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EFFECT OF SOME MICROELEMENTS AND BIOLOGICAL CONTROL AGENTS IN CONTROL OF TOMATO SEEDLING DAMPING-OFF CAUSED BY *RHIZOCTONIA SOLANI* KUHN

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SUMMARY

This study was conducted at plant protection Department/College of Agriculture/ University . of Basrah to evaluate the efficiency of some Biological and chemical factors and there interaction in control Tomato damping-off disease caused by *Rhizoctonia solani*. The results showed that biological control agent (*Trichoderma harzianum* ; *Bacillus subtilis*) have a high antagonistic ability in inhibition the growth of *R.solani*. It was also found that Flutolanil fungicide, copper and silicon elements were completely inhibited the growth of the pathogenic fungus at 30, 200, 500 ppm respectively. Pot experiment in Glass hose showed that *T.harzianum* + *B.subtilis* + Flutolanil (T + B + F) reavealed higest percentage of seed germenation reached to 100% compared with 60% for pathogen treatment. On the other hand T + B + F treatment reduced the percentage of damping-off rateto zero % compared with 45.395% of pathogen fungus *R.solani*. The result also showed that the use of biological agent as seed coating and chemical agent led to increased the concentration of NPK in tomato plant

Introduction

Rhizoctonia solani is a soil borne fungi it causes several diseases for different economical crops and vegetables such as seed decay and pre and post emergency damping-off. It also causes root rot and infect fruit in contact with soil surface (22). The control of *R.solani* is difficult because of a wide host range of the fungus and its efficiency to survive a long time as resting sclerotia or saprophyte on organic matter (14). Several strategies were used against soil borne pathogen, chemical control is the most widely used method because it is easy and faster in its effect compared to other methods, but extreme use of fungicide led to emergency of many problems such as resistance and its impact on non-target organisms (34). Therfor efforts of researchers are directed to use

microorganism such as *Trichoderma spp, Pseudomonas spp*, and *Bacillus spp* to control plant disease especially root infecting fungi (23).

T.harzianum and *B.subtilis* are widely used to control of seedling damping-off caused by *R.solani* (1; 13) on the other hand nutrient won a good deal of attention as indicated by numerous studies to have a positive role in reducing the damage of many plant diseases (18, 32). Because of the losses caused by Tomato seedling damping-off disease particularly in winter planting in Basrah provence, as statistical studies indicate that the incidence of disease ranging from 9.4-29% in Umm Qaser, Safwan and Zuber fields according to planting times (31; 5). The aim of this study was to evaluate the efficiency of some Biological control agents; microelement and fungicide flutolanin and their interaction in control of Tomato seedling cased by *R.solani*.

Materials and Methods

1:- Antagonism between *Trichoderma harzianum* and *Rhizoctonia solani*

Isolate of *R.solani* was obtained from Tomato seedlings infected with damping-off disease collected from Zuber farms. *T.harzianum* was obtained from the laboratory of plant pathology/plant protection Department/Agricultural/ College/ University of Basrah.

The antagonism ability of *T.harzianum* against *R.solani* was conducted according to Aghighi *et al.*(2).

R.solani myceialel discs 0.5 cm dimeter taken fromagrowing culture margin of the fungus were placed in the center of PDA plates and at 3 cm distance from it, the *T.harzianum* discs (diameter of 0.5 cm) were placed. Control treatment included *R.solani* mycelia plugs was placed in center on non. *T.harzianum* inoculated PDA plates. All plates were incubated at 25C° foe 7 days. The antagonistic ability of *T.harzianum* was measured according to the following formula:-

C=A-B

C= the inhibition percentage

A= the distance between the pathogenic fungus and Biocontrol agent(3 cm)

B= the radial growth of pathogenic fungus in dual culture.

Therefore:-

1- Weak inhibition if the C value $\geq$ 0.9 cm
2- Moderate inhibition if the value from 1-1.9 cm
3- Strong inhibition if the C value $\leq$ 2 cm

This study were done by three replicate for each treatment.

