old colony of *F. oxysporum* was placed at the center of each plate. Three replicates were performed for each concentration. A control treatment containing PDA without the fungicide was included. The plates were incubated at  $25 \pm 2^\circ\text{C}$ . The final results were recorded by measuring the diameter of the fungal colonies in two perpendicular directions. After 5 days, the percentage of fungal inhibition was calculated.

### Evaluation of the Efficacy of Induced Systemic Resistance Inducers in Protecting Tomato Plants from *Fusarium oxysporum* Infection and Their Impact on Yield Traits

This experiment was conducted under greenhouse conditions at the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad. Sterilized plastic pots were treated with 1% sodium hypochlorite solution for 5 minutes, then filled with 3 kg of sterilized soil mixture. The sterilization process was repeated twice with a 24-hour interval. A completely randomized design (CRD) was used, with 17 treatments and 3 replicates per treatment. Data were analyzed using the Least Significant Difference (LSD) test at a 5% probability level. The treatments included

Disease severity measured depending on disease index below

0 = not infected

1 = yellowing 1-25 %

2 = yellowing and wilt 26-50%

3 = yellowing and wilt 51-75%

4 = wilt all plant

$$\text{Disease severity (\%)} = \frac{\text{No. of plants in class}(0 \times 0) + \dots + \text{No. of plants in class}(4 \times 4)}{\text{Total no of All examined plants} \times \text{highest class}} \times 100.$$

Total protein plants was determined in the leaves after 50 days of inoculation with the pathogen. The leaves were collected from each replicate from different treatments separately. The leaver was washed with water, and left to dry by placing them in paper bags in an electric oven at a temperature of  $50^\circ\text{C}$ , for 48 hours. The samples were ground using an electric grinder. 0.2 g from each sample taken and digested using sulfuric acid, and perchloric acid, according to the method of (Tkachuk,1977), The percentage of total nitrogen estimated using the Microkeldahl equipment, and by multiplying the percentage of nitrogen by the conversion factor (6.25). The percentages of total protein obtained in the leaves according to the method followed by (Cresser and Parsons ,1979).

Chlorophyll content in the leaves of plants from different treatments was measured using a SPAD meter. To assess some biochemical properties related to inducing resistance in tomato plants against the pathogen FOL; Samples were taken from leaves of plants treated with the aforementioned factors, which are believed

to induce plant resistance to the disease. The activity of the PPO enzyme in the leaves was estimated according to the method of Ohja and Chatterjee (2012) after 7 and 14 days of inoculating the pots with the pathogen. Fresh and dry weights of the plants were also measured after uprooting and washing the root system with running water to remove adhering soil. The plants were then placed in paper bags and dried in an electric oven at 60°C for 48 hours."

## RESULTS AND DISCUSSION:

### Isolation and Identification of the Pathogen

Isolation and identification results revealed the presence of nine isolates of *Fusarium oxysporum*, as shown in Figure 1. These isolates varied in growth rate and mycelial density on PDA medium. They exhibited a color range from cottony white to dark pink or purple and possessed three main types of asexual reproductive structures. Firstly, single-celled microconidia, usually produced on short solitary conidiophores, were characterized by their cylindrical to oval or kidney-shaped appearance. They often formed false heads on short solitary conidiophores (monophialides). Secondly, macroconidia were multicellular (3-6 cells), spindle-shaped with pointed ends, and sometimes curved. They were produced on branched conidiophores that aggregated or coalesced to form sporodochia. Thirdly, chlamydospores were produced singly or in pairs in short lateral branches or within the mycelium, sometimes forming chains. These thick-walled or thin-walled chlamydospores served as survival structures under adverse conditions. (Booth, 1977; Leslie and Summerell, 2006; Soleha et al., 2022).

