Evaluation of the efficacy of β-glucanase and the biopharmaceutical Novo-Treat in the integrated control of Verticillium wilt disease caused by *Verticillium dahliae* on olive seedlings

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Abstract

This study was conducted in the laboratories of the College of Agriculture and the research station of the Plant Protection Department - Tikrit University, which aimed to evaluate the effectiveness of the beta-glucanase and the biopharmaceutical Novo-treat in the integrated control against Verticillium wilt disease on olive seedlings for the 1/10/2025-1/5/2026 season. The initial diagnosis results of the fungi isolated from olive seedlings that showed symptoms of wilt and yellowing infection in Salah Al-Din province showed that the causative agent of the disease is the fungus *Verticillium dahlia*, which was molecularly identified with 100% conformity to the fungal strains registered in the NCBI gene bank, and the isolate was deposited under accession number PX682723.1. Field application results showed that the treatment (β-glucanase + Novo-treat + Beltanol) for the Nabali olive variety resulted in the highest peroxidase activity, reaching 2.71 units/ml, compared to the lowest activity of 0.12 units/ml in the control treatment for the Ashrasi and Ba'ashiqi varieties. The Nabali olive variety also recorded the highest polyphenol oxidase activity, reaching 2.45 units/ml, compared to the lowest activity of 2.37 units/ml in the Ba'ashiqi control treatment. Interaction results showed that the treatment (β-glucanase + Novo-treat + Beltanol) for the Nabali olive variety resulted in the lowest infection severity of 0.11, compared to the highest infection severity of 0.95 in the pathogen treatment for the Ba'ashiqi variety. The same treatment also showed the highest plant height, number of branches and leaves, leaf area, chlorophyll, and total dry weight for the Nabali olive variety, reaching 72.84 cm, 13.96 branches, 131.05 leaves, 7.45 cm^2, 33.27 spad., and 24.46 g, respectively. This contrasts with the lowest indicators in the pathogenic fungus-only treatment for the Ba'ashiqi variety, which reached 37.97 cm, 4.74 branches, 3.22 leaves, 3.3 cm^2, 15.58 spad., and 7.5 g, respectively.

Keywords: Verticillium wilt, *Verticillium dahliae* fungus, beta-glucanase enzyme, Novo-Treat biopharmaceutical, integrated pest management, olive.

Introduction

The olive tree (*Olea europaea*) is a perennial evergreen belonging to the olive family, Oleaceae. The importance of the olive fruit lies in its high calorie content and nutritional value. It contains a high

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percentage of vitamins and minerals, a high percentage of monounsaturated fatty acids, and phenolic compounds with antioxidant, blood pressure-lowering, anti-atherosclerotic, anti-inflammatory, blood sugar-lowering, and cholesterol-lowering properties (30). Olive trees are susceptible to numerous pathogens during their growth stages, causing severe damage or even death. *Verticillium dahliae*, the fungus causing Verticillium wilt, is a Ascomycetetous species that inflicts significant economic losses due to its very wide host range, encompassing over 300–400 plant species. Its sclerotia can remain viable for up to 14 years in the absence of a host or under unfavorable conditions (25). The severity of this disease lies in its significant impact on olive tree productivity, with recorded losses ranged 75–89% (16). The disease also negatively affects the quality of olive oil, particularly the volatile compounds and phenols responsible for its distinctive flavor and aroma, thus reducing its market value (15).

One of the most important enzymes inhibiting fungal growth is beta-1,3-glucanase, which hydrolyzes the 1,3-β-D-glucosidic bonds in beta-1, 3-glucan, a key component of the fungal cell wall. Beta-1,3-glucanase is a type of pathogenic protein that functions in two ways: first, by hydrolyzing beta-1,3-glucan, a major structural component of fungal cell walls; and second, by stimulating the release of immune stimuli associated with the cell wall, which in turn trigger defensive responses (40). Novo-Treat is a biopharmaceutical used to treat soilborne diseases, stimulate plant growth, and increase resistance against fungal and bacterial pests. This is due to its content of 24 natural antibiotics that act as stimulants at multiple sites (1).

Given the importance of olive trees and to develop some solutions to combat Verticillium wilt disease, the current study aimed to isolate and identify the pathogen *V. dahlia* from infected olive seedlings and evaluate the effectiveness of beta-1,3-glucanase in conjunction with the organic preparation Novo-Treat and the pesticide Beltanol in inhibiting the pathogen at the applied level.

Materials and Methods

Isolation and Molecular Identification of the Pathogenic Fungus *Verticillium dahliae*

After isolating *Verticillium dahliae* from the roots of an olive seedling exhibiting symptoms of wilting, yellowing, and leaf drop, molecular diagnosis was performed using nucleotide sequencing of the ITS region. A 3-day-old fungal colony (250 mg) was used. Genomic DNA was extracted using the ZR Fungal/Bacterial/Yeast DNA Mini Prep™ kit, provided by ZR USA. The ITS region was amplified using polymerase chain reaction (PCR) with the following primer pairs: ITS1 as a forward primer 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4 as a reverse primer 5′-TCCTCCGCTTATTGATATGC-3′, according to (38). The primers were provided by Integrated DNA Technologies, Canada. The polymerase chain reaction (PCR) was performed using a 25 µL volume of PCR with 1.5 µL of DNA, 5 µL of Taq PCR PreMix, and 10 pc/mL (1 µL) of each primer, then rounded up to 25 µL with distilled water. The reaction program consisted of initial denaturation of the strands at 95°C for 5 minutes, 37 cycles, each including: denaturation of the strands at 95°C for 45 seconds, annealing at 95°C for

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45 seconds, followed by annealing at 58°C for 45 seconds, and an elongation at 72°C for 45 seconds. Then the program was finalized with an elongation at 72°C for 7 minutes. A thermochromic PCR system (Applied Biosystems Gene-amp PCR System 9700) was used for amplification. The polymerase chain reaction (PCR) product was run using electrophoresis on agarose gel (1.5%), and the PCR product beams were then visualized using ultraviolet light at a wavelength of (302 nm) after treatment with Korean Intron red dye. The nucleotide sequences of the amplified gene were determined by polymerase chain reaction (PCR) after amplification of the ITS region. The PCR products were sent to Sequencing to the Korean Biotechnology Laboratory (Applied Biosystems 3730XL, DNA sequencing machine). The results were compared using an online software program (Base Local Alignment Search Tool - BLAST). BLAST matched the nucleotide sequences of the fungal isolate with those of strains registered in the National Center for Biotechnology Information (NCBI) database. The sequences of fungal isolate was deposited at the NCBI database, and a phylogenetic tree was constructed to confirm the close relationship of the local isolate to its global counterparts.

Field Application

This experiment was conducted during the spring season on October 1, 2025, at the Plant Protection Department's research station using a completely randomized design (RCD). The field soil was cleaned, plowed, and harrowed. The soil was sterilized with 5% formalin (37%) and then covered with plastic film for 10 days. After that, the film was removed, and the soil was aerated for 3 days to allow the formalin to evaporate. The soil was then packed into pots at a rate of 4 kg/pot.

Transactions of the Experiment

Factor 1: Three olive varieties (Ashrasi, Ba'ashiqi, and Nabali)

Factor 2: The experiment included the following combinations:

Control (Healthy Plants)

Pathogenic Fungus (V.d) *V. dahlia*

(V.d) + β-glucanase

(V.d) + Novo-treat

(V.d) + Beltanol

(V.d) + β-glucanase + Novo-treat

(V.d) + β-glucanase + Beltanol

(V.d) + β-glucanase + Novo-treat + Beltanol

The Transactions were distributed with 8 combinations per variety (3 varieties) with 6 replicates per treatment, so that the number of experimental units became 144 units. The fungal pathogen spore suspension was added to the Transactions that included it at a rate of 100 ml (10^8 spores/ml) per pot. Also, the enzyme purified from the fungus *Trichoderma longibrachiatum*, which was obtained from the laboratories of the Plant Protection Department - College of Agriculture - Tikrit University, and the preparation Novo-Treat (which was prepared from the local markets and is produced by Atoms Canada) were added at a rate of 100 ml (5% concentration) per pot for the Transactions that included it. Also, the pesticide Beltanol was added at a rate of 100 ml (0.2% concentration). As for the combinations, they were added in a total volume of 100 ml prepared from 50 ml + 50

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ml of each substance for the binary combinations and 33.33 ml of each substance for the ternary combinations.

Studied Characteristics

Infection Severity Assessment

According to equation (20), infection severity was calculated for each treatment based on the appearance of the infection and the pathological index developed in this study.

Pathological Index

Based on the recorded symptoms of the disease patterns, the following pathological index was developed in this study:

0: Healthy plants (no disease symptoms)

1: Yellowing and wilting of less than 25% of the plant

2: Yellowing and wilting of 25-50% of the plant + 25-50% leaf drop

3: Yellowing and wilting of 50-75% of the plant + 50-75% leaf drop

4: Complete yellowing and wilting followed by complete leaf drop + plant death

Estimation of Some Systemic Plant Resistance Indicators

Preparation of Root Enzyme Extract

One gram of roots was taken for each treatment, washed thoroughly, cut into small pieces, and crushed in a ceramic mortar placed in an ice bath. 5 ml of phosphate binder solution (pH 6) was filtered through filter paper, then placed in tubes and centrifuged in a refrigerated centrifuge (4°C) at 10,000 rpm for 5 minutes to separate the filtrate from the precipitate. The plant filtrate, representing the crude enzyme, was then collected.

Peroxidase enzyme estimation

Peroxidase enzyme was determined according to the method of (13) by measuring the absorbance at 470 nm of the reaction mixture consisting of 2.5 ml of hydrogen peroxide solution ($H_2 O_2$) and 0.1 ml of the enzyme extract for each treatment. The enzyme unit was defined as the change in absorbance of 0.01 nm per minute.

Polyphenol oxidase enzyme estimation

Polyphenol oxidase enzyme was determined according to the method of (19) by measuring the absorbance at 470 nm. The reaction mixture consisting of 2.5 ml of Chatechol solution and 0.1 ml of enzyme extract for each treatment, and the enzyme unit was defined as the change in absorbance of 0.01 per minute.

Vegetative markers

Estimation of Dry Vegetative Weights

Samples were randomly taken from the field from the replicates. The plants were then cleaned and washed with water to remove any soil residue from the roots. The weights of the plants (wet) were measured, and then the plants were dried in the sun until the weight stabilized. The dry weight (in grams) was then calculated using a sensitive balance.

Estimation of Chlorophyll

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The chlorophyll content in the leaves was estimated using a chlorophyll meter. Three plants were randomly selected from each replicate, and the leaves were placed in the meter and automatically punctured to ensure that they could not be reused in subsequent readings.

Leaf Area

According to the equation (24), leaf area was estimated by measuring leaf length and widest leaf width:

$A = 0.68 \times L \times W$

Where:

A = Leaf Area (cm^2)

L = Leaf Length (cm)

W = Widest Leaf Width (cm)

The coefficient 0.68 is approximately suitable for olive leaves in many studies.

Statistical Analysis:

The field experiment was conducted using a completely randomized block design (RCBD). Data were analyzed using the SPSS statistical software. Treatment means and interactions were compared using the Least Significant Difference (LSD) test and Duncan's multiple range test at a probability level of 5% (4).

Results and Discussion

The molecular diagnosis of the pathogenic fungus isolated from an olive seedling exhibiting symptoms of Verticillium wilt disease revealed that the causative agent is the fungus Verticillium dahlia. It showed a 99.4-100% match with fungal strains registered in the NCBI gene bank, and the isolate was deposited under accession number PX682723.1. Figure 1 shows the phylogenetic tree, confirming that the isolated fungus belongs to cluster 2, which contains only the species *Verticillium dahlia.*

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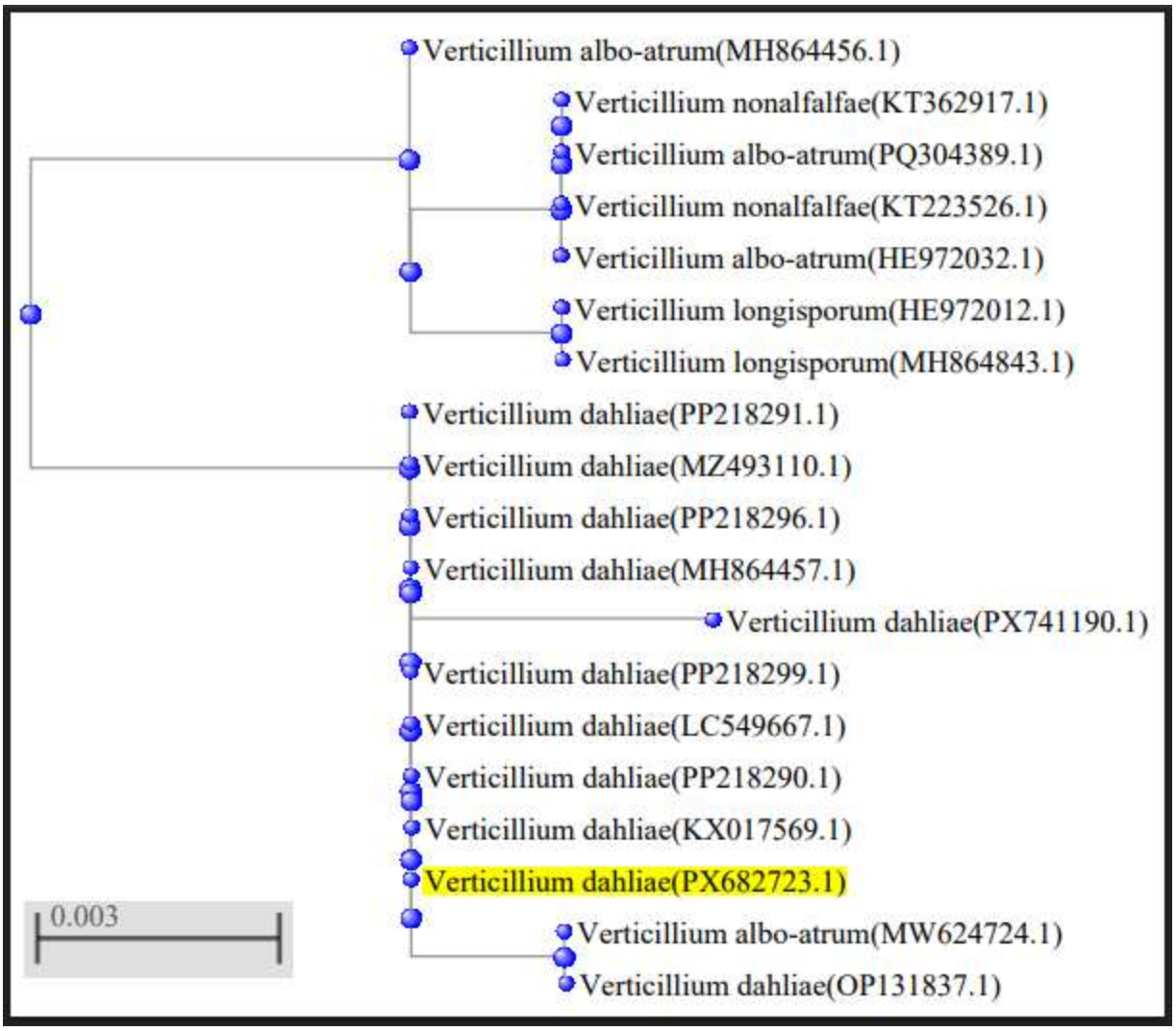


Figure 1 Phylogenetic tree according minimum evaluation, showing the fungus *Verticillium dahlia* (PX682723.1) position among globally recorded strains

Effect of β-glucanase enzyme compatibility with Novo-treat and Beltanol fungicide on V. dahliae infection parameters and growth in three olive varieties.

Infection severity:

Table (1) shows the compatibility of β-glucanase with Novo-treat and Beltanol fungicide in the severity of infection by the pathogenic fungus *V. dahliae*, pathogen, in three olive seedling varieties. At the average treatment level, the β-glucanase treatment showed the lowest infection severity at 0.4, compared to the highest infection severity in the fungus-only treatment at 0.89. At the variety level, the Nabali variety recorded the lowest infection severity at 0.29, while the Ba'shiqi variety recorded the highest at 0.37. The interaction results showed that the (β-glucanase + Novo-treat + Beltanol) treatment for the Nabali olive variety. The lowest infection severity, 0.11, was observed, compared to the highest infection

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severity of 0.95 in the treatment of the pathogenic fungus of the Ba'shiqi variety.

Table (1) Effect of β-glucanase compatibility with the Novo-Treat and the fungicide Beltanol on the severity of infection by the pathogenic fungus *V. dahliae*, in three olive seedling varieties.

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
0	0	0	0	Control (healthy plants)
0.89	0.95	0.91	0.82	Pathogenic fungus V.dahlia (V.d)
0.4	0.44	0.41	0.35	(V.d)+ β – glucanase
0.32	0.35	0.31	0.29	(V.d)+ Novo – treat
0.34	0.37	0.34	0.31	(V.d)+ Beltanol
0.29	0.33	0.29	0.24	(V.d) + β – glucanase + Novo – treat
0.27	0.31	0.27	0.23	(V.d) + β – glucanase + Beltanol
0.15	0.19	0.15	0.11	(V.d) + β – glucanase + Novo – treat + Beltanol
	0.37	0.34	0.29	Average of varieties
Transactions: 0.07, varieties: 0.11, interaction: 0.13				LSD 0.05

The reason for the high percentage and severity of infection in treatment with the pathogenic fungus *V. dahliae* alone is attributed to the absence of any biological or chemical control agent capable of limiting the growth of the fungus or inhibiting its colonization of plant tissues. This allowed the fungus to penetrate the roots and colonize the xylem vessels with high efficiency, causing blockage of the vessels by mycelium or microsclerotia and the gelatinous substances secreted by the plant in response to infection. This results in an impediment to the transfer of water and nutrients to the vegetative parts, so symptoms of wilting, yellowing, and leaf drop appear, and the severity of the infection increases. Also, the continued multiplication of the fungus inside the vascular tissues increases the development of the disease and leads to higher infection severity values compared to Transactions that included biological or chemical control methods. This is consistent with other studies by (27, 35). In addition to the ability of the pathogenic fungus to secrete enzymes that break down cellulose and protein, which cause root rot, the decrease in the percentage and severity of infection in treatment with the glucanase, Novo-Treat, and Beltanol is due to the fact

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that the glucanase directly affects the pathogenic fungus. The enzyme is one of the hydrolytic enzymes that breaks down the glucan polymer, the second most important main component in the cell walls of the pathogenic fungus, which leads to weakening the fungus and its death due to the disruption of permeability and the exposure of the fungal cell contents to the surrounding environmental conditions. As for the organic preparation Novo-treat, which contains several antibiotics, most of these antibiotics work to increase the permeability of the plasma membrane in the fungal cell, as the antibiotic molecules bind to the phospholipids present in the plasma membrane, forming complexes that lead to the formation of ion channels inside the membrane. This results in the leakage of ions and cellular compounds, the collapse of osmotic balance, then the rupture of the membrane and the death of the cell. It may also stimulate systemic resistance in plants (ISR), which enhances the plant's resistance to pathogenic fungi. Beltanol, on the other hand, has a stimulating effect on plants and is effective against many pathogenic fungi. Its effectiveness is activated by the binding of the active ingredient, 8-Hydroxyquinoline sulfate, to essential fungal metal ions such as copper, iron, and zinc. These elements are necessary for the activity of many fungal enzymes. Depriving the fungus of these elements disrupts vital metabolic processes, stops fungal hyphae growth, and prevents the pathogen's reproduction, ultimately leading to its death or inhibiting its development within the plant. The fungicide is also characterized by its rapid absorption and translocation within the plant, allowing it to attack fungi located inside plant vessels, not just on the plant surface. These findings are consistent with those of (8, 23, 28, 34).

Peroxidase Activity

Table (2) shows the compatibility of the β-glucanase with the Novo-Treat preparation and the fungicide Beltanol in peroxidase activity for three olive varieties infected with the pathogenic fungus *V. dahliae*. At the average treatment level, the triple combination (β-glucanase + Novo-Treat + Beltanol) showed the highest activity at 2.67 units/ml, while the fungus-only treatment recorded the lowest activity at 2.03 units/ml. At the variety level, the Nabali variety recorded the highest peroxidase activity at 2 units/ml. The interaction results showed that the (β-glucanase + Novo-Treat + Beltanol) treatment for the Nabali olive variety yielded the highest activity at 2.71 units/ml, compared to the lowest activity of 1.92 units/ml for the fungus-only treatment for the Ba'shiqi variety.

Table (2) Effect of the compatibility of the β-glucanase with the Novo-treat and Beltanol on the peroxidase activity (units/ml) of three varieties of olive seedlings under conditions of infection with the pathogenic fungus *V. dahlia*.

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
0.13	0.12	0.12	0.14	Control (healthy plants)
2.03	1.92	2.03	2.15	Pathogenic fungus V.dahlia (V.d)

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2.05	1.97	2.06	2.11	(V.d)+ β – glucanase
2.10	2.01	2.09	2.21	(V.d)+ Novo – treat
2.03	1.95	2.05	2.09	(V.d)+ Beltanol
2.2	2.11	2.19	2.3	(V.d) + β – glucanase + Novo – treat
2.14	2.07	2.07	2.27	(V.d) + β – glucanase + Beltanol
2.67	2.63	2.66	2.71	(V.d) + β – glucanase + Novo – treat + Beltanol
	1.85	1.91	2.0	Average of varieties
Transactions: 0.072, varieties: 0.074, interaction; 0.081				LSD 0.05

The results show a significant increase in the peroxidase activity of the treated seedlings (β-glucanase + Novo-Treat + Beltanol). This result explains the role of the β-glucanase, which stimulates the plant's defense response by releasing immune-stimulating molecules from the fungal wall as a result of the breakdown of beta-glucans present in the fungal wall. Consequently, the activity of the peroxidase increases along with the rest of the defense enzymes. As for the bio-preparation Novo-Treat, it may activate systemic induced resistance (ISR), in addition to the Beltanol, which reduces fungal growth and decreases the severity of infection. Consequently, peroxidase activity increases in the early stages as a result of the plant's response, and then it may decrease later with the decrease in disease pressure after controlling the fungus. This is consistent with (6, 39).In addition to the increased activity in the treatment with the pathogenic fungus V. dahlia compared to the control (healthy) treatment, this can be explained by the fact that the pathogen induces resistance in the infected plant. As a result of the plant's response to infection, several resistance factors appear, including pathogenesis-related proteins, which are a natural reaction of the plant to plant diseases. These results are consistent with (11, 14, 32). Peroxidase is one of the active defense mechanisms in the plant that responds to infection by pathogens such as V. dahlia. It stimulates lignin formation and strengthens the cell wall, which is consistent with the study by (31). Peroxidase is also an important indicator of plant infection by pathogens (3).

Polyphenol Oxidase Activity

Table (3) shows the compatibility of the β-glucanase with the Novo-Treat and Beltanol in the activity of polyphenol oxidase in three olive varieties infected with the pathogenic fungus V. dahliae. The results at the average treatment level showed the superiority of the triple combination treatment (β-glucanase + Novo-Treat + Beltanol), recording the highest activity at 2.41 units/ml, compared to the lowest activity in the fungus-only treatment at 1.77 units/ml. At the variety level, the results showed that the Nabali variety recorded the highest activity of this enzyme at 1.76 units/ml, while the interaction results showed that the (β-

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glucanase + Novo-Treat + Beltanol) treatment for the Nabali olive variety gave the highest activity at 2.45 units/ml, compared to the lowest activity at 1.8 units/ml in the β-glucanase enzyme treatment. Glucanase activity of the Ashrasi variety.

