



ANTI-FUNGAL ACTIVITY OF SOME NUTRIENTS PLANTS AND *TRICHODERMA HARZIANUM* AGAINST *F. SOLANI* CAUSED DAMPING OFF DISEASE ON CANTALOUPE.

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ABSTRACTS

This study was initiated at the Organic Agriculture center/ Department of plant protection– Ministry of Agriculture, to assess the efficacy of some Eco-friendly materials individually or in an integration to control *Fusarium* rot root disease in melon caused by *Fusarium solani*. Results of greenhouse condition showed that combination treatment $\text{CaSiO}_3 + \text{Trichoderma harzianum} + \text{FeSO}_4$ was the best decreased disease incidence and severity to 0.00 and 0.00 % respectively compared with control (pathogen only) caused 86.67 and 77.70% respectively but not signification differences neither CaSiO_3 alone and $\text{CaSiO}_3 + \text{Trichoderma harzianum}$ treatments, besides, it increased plant fresh and dry weights compared to other combinations. Results indicated that treated plant produced after 14d and 21d of poly phenol oxidase enzyme, treatment of *Trichoderma harzianum* + $\text{CaSiO}_3 + \text{FeSO}_4$ showed superiority in inducing poly phenol oxidase enzyme. The change in light absorbance/ min/g. Fresh weight of melon was (88.67 ,90.33) respectively, compared to control treatment (pathogenic fungus) the activity of poly phenol oxidase was (62.80, 59.33) respectively.

Key words: *Trichoderma harzianum*, *Fusarium solani*, Calcium silicate CaSiO_3 and iron sulfate FeSO_4

الفعالية التثبيطية لبعض العناصر الغذائية والفطر *Trichoderma harzianum* ضد الفطر *Fusarium solani* المسبب لمرض موت البادرات على البطيخ

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الخلاصة

اجريت هذه الدراسة في مركز الزراعة العضوية/ دائرة وقاية المزروعات/ وزارة الزراعة/ بغداد خلال الموسم 2021-2022م لتقييم كفاءة بعض المواد الصديقة للبيئة بصورة منفردة او بالخلط فيما بينها في الحد من مرض تعفن الجذور الفيوزاري في البطيخ المتسبب عن الفطر *Fusarium solani*. اظهرت النتائج في ظروف البيت البلاستيكي ان معاملة التكامل بين $\text{CaSiO}_3 + \text{Trichoderma harzianum} + \text{FeSO}_4$ حققت اقل نسبة وشدة اصابة بلغت 0.00 و 0.00 % على التوالي، الا انها لم تختلف معنوياً عن معاملات اضافة CaSiO_3 بمفردها ومعاملة $\text{CaSiO}_3 + \text{Trichoderma harzianum}$ والتي بلغت فيها النسبة المئوية للاصابة الكلية والشدة 6.67، 13.3 و 2.78، 5.55 % على التوالي مقارنة مع معاملة المقارنة (فطر ممرض فقط) اذ حققت 86.67 و 77.70 % على التوالي، فضلاً عن تسببها في زيادة الوزن الطري والجاف للنبات معنوياً على باقي المعاملات الاخرى، وبينت النتائج بعد 14 و 21 يوم من الزراعة تفوق معاملات التكامل بين المقاوم الاحيائي $\text{CaSiO}_3 + \text{Trichoderma harzianum} + \text{FeSO}_4$ بزيادة فعالية انزيم Poly phenol oxidase مقدراً على اساس التغير في الامتصاص الضوئي/ دقيقة/ غم وزن طري من نباتات البطيخ ليبلغ 88.67 ، 90.33 على التوالي قياساً بمعاملة المقارنة (فطر ممرض بمفرده) والتي بلغت (59.33، 62.80) على التوالي.

الكلمات المفتاحية : *Trichoderma harzianum* ، *F.solani* ، سليكات الكالسيوم CaSiO_3 ، كبريتات الحديد FeSO_4



INTRODUCTION

Melon *Cucumis melo* a species belonging to the *Cucurbitaceae* family, is an important economic crop grown in large areas (Mabaleha *et al.*, 2007). Iraq ranks 12th globally and 2nd amongst Arabic countries After Morocco in melon cultivation. In 2021, Iraq cultivated 63,089 dunums producing 205175 tons (Central Directorate of Agricultural Statistics, 2021; Al-Juboori *et al.*, 2018). Diseases are one of the biological factors limiting melon production (Al-Ani *et al.*, 2009), especially those caused by soil fungi, including several species of the genus *Fusarium* spp (Matloob *et al.*, 2017). *Fusarium* root rot diseases are caused by several species including *F. solani*, *F. avenaceum*, *F. oxysporum*, *F. graminearum* and *F. tricinctum* (Okello & Mathew, 2019.). This pathogenic fungus damages the plant parts below the soil surface, and weakens the vegetative system (Zheng *et al.*, 2018). Although chemical control is a quick and effective way to minimize diseases, but it cannot be adopted as a long term controlling strategy as the excessive use of fungicides can impact human health and the environment (Camilo- Cotrim *et al.*, 2022). Accordingly, alternative controlling approaches have been considered, including the use of microorganisms as biological control agents to minimize the pathogen's inoculum and improve production quantity and quality (Mosa & Hassan, 2023). The fungus *Trichoderma harzianum* has been widely used to control pathogenic fungi (Saeed & Juber 2017; Yasin *et al.*, 2021; Kareem *et al.*, 2020; Abdul-Karim *et al.*, 2021; Kassoub & Hassan, 2022) as it has the inhibitory activity and highly competitive against pathogens. Besides it is stimulating the growth and defense mechanisms of the plant host (Hassan *et al.*, 2021; Kassoub, 2022; Al-Awabid & Yass, 2023). Using nutrient elements against plant pathogens is another controlling approach alternative to chemical control. (Abdel Aal *et al.* 2019) found that calcium silicate could decrease the incidence of Septoria leaf spot disease on peanut leaves. (Fleurat-Lessard *et al.*, 2011) found that FeSO_4 could inhibit the fungal growth of *Botrytis cinerea*, *Eutypa lata*, *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum*, *Deluxeia seriata* and *Neofusicoccum parvum* on PDA medium at concentrations ranged 5-20 mMol/ L. (Aznar *et al.*, 2015) indicated that using iron can enhance plant immunity, through phenolic compounds and proteins production against pathogens, in addition to increasing photosynthesis. Therefore, the study aimed to test the antagonistic activity of some eco-friendly materials against the cause of Cantaloupe root rot disease under greenhouse conditions.

MATERIALS AND METHODS

Source of inoculum

An isolate of the pathogenic fungus *Fusarium solani* (Acc codes OQ689863 and OQ689864) was obtained from the Plant disease laboratory of the Department of Plant Protection - College of Agricultural Engineering Sciences/University of Baghdad. Its pathogenicity was confirmed.

Inhibitory activity assessment of Bio-Agent and nutrients against *Fusarium solani* growth on melon plants in pots.

The experiment was carried out in a greenhouse of the Organic Farming Centre Laboratory/ Department of Crop Protection/Ministry of Agriculture. Soil mixed with peat moss at 1:2 (weight/weight), was autoclaved for 20m twice, at interval of 24h.

Two kg capacity pots were sterilized with 5% sodium hypochlorite solution for 3m, sun dried and the following treatments were applied:



- 1- Without adding pathogen.
- 2- *F. solani*.
- 3- CaSiO_3 .
- 4- FeSO_4 .
- 5- *Trichoderma harzianum*.
- 6- $\text{FeSO}_4 + \text{CaSiO}_3$.
- 7- *Trichoderma harzianum* + CaSiO_3 .
- 8- *Trichoderma harzianum* + FeSO_4 .
- 9- *Trichoderma harzianum* + $\text{CaSiO}_3 + \text{FeSO}_4$.
- 10- $\text{CaSiO}_3 + \text{F. solani}$.
- 11- $\text{FeSO}_4 + \text{F. solani}$.
- 12- *Trichoderma harzianum* + *F. solani*.
- 13- $\text{CaSiO}_3 + \text{FeSO}_4 + \text{F. solani}$.
- 14- *Trichoderma harzianum* + $\text{CaSiO}_3 + \text{F. solani}$.
- 15- *Trichoderma harzianum* + $\text{FeSO}_4 + \text{F. solani}$.
- 16- *Trichoderma harzianum* + $\text{FeSO}_4 + \text{CaSiO}_3 + \text{F. solani}$.
- 17- chemical pesticide (Uniform) + *F. solani*.

Soil in pots was contaminated with fungus pathogen inoculum loaded on local millet seeds previously sterilized by autoclave at rate of 30g /pot soil. For control treatment, Pathogen free millet seeds were added. To ensure pathogen vitality, treatments watered and covered with perforated polyethylene bags for 3d, to maintain a suitable moisture level. Seeds of a local Cantaloupe type melon variety, superficially sterilized with a 3% sodium hypochlorite solution for one minute, and then washed with sterile water were sowed at 5 seeds/pot after each treatment.

About 0.5g per pot of *T. harzianum* at 1×10^9 spores/g concentration was added and mixed well with the soil. CaSiO_3 was added at rate of 500 mg/l and 50 ml/pot concentration based on the best treatment (data not shown to first researcher). Fifty 50 per pot of chemical pesticide Uniform 446 SE, produced by Syngenta, AE (mefenoxam 124 g/L+ azoxystrobin 322 g/L) at 1 ml/L concentration was added to the soil following the recommended doze). FeSO_4 was added at a concentration of 1000 mg/L and rate of 50 ml/pot, according to best treatment (data not shown to first researcher).

Pathogen free control treatments using *T. harzianum*, CaSiO_3 and FeSO_4 , were included. A half of each concentration was used combination treatments. The experiment was designed based on Completely Randomized Design (CRD), with 17 treatments and three replications each. L.S.D test at the probability level of 0.05 was used. After the germination of all seeds in control treatment, seed rot and post-emergence damping off percentages were calculated. Recoding the percentage of post-emergence damping off was after 10d and continued up to 30d of sowing. The pathogen was isolated from infected roots, grown on PDA medium, and examined under compound microscope. After 60d of cultivation, three plants were taken from each replicate, and the disease severity was estimated according to 5 degree pathogenicity scale as follows (Mousa, 2022):

0 = healthy

1= 1-25% of the root rot

2= 26-50% of the root rot



3=51-75% of the root rot

4=76-100% of the root rot

The disease severity percentage was calculated according to **McKinney equation (1923)** as follows:

$$\text{Infection severity} = \frac{(\text{plant of 0 scale} \times 0) + \dots + (\text{plant of 4 scale} \times 4)}{\text{total plants} \times 4} \times 100$$

To calculate the fresh and dry weights, 3 plants were collected from each replicate, plant roots were washed with tap water to remove the soil placed in paper bags and dried in an electric oven at 40 ° C until the weight was stabilized. Some biochemical parameters were tested to identify induced systemic resistance in seedlings against the pathogenic fungus. Leaf samples were collected from plants treated with previously mentioned bio-agents, inoculated with *F. solani* and thought to be systemically resistance induced against the disease after 14d and 21d of inoculation. PPO was estimated following the method previously described (**Ohja & Chatterjee 2012**).

RESULTS AND DISCUSSION

Inhibitory activity assessment of Bio-Agent and nutrients against *Fusarium solani* growth on melon plants in pots.

All treatments could decrease the rate of infection and disease severity of the *F. solani* compared to pathogenic fungus only (control).

All treatments provided a good protection to melon plants against infection in pre- and post-emergence stage with incidence ranged 0.00 - 13.33%, and 0.00 to 20.00%, respectively, compared to pathogenic fungus treatment alone, which scored 26.67-60.0%, respectively (Table 1).

The combination of *T. harzianum* with CaSiO_3 and FeSO_4 was the best treatment when inhibited the fungal pathogen infection both in pre- and post-emergence plant's stages as well as the overall infection rate among other treatments. However, it did not differ significantly from CaSiO_3 , and *T. harzianum* with CaSiO_3 treatments which scored (6.7, 13.3) % the infection rate of pre- and post-emergence infection rates, respectively. Whereas, Uniform pesticide treatment was the least effective when reduced infection in pre- and post-emergence stages, so the total infection rate was scored (46.63) %.

Data showed that all treatments reduced infections severity of melon seeds rot and damping off. *T. harzianum*, CaSiO_3 and FeSO_4 combination scored an infection severity up to 0.00%, but it did not differ significantly from CaSiO_3 , *T. harzianum* with CaSiO_3 treatments (in the presence of pathogenic fungus), which scored (2.78, 5.55) % respectively. The high infectivity and disease severity percentages (Figure 1 A and B) may be related to compounds produced by the pathogenic fungus including secondary metabolites, toxins, and enzymes that degrade pectin and cellulose in the early stages of infection, mainly, chitinase, cellulolytic, pectolytic and protease enzymes (**Al-Mayahi & Hassan, 2021**).

Similarly, symptoms on plants by infected fungus *F. solani* are caused by secondary metabolic compounds transferred to the root system, and these compounds include fusaric acid, javanic acid, polypeptide toxin, and anhydro fusarbin (**Kassoub & Hassan, 2022**). *T. harzianum* inhibitory activity against *F. solani* may be through direct parasitism on the pathogenic fungus through longing, twining and inter-penetration mechanisms and producing enzymes that decompose walls, inhibiting the growth of the pathogenic fungus and reducing



infection (Yao *et al.*, 2023). It can also change the structure of the root system by increasing root cell wall thickness, as well as increasing the availability of plant nutrients, which increase the plant resistance to environmental stresses (Janardan *et al.*, 2011). CaSiO₃ reduction of the infection rate and disease severity may be either through physical inhibition due to preventing the penetration of germination tube of the pathogen into plant epidermis cells or through silicon accumulation around the fungal hyphae, which forms a barrier to protect plant cells (Abd-El Kareem *et al.*, 2019). In the other hand, CaSiO₃ stimulates chitinase activity and speed up peroxidase and polyphenol oxidase enzymes activity after infection. (Abdel Aal *et al.*, 2019).

The effect of iron sulphate FeSO₄ in reducing infection rate and severity is due to the role of iron in producing reactive oxygen species (ROS), which interact with hormone signals and stimulate cellular responses that strengthen plant cells against pathogens (Saleem *et al.*, 2022).

Table (1): Nutrients and bio-agent effect on pathogenic fungus *Fusarium solani* growth in pots.

Treatment	Infection %			Severity %
	Pre-emergence	Post-emergence	total	
Control	0	0	0	0
<i>F. solani</i>	26.67	60.0	86.67	77.70
CaSiO ₃	0	0	0	0
FeSO ₄	0	0	0	0
<i>T. harzianum</i>	0	0	0	0
FeSO ₄ + CaSiO ₃	0	0	0	0
<i>T. harzianum</i> + CaSiO ₃	0	0	0	0
<i>T. harzianum</i> + FeSO ₄	0	0	0	0
<i>T. harzianum</i> + FeSO ₄ + CaSiO ₃	0	0	0	0
+CaSiO ₃ <i>F. solani</i>	0	6.7	6.7	2.78
FeSO ₄ + <i>F. solani</i>	13.33	13.3	26.63	13.77
<i>T. harzianum</i> + <i>F. solani</i>	13.33	20.0	33.33	16.66
FeSO ₄ + CaSiO ₃ + <i>F. solani</i>	6.67	6.7	13.37	8.33
<i>T. harzianum</i> + CaSiO ₃ + <i>F. solani</i>	0	13.3	13.3	5.55
<i>T. harzianum</i> + FeSO ₄ + <i>F. solani</i>	6.67	13.3	19.97	8.33
<i>F. solani</i> + FeSO ₄ + CaSiO ₃ + <i>T. harzianum</i>	0	0	0	0
Uniform + <i>F. solani</i>	13.33	33.3	46.63	22.22
L.S.D _{0.05}	11.383	13.94		7.80

- Each number in the table is an average for 3 replicates.



Figure (1): The effect of some Bio-agents on the rate and severity infection of melon rot seeds disease and damping off, and their effect on the root system of plants.

- A)** The vegetative system of some treatments from right to left (*T. harzianum* + CaSiO_3 + FeSO_4 + *F. solani* Combination treatment, CaSiO_3 + *F. solani* treatment, CaSiO_3 + *T. harzianum* + *F. solani* treatment, control treatment (without pathogenic fungus), treatment with pathogenic fungus alone)
- B)** The root system of some treatments from right to left (pathogenic fungus alone, control treatment "without pathogenic fungus", CaSiO_3 + *T. harzianum* + *F. solani* treatment, CaSiO_3 + *F. solani*, *T. T. harzianum* + CaSiO_3 + FeSO_4 + *F. solani* treatment)

The (FeSO_4 + CaSiO_3 +*T.harzianum*) combination treatment scored the highest fresh and dry weights of plants contaminated with the pathogenic fungus which were (15.80, 5.97) g/plant, respectively, followed by (CaSiO_3 + *T.harzianum*) scoring (14.10, 5.30) g/plant, respectively, compared to control treatment (without the pathogenic fungus and pathogenic fungus only), which scored (4.13, 12.50 and 2.30, 6.80) g/plant, respectively (Figures 2). Similarly, it was stated that *T. harzianum* associated with plant roots improves plant growth by increasing water and nutrients uptake including phosphorus, manganese, nitrogen, magnesium, sodium and calcium. It also works to increase vegetative and flowering growth, the production quality and quantity, as well as metabolic compounds production that promote plant growth in the soil, within bio-fertilizer concept (Al-luhaiby & Hassan, 2020). Silicon increases plant growth, development and production, as it is a useful or semi-essential nutrient for plants (Coskun *et al.*, 2019).

Whereas, iron and sulphate increase plant growth, improve metabolism (chlorophyll and carotenoids), antioxidant enzymatic and non-enzymatic activity and reduce oxidative stress (Saleem *et al.*, 2022; Alheety *et al.*, 2022).

Ahmed & Alaridhee (2013) found a significant increase in the concentration of iron in wheat leaves, which had a positive effect on plant height, number of ears, grain weight and yield.

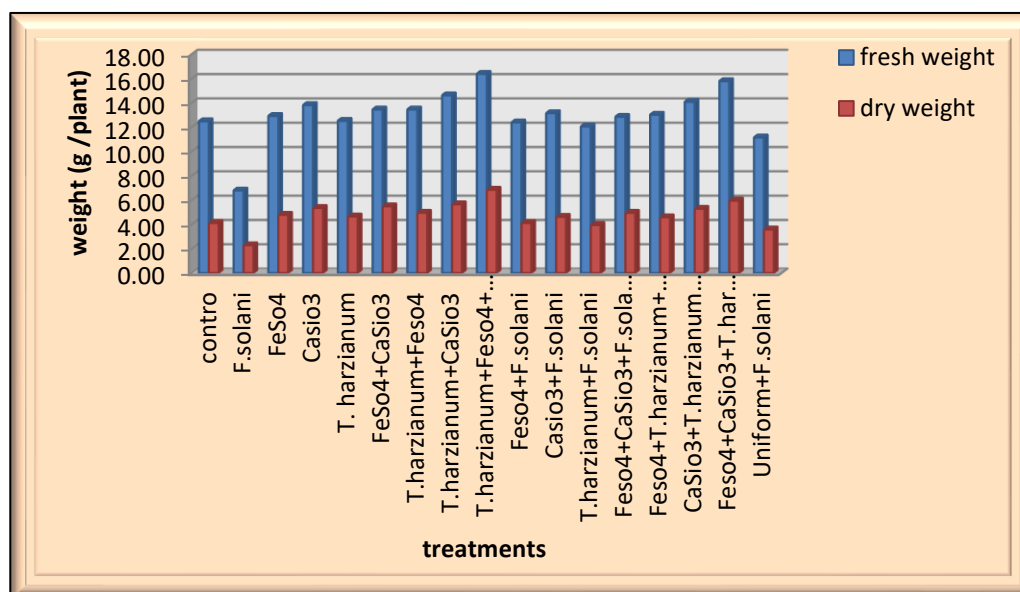


Figure (2): Effect of Nutrients and bio-agent on fresh and dry weights of melon plants

L.S.D_{0.05} for fresh weight = 0.804

L.S.D_{0.05} for dry weight = 0.7362

There were significant differences in Polyphenol Oxidase enzyme (PPO) activity estimated based on changing rate in light absorption / min / gm fresh weight in the melon. The used factors could enhance (PPO) enzyme activity compared to control treatment (pathogenic fungus only). Therefore, the study aimed to test the antagonistic activity of some eco-friendly materials against the pathogenic fungus *F. solani* under laboratory conditions. The highest activity of the enzyme scored 88.67 of *T. harzianum* + FeSO_4 + CaSiO_3 + *F.solani* treatment, and 84.33 of CaSiO_3 + *T. harzianum* + *F. solani* treatment 14d of adding the pathogen. The highest activity of enzyme was scored 21d after adding the pathogen to *T. harzianum* + FeSO_4 + CaSiO_3 + *F.solani* treatment scoring 90.33 followed by other treatments. Similarly, bio-agents could inhibit pathogen enzymes by suppressing those degrading the plant host cell walls and causing infection (**Ruangwong et al., 2021**). *T. harzianum* also produces a number of enzymes, such as Chitinase, Protease, and B-glucanases, which break down the cell walls of pathogenic fungi. In addition to increase plant-inducing enzymes, including Peroxidase and Polyphenol Oxidase, related to defense mechanisms in plants (**Chakraborty & Chatterjee, 2007**)

Additionally, silicon could activate defense-related enzymes, stimulate the production of an anti-pathogen compound, and activate the expression of defense-related genes (**Wang et al., 2017**).

When tested the effect of calcium silicate on Cercospora leaf spot disease on peanuts, (**Abdel Aal et al., 2019**) reported a relationship between induced resistance and some biochemical changes that including peroxidase and polyphenol oxidase.



Table (2): Nutrients and bio-agent effect on PPO enzyme activity in melon plants in pots 14d and 21d after adding the pathogen.

treatments	(PPO) after 14d	(PPO) after 21d
Control	40.50	38.67
<i>F. solani</i>	62.80	59.33
CaSiO ₃	80.07	79.33
FeSO ₄	75.33	72.67
<i>T. harzianum</i>	70.43	68.67
FeSO ₄ + CaSiO ₃	80.77	80.00
<i>T. harzianum</i> + CaSiO ₃	80.33	77.33
<i>T. harzianum</i> + FeSO ₄	75.83	73.67
<i>T. harzianum</i> + FeSO ₄ + CaSiO ₃	81.00	79.33
+CaSiO ₃ <i>F. solani</i>	83.80	84.67
FeSO ₄ + <i>F. solani</i>	80.40	82.33
<i>T. harzianum</i> + <i>F. solani</i>	74.33	71.67
FeSO ₄ + CaSiO ₃ + <i>F. solani</i>	84.33	86.33
<i>T. harzianum</i> + CaSiO ₃ + <i>F. solani</i>	82.67	83.67
<i>T. harzianum</i> + FeSO ₄ + <i>F. solani</i>	82.33	83.33
<i>T. harzianum</i> + FeSO ₄ + CaSiO ₃ + <i>F. solani</i>	88.67	90.33
Uniform + <i>F. solani</i>	37.00	33.67
L.S.D _{0.05}	1.445	1.470

CONCLUSION

Using a combination of (calcium silicate CaSiO₃, iron sulfate FeSO₄ and bioagent *Trichoderma harzianum*) played a significant effective role against the pathogen of *Fusarium solani*, and it was reflected positively on the plant growth, thus it can be used as an important and secure alternative of chemical pesticides.



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