2:- Effect of *B. subtilis* on the growth of *R. solani* and *T. harzianum*

B.subtilis was obtained from Biology Department, College of Science/ University of Basrah. Petri dishes containing PDA were inoculated with *B.subtlis* culture (48 hours age) grown on nutrient broth by placing four drops on the outline of perpendicular lines at a distances of 1 cm from the dish edge, plates were incubated at 25 C° for 48 hours (29) after that plates were inoculated with disc (0.5cm) of *R.solani* (4 days Age) control treatment included plates inoculated with *R.solani* alone. All plates were incubated at 25 C for 7 days and the percentage of inhibition was measured according to following formula

$$\% \text{ inhibition } = \frac{R1-R2}{R1} \times 100$$

R_1= The growth rate of the pathogenic fungus at control treatment
R_2= The growth rate of the pathogenic fungus at dual culture

3:- Effect of different concentration of the fungicide Flutolanil on the growth of *R.solani* and *T.harzianum*

Flutolanil 25% N-[3-(l-methyl ethoxy) phenyl}-2-trifluoro-methyl) Benzamide. Produced by Nihon, Nohyaku Company/Japan. Was used. Stock solution of flutolanil (250 ppm as active ingredient) was prepared. Aliqnet from the stock solution was transferred to 250 ml flasks containing 100 ml of PDA to obtain 0, 10,20 and 30 ppm as ai of flutolnil. The media was poured in sterile petridishes then center of each dish inoculated with 0.5 cm disc taken from *R.solani* and *T.harzianum* cultures individually. Control treatment included inoculated PDA petridishes free from fungicides with each fungus. Experiments was conducted in three replicated. Percentage of growth inhibition were calculated according to the previous formula

4:- Effect of some microelements (sodium silicate and copper sulfate) on the growth of *R.solani* and *T.harzianum*

Stock solution (1000 ppm) was prepared from sodium silicate and copper sulfate, small quantities of each solution were transferred to conical flasks containing 100 ml of PDA in order to obtain 0, 100, 200 300, 400, 500 ppm from sodium silicate and 0, 75, 100, 175, 200 ppm from copper sulfate.Amedium containing each elements poured in sterile petridishes, after it being solldified, center of each dishes were inoculated with 0.5 cm discs taken from *R.solani* and *T.harzianum* cultures. Control treatment included PDA free from elements used inoculated with each fungus. All dishes incubated at 25 C°

for 7 days. Experiment replicated three time and growth inhibition of each fungus calculated according to the previous formula.

5:- Effect of microelement and Biological control agent in control of *R.solani*

Loamy soil sterilized by acommercial formalin was used in this experiment. Soil were left 10 days after sterilization then were distributed in appropriate size pots. Some pots were infested with *R.solani* inoculums (the fungus grown on wheat seeds) by 2% (w/w). pots were irrigated and left for three days. After that the fungicide flutolanil were added to the pots soil in concentration of 2g/L (10 ml/pot)Treatments included biological agent was used as seed coating. Preparation of each agent were prepared by using kaolin clay; Arabic gum and suspension of *T.harzianum* in concentration of 1×10^8 spore/ml or suspension of *B.subtilis* at concentration of 2.5×10^8 cfu/ml in ratio of 1:2:1 respectively. Tomato seeds were dipped in *T.harzianum* or *B.subtilis* preparation or *T.harzianum* plus *B.subtilis* preparation. Seed were dried at laboratory temperature. pots soil were planted with Tomato seeds coated with biological agent in rate of 10 seeds for pot. The experiment included the following treatments

1- Sterile soil (control treatment)
2- Soil infested with *R.solani*
3- Sterile soil plus $CuSO_4$ (200 mg/Kg soil)
4- Sterile soil plus Si (500 mg/Kg soil)
5- Sterile soil plus flutolanil (2 g/l) (100ml/pot)
6- *R.solani* + $CuSO_4$
7- *R.solani* + Si (sodium silicate)
8- *R.solani* + flutolanil
9- *R.solani* + $CuSO_4$ + *T.harzianum* (seed coated)
10- *R.solani* + Si + *T.harzianum*
11- *R.solani* + $CuSO_4$ + *B.subtilis*
12- *R.solani* + Si + *B.subtilis*
13- *R.solani* + flutolanil + *B.subtilis*
14- *R.solani* + flutolanil + *B.subtilis*
15- *R.solani* + $CuSO_4$ + (*T.harzianum* + *B.subtilis*)
16- *R.solani* + Si + (*T.harzianum* + *B.subtilis*)
17- *R.solani* + flutolanil + (*T.harzianum* + *B.subtilis*)

The experimentwas carried out with three replicates for each treatment the following measurements were taken

1- % of germination
2- % of seedling damping-off

3- % of the occurence of pathogenic fungus in the roots

The experiment was re-conducted in the field and at the end of the experiment concentration of the NPK in tomato leaves was measured according to Page *et al*. (29); Murphy and Riley (27).

Results and Discussion

1:- Effect of *T.harzianum* on the growth of *R.solani*

Results of this experiment showed that *T.harzianum* had a high antagonistic ability against *R.saloni* (Fig 1), with an inhibition zone reach 3 cm according to Aghighi *et al*.(2) scale. This result was compatible with many studies stated that the ability of *T.harzianum* in the inhibition growth of *R.solani* is due to different mechanisms such as production of toxic metabolites (6) or production of enzymes like B-1,3 glucanases and chitinases which break down the fungal cell-wall (32) or cellulases (35) or due to direct parasitism on fungal mycelium (6)

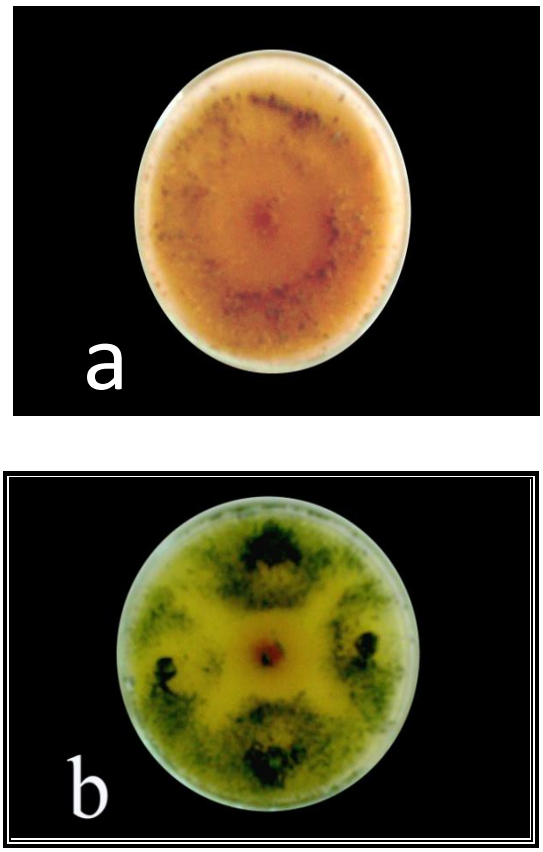

Fig (1). Effect of *T.harzianum* on the growth of *R.solani*
a= control, b=*R.solani* and *T.harzianum*

2:-Effect of B. subtilis on the growth of *R.solani* and *T. harzianum*

The results showed that the bacteria *B.subtilis* was completely inhibited the growth of fungus *R.solani* as inhibition percentage reached 100% (Fig 2). This result are agreed with previous studies which refer the antagonistic ability of *B. subtilis* to production of many antibiotics such as subtiline, bactracin, lturin and surfactin or to its ability to production cell wall degrading enzymes such as endochitinase, proteases and B-1,3-glcanases which break down the fungal cell wall (25; 28) On the other hand results of this test showed that *B.subtilis* had moderate ability in inhibition the growth of *T.harzianum* on PDA as percentage inhibition reached 55.5% and this may be due to some mechanisms enable the fungus *T.harzianum* to avoid enzymes and toxins produced by *B.subtilis*. As noted from this experiment the absence of an obvious effect of *T.harzianum* on growth of *B.subtilis*.

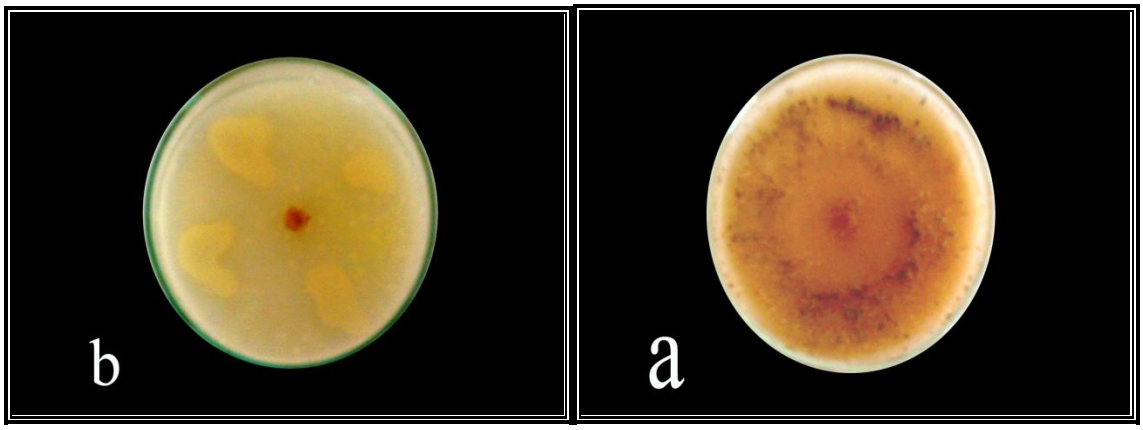

Fig (2). effect of *B.subtilis* on the growth of *R.solani*
a= control, b=*R.solani* and *B.subtilis*

3:- Effect of fungicide flutolanil 25% in the growth of *R.solani* and *T.harzianum*

Results of this experiment (Table 1) showed that effect of fungicide flutolani 25% was varied according to the concentration used, as inhibition percentage reach 100%, when using concentration of 30 mg/L as ai

This results are compatible with previous studies pointed to the effectiveness of the fungicide flutolani in the growth inhibition of *R.solani* (19). The ability of flutolanil in inhibition the growth of *R.solani* may be due to its ability to prevent synthesis of fungal cell wall (24) on the other hand results of this experiment showed that fungicide flutolanil did not affect significantly the growth of

T.harzianum especially at low concentration (10 and 20 mg/L) as inhibition percentage reached 4.4 and 27.7% respectively.

Table (1). Effect of fungicide Flutolanil on the growth of *R .solani* and *T.harzianum*

Concentrations(mg/L)	% of inhibition	
	R .solani	***T.harzianum***
0	0	0
10	77.7	4.4
20	94.4	27.7
30	100	50
R.L.S.D (0.01)	0.8	9.1

4:-Effect of copper sulfate 0n the growth of *R.solani* and *T.harzianum*

The results of this experiment (Table 2) showed that copper sulfate significantly reduced the growth of *R.solani* at all concentration tested compared with control treatment. As it showed a concentration 200 mg/L gave highest percentage of inhibition reached 100%. This results was inconformity with other studies noted to the effectiveness of copper in the inhibition of many fungi like *Pytophthora capsici* (15) and *Fusarium oxysporum* (3) and *Pythium aphanidermatum* (16). The effectiveness of copper may be referred to copper ion which has the ability to link with some chemicals like amine and carboxyl groups existing in many important enzymes in the fungal cells (4). On the other hand results of this experiment showed that the copper did not effect the growth of *T.harzianum* but high concentration of copper caused changing in the colour and shape of fungal colony.

Table (2). Effect of copper sulfate on the growth of *R.solani* and *T.harzianum*

Concentration mg/L	% inhibition	
	R.solani	***T.harzianum***
0	0	0
75	61.1	0
100	66.6	0
125	72.2	0
150	83.3	0
175	88.8	0
200	100	0
R.L.S.D (0.01)	6.2	0

5:- Effect of sodium silicate on the growth of *R.solani* and *T.harzianum*

Results of this experiment (Table 3) showed that all concentration used of silicon inhibited the growth of *R.solani* but the % of inhibition increased as silicon concentration used increased were 500 mg/L gave the highest inhibition reached 100% while the % of inhibition for other concentrations (100-400 mg/ml) ranged between 5.5-88.8%. Studies on the impact of silicon on the growth of plant pathogenic fungi are very few. However Belanger (7) pointed out that the silicon used at concentration 500mg/L completely inhibited the growth of *Pythium aphanidermatum*. Cherif *et al*.(9) also noted that silicon element may be accumulated in the fungal cells and that causes disturbance to enzyme function. On the other hand all concentration of silicon used did not affect the growth of *T.harzianum* compared with control treatment but the fungal colony of biofungus lost its circular regular shape.

Table (3). Effect of sodium silicate on the growth of *R.solani*

Concentration mg/L	% inhibition
0	0
100	5.5
200	61.1
300	83/3
400	88.8
500	100
R.L.S.D (0.01)	3.7

6:- Effect of chemical and biological treatment and there interaction on seed germination and tomato seedling damping-off of tomato

The results in Table (4) showed that the pathogenic fungus *R.solani* had significant effect on tomato seed germination which reached 60% compared to 100% in control treatment (Sterile soil free from *R.solani*) However coating of tomato seeds with biological control agent (*B.subtilis*, *T.harzianum*) alone or with combination with chemical agent reduced the negative effect of *R.solani* as % of seed germination increased from 60% in *R.solani* treatment to 100% in (T + B) + F + R and T + F+R treatments while seed germination ranged from 73.7-90% for other treatments. The increase of seed germination in treatments involved *T.harzianum* may be due to the ability of this fungus to stimulate seed germination through excreted of several enzymes and growth regulators (6) or may be due to possession of *T.harzianum* to one or more mechanisms such as competition for nutrient and space or mycoparasitism or production of enzymes and antibiotics which all acts as inhibitory agent to pathogenic fungus (6; 16). The results also showed that treatments involved *B.subtilis* increased seed germination compared with pathogenic fungus treatment (*R.solani* alone) and this may be due to possession of *B.subtilis* to several mechanism such as production of antibiotics and enzymes which inhibited the growth of pathogenic fungus (26). As for the role of fungicide flutolanil and microelement in increasing the % of germination it may be due direct effect of these chemical element on pathogenic fungus through its ability to interfere with essential enzymes in fungus cells specially which contain amino and thiol groups (10; 7). The results of this experiment also showed that there were significant differences between biological, chemical treatments and pathogenic fungus treatment (*R.solani*) in percentage of seeding damping-off. As seeding damping-off were reduced from 45.39 % in pathogenic fungus treatment to zero % in (T + B) + F + R and T + F + R treatment while the % of seedling damping-off reached 11.12% in (T + B) + Cu + R. The results showed that coating tomato seeds with biological agent in compenation with chemical factors gave better results in reduction of % seedling damping-off and this may be due to possession of *T.harzianum* and *B.subtilis* to several mechanisms that effect the pathogenic fungus as mentioned by (13; 6; 26).

Table (4). Effect of biological and chemical treatment and their interaction on seed germination and seedling damping-off

Treatments	% germination	% Seedling damping-off
Control (sterilized soil only)	100*	0
Contaminated soil with fungus R.	60	45.39*
Sterilize soil plus Cu	100	0
Sterilize soil plus Si	199	0
Sterilize soil plus F	100	0
Cu+Rs	76.56	15.28
Si+Rs	60	27.30
F+Rs	80	16.67
T+Cu+Rs	75.34	12.04
T+Si+Rs	66.67	20.63
B+Cu+Rs	70	14.28
B+Si+Rs	60	16.98
T+F+Rs	100	0
B+F+Rs	83.34	11.58
(T+B)+Cu+Rs	90	11.12
(T+B)+Si+Rs	86.67	15.28
(T+B)+F+Rs	100	0
R.L.S/D (0.05)	6.878	13.02

- **Three replicates average**
- **T= *Trichoderma harzianum***
- **Rs= *R.solani***
- **B=*Bacillus subtilis***
- **F= flutolanil 25%**
- **T+B= (*Trichoderma harzianum* + *Bacillus subtilis*)**
- **Si= Silicon**
- **Cu= Copper**

7:- Effect of biological and chemical treatment and their interaction on occurrence of *R.solani* in tomato roots.

Results of this study (Table 5) indicated that there was significant reduction in occurrence percentage of pathogenic fungus in tomato seedling roots in treatment involved companition between biological agent and chemical agent agreed with control treatment as percentage of *R.solani* occurrence in seedling roots reduced from 40% in pathogenic fungus treatment to 23.4 and 30% in T+F+R and (T + B) + F + R respectively. As indicated in results of the same table the best treatment in reducing the occurance of *R.solani* in the seedling root was the treatment involved participation of *T.harzianum* or *B.subtilis* plus fungicide flutolanil. The high occurance of *R.solani* in treatments involved Si and Cu may be due to leaching of these elements during irrigation. This results are compatible with previous studies pointed out that *T.harzianum* and *B.subtlis*

possession several mechanisms such as competition and antibiosis or mycoparasitism that inhibited the pathogenic fungus (11; 8; 20).

Table (5). effect of biological and chemical treatment and their interaction on *R.solani* occurrence on the tomato roots.

Treatments	% occurrence of *R.solani*
Control (sterilized soil only)	0
Contaminated soil with fungus R.	90*
Sterilize soil plus Cu	0
Sterilize soil plus Si	0
Sterilize soil plus F	0
Cu+Rs	73.34
Si+Rs	73.34
F+Rs	46.67
T+Cu+Rs	33.34
T+Si+Rs	36.67
B+Cu+Rs	33.34
B+Si+Rs	40
T+F+Rs	23.34
B+F+Rs	30
(T+B)+Cu+Rs	33.34
(T+B)+Si+Rs	36.67
(T+B)+F+Rs	30
R.L.S.D (0.05)	6.117

- **Three replicates average**
- **T= *Trichoderma harzianum***
- **Rs= *R.solani***
- **B=*Bacillus subtilis***
- **F= flutolanil 25%**
- **T+B= (*Trichoderma harzianum* + Bacillus subtilis)**
- **Si= Silicon**
- **Cu= Copper**

8:-Effect of biological and chemical treatment and their interaction on NPK content in tomato plants

Results of Table (6) indicated that all biological and chemical treatment increased nitrogen content in tomato leaves compared with pathogen treatment (*R.solani*) (except for Cu treatment) , but the best treatment in increasing N content was T + F + R which reached 4.865% compared with 2.215 for pathogen treatment other treatment varied in their increasing % of N content in the leaves. It was also showed from the same table that (T + B) + F + R treatment increased the % of P to 0.887% compared with 0.578% for pathogen treatment. On the

other hand (T + B) + F + R treatment caused asignificant increase in % of potassium compared with pathogen treatment. The high level of macroelement in tomato leaves may be due to the role of Biological agent in protection of the tomato roots to be infected with *R.solani*. This result was compatible with Ma and Takahashi (21) and El-Hussieni (12).

Table (6). Effect of biological and chemical treatment and their interaction on NPK content in tomato leaves

Treatments	N%	P%	K%
Control (sterilized soil only)	3.305*	0.578*	1.680*
Contaminated soil with fungus R.	2.215	0.495	0.625
Sterilize soil plus Cu	3.145	0.539	2.175
Sterilize soil plus Si	4.575	0.576	2.210
Sterilize soil plus F	3.485	0.643	2.110
Cu+Rs	3.320	0.643	2.450
Si+Rs	3.605	0.778	2.630
F+Rs	3.475	0.845	2.150
T+Cu+Rs	4.459	0.822	3.440
T+Si+Rs	3.300	0.636	3.210
B+Cu+Rs	4.165	0.754	3.450
B+Si+Rs	3.815	0.688	3.350
T+F+Rs	4.885	0.855	3.930
B+F+Rs	4.115	0.487	3.653
(T+B)+Cu+Rs	4.485	0.818	4.580
(T+B)+Si+Rs	3.450	0.742	4.495
(T+B)+F+Rs	4.140	0.887	4.825
R.L.S/D (0.05)	1.013	0.037	0.764

- **Three replicates average**
- **T= *Trichoderma harzianum***
- **Rs= *R.solani***
- **B=*Bacillus subtilis***
- **F= flutolanil 25%**
- **T+B= (*Trichoderma harzianum* + Bacillus subtilis)**
- **Si= Silicon**
- **Cu= Copper**

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تأثير بعض العناصر المعدنية الصغرى وعناصر المكافحة الإحيائية في مكافحة مرض سقوط بادرات الطماطةتسبب عن الفطر *Rhizoctonia solani* Kuhn

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الخلاصة

أجريت هذه الدراسة في قسم وقاية النبات (كلية الزراعة/جامعة البصرة) بهدف تقييم فاعلية بعض العوامل الإحيائية والكيميائية والتداخل فيما بينها في مكافحة مرض سقوط بادرات الطماطا المتسبب عن الفطر *Rhizoctonia solani* (Kuhn) وأظهرت النتائج أن لعاملي المقاومة الإحيائية *Trichoderma harzianum* و*Bacillus subtilis* قدرة عالية في تثبيط نمو الفطر *R.solani* كما أدى استخدام المبيد فلوتولانيل بتركيز ٣٠ ملغم/لتر وسط زرعي وعنصر النحاس بتركيز ٢٠٠ ملغم/لتر وعنصر السليكون بتركيز ٣٠ ملغم/لتر إلى تثبيط نمو الفطر الممرض بشكل كامل . وأظهرت تجربة الأصص داخل البيت الزجاجي أن استخدام الفطر *T.harzianum* + البكتريا *B.subtilis* + المبيد فلوتولانيل أعطت أفضل % لإنبات البذور بلغت ١٠٠% قياساً بـ ٦٠% لمعاملة الفطر الممرض كما أدت هذه المعاملة إلى خفض النسبة المئوية لموت البادرات من ٤٥.٣٩% إلى الصفر % (وجاءت نتائج تجربة الحقل معززة لنتائج تجربة الأصص إذ حققت معاملة المبيد فلوتولانيل + الفطر *T.harzianum* + البكتريا *B.subtilis* ومعاملة النحاس + البكتريا *B.subtilis* خفضاً معنوياً في % لموت البادرات بلغت صفر % قياساً بـ ٤١.٨٨% لمعاملة الفطر الممرض كما أدى استخدام عناصر المكافحة الإحيائية والكيميائية إلى زيادة محتوى النبات من العناصر الكبرى NPK.

*** البحث مستل من رسالة ماجستير للباحث الثاني**