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Antifungal Activity of *Brevundimonas diminuta* Against *Fusarium oxysporum* on Tomato Plants under Greenhouse Condition

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Abstract

Eighty one bacterial isolates were obtained from 53 soil samples of different plants rhizosphere. All the isolated bacterial were screened for antifungal effect against *Fusarium oxysporum*. Three isolates gave antifungal activity with inhibition zone ranged between (0.5-2.5 cm). Two isolates (Bd1 and Bd2) were *Brevundimonas diminuta*, while the third(Pf1) was *Pseudomonas fluorescence* .*B.diminuta*(Bd1) which used in this study isolated from *Raphanus sativus* gave the highest inhibitionzone against *F. oxysporum*.In green house experiment , seedling of tomato cultivar *Lycopersicon esculentum* . var. *commune* were treated with two concentration 10^{12} cell/ml(B^{12}) ,10^{14} cell/ml (B^{14}) of *B.diminuta* (Bd1) and planted in soil mixed with10^{6} spores/gm(F) of *F. oxysporum* one day before seedling planting. . The growth parameters(percentage in leaf area , total chlorophyll , percentage for fresh and dry weight) were recorded after 60 days.Also ,FLC analysis were used todetectthe hormones in plant leaves. The result showedsignificant increased in growth characters by (FB^{14}) treatmentcompared with (CF) treatment(tomato plant infected by*F. oxysporum* only). The plant growth parameters values correspondingly increased by increasing bacterial dose. While the FLC analysis for the hormones showed increasing percentage for the Indole acetic acid , gibberellic acid and cytokinine group (Kintein ,Zatain and Benzyl adenine) under FB^{14} treatment compared with CF treatment , respectively . Beside ,The Abscisic acid hormone which promote for plant resisting against fungi was significant increasedwith FB^{14} treatment .The *B. diminuta* (Bd1) is reported for the first time prove effective useful for their establishment and proliferation in soil for antifungal effect against *F.oxysporum*.Also, this is first time using *B. diminuta* (Bd1) cultural as well as plant growth promoting rhizobacteria (PGPR) functional for tomato crop.

Keywords: *Brevundimon asdiminuta*, PGPR , biocontrol , antifungals, tomato plant.

الفعالية التثبيطية لبكتريا *Brevundimonas diminuta* ضد الفطر *Fusarium oxysporum* على نباتات الطماطة تحت ظروف البيت الزجاجