RESEARCH | Egyptian Journal of Agricultural Research | PLANT PATHOLOGY

Beneficial effects of *Trichoderma viride* and salicylic acid against Fusarium wilt in tomato

Mohamed E. Khalil
Address:
Plant Pathology Reserach Institute, Agricultural Research Center, Giza, Egypt.
*Corresponding author: ***Mohamed E. Khalil,*** mohamedeffat631@gmail.com
Received: 12-12-2020; **Accepted**: 26-1-2020; **Published**: 27-2-2021 10.21608/ejar.2021.53385.1054

ABSTRACT
Tomatoes are among the most economical vegetable crops that can be affected by many diseases, for example Fusarium wilt disease, which is caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici*, which is considered one of the most common diseases affecting tomato plants. Stimulating the chemical defence system of tomato plants to resist fungal pathogens is the primary goal of any pathogen control process. Biocontrol and chemical inducers represent an important strategy to stimulate the plant defence system, especially when applied together. In the current study, the efficacy of the following treatments, for example, *Trichoderma viride* and salicylic acid (SA), alone or in combination, was evaluated compared to the effectiveness of the recommended fungicide Topsin-M 70%, which was used in the recommended dose as a new strategy to increase the defence efficiency of tomatoes to combat Fusarium wilt disease under greenhouse and field conditions. The various physiological defensive changes were studied in addition to estimating some enzymes associated with the chemical defence system of tomato plants such as polyphenol oxidase, peroxidase and hydrolysis enzyme (chitinase) and as well as estimation of total dissolved phenols). Under greenhouse conditions, tomato plants were artificially infected with *F. oxysporum* f. sp. *lycopersici* and left to normal infection under field conditions. Initially and under greenhouse conditions, tomato plants were inoculated with *T. viride* in one of two methods (dipping seedling roots and/or soil treatment) and/or spraying them daily for a week with SA, alone or together, one week before inoculation with the pathogen. The same treatments were performed on tomato plants that were left for natural infection under field conditions, after which the plants were collected 35 days after infection with the pathogen. The results obtained confirmed that all the treatments used led to the protection of tomato plants completely from infection, as the risk of disease decreased significantly, also, the level of all physiological estimates specified changed significantly as a result of the use of anti-fungi and chemical inducers, as many defence compounds were shown, which resulted in the activation of the chemical defence system in tomato plants against the attack of pathogens. These parameters are applied to all these measures, not only to reach their stimulation within the infected plants, but also to exceed their contents in healthy plants. These treatments also led to a significant increase in the growth variables and the total yield of the plant.
Keywords: Tomato, Fusarium wilt, *Trichoderma viride*, salicylic acid, phenolic compounds, peroxidase, polyphenol oxidase, chitinase

INTRODUCTION
Tomato (*Lycopersicon esculentum* Mill.) is one of the most economically important vegetable plants that can be grown in Egypt in greenhouses and open fields, where it is used for human nutrition either fresh or processed (Abdel Karim, *et al.,* 2006). The economic importance of the tomato crop is not only due to its economic value, but also due to its nutritional value, as tomato plants contain antioxidant compounds, such as vitamin C and carotenoids. Tomatoes are currently used as a therapeutic method for treating human cancer, as they contain lycopene, which has proven to be an effective treatment for prostate cancer (Giovanucci, 1999). The Egyptian production of tomato crop grown in greenhouses and open fields It occupied the fifth place at the level of global production of tomatoes, representing about 4. 86% of the total global production for the year 2014, as well as the Egyptian production of tomatoes occupied the first place at the level of African production, which was estimated at about (43.11%) of World total production. In the current years, the amount of tomato harvest in Egypt has increased, reaching almost 8.3 million tons, produced from 52,8854. 94 fed, with an average production of 15.69 tons / fed (FAO, 2017). Moreover, tomato plants are infected with many fungal pathogens, especially when the relative humidity in the air increases, which in turn affects the ripening of fruits (Morsi, *et al.,* 2009 and Abdel Moneim, 2010). The fungus infection causes severe damage to tomatoes, as it cannot be easily controlled as the fungal pathogens are transmitted within the cultivated seeds as well as via contaminated soil (Dohroo, 1988). Fusarium wilt disease, caused by *F. oxysporum f. sp. lycopersici* is one of the most dangerous diseases affecting tomatoes as it affects their productivity, which leads to a significant decrease in the yield by an estimated 25% (Abdel-Monaim, 2012). Fusarium wilt disease in greenhouses and open fields was controlled in many ways, for example cultivation of resistant varieties, agricultural procedures, pesticides and fumigants, however the damage remained significant. The difficulty in controlling *F. oxysporum f. sp. lycopersici* encouraged researchers to find other alternative methods that were more effective and safer (Hibar, *et al.,* 2007). Generally, fungal diseases are controlled by the use of fungicides, which have a harmful effect on human health and the environment, (Rauf, 2000). Therefore, it is advised to use other alternative methods to combat fungal diseases, for example the use of antibodies and resistant plants to reduce the harmful effect of the use of fungicides on humans, animals and the environment (Ragab, *et al.,* 2009; Punja, *et al.,* 2017 and Abou-Zeid, *et al.,* 2018). The use of plant resistance induction as an alternative and safe

method in combating fungal diseases has garnered a lot of attention when applied, as it increased the plants' resistance against disease infection. Induction of chemical resistance in host plants through the use of antimicrobials and their bioactive products has currently gained high popularity in modern agricultural procedures (Mishra *et al.,* 2014).
The current study aim to evaluate the efficacy of the antagonist (*T. viride*, and salicylic acid) used alone or in combination to combat Fusarium wilt disease of tomato plants. Also, studying the side effect of its use on biochemical changes that may lead to stimulating the chemical defence system of plants against pathogens.

MATERIALS AND METHODS

The source of the pathogen, the antagonist and the tomato seeds:
The fungus *Fusarium oxysporum* f. sp. *lycopersici* was isolated from tomato plants (cv. Super Strain B), with obvious wilting symptoms witch cultivated in Qalyubia Governorate. The fungus was then purified and defined according to Hansen (1926); Ricker and Ricker, (1936) and Giman, (1957). Meanwhile, a pure isolate of the antagonist, namely *Trichoderma viride*, was obtained by the Department of Integrated Pest Management (IPMD), Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The pathogen and the antagonist were stored on potato dextrose agar (PDA) medium at 4 ° C until their use. However, tomato seeds (cv. Super Strain B), were brought from Vegetable Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt in 2019.

Effectiveness of *Trichoderma viride*, salicylic acid alone or in combination and the fungicide "Topsin-M 70%" on the tomato wilt disease and measurements of the growth of tomato plants:

Under the conditions of plastic houses:
The potted experiment was designed under greenhouse conditions, in Vegetable Diseases Research Department, Plant Diseases Research Institute, Agricultural Research Center, Giza, Egypt to study the efficacy of *T. viride* antibody and chemical inducer salicylic acid SA C7 H6 O3 0. 05-0 1%, alone or in combination, on tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici*. In this experiment, 25 cm of plastic pots were used, which contained 5 kg of sterile clay soil. *F. oxysporum* f. sp. *lycopersici* was grown on potato dextrose agar at 27 ± 1 ° C for seven days. A suspension Spores of the pathogen fungus was prepared at a concentration of (107cfu / ml), where the germs were counted with a hemocytometer slide. After that, 10 ml From a suspended pathogen fungus spores was added to each pot as a soil treatment according to Houssien et al. (2010). The *T. viride* antibody was grown on a glucose fermentation medium (GFM) (Brain and Hemning, 1945). at 25 ± 1 ° C for seven days. After that, a suspension of the antifungal spores was prepared at a concentration of 108 cfu / mL as described by Verma et al. (2007). The anti- fungus *T. viride* was used in two ways to study its effectiveness on tomato wilt disease. The first method, the antifungal spore's suspension (T1) was used as a soil treatment where 10 ml was added to each pot of the previously prepared concentration one week before the soil was inoculated with the pathogen spores. Whereas, the second method (T2) was carried out by dipping the roots of tomato seedlings, whose seeds were planted in potteries and leaving them until the plants had two real leaves, for a period of approximately 30 minutes, in 50 ml of the same concentration of antufungal that was used in the first method, before transporting To the pots inoculated with the pathogen. Meanwhile, tomato seedlings were sprayed well with a chemical inducer solution of SA at a concentration of 200 mM, every 24 hours for a week so that tomato leaves can absorb the acid sufficiently before pollinating the soil with the pathogen. Also, the recommended concentration (1.5 g/L) for the fungicide Topsin-M 70%" Thiophanate-Methyl was used by dipping the roots of tomato seedlings for 30 minutes prior to planting. Five tomato seedlings (cv. Super strain B) were transferred into each pot, and five pots were identified as duplicates for each treatment in a completely random template design.
The experiment was designed to include:
1) Non infested soil (control.,
2) soil treated with *F. oxysporum* only.,
3) SA (200 mM.,
4) *T. viride* (10^8 cfu/mL T1.,
5) *T. viride* (10^8 cfu/mL T2.
6) SA + "*T. viride" T1).*
7) SA +; "*T. viride" T2).*
8)(fungicide "Topsin-M 70 %" 1. 5 g/L used as a positive control).
Generally, all experiment pots were fertilized with NPK soluble mineral fertilizer (1: 1: 1) and watered the pots as needed. At the end of the experiment, disease severity was estimated. Also, some growth measures such as plant height (cm) and fresh and dry weight (g/plant) of shoot were estimated.

Measuring the severity of the disease
The incidence of disease severity was estimated on the scale reported by Song et al. (2004) which consists of five classifications, which range from 0-4, as these classifications express the degree of wilting. Where, the number zero expresses that the plant is healthy and does not have symptoms of wilt infection. Moreover, the number four expresses plants that have contracted the disease and exhibit symptoms of wilting on all their parts. The numbers from the scale 1 to 3 express different measures of wilt symptoms, which express the degree of severity of the disease. Where, the number 1 - expresses the percentage of plant leaves staining yellow, which ranges between 1-20%. While, the number 2 expresses the yellow colouration of the plant leaves, ranging from 21-40%. On the other hand, the third number expresses the percentage of plant leaves yellowing, ranging between 41-60%. The researcher mentioned that the disease severity rate can be calculated by the

following equation:
Percentage of disease incidence = range x number of affected plants
Highest scale × total number of plants

Under field conditions:
The field experiment was carried out under normal infection conditions of *F. oxysporum* f. sp. *lycopersici* in the Mitt Kenana village, Toukh, Qalyubia Governorate, during the growing seasons 2019 and 2020 to assess the effectiveness of the anti-fungus *T. viride* and the chemical inducer of SA alone or in combination) to control wilt disease in tomato plants and its effect on plant growth parameters and fruit weights. The experiment was planned on the of based a randomized, complete block design containing three replicates. The area of the experimental unit was calculated as it was 10.5 m 2 (3.5 x 3 m). As each unit contained 12 rows, each row was 3.5 meters long and 25 cm wide. Each row included approximately 15 tomato seedlings (cv. Super Strain B). Tomato plants were treated with the treatments described above. The comparison group of plants was treated with water only, without any additional treatments. The experimental plots were fertilized with NPK mineral fertilizers according to the recommendations of the Egyptian Ministry of Agriculture. The rate of disease severity was estimated as previously reported. Five weeks after infection with the pathogen, the tomato plants were cut back and the following measurements were estimated:
1- Plant height (cm).
2- Fresh and dry weights of the plant(g/plant).
3- Estimating the amount of the yield by estimating the weight of the fruits (Kg/Plant).
4- Estimation of some biochemical and enzyme changes that are related to stimulating the chemical defence activity of tomatoes.

Estimation of some chemical changes related to stimulating the chemical defence activity of tomatoes:
Estimation of total phenols content:
Soluble Phenols extract was obtained from treated tomato seedlings which were cultivated under field conditions during the two growing seasons 2019-2020 as described by Hsu et al. (2003). Five gram extract sample was taken From every transaction, And it was mixed up with 80 ml methanol and kept overnight. The solution was filtered through four layers of cheesecloth and the product was diluted to 100 ml for later use to determine the total dissolved Phenols content according to Slinkard and Singleton (1997), a volume of the prepared solution was taken for the determination of about 200 microliter and mixed with 1. 4 ml of distilled water, and 0. 1 ml of 50% (1N) Folin-Ciocalteu phenol reagent. After of time period at least 30 seconds and not exceeding 8 minutes, the chemical substance (sodium carbonate 20%) was added at 0.3 ml w / v. Then, the mixture was left for 2 hours until it could complete the reaction and after vortexing for a short time, the color was developed and the change in absorbance was estimated at 765 nm in a spectrophotometer. The total dissolved phenol content was standardized against tannic acid and the values of the change in absorbance were converted to mg of phenols per 100 grams of fresh weight tissue. Moreover, each value obtained is an average of three replicates.

Determination of oxidative and hydrolytic enzymes:
A sample was taken from tomato plants that were treated with the aforementioned treatments, to estimate the activity of polyphenol oxidase, peroxidase and hydrolytic enzymes (chitinase). The enzymatic extract was obtained from the combined plants as described by Maxwell and Bateman (1967). The activity of the enzymes polyphenol oxidase, peroxidase and chitinase was determined using a spectrophotometer. The cuvette contained the same solution, but the solution was boiled off to inhibit the enzyme activity. For peroxidase, data readings were recorded every 15 seconds for 2.5 minutes at 425 nm while, for polyphenol oxidase every 30 seconds for 5 minutes at 495 nm.

a- Determination of polyphenol oxidase activity:
Polyphenol oxidase activity was determined according to the method described by Maxwell and Bateman (1967). In the beginning, an amount of crude enzyme was applied about 0.5 ml, then, an amount of sodium phosphate buffer was applied at a pH 7 its size 0.5 ml, and then 0.5 ml of catechol was placed and they were mixed together, and it was supplemented Final volume by sterile distilled water to 3 ml. Polyphenol oxidase activity was expressed by the change in absorbance / minute at the optical density of 495 nm. Moreover, each value obtained was taken from the mean of three replicates.

b. Estimate peroxidase activity:
The peroxidase activity was estimated according to Allam and Hollis (1972). In this method, the oxidation of pyrogallol was calculated and converted to pyrogalline in the presence of H_2O_2 at a wavelength of 425 nm. The cuvette sample was formed from sodium phosphate buffer at pH 7, at 0.5 ml at 0.1 M, enzyme extract at 0.3 ml, pyrogallol at 0.3 ml at 0.05 M, and H2O2 1%. at 0.1 ml, The previous components were supplemented with sterile distilled water until it reached a volume of 3 ml. The peroxidase enzyme activity was differentiated as the change in absorption / minute. Based on the above, each value obtained is a mean of the values of three replicates.

c. Estimation of chitinase activity:
The reactant of colloidal chitin was obtained from chitin that was ground according to the method described by Ried and Ogryd-Ziak (1981). Enzyme activity was characterized as µmoles GLCc NAc g / mL at 575 nm - 1 g^{-1} fresh weight of tissue. Enzyme activity was characterized as enzyme / mg protein unit. Each value obtained, as mentioned in the other experiments, was an average of the values of three replicates.

Statistical analyzes:
The results obtained for appropriate statistical analysis were set using the MSTAT-C program (MSTAT-C, 1991), while the means were compared using ANOVA where LSD (P≤0.05).

RESULTS

Effectiveness of *Trichoderma viride*, salicylic acid alone or in combination and the fungicide "Topsin-M 70%" on the tomato wilt disease and measurements of the growth of tomato plants:

Under the conditions of plastic houses:

The results obtained and shown in **Table 1** show that by using both the antagonist *T. viride* (which was used in two different ways T1 and T2) and the chemical inducer SA alone or in combination and the fungicide Topsin-M 70%, the severity of wilt infection on tomato plants was reduced compared to the comparison treatment. In this study, which was conducted under greenhouse conditions, tomato seedlings were treated with the antagonist (*T. viride*) by both methods (T1 and T2) combined with the chemical inducer (salicylic acid (SA), as this treatment reduced the severity of wilt infection compared to With the use of these treatments alone. It was noted from the results that when using the chemical inducer, it had an effective effect in reducing the severity of the disease more than using the antagonist (T1 and T2) when applying these treatments alone, where the infection severity ratio was recorded as follows 3.00%, 3.33% and 3.66%, respectively. On the other hand, when tomato plants were treated with the topsin-M 70% fungicide And (the antibody *T. viride* (T 1 and chemical inducer) SA) combined together, the highest protection of the plants against infection with the pathogen was recorded, where the severity ratio reached Infection is 1.66% and 2.00%, respectively, followed by treatment of tomato plants By chemical inductance SA in combination with anti-object *T. viride* (T2), where the severity of infection was recorded as follows 2.33%, while when tomato plants were treated with the anti-organism *T. viride* by both methods (T1 and T2) singly have scored a Say protect the plants against infection compared to other transactions.
Moreover, when using the antibody with both methods (T1 and T2) and chemical inducer (SA) alone or in combination, also, the use of the topsin-M 70% fungicide, these treatments recorded a significant increase in some measures of growth such as plant height , fresh and dry weights of shoots whether in the presence or absence of the pathogen. It was observed from the results that the use of the antibody with both methods combined with the chemical inducer had a high significant increase in plant height, fresh and dry weights more than using either of them separately. Also, it was observed from the results that when using the chemical inducer, it increased the plant height, and fresh and dry weights more than the used antibody (T1 and T2) when using these treatments alone compared to other transactions.
In general, when anti-object *T. viride* (T1) and the chemical inducer SA were used together, this treatment recorded the highest plant height, fresh and dry weights for tomato plants, whether in uninfected or infected soils, followed by treatment of tomato plants with chemical inducement SA combined with The antibody *T. viride* (T2) compared to the Topsin-M 70% fungicide, which recorded 28.66 cm, 7.00 and 3.22 g/ plant, respectively.

Table 1. Effectiveness of *Trichoderma viride*, salicylic acid alone or in combination and fungicide Topsin-M 70% on tomato wilt disease and growth measurements plant height (cm), fresh and dry weights (g / plant) under greenhouse conditions.

Treatments		Disease severity %	Plant height (cm)	Fresh weight (G/P)	Dry weight (G/P)
Control)		0.00	33.33	7.33	3.50
F. oxysporum		5.50	15.66	4.33	1.33
Salicylic acid SA (200 mM)		3.00	23.66	6.33	2.7 0
T. viride (10^8 cfu/mL) (T1)*		3.33	20.33	6.00	2.66
T. viride (10^8 cfu/mL) (T2)*		3.66	20.66	5.66	2.33
SA + T*1*		2.00	27.33	6.80	3.00
SA + *(t2)*		2.33	26.66	6.66	2.90
Topsin-M 70 % 1.5 g/L		1.66	28.66	7.00	3.22
L.S.D. at 5%		0.29	1.77	0.90	0.57

*While T1 is soil treatment and T2 is seedlings treatment

Under field conditions:
The effectiveness of the chemical inducer SA and the antagonist *T. viride* (which was used in two different ways **T1** and **T2**) was estimated alone or in combination, and also, the fungicide Topsin-M 70% on tomato wilt disease, under field conditions during the two growing seasons 2019-2020. The results in Table 2 clearly showed that all the treatments that were used resulted in a high significant decrease in the severity of wilt disease infection compared to the comparison treatment. It was observed from the results that when the tomato plants were treated with the antufungal using either of the two methods and the chemical stimulus combined together, the severity of the disease was significantly reduced compared to using each agent alone. Moreover, when tomato plants were treated with either the antufungal (either of the two methods) or the

chemical inducer separately, the results indicated that the chemical inducer (SA) was more effective in reducing the severity of the wilt infection than the antufungal (**T1** and **T2**. On the other hand, when mixing both the antufungal *T. viride* (**T1**) with the chemical inducer SA, this treatment significantly increased the severity of the wilt infection, along with the use of the topsin-M 70% fungicide in both seasons, the infection severity ratio was 1.16% and 0.63% on average, respectively, compared to 4.49% on average in the comparison treatment in both seasons, respectively. On the contrary, when tomato plants were treated with antufungal *T. viride* by both methods (**T1** and **T2**), the severity of the disease was reduced, but by less than the other treatments, as the results were (3.16% and 3.66% on average during two seasons, respectively.

From the same table, it was noted from the results that when studying the effectiveness of the same previously mentioned treatments on some measures of growth of tomato plants and fruit weight, under field conditions during the growing seasons 2019 and 2020. The chemical inducer alone in the treatment of tomato plants had a greater effect on increasing the growth measures greater than the use of the antufungal, by both methods (**T1** and **T2**). On the other hand, when combining both the chemical inducer SA and the antufungal *T. viride* The two methods (t1 and t2) had the most effect on plant height along with the topsin-M fungicide 70% where the results obtained were 65.33, 62.94 and 72.44 cm on mean in both seasons, respectively, compared to the comparison treatment.

In addition, these transactions gave uniformity It was significantly higher in fresh and dry weights, as it recorded 251.44, 245.44, 303.44, 51.44, 48.33 and 56.44 g / plant on average in both seasons, respectively, compared to the comparison treatment. Whereas, when tomato plants were treated with *T. viride*, using both methods (**T1** and **T2**) alone, this gave less effect on the growth parameters in both seasons compared to other treatments.

In addition, the results in **Table (2)** showed that when the tomato plants were treated with the fungicide, this gave a significant increase in the weight of the fruits, followed by the treatment of the tomato plants with the antufungal mixture and the chemical inducer. It was also observed that when tomato plants were treated with chemical inducement SA alone, the weight of the fruits increased higher than the weight obtained when the plants were treated with *T. viride* by both methods (**T1** and **T2**). However, this increase was not significant compared to other transactions.

Table 2. Effectiveness of *Trichoderma viride* and salicylic acid, alone or in combination, and fungicide Topsin-M 70% on tomato wilt disease and growth measurements (plant height cm, fresh and dry weight g / plant) and fruit weights (Kg / plant) under field conditions during the 2019-2020 seasons.

Treatments	Disease severity %			Plant height (cm)			Fresh weight (G/P)			Dry weight (G/P)			Fruits weights (Kg/p)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Control	4.33	4.66	4.49	40.00	40.44	40.22	122.00	122.44	122.22	25.22	25.00	25.11	1.0 0	0.9 0	0.95
Salicylic acid SA (200 mM)	2.66	2.00	2.33	60.33	62.55	61.44	205.33	206.00	205.67	44.33	44.55	44.44	2.1 0	2.2 0	2.15
T. viride (10^8 cfu/mL) (T1)*	3.00	3.33	3.16	58.00	58.66	58.33	199.22	200.66	199.94	42.00	42.66	42.33	2.00	2.20	2.10
T. viride (10^8 cfu/mL) (T2)*	3.66	3.66	3.66	55.22	57.00	56.11	190.55	190.33	190.44	40.22	38.00	39.11	1.90	1.90	1.90
SA + *T1*	1.33	1.00	1.16	65.44	65.22	65.33	250.22	252.66	251.44	50.66	52.22	51.44	2.50	2.30	2.40
SA + T2	1.66	2.00	2.33	63.55	62.33	62.94	245.11	245.77	245.44	49.00	47.66	48.33	2.30	2.10	2.20
Topsin-M 70 % 1.5 g/L	0.66	0.60	0.63	71.33	73.55	72.44	300.66	306.22	303.44	55.66	57.22	56.44	2.60	2.40	2.50
L.S.D. at 5%			0.28			5.34			15.67			4.22			0.22

*While T1 is soil treatment and T2 is seedlings treatment

Estimation of some chemical changes related to stimulating the chemical defence activity of tomatoes:

Estimation of total phenols content:

The effectiveness of the antufungal *T. viride*, used by both methods (T1 and T2), the chemical inducer of salicylic acid (SA) alone or together, and the fungicide Topsin-M 70%) was studied on the content of total dissolved phenols present in the leaves of tomato plants infected with wilt under Field conditions during the two growing seasons 2019-2020. The results in **Table (3)** showed that when tomato plants were treated with the antufungal and used with both methods and the chemical induction alone or in combination, as well as, when using the fungicide, this led to an increase in the total amount of dissolved phenols compared to the comparison treatment. There was a significant increase in the total dissolved phenols in the leaves of tomato plants infected with wilt when treated with the chemical inducer of salicylic acid (SA), as this treatment increased the content of total dissolved phenols by a greater amount than using the antifungus in both methods (T1 and T2) when Use these transactions individually during both growing seasons. In addition, the highest value of total dissolved phenols was obtained in the leaves of tomato plants infected with wilt when treated with the fungicide topsin-M 70%. Next, the plants were treated with the chemical inducer SA in combination with the antufungal *T. viride*, used by both methods (T1. and T2) in both growing seasons. On the contrary, the lowest value of total dissolved phenols was recorded in the leaves of tomato plants when it was treated with antufungal *T. viride*, which was used by both methods (T1 and T2) in both growing seasons compared to the other treatments.

Table 3: The efficacy of *Trichoderma viride*, salicylic acid applied alone or together, and the fungicide Topsin-M 70% on total soluble phenol content (mg / 100 g dry weight) in tomato leaves affected with wilt Under field conditions during the two growing seasons 2019-2020.

Treatments	**Total phenol (mg/ 100g fresh weight of tomato leaves)**		
	2019	**2020**	**Mean**
Control	72.28	74.93	73.61
Salicylic acid SA(200 mM)	126.75	127.18	126.95
T. viride* (10^8 cfu/mL) (T1)	115.41	116.65	116.03
T. viride* (10^8 cfu/mL) (T2)	112.12	113.33	112.73
SA + *(T1)*	138.90	140.37	139.64
SA + *(T2)*	132.22	133.42	132.82
Topsin-M 70 % 1.5 g/L	142.56	143.67	143.12

*While T1 is soil treatment and T2 is seedlings treatment

Determination of oxidative and hydrolytic enzymes:

The results in **Table (4)** showed that tomato plants that were treated with the antufungal *T. viride* and used by both methods (T1 and T2) and the chemical inducer of SA alone or combined together and the fungicide Topsin-M 70% led to an increase In polyphenol oxidase enzyme activity compared to comparison treatment. In addition, a significant increase in the activity of the enzyme polyphenol oxidase was observed in the leaves of tomato plants afflicted with wilt, which were treated with chemical induction of salicylic acid in both growing seasons, respectively, more than the use of the antufungal, which is used in both methods (T1 and T2) when using them individually. On the other hand, the highest activity of polyphenol oxidase was recorded in leaves of tomato plants infected with wilt and that were treated with the fungicide topsin-M 70%, followed by treatment of the plants with chemical inducer SA + T. viride (T1 and T2) in both growing seasons respectively. While, the lowest activity of polyphenol oxidase enzyme was recorded when tomato plants were treated with *T. viride* (T1 and T2) when they were used individually in both growing seasons respectively compared to the other treatments.

In addition, it was observed from the results in the same table to record a high increase in the activity of the peroxidase enzyme in the leaves of tomato plants infected with wilt, which was treated with the same treatments mentioned above compared to the comparison treatment. On the other hand, when tomato plants were treated with the chemical inducer of salicylic acid, an increase in the activity of the peroxidase enzyme was observed compared to using the antifungal in either of the two methods (**T1** and **T2**) alone in both growing seasons, respectively. At the same time, the maximum increase in peroxidase activity was recorded in the leaves of tomato plants infected with wilt and treated with the fungicide Topsin -M (70%), followed by treatment of the plants with chemical inducer SA in combination with the antufungal *T. viride* by two treatments (**T1** and **T2**) in Both growing seasons in a row. In addition, the lowest peroxidase activity was recorded in tomato leaves infected with wilt and treated with antufungal *T. viride* by either of the two methods (**T1** and **T2**) in both growing seasons, respectively, compared to the other treatments.

Also, the results in **Table (4)** show an increase in the activity of the enzyme chitinase in the leaves of tomato plants infected with wilt, which were treated with both the antufungal and the chemical inducer separately or together, as well as the fungicide. Moreover, a significant increase in chitinase enzyme activity was recorded in leaves of tomato plants infected with wilt, which were treated with chemical inducement of salicylic acid in both seasons, respectively, compared to treatment of the plants with the antifungal used by any of the two methods (T1 and T2) when applying these treatments individually. Also, the highest value was recorded for chitinase activity in tomato leaves infected with wilt when treated with chemical inducer SA combined with antufungal *T. viride* used by any of the two methods (T1 and T2) and the fungicide Topsin-M (70%) in both growing seasons the same.

Table 4: Effectiveness of *Trichoderma viride*, salicylic acid alone or in combination, and the fungicide Topsin-M 70% on polyphenol oxidase and peroxidase and hydrolysis enzyme (chitinase) activity in tomato leaves infected with wilt under field conditions during the two growing seasons 2019-2020.

Treatments	**Polyphenol oxidase (m/g f. w)**		**Peroxidase (m/g f. w)**		**Chitinase (m/g f. w)**	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**
Control	1.42	1.62	2.14	2.29	1.64	1.83
Salicylic acid SA(200 mM)	3.13	3.56	3.35	3.54	3.16	3.81
T. viride* (10^8 cfu/mL) (T1)	2.51	2.72	2.71	3.11	2.76	2.96
T. viride* (10^8 cfu/mL) (T2)	2.25	2.31	2.37	2.64	2.45	2.54
SA + *(T1)*	3.62	4.22	3.96	4.42	3.85	4.52
SA + *(T2)*	3.46	3.97	3.73	4.12	3.76	4.13
Topsin-M 70 % 1.5 g/L	3.82	4.73	4.65	4.94	4.12	4.96

*While T1 is soil treatment and T2 is seedlings treatment

DISCUSSION

Fusarium oxysporum is one of the most dangerous pathogens that infect plants, causing it to cause wilt disease, as it spreads widely in agricultural soil, causing serious problems, especially for global production of tomatoes. (Pasco *et al*., 2017) he has been mentioned that, tomato wilt disease caused by *F. oxysporum* f. sp*. lycopersici* was the main reason for the reduction in the global tomato yield. In recent times, biological control and chemical inducers have been known as one of the safe alternatives to fungicides used in controlling plant diseases, which play an important role in activating the chemical defence system of plants. (Wahba *et al.,* 2016 and Pascoe *et al.,* 2017) both have explained that, the use of the antibody *T. harzianum* in tomato wilt control caused by *F. semitectum* reduced the incidence of infection by up to 100%. On the other hand, salicylic acid is a compound effective in controlling wilt disease, they have decided (Gunes *et al.,* 2007; Abdel-Moneim *et al.,* 2011 and Akram and Anjum, 2011), that, the use of chemical inducers in controlling wilt disease has resulted in a significant reduction the severity of the disease in tomato plants. When, studying the reaction of the antibody and the chemical inducer in activating the chemical defence system of tomato plants under field conditions, the results obtained showed that the total amount of dissolved phenols increased significantly in the leaves of all the treated tomato plants, when using a suspension Spores of the antufungal *T. viride* (T1) by treating the soil was more effective than using it by dipping the roots of the seedlings in its solution (T2), as it increased the amount of total dissolved phenols. The results obtained also indicated that the accumulation of total dissolved phenols was reduced in a comparison treatment. It is clear that the treatment of tomato plants with the antibody *T. viride* and the chemical inducer of salicylic acid induced tomato plants to form total dissolved phenols not only to reach their content within the infested plant, but also to exceed their content in healthy plants. These results are in agreement with those reported by Guleria et al. (2005); Ali et al. (2007); El- Khallal (2007) and Abo-Elyousr et al. (2009), where they clarified that there may be a positive relationship between the total dissolved phenols obtained and the use of both salicylic acid with *Trichoderma*, and this study agreed with what previous researchers had mentioned, but only in the case of treatment by using the method used with (T1) in order to reduce the use of the fungicide if the conditions required it to be used, the results showed that when tomato plants were treated with T1 + SA, they led to an increase in the amount of total dissolved phenols higher than that obtained when tomato plants were treated with T2 + SA, compared to the comparison treatment. The same researchers also showed that the accumulation of phenolic compounds at the site of infection as a result of treatment with the antagonist and the inducing agent was related to the restriction of pathogen development, because these compounds are toxic to pathogens. As for the reaction of phenolic compounds inside plants, (Abo-Elyousr *et al.,* 2009), he indicated that, many phenolic compounds have a function within the plant as anti-pathogens, as precursors to structural polymers such as lignin, or as signaling molecules. Also,These compounds may prevent the pathogen from progressing by increasing the mechanical plant cell wall strength. On the other hand, when studying the effectiveness of the antibody and the chemical inducing agent on increasing the activity of the chemical defence system of the tomato plants by estimating the activity of some oxidizing enzymes, the results showed that there was a high increase in the activity of the enzyme polyphenol oxidase inside the tomato plants, under the conditions of fields, which treated with salicylic acid + (T1 and T2), in contrast, had the lowest record of polyphenol oxidase enzyme activity when treated tomato plants with *T. viride* (T1) and *T. viride* (T2) alone, respectively, compared to the other treatments. Furthermore, the highest activity of peroxidase enzyme when tomato plants were treated by using the anti-organism and the induced agent together was recorded, while the lowest activity of the peroxidase enzyme was recorded when the tomato plants were treated with the *T. viride* antibody (T1 and T2) alone compared to other treatments. Saying that tomato plants treated with biological and abiotic agents may induce some mechanisms of the plants' like chemical defence system, such as increasing the activity of oxidizing enzymes (polyphenol oxidase and proxidase) and hydrolytic enzymes (chitinase) as these compounds are responsible for increasing the chemo-defence activity of the plants against pathogens. This view is consistent with Gail et al. (2007) and Jayalakshmi et al. (2009), where, they reported that there was an increase in the activity of oxidative enzymes (polyphenol oxidase and peroxidase) after treatment of the plants with the antibody *T. harzianum*. On the other hand, both have explained El- Khallal, (2007) and Mandal, *et al.,* (2009), where, they indicated an occurrence a significant increase in the activity of polyphenol oxidase enzyme when the plants were treated by foliar spray with salicylic acid. To explain the reaction of the oxidizing enzymes, (Ramamoorthy, *et al.,* 2002; El-Khallal, 2007; Latha, *et al*., 2009; Abd-El-Khair *et al.,* 2011 and Surekha *et al*., 2014), they noted that the use of the antagonist *Trichoderma spp*. on wilted beans to induce chitinase, peroxidase, and polyphenol oxidase enzymes, which are responsible for increasing the chemo-defensive activity in the plant by preventing the pathogen progression within the bean plant. Conti et al. (1974); Yamamoto and Tani (1978) and Arora (1979), were discussed the reaction of oxidative enzymes inside plants where they are mentioned that oxidative enzymes such as polyphenol oxidase and peroxidase are responsible for the formation of lignin inside the plant cell as well as they oxidize phenols and convert them into quinines that are more toxic to pathogens, while working Other oxidizing phenols create protective barriers that reinforce the plant cell wall structure. Both have also explained Yedidia et al. (2003) and Newman Sally et al. (2011), that, the enzyme polyphenol oxidase cooperates with lignin in inducing the oxidation of phenolic compounds that depend on oxygen to quinine, as this process protects plant tissues from damage and infection with pathogens. (Yoshida *et al.,* 2003), he has indicated that, the peroxidase enzyme has a major role in ethylene biosynthesis and the construction of the components of the plant cell wall from lignin and suberin, as these components affect the wall thickness and thus increase the resistance of plants against infection with fungal pathogens, as peroxides enter the formation of lignin, polymerize glycoproteins rich in hydroxyproline, and regulate the elongation of the cell wall. The results obtained also showed an increase in the activity of the enzyme chitinase, where the highest value of the enzyme activity was recorded in tomato plants infected with *F. oxysporum* when treated with salicylic acid + (T1 and T2) and Topsin-M (70%). These results are consistent with both Schlumbaum et al. (1986); Ham et al. (1991); Leah et al. (1991) and Velazhahan et al. (2003), where, they reported that, chitinases play an important role in the analysis of chitin, which is a major component of fungal cell walls, which leads to direct inhibition of the growth of many fungal pathogens.

Conclusion

It can be concluded that the group of antagonists and chemical inducers can be used as integrated alternatives promising in the control of fusarium wilt disease in tomato plants. Therefore, in this study, new methods were followed in combating tomato plant wilt disease that depend mainly on activating the chemical defence system of plants against fungal pathogens, where biotic and abiotic agents such as *Trichoderma viride* antibody were used as a biological control agent, also, salicylic acid as a chemical stimulus, these agents are used to combat fungal diseases.

Conflict of Interest: The authors declare no conflict of interest

REFERENCES

Abd-El-Kareem, F., El-Mougy, N. S., El-Gamal, N. G., & Fotouh, Y. O. (2006). Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. *Res. J. Agric. Biol. Sci*, *2*(4), 147-152.

Abd-El-Khair, H., Khalifa, R. K. M., & Haggag, K. H. (2010). Effect of Trichoderma species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants. *J Am Sci*, *6*(9), 486-497

Abdel-Monaim, M. F. (2010). Induced systemic resistance in tomato plants against Fusarium wilt disease. Minia 2nd Conf. *Agric. Environ. Sci*, 253-263.

Abdel-Fattah, G. M., & Al-Amri, S. M. (2012). Induced systemic resistance in tomato plants against Fusarium oxysporum f. sp. lycopersici by different kinds of compost. *African Journal of Biotechnology*, *11*(61), 12454-2463.

Abdel-Monaim, M. F., Ismail, M. E., & Morsy, K. M. (2011). Induction of systemic resistance of benzothiadiazole and humic acid in soybean plants against Fusarium wilt disease. *Mycobiology*, *39*(4), 290-298.

Abo-Elyousr, K. A., Hashem, M., & Ali, E. H. (2009). Integrated control of cotton root rot disease by mixing fungal biocontrol agents and resistance inducers. *Crop Protection*, *28*(4), 295-301.

Abou-Zeid, N. M., Mahmoud, Noher, A., Mohamed, S. Abbas, Amira, Sh. Soliman and Eid, Hend, T. (2018). Induction of systemic resistance against Tomato wilt disease under greenhouse conditions in Egypt. Research Article Bioscience Research, 2018 15(4): 4559-4570.

Akram, W., & Anjum, T. (2011). Use of bioagents and synthetic chemicals for induction of systemic resistance in tomato against diseases. *Int. Res. J. Agric. Sci. Soil Sci*, *1*, 286-292.

Ali, M. B., Hahn, E. J., & Paek, K. Y. (2007). Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in Panax ginseng bioreactor root suspension cultures. *Molecules*, *12*(3), 607-621.

Allam, A. I., & Hollis, J. P. (1972). Sulfide inhibition of oxidases in rice roots. *Phytopathology*

Arora, Y. K. (1979). *Changes in the enzymes involved in polyphenol metabolism in mung bean infected with Rhizoctonia solani* (Doctoral dissertation, Ph. D. Thesis. Punjab Agricultural University, Ludhiana, India).

Kannan, K., Tanabe, S., Giesy, J. P., & Tatsukawa, R. (1997). Organochlorine pesticides and polychlorinated biphenyls in foodstuffs from Asian and oceanic countries. *Reviews of environmental contamination and toxicology*, 1-55.

Basco, M. J., Bisen, K., Keswani, C., & Singh, H. B. (2017). Biological management of Fusarium wilt of tomato using biofortified vermicompost. *Mycosphere*, *8*(3), 467-483.

Brian, P. W., & Hemming, H. G. (1945). Gliotoxin, a fungistatic metabolic product of Trichoderma viride. *Annals of Applied Biology*, *32*(3), 214-220.

Conti, G. G. (1974). Phenylalanine ammonia-lyase and polyphenoloxidase activities correlated with necrogenesis in cauliflower mosaic virus infection.

Dohroo, N. P. (1988). Germplasm reaction of cauliflower to stalk rot (Sclerotinia sclerotiorum). *Indian J. Plant Pathol*, *6*(2), 144.

El-Khallal, S. M. (2007). Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 1-Changes in growth, some metabolic activities and endogenous hormones related to defence mechanism. *Aust J Basic Appl Sci*, *1*(4), 691-705.

El-Khallal, S. M. (2007). Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 1-Changes in growth, some metabolic activities and endogenous hormones related to defence mechanism. *Aust J Basic Appl Sci*, *1*(4), 691-705.

Bernstad, A. K., Cánovas, A., & Valle, R. (2017). Consideration of food wastage along the supply chain in lifecycle assessments: A mini-review based on the case of tomatoes. *Waste Management & Research*, *35*(1), 29-39.

Gailite, A., Samsone, I., & Ievinsh, G. (2005). Ethylene is involved in Trichoderma-induced resistance of bean plants against Pseudomonas syringae. *Acta Universitatis Latviensis*, *691*, 59-70.

Gilman, J. C. (1957). A manual of Soil Fungi Lowa State College Press. *Anes, Iowa USA pp*, *392*.

Giovannucci, E. (1999). Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Journal of the national cancer institute*, *91*(4), 317-331.

Guleria, S., Sohal, B. S., & Mann, A. P. S. (2005). Salicylic acid treatment and/or Erysiphe polygoni inoculation on phenylalanine ammonia-lyase and peroxidase content and accumulation of phenolics in pea leaves. *Journal of vegetable science*, *11*(2), 71-79.

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E. G., & Cicek, N. (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity. *Journal of Plant Physiology*, *164*(6), 728-736.

Ham, K. S., Kauffmann, S., Albersheim, P., & Darvill, A. G. (1991). Host pathogen Interactions XXXIX. A soybean pathogenesis-related protein with b-1, 3-glucanase activity releases phytoalexin elicitor-active heat stable fragments from fungal walls. *Mol. Plant-Microbe Interact*, *4*, 545-552.

Hansen, H. N. (1926). A simple method of obtaining singlespore cultures. *Science*, *64*(1659), 384-384.

Ojha, S., & Chatterjee, N. C. (2012). Induction of resistance in tomato plants against Fusarium oxysporum f. sp. lycopersici mediated through salicylic acid and Trichoderma harzianum. *Journal of plant protection research*, *52*(2).

Houssien, A. A., Ahmed, S. M., & Ismail, A. A. (2010). Activation of tomato plant defense response against Fusarium wilt disease using Trichoderma harzianum and salicylic acid under greenhouse conditions. *Res. J. Agric. Biol. Sci*, *6*(3), 328-338.

Hsu, C. L., Chen, W., Weng, Y. M., & Tseng, C. Y. (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food chemistry*, *83*(1), 85-92.

Jayalakshmi, S. K., Raju, S., Usha-Rani, S., Benagi, V. I. and Sreeramulu, K. (2009). *Trichoderma harzianum* L1 as a potential source for lytic enzymes and elicitor of defence responses in chickpea (Cicer arietinum L.) against wilt disease caused by *Fusarium oxysporum* f. sp. ciceri. Australian Journal of Crop Science, 3(1): 44-52.

Latha, P., Anand, T., Ragupathi, N., Prakasam, V., & Samiyappan, R. (2009). Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against Alternaria solani. *Biological Control*, *50*(2), 85-93.

Leah, R., Tommerup, H., Svendsen, I. B., & Mundy, J. (1991). Biochemical and molecular characterization of three barley seed proteins with antifungal properties. *Journal of Biological Chemistry*, *266*(3), 1564-1573.

Mandal, S., Mallick, N., & Mitra, A. (2009). Salicylic acid-induced resistance to Fusarium oxysporum f. sp. lycopersici in tomato. *Plant physiology and Biochemistry*, *47*(7), 642-649.

Maxwell, D. P., & Bateman, D. F. (1967). Changes in the activities of some oxidases in extracts of Rhizoctonia-infected bean hypocotyls in relation to lesion maturation. *Phytopathology*, *57*(5).

Mishra, A. K., Morang, P., Deka, M., Kumar, N. S. and Kumar, D. B. S. (2014). Plant growth-promoting rhizobacterial strain-mediated induced systemic resistance in tea (Camellia sinensis (L.) O. Kuntze) through defence-related enzymes against brown root rot and charcoal stump rot.Appl. Biochem. Biotechnol., 174(2): 506-521.

Morsy, E. M., Abdel-Kawi, K. A., & Khalil, M. N. A. (2009). Efficiency of Trichoderma viride and Bacillus subtilis as biocontrol agents gainst Fusarium solani on tomato plants. *Egyptian Journal of Phytopathology*, *37*(1), 47-57.

Nouman, W., Siddiqui, M. T., & Basra, S. M. A. (2012). Moringa oleifera leaf extract: An innovative priming tool for rangeland grasses. *Turkish Journal of Agriculture and Forestry*, *36*(1), 65-75.

Newman, S. M., Tantasawat, P., & Steffens, J. C. (2011). Tomato polyphenol oxidase B is spatially and temporally regulated during development and in response to ethylene. *Molecules*, *16*(1), 493-517.

Punja, Z. K., Utkhede, R., & Arora, D. (2004). Biological control of fungal diseases on vegetable crops with fungi and yeasts. *Fungal biotechnology in agricultural, food, and environmental applications*, *21*, 157-171.

Ragab, M. M., Saber, M. M., El-Morsy, S. A., & El-Aziz, A. R. M. A. (2009). Induction of systemic resistance against root rot of basil using some chemical inducers. *Egyptian Journal of Phytopathology*, *37*(1), 59-70.

Ramamoorthy, V., Raguchander, T., & Samiyappan, R. (2002). Induction of defense-related proteins in tomato roots treated with Pseudomonas fluorescens Pf1 and Fusarium oxysporum f. sp. lycopersici. *Plant and soil*, *239*(1), 55-68.

Ricker, A. J., & Ricker, R. S. (1936). Introduction to research on plant diseases. Jon. S. *Swift Co. Inc., St. Lovis, Chicago, New York*, *117*.

Rauf, B. A. (2000). Seed-borne disease problems of legume crops in Pakistan. *Pakistan Journal of Scientific and Industrial Research*, *43*(4), 249-254.

Reid, J. D., & Ogrydziak, D. M. (1981). Chitinase-overproducing mutant of Serratia marcescens. *Applied and Environmental Microbiology*, *41*(3), 664-669.

Schlumbaum, A., Mauch, F., Vögeli, U., & Boller, T. (1986). Plant chitinases are potent inhibitors of fungal growth. *Nature*, *324*(6095), 365-367.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture*, *28*(1), 49-55.

Song, W., Zhou, L., Yang, C., Cao, X., Zhang, L., & Liu, X. (2004). Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. *Crop protection*, *23*(3), 243-247.

Surekha, C. H., Neelapu, N. R. R., Prasad, B. S., & Ganesh, P. S. (2014). Induction of defense enzymes and phenolic content by Trichoderma viride in Vigna mungo infested with Fusarium oxysporum and Alternaria alternata. *International Journal of Agricultural Science and Research*, *4*(4), 31-40.

Surekha, C. H., Neelapu, N. R. R., Prasad, B. S., & Ganesh, P. S. (2014). Induction of defense enzymes and phenolic content by Trichoderma viride in Vigna mungo infested with Fusarium oxysporum and Alternaria alternata. *International Journal of Agricultural Science and Research*, *4*(4), 31-40.

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., & Valero, J. R. (2007). Antagonistic fungi, Trichoderma spp.: panoply of biological control. *Biochemical Engineering Journal*, *37*(1), 1-20.

Wahba, Z. H., & Barakat, O. S. (2006). The Role of Antagonism in the Rhizospheric Region for Chaetomium globosum and Trichoderma harzianum against Fusarium spp. Attacking Tomato Plant. *Egyptian Academic Journal of Biological Sciences, H. Botany*, *7*(1), 27-36.

Tani, T., & Yamamoto, H. (1978). Nucleic acid and protein synthesis in association with the resistance of oat leaves to crown rust. *Physiological Plant Pathology*, *12*(1), 113-121.

Yedidia, I., Shoresh, M., Kerem, Z., Benhamou, N., Kapulnik, Y., & Chet, I. (2003). Concomitant induction of systemic resistance to Pseudomonas syringae pv. lachrymans in cucumber by Trichoderma asperellum (T-203) and accumulation of phytoalexins. *Applied and Environmental Microbiology*, *69*(12), 7343-7353.

Yoshida, K., Kaothien, P., Matsui, T., Kawaoka, A., & Shinmyo, A. (2003). Molecular biology and application of plant peroxidase genes. *Applied microbiology and Biotechnology*, *60*(6), 665-670.

التأثيرات المفيدة لـ *Trichoderma viride* وحمض الساليسيليك ضد ذبول الفيوزاريوم في الطماطم

محمد عفت خليل
قسم بحوث المكافحة المتكاملة- معهد بحوث أمراض النباتات- مركز البحوث الزراعية- الجيزة- مصر
*بريد المؤلف المراسل: mohamedeffat631@gmail.com

الملخص العربى

تعتبر الطماطم من أكثر محاصيل الخضر الاقتصادية التي يمكن إصابتها بالكثير من الأمراض على سبيل المثال مرض الذبول الفيوزاريوم والذي يسببة فطر *"Fusarium oxysporum f. sp. lycopersici"* حيث يعتبر هذا المرض من أخطر الأمراض التي تصيب نباتات الطماطم. إن حث النظام الدفاعي الكيماوي لنبات الطماطم لمقاومة المسببات المرضية الفطرية هو الهدف الأساسي لأي عملية لمكافحة المسببات المرضية. تمثل المكافحة الحيوية والمستحثات الكيماوية استراتيجية مهمة لحث النظام الدفاعي للنبات ، خصوصا عند تطبيقهما معًا. في الدراسة الحالية ، تم تقدير فاعلية المعاملات التالية على سبيل المثال الفطر المضاد *"Trichoderma viride"* وحمض الساليسيليك منفردين أو مجتمعين معا, مقارنة بفاعلية المبيد الفطري الموصى به "Topsin-M 70%" والذي تم إستخدامه بالجرعة الموصى بها كإستراتيجية جديدة لرفع كفائة الطماطم الدفاعية لمكافحة مرض الذبول الفيوزاريوم تحت ظروف البيوت المحمية والظروف الحقلية. تم دراسة التغيرات الدفاعية الفسيولوجية المختلفة بالإضافة إلى تقدير بعض الإنزيمات المرتبطة بالنظام الدفاعي الكيماوي لنبات الطماطم مثل بوليفينول أوكسيديز ، بيروكسيداز وإنزيم التحلل المائي (تشيتيناز) وكذلك تقدير الفينولات الكلية الذائبة). تحت ظروف البيوت المحمية, تم إصابة نبات الطماطم صناعيا بالفطر *"F. oxysporum f. sp. lycopersici"* وتركت للإصابة الطبيعية تحت الظروف الحقلية. في البداية وتحت ظروف البيوت المحمية, تم تلقيح نباتات الطماطم بالفطر المضاد *"T. viride"* بإحدى الطريقتين)غمس جذور الشتلات / أو معاملة التربة) أو رشها يوميًا لمدة أسبوع بالمستحث الكيماوي SA) منفردين أو مجتمعين معا قبل أسبوع واحد من التلقيح بالفطر الممرض .(أجريت نفس المعاملات على نباتات الطماطم التي تركت للعدوى الطبيعية تحت الظروف الحقلية, بعد ذلك تم جمع النباتات بعد 35 يومًا من الإصابة بالفطر الممرض. وأكدت النتائج المتحصل عليها أن جميع العلاجات المستخدمة أدت إلى حماية نباتات الطماطم بشكل كامل من الإصابة ، حيث انخفضت نسبة خطورة المرض بشكل كبير, كذلك تغير مستوى جميع التقديرات الفسيولوجية المحددة بشكل ملحوظ نتيجة لإستخدام الفطر المضاد والمستحث الكيماوي حيث أظهر العديد من المركبات الدفاعية مما نتج عنه تنشيط النظام الدفاعي الكيماوي في نباتات الطماطم ضد هجوم الكائنات الممرضة. يتم تطبيق هذه المعاملات على كل هذه القياسات ليس فقط للوصول لتحفيزيها داخل النبتات المصابة ولكن أيضًا يتجاوز محتوياتها في النباتات السليمة. كما أدت هذه المعاملات إلى زيادة معنوية في متغيرات النمو وكمية المحصول الكلي للنبات.

الكلمـات المفتاحيـة: الطمـاطم، فيوزاريـوام، تريكوديرمـا، حمـض الساليسـيليك ، محتـوى المركبـات الفينوليـة ، بيروكسيديز ، بوليفينول أوكسيديز ، إنزيم الكيتيناز