Table (3) Effect of β-glucanase compatibility with the Novo-Treat preparation and Beltanol on polyphenol oxidase (PPO) activity (units/ml) of three olive seedling varieties under conditions of infection with the pathogenic fungus *V. dahliae*

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
0.07	0.07	0.07	0.08	Control (healthy plants)
1.77	1.66	1.77	1.89	Pathogenic fungus V.dahlia (V.d)
1.79	1.71	1.8	1.85	(V.d)+ β – glucanase
1.84	1.74	1.83	1.95	(V.d)+ Novo – treat
1.77	1.69	1.78	1.83	(V.d)+ Beltanol
1.94	1.85	1.93	2.04	(V.d) + β – glucanase + Novo – treat
1.89	1.83	1.82	2.01	(V.d) + β – glucanase + Beltanol
2.41	2.37	2.40	2.45	(V.d) + β – glucanase + Novo – treat + Beltanol
	1.62	1.68	1.76	Average of varieties
Transactions: 0.041, varieties: 0.037, interaction: 0.076				LSD 0.05

The results proved that there is an increase in the activity of Polyphenol oxidase of the treated seedlings with (β-glucanase + Novo-treat + Beltanol). This indicates the existence of a synergistic effect of the enzyme with the pesticide and the preparation in stimulating the activity of Polyphenol oxidase, as this interaction was reflected in the increase of the activity of the enzyme. This is evidence of the induction of systemic resistance in olive seedlings, and this is consistent with the study of (12, 37). also noted the enzyme's interaction with certain cell wall proteins to form cross-links and multiple compounds, which increases cell wall rigidity (7, 21). The high specific activity of the PPO enzyme is attributed to its role as part of the plant's natural defense response when exposed to pathogens. Stimulating defense mechanisms leads to

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increased activity of defense enzymes, including polyphenol oxidase, in conjunction with the induction of pathogenesis-related proteins, which contributes to enhancing systemic resistance and limiting infection development. This result is consistent also with (36). Studies have indicated that the high activity of the Polyphenol oxidase enzyme is associated with a high level of resistance in plants infected with the pathogenic fungus as a primary response to fungal invasion. One of the most important mechanisms of action of this enzyme is the oxidation of catechols and phenolic compounds into reactive quinones, which contribute to the polymerization of phenolic compounds and strengthen the cell wall. It also indirectly helps in the precipitation of lignin and suberin at the site of infection, thus limiting disease spread. These results are consistent with those of (18, 33).

Number of Leaves

Table (4) shows the effect of the β-glucanase enzyme's interaction with the Novo-Treat preparation and the fungicide Beltanol on the number of leaves in three olive seedling varieties under conditions of infection with the pathogenic fungus *V. dahliae*. At the average treatment level, the triple combination treatment showed the highest number of leaves, reaching 127.92, compared to the lowest number of leaves in the fungus-only treatment, which reached 27.16. At the variety level, the results showed that the Nabali variety recorded the highest number of leaves, reaching 114.86. The interaction results showed that the triple treatment for the Nabali variety resulted in the highest number of leaves (131.05) compared to the lowest (3.22) in the treatment with only the pathogenic fungus for the Ba'shiqi variety.

Table (4) Effect of β-glucanase compatibility with the Novo-Treat and Beltanol on the number of leaves in three olive seedling varieties under conditions of infection with the pathogenic fungus *V. dahlia*.

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
148.75	147.28	148.12	150.85	Control (healthy plants)
27.16	3.22	23 .5	51.09	Pathogenic fungus *V.dahlia* (V.d)
81.12	58.58	80.28	104.51	(V.d)+ β – glucanase
95.79	65.77	95.73	125.87	(V.d)+ Novo – treat

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88.44	62.68	88.28	114.36	(V.d)+ Beltanol
116.96	111.9	115.93	123.06	(V.d) + β – glucanase + Novo – treat
103.49	93.41	98.95	118.12	(V.d) + β – glucanase + Beltanol
127.92	126.31	126.4	131.05	(V.d) + β – glucanase + Novo – treat + Beltanol
	83.64	107.67	114.86	Average of varieties
Transactions: 7.04, varieties: 7.71, interaction: 9.04				LSD 0.05

Leaf Area

Table (5) shows the effect of the β-glucanase enzyme's interaction with the Novo-Treat preparation and the fungicide Beltanol on the leaf area of three olive seedling varieties under infection with the pathogenic fungus V. dahliae,. At the average treatment level, the combination treatment (β-glucanase + Novo-Treat + Beltanol) recorded the highest leaf area at 6.90 cm, while the fungus-only treatment recorded the lowest leaf area at 3.50 cm. At the variety level, the Nabali variety showed the highest average leaf area at 6.01 cm. Regarding the interaction results, the highest leaf area was recorded in the triple combination treatment for the Nabali variety, reaching 7.45 cm, while the lowest leaf area was in the fungus-only treatment, at 3.69 cm and 3.3 cm for the Nabali and Ba'ashiqi varieties, respectively.

Table (5) Effect of compatibility of β-glucanase with the Novo-treat and Beltanol on the leaf area (cm) of three varieties of olive seedlings under conditions of infection with the pathogenic fungus V. dahliae,

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
8.29	8.13	8.21	8.54	Control (healthy plants)
3.50	3.3	5. 20	3.69	Pathogenic fungus V.dahlia (V.d)
5.07	4.75	5.02	5.44	(V.d)+ β – glucanase
5.18	4.89	5.15	5.49	(V.d)+ Novo – treat
5.2	4.86	5.22	5.52	(V.d)+ Beltanol

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5.77	5.56	5.69	6.05	(V.d) + β – glucanase + Novo – treat
5.39	5.02	5.21	5.93	(V.d) + β – glucanase + Beltanol
6.90	6.4	6.84	7.45	(V.d) + β – glucanase + Novo – treat + Beltanol
	5.36	5.91	6.01	Average of varieties
Transactions: 0.55, varieties: 0.52, interaction: 0.68				LSD 0.05

The reason for the increase in leaf area of seedlings in the triple combination treatment is due to the efficiency of the β-1,3-glucanase in breaking down the β-1,3-glucan compounds that make up the cell wall of the pathogenic fungus, which weakened its growth and reduced the severity of the infection. In addition, the Novo-treat preparation and the pesticide Beltanol played a role in enhancing the efficiency of control and reducing the damage caused by the pathogenic fungus to the plant, which led to an improvement in the physiological condition of the plant and the investment of nutrients and energy in growth, which led to an increase in leaf area compared to the treatment of the pathogenic fungus alone. These results are consistent with Lorito et al. (1994) and (10 , 17). The superiority of the treatment (V.d) + β-glucanase + Novo-treat + Beltanol over the pathogen treatment alone is attributed to the synergistic effect of the treatment components. This synergistic effect led to a reduction in infection severity and a decrease in fungal damage to the root system. As a result, the plant's water and nutrient uptake was improved, along with enhanced photosynthesis and the utilization of its byproducts for growth. This positively impacted the plant's physiological state, leading to higher vegetative growth indicators compared to plants treated with the pathogen alone. These results are consistent with those of (26).

Chlorophyll content

Table (6) shows the effect of the β-glucanase enzyme's interaction with the Novo-Treat preparation and the fungicide Beltanol on the chlorophyll of three olive seedling varieties under conditions of infection with the pathogenic fungus V. dahliae,. At the average treatment level, the triple treatment (β-glucanase + Novo-Treat + Beltanol) recorded the highest chlorophyll content at 31.36 SPAD, while the fungus-only treatment recorded the lowest content at 16.10 SPAD. At the average variety level, the Nabali variety exhibited the highest chlorophyll content at 29.23 SPAD. The results of the interactions also showed that the triple treatment (β-glucanase + Novo-treat + Beltanol) for the Nabali variety achieved the highest chlorophyll content, reaching 33.27 spad, while the treatment with the pathogenic fungus recorded the lowest chlorophyll content in the Ba'shiqi and Ashrasi varieties, at 15.58 spad and 15.62 spad, respectively.

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Table (6) Effect of the β-glucanase enzyme's interaction with the Novo-treat and Beltanol on chlorophyll (spad) in three olive seedling varieties under infection conditions with the pathogenic fungus *V. dahlia*.

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
34.78	34.64	34.68	35.01	Control (healthy plants)
16.10	15.58	15.62	17.11	Pathogenic fungus V.dahlia (V.d)
25.79	23.37	26.14	27.86	(V.d)+ β – glucanase
28.86	27.51	29.07	30.01	(V.d)+ Novo – treat
27.64	26.28	27.64	28.99	(V.d)+ Beltanol
30.10	29.44	29.72	31.15	(V.d) + β – glucanase + Novo – treat
29.52	28.98	29.11	30.47	(V.d) + β – glucanase + Beltanol
31.36	29.85	30.96	33.27	(V.d) + β – glucanase + Novo – treat + Beltanol
	26.96	27.87	29.23	Average of varieties
Transactions: 1.20 varieties: 1.11, interaction: 1.35				LSD 0.05

The increased chlorophyll content in the treatment ((V.d) + β-glucanase + Novo-treat + Beltanol)) can be attributed to the complementary effect of its components in reducing the severity of infection by the fungus V. dahliae. The enzyme β-1,3-glucanase led to the breakdown of β-1,3-glucan, the main component of the fungus's cell wall, thus limiting its growth and spread. Meanwhile, the bio-preparation Novo-treat contributed to stimulating plant resistance and the secretion of plant growth regulators, as well as its role in improving nutrient absorption. The fungicide Beltanol worked to reduce the pathogenic density of the fungus. This complementary effect led to the preservation of the integrity of the chloroplasts, the reduction of chlorophyll pigment damage, and the continuation of photosynthesis with high efficiency, which was reflected in the increased chlorophyll content in the treated plants. The reduction in disease severity also maintained the efficiency of photosynthesis and reduced the oxidative stress on the plant. The decrease in chlorophyll content in the treatment with the fungus V. dahliae alone is attributed to the fungus colonizing the xylem vessels and causing their blockage with mycelium and gummy secretions. This impedes the transfer

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of water and minerals to the leaves, resulting in water stress and an increase in the formation of reactive oxygen species (ROS). These ROS lead to the oxidation and decomposition of chlorophyll molecules, damage to chloroplasts, and a decrease in photosynthetic efficiency. This is reflected in the appearance of yellowing, wilting, and a decrease in chlorophyll content in infected plants. These results are consistent with those of Gharbi et al. (2017);); and Alvera-Azcárate et al. (2014). (5, 22,)

Dry weight of the shoot system

The results shown in Table (7) demonstrate the effect of the β-glucanase enzyme's interaction with the Novo-Treat and Beltanol on the dry weight of the shoot system of three olive seedling varieties under conditions of infection with the pathogenic fungus *V. dahliae*, At the treatment level, the combination treatment (β-glucanase + Novo-Treat + Beltanol) recorded the highest dry weight at 23.83 g, while the fungal treatment recorded the lowest dry weight at 10.5 g. At the variety level, the Nabali variety recorded the highest weight at 21.05 g. Regarding the results of interactions, the treatment (β-glucanase + Novo-Treat + Beltanol) resulted in the highest weight at 24.46 g in the Nabali variety, compared to the lowest weight of 7.5 g in the pathogen treatment for the Ba'shiqi variety.

Table (7) Effect of β-glucanase enzyme compatibility with the Novo-Treat and Beltanol on the dry weight of the shoot system (g) of three olive seedling varieties under conditions of infection with the pathogenic fungus V. dahliae,

Average of Transactions	olive seedling varieties			Transactions
	Ba'shiqi	Ashrasi	Nabali	
25.08	24.87	25.01	25.36	Control (healthy plants)
10.5	7.5	9. 21	13.5	Pathogenic fungus V.dahlia (V.d)
16.22	11.95	16.31	20.4	(V.d)+ β – glucanase
17.58	14.23	17.84	20.67	(V.d)+ Novo – treat
16.21	11.97	16.29	20.36	(V.d)+ Beltanol
21.13	20.18	21.09	22.12	(V.d) + β – glucanase + Novo – treat
20.51	19.52	20.49	21.52	(V.d) + β – glucanase + Beltanol
23.83	23.51	23.52	24.46	(V.d) + β – glucanase + Novo – treat + Beltanol

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	16.72	20.08	21.05	Average of varieties
Transactions: 1.32, varieties: 1.41, interaction: 1.65				LSD 0.05

The most important reason for the increase in vegetative dry weight is likely due to the ability of the β-1,3-glucanase enzyme to weaken the cell wall of the pathogenic fungus and break down its components, thus reducing infection severity and minimizing damage to the plant. This allows the plant to utilize more photosynthetic products and energy for growth and dry matter accumulation. Furthermore, the enzyme's compatibility with the preparation Novo-treat and the fungicide Beltanol contributed to enhancing the effectiveness of pathogen control within integrated pest management programs, which positively impacted the increase in vegetative dry weight. These results are consistent with those of (9, 29), who demonstrated that increased β-1,3-glucanase enzyme activity enhanced the efficiency of biological control and reduced infection severity, leading to improved plant growth. The reason for the decrease in root weight in the fungal treatment may be due to the fungus attacking the root hairs, leading to their breakage and a significant reduction in their number, thus decreasing the overall root weight. (2) reported similar results. The increase in the dry weight of the shoot system reflects the superiority of the aforementioned vegetative growth parameters due to the resistance factors under investigation, which reduce damage from the fungal pathogen on the one hand and promote plant growth on the other.

Conclusions

The fungus *Verticillium dahliae* is the main causative agent of wilt and yellowing disease in the olive seedlings, and its molecular identification was confirmed with a perfect match to internationally registered strains. The triple integrated control treatment (beta-glucanase with Novo-treat and the fungicide Beltanol) proved significantly superior to the other Transactions, as it increased the activity of peroxidase and polyphenol oxidase associated with plant resistance. This treatment also reduced disease severity to the lowest level compared to the highest severity recorded with the treatment of the pathogen alone. Furthermore, it excelled in improving all vegetative growth indicators (height, number of branches and leaves, leaf area, chlorophyll content, and total dry weight), particularly in the Nabali olive variety.

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