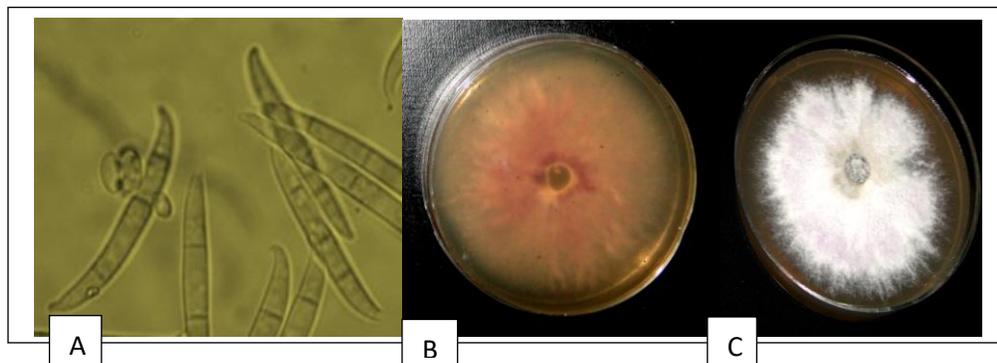


Figure 1: Morphological characteristics of *F.oxysporum*.

**A: Macroconidia under microscope B: Pigment produced by a fungus isolate C: The nature and form of fungal growth.**

### Effect of *F. oxysporum* on Tomato Seed Germination in Pots

All nine *F. oxysporum* isolates were pathogenic, causing a significant reduction in tomato seed germination compared to the control treatment (without the fungus), which had a 100% germination rate (Table1). Isolate FOL4 significantly reduced germination to only 3.3%. Although all tested isolates were capable of causing damping-off in tomato seedlings, with a variation in their virulence. These findings align with previous studies that have reported varying degrees of pathogenicity among *F. oxysporum* isolates. The high disease incidence could be attributed to the extensive growth of fungal mycelium within the vascular tissues, obstructing water and nutrient transport to the leaves, and the production of toxins that degrade plant cell walls.

These isolates also had a severe impact on plant growth compared to the control treatment, likely due to the presence of fungal mycelium in the xylem vessels, leading to disease development as a result of vessel

blockage. Additionally, the fungus can induce the accumulation of plant growth regulators such as ethylene and abscisic acid, while decreasing cytokinin levels. These disruptions in plant growth regulators can lead to disturbances in other metabolic processes in infected plant cells (Ortiz, 2014), resulting in reduced shoot and root growth. Furthermore, the fungus secretes various cell wall-degrading enzymes, increasing the likelihood of vessel blockage, negatively affecting photosynthesis, and subsequently reducing plant vigor. Moreover, the fungus produces toxins such as fusaric acid, which reduces cellular respiration rates in plant cells, causing disruptions in other vital plant processes and leading to impaired growth and vigor (Bryla et al., 2022; Wore, 2023).

**Table 1: Pathogenicity test of *Fusarium oxysporum* isolates on tomato seeds in vivo**

Isolate Code	% Germination
FOL1	43.3
FOL2	53.3
FOL3	23.3
FOL4	3.3
FOL5	43.3
FOL6	26.7
FOL7	33.3
FOL8	13.3
FOL9	6.7
Control	100.0
L.S.D <sub>0.05</sub>	9.33

- Each value in the table represents the mean of three replicates.

#### **In Vitro Assessment of the Effect of Amino Acids on the Growth of the Pathogen *F. oxysporum***

The results in (Table 2) demonstrated significant differences in the inhibition rates of the pathogen when treated with the studied concentrations of proline, glutamate, and tryptophan amino acids compared to the control treatment (0.0%). The results showed that proline, at all three concentrations of 500, 1000, and 1500 mg/L, was most effective in inhibiting the growth of the pathogen, achieving inhibition rates of 51.11%, 100%, and 100%, respectively. Glutamic acid, on the other hand, achieved lower but still significant inhibition rates of 19.26%, 44.45%, and 86.67% at the same concentrations. Tryptophan showed inhibition rates of 22.22%, 40.37%, and 88.89% at concentrations of 500, 1000, and 1500 mg/L, respectively.

The results also indicated a direct relationship between the tested concentrations and the inhibition rates of the pathogen's growth, with higher concentrations leading to greater inhibition. Proline at a concentration of 1000 mg/L achieved complete inhibition (100%). These results support the findings of Raj et al. (2004) and Jastrzebowski and Gabriel (2015), who reported the inhibitory effects of proline against fungi on PDA

medium. The superior effect of proline compared to glutamate and tryptophan in inhibiting fungal growth can be attributed to its direct action as an inhibitor of vital processes necessary for pathogen growth, thus hindering its growth and leading to its death.

Amino acids can also induce the production of metabolites that scavenge reactive oxygen species, acting as antioxidants and damaging fungal hyphae and new growth, thereby preventing fungal spread. Numerous studies have demonstrated the effectiveness of amino acids in inhibiting the growth of pathogens (Zhao et al., 1998; Jastrzebowski and Gabriel, 2015; Parthasarathy et al., 2021).

**Table 2: Effect of Amino Acids on the Growth of the Pathogenic Fungus in PDA Medium**

Acids	Concentration (mg/L)	Average colony diameter (cm)	% Inhibition
Proline	0	9.00	0.00
	500	4.40	51.11
	1000	0.00	100.00
	1500	0.00	100.00
Tryptophane	0	9.00	0.00
	500	7.00	22.22
	1000	5.36	40.37
	1500	1.00	88.89
Glutamate	0	9.00	0.00
	500	7.26	19.26
	1000	5.00	44.45
	1500	1.20	86.67
L.S.D. <sub>0.05</sub>	Acids: 0.42 Concentrations:0.48 Interaction:0.84		Acids: 4.69 Concentrations:5.42 Interaction:9.39

- Each value in the table represents the mean of three replicates.

### Evaluation of the Toxic Effect of Beltanol Fungicide against the Pathogenic Fungus *F. oxysporum* on PDA Medium

The results of this test showed that the use of Beltanol fungicide at concentrations of 0, 500, 1000, 1500, and 2000 micrograms per liter inhibited the growth of the pathogenic fungus, but with varying degrees. The concentration of 2000 micrograms per liter gave an inhibition rate of 100% compared to the control treatment without the addition of the fungicide to the PDA medium, which had an inhibition rate of 0% (Table 3). This result confirms the high ability of this fungicide to control pathogens, through its mode of action. The active ingredient, Chinosol, binds with heavy metals and forms a complex that is difficult for the pathogen to absorb. Or, the fungicide forms chelate compounds with copper in the host tissues, which facilitates its passage into the pathogen's cells, where it is then released and kills the pathogen, as indicated by Meister (2000). This result is consistent with the findings of Radhi (Al-Mayahi, 2020), who obtained an inhibition rate of 100% when using Beltanol fungicide at a concentration of 1500 micrograms per liter against the pathogenic fungus *F. solani*.

These results are in line with several studies and research that indicated the ability of this fungicide to control pathogens, especially soilborne pathogens. Treatment with Beltanol at a concentration of 1-1.5 ml/L led to 100% inhibition of mycelial growth of *F. solani*, *R. solani*, and *Macrophomina phaseolina* isolates compared to the control treatment (Matloub, 2012; Mohsen and Al-Kaabi, 2015; Radhi, 2017).

**Table 3: Effect of Beltanol Fungicide on the Pathogenic Fungus *F. oxysporum***

Concentrations (µg/L)	Average colony diameter (cm)	%Inhibition
0	9.00	0.00
500	6.80	24.44
1000	4.43	50.74
1500	1.10	87.78
2000	0.00	100.00
L.S.D. <sub>.0.05</sub>	0.47	5.29

- Each value in the table represents the mean of three replicates

### Effect of Some Amino Acids on Reducing the Incidence and Severity of Tomato Root Rot and Fusarium Wilt under Pot Conditions

All the amino acids (Proline, Tryptophan, and Glutamate), when used individually or in combination in treatments inoculated with the pathogenic fungus, had an effect in reducing the incidence and severity of the disease compared to the control treatment inoculated with the fungus alone (Table 4). The combination treatment of Proline, Tryptophan, and Glutamate achieved complete prevention of infection by the pathogenic fungus, with an incidence and severity of 0.00% for both, compared to the control treatment

which reached 83.33% and 73.43%, respectively, but this was not significantly different from the combination treatment of Proline and Tryptophan, the combination treatment of Proline and Glutamate, and the combination treatment of Tryptophan and Glutamate, which reached 0.00%, 0.00%, and 3.33%, 2.13%, and 6.67%, 3.87%, respectively. The other treatments followed. The combination treatment of Proline, Tryptophan, and Glutamate with the pathogen outperformed the rest of the treatments by achieving the highest increase in the average fresh and dry weight, which reached 7.76 and 3.40 g/plant, respectively (Figure 2), followed by the other treatments. Many studies have indicated that these acids possess high efficacy in combating many plant pathogens (Raj et al.,2004 ; Deng et al.2023).The role of Proline in resisting the pathogen is through reducing the destructive oxidation effects on metabolic activities resulting from the influence of pathogens (Kim et al,2021; Parthasarathy et al,2021). Qu et al. (2020) indicated that Proline works to increase the effectiveness of anti-oxidation enzymes in the plant, such as Peroxidase and Catalase. The mechanism of adding Proline in combating pathogens has been explained by improving plant growth as a result of inducing the activity of Chitinase, Glucanase, and other enzymes in what is known as induced resistance. This makes the plants grow well and then increases their ability to benefit from the nutrients in the soil and their ability to achieve high resistance to diseases and pests.

The role of Proline, Tryptophan, and Glutamate in reducing the incidence and severity of infection with the pathogenic fungus is attributed to their effective role in protecting tomato plants by raising the plants' self-resistance to infection with the pathogenic fungus by increasing the plants' content of nutrients such as Fe<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, B<sup>3+</sup>, Cu, N, P, K and stimulating plant defense enzymes, as well as encouraging the growth and reproduction of beneficial and desirable microorganisms such as yeasts and algae in the soil solution, which is positively reflected in increasing the resistance of tomato plants to the pathogen ( Parthasarathy et al.,2021; Zhao et al.,2022).

While Proline works to increase the efficiency of roots in absorbing nutrients, in addition to increasing photosynthesis (Kim et al.,2021). The positive effect of Tryptophan may be explained by its role in increasing the concentration of nitrogen in the leaves or its role in increasing the concentration of chlorophyll by providing the nutrients involved in its composition or direct participation in its synthesis, and also its role in preserving the chlorophyll formed in the leaves from oxidation. Or the reason may be due to the superiority of plants treated with Glutamate in increasing the dry weight of the root and shoot systems because it has a role similar to growth regulators Qiu et al.,2020; Zhao et al.,2022), in addition to its role in reducing heat stress and stimulating respiration and cell division processes and enters into the electron transport system and protects chloroplasts from oxidation (Deng et al.,2023), as it also works to increase the efficiency of photosynthesis.

N.	Treatment	% Disease Incidence	% Disease severity
1	Control	0.00	0.00
2	<i>F.oxysporum</i> (FO)	83.33	73.43
3	Prolin(P)	0.00	0.00
4	Glutamate(G)	0.00	0.00
5	Tryptophan(T)	0.00	0.00
6	P+G	0.00	0.00

7	P+T	0.00	0.00
8	G+T	0.00	0.00
9	P+G+T	0.00	0.00
10	P+FO	13.33	8.87
11	G+FO	23.33	13.37
12	T+FO	26.67	16.77
13	P+G+FO	0.00	0.00
14	P+T+FO	3.33	2.13
15	G+T+FO	6.67	3.87
16	P+T+G+FO	0.00	0.00
17	Beltanol+FO	33.33	27.33
	L.S.D <sub>0.05</sub>	6.14	2.99

- Each value in the table represents the mean of three replicates

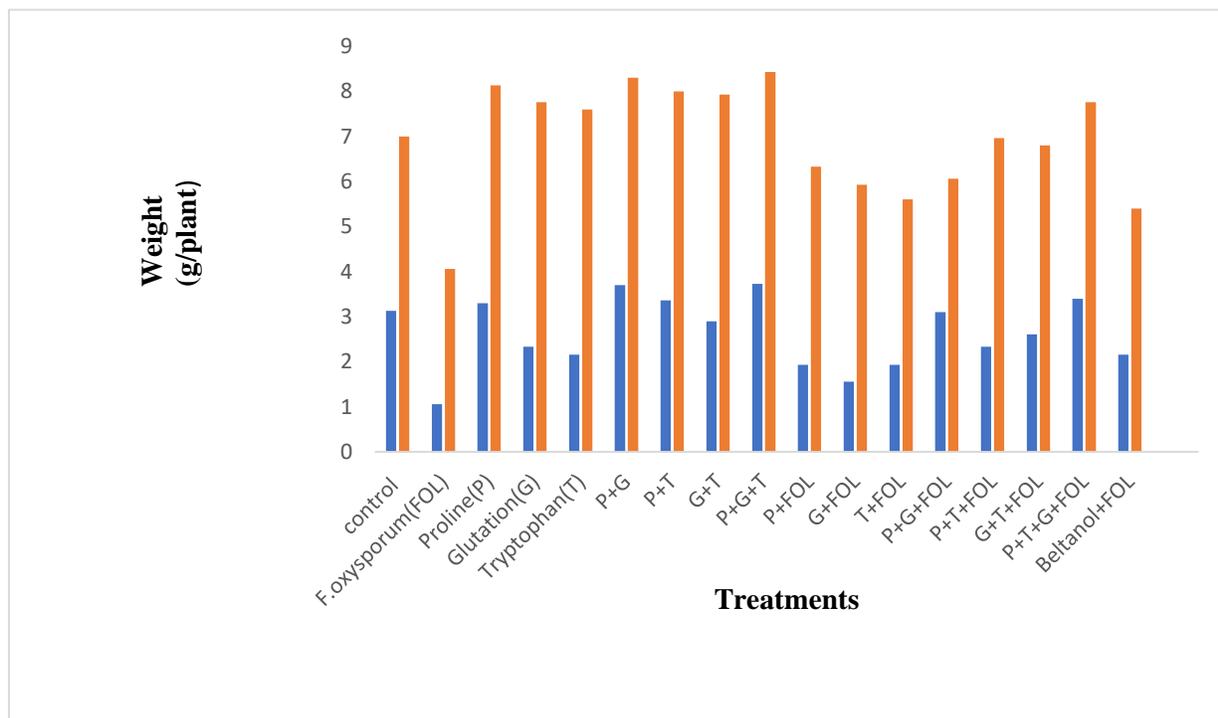


Figure 2: Effect of some amino acids on some growth characteristics of tomato plants infected with *F.oxysporum*

L.S.D<sub>0.05</sub> for Fresh Weight: 0.5, and L.S.D<sub>0.05</sub> for Dry Weight: 0.30

The effect of the treatments on the average percentage of protein in tomato plants was statistically significant. All treatments showed a significant increase in the average percentage of protein (Figure 3) compared with the two control treatments (pathogenic fungus only) and (without pathogenic fungus), which were 13.37 and 6.40%, respectively. The highest percentage of protein was in the combination treatment of Proline, Tryptophan, and Glutamate with the fungus, as it was 24.67%, which was not significantly different from the combination treatment of Proline and Tryptophan, which was 23.33%, followed by the other treatments. It is observed from the results above that the effect of Proline was clear, and this is due to the increase in chlorophyll content in the leaves of the tomato plants treated with Proline (Figure 4), which is attributed to its role in increasing the concentration of nitrogen in the leaves, which is involved in the composition of chlorophyll, or it plays a role in increasing the concentration of chlorophyll by providing the nutrients involved in its composition or its direct participation in its synthesis, as well as its important role in preserving the chlorophyll formed in the leaves from oxidation as an antioxidant (Deng et al.,2023). As for the role of Proline, Tryptophan, and Glutamate in increasing the plant's content of amino acids and sugars and their positive effect on the synthesis of chlorophyll pigment and preventing its decomposition. This result is consistent with what was mentioned by (Kim et al.,2021), who confirmed the role of amino acids in increasing the amount of total chlorophyll in treated plants, increasing the amount of amino acids and sugars, as well as their role in increasing the activity and concentration of a number of plant enzymes, which increases the speed of metabolic reactions within the plant, in addition to their great importance in the positive impact on photosynthetic pigments and the amount of total soluble amino acids and sugars and carbohydrates, and this in turn works to increase plant height, number of leaves and the amount of yield. There were significant differences in the activity rate of the enzyme (PPO) (Polyphenol Oxidase) estimated on the basis of the rate of change in optical absorption/minute/g fresh weight in the tomato plant. All factors could induced systemic resistance in treated plants when the activity of the enzyme PPO increased, compared to the control treatment (pathogenic fungi only) (Figure 5). The highest enzyme activity scored 66.56 rate of change in absorption / minute / g fresh weight, 7 days after adding the fungal inoculum in the treatment of a pathogenic fungus, followed by other treatments. The mixture FOL +P +G + T scored the highest 68.63 (rate of change in absorption / minute / g fresh weight) activity, 14 days post treatment, followed by other treatments.

Similarly amino acids agents inhibited pathogen enzymes by suppressing the action of pathogen enzymes responsible for the plant's pathogenic ability, which include enzymes that degrade the plant host's cell walls and cause infection (Zhao et al.,2022).

The PPO activity in the plant defense mechanism is triggered by plant pathogens. This enzyme one of compounds that stimulate the formation of mechanical defenses in plants through increasing the thickness of the plant cell wall resulted from the deposition and polymerization of infection-related proteins, lignin and suberin. The cell wall, therefore, can resist the penetration of pathogens (Deng et al.,2023). The PPO enzyme prevents pathogens from attacking the plant through oxidizing the natural phenols present in the plant tissues to produce quinones, initiating a series of polymerization reactions and resulting in the production of melanins and the formation a hypersensitive reaction by stimulating plant resistance against pathogenic fungi (Parthasarathy et al.,2021; Deng et al.,2023).

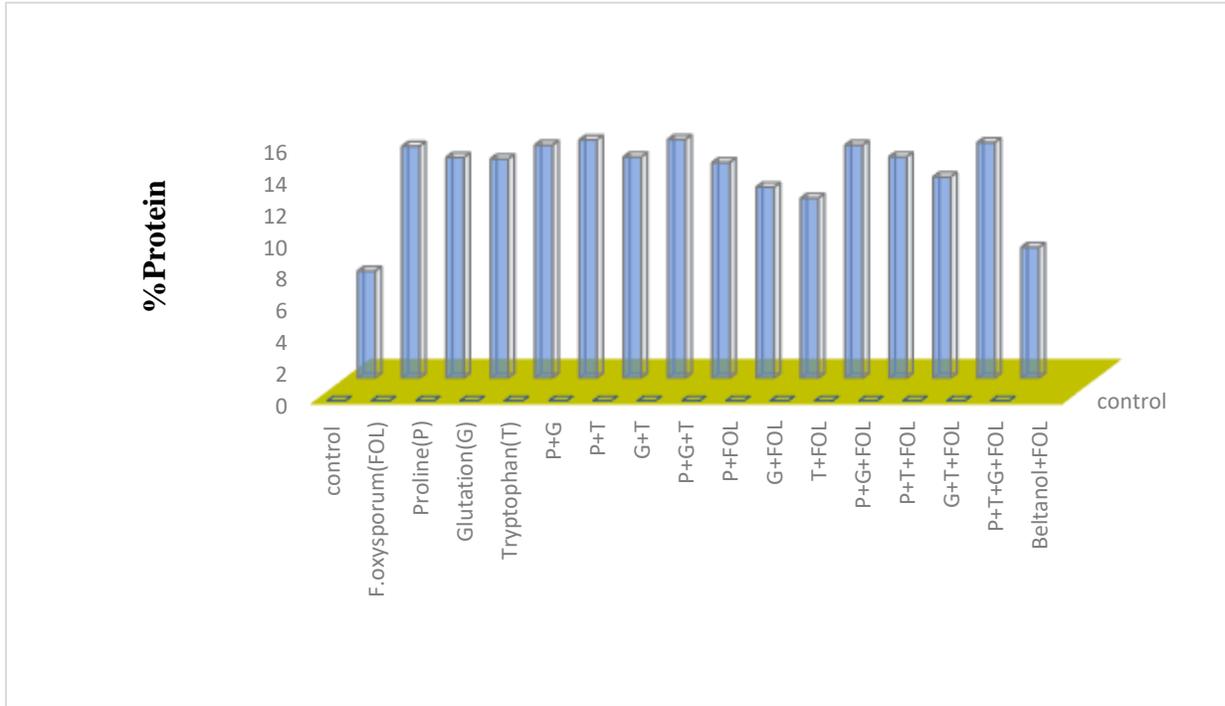


Figure 3: Effect of some amino acids on total protein .L.S.D<sub>0.05</sub>: 0.40

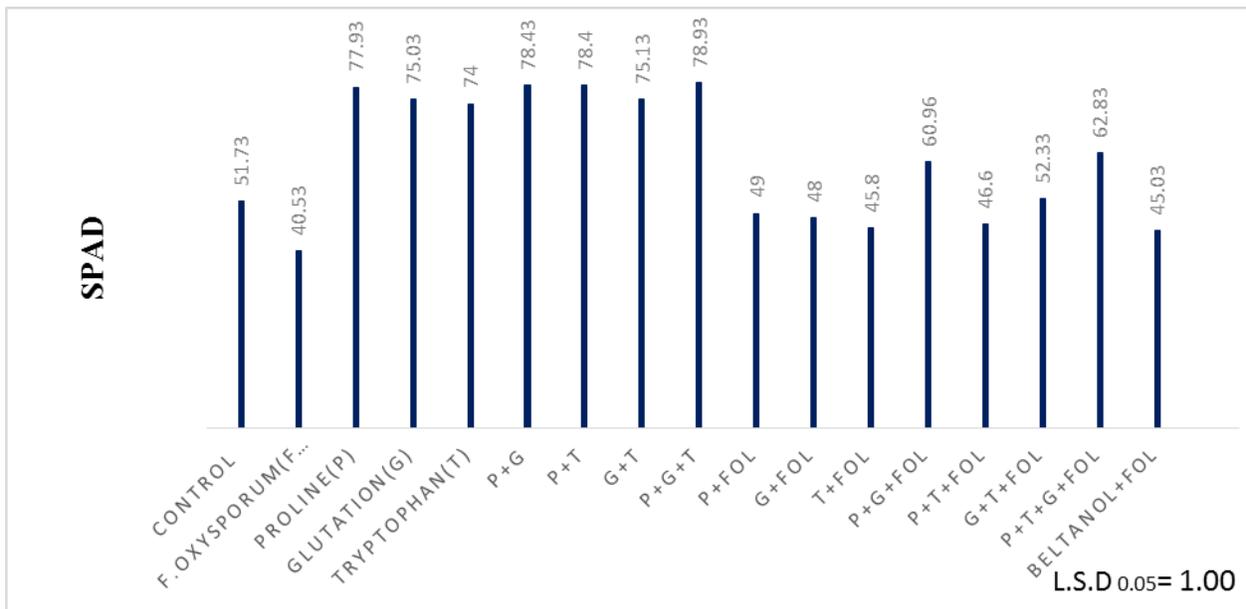


Figure 4: Effect of Amino acids on Chlorophyll Content in Tomato Plants under greenhouse conditions

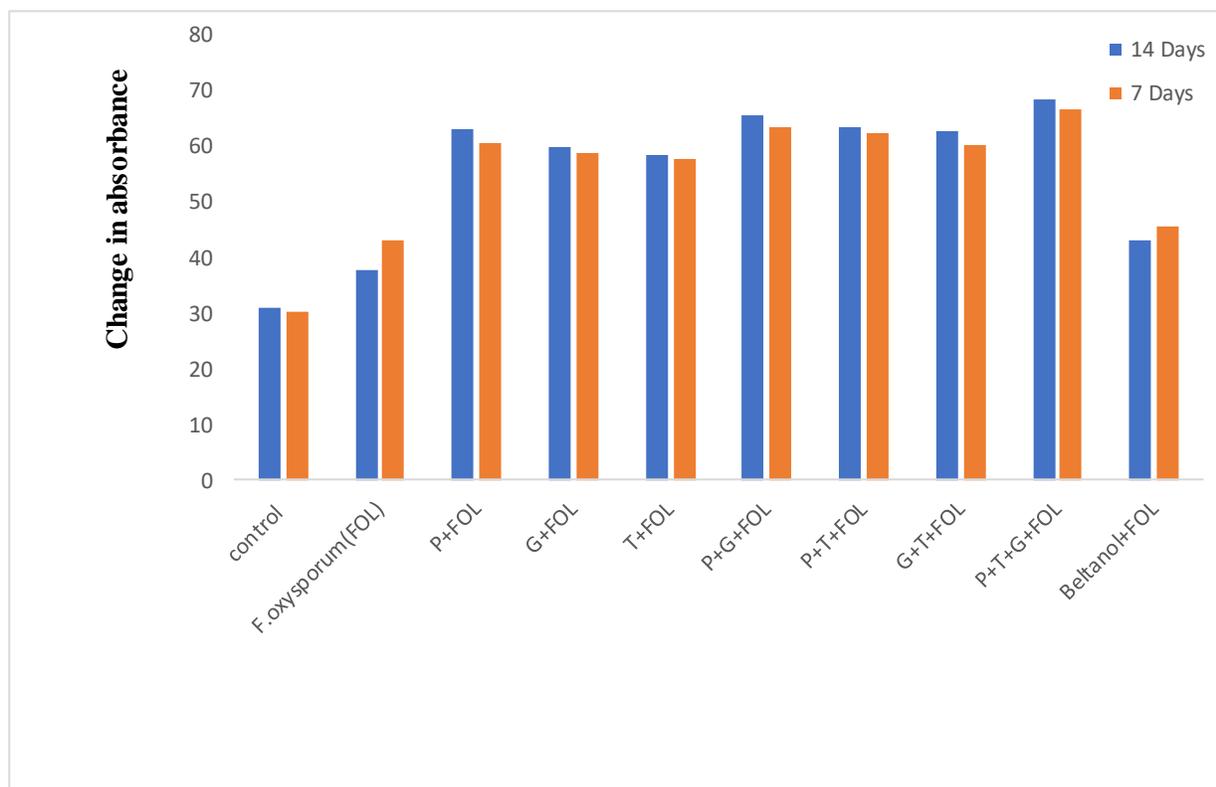


Figure 5: The activity of the poly phenol oxidase enzyme (PPO) (Change in absorbance  $\text{min}^{-1}\text{g}^{-1}$  fresh tissue weight after 7 and 14 days of adding the pathogen to the different treatment.  $\text{LSD}_{0.05}$  For 7 Days: 0.85, and  $\text{LSD}_{0.05}$  For 14 Days :0.70 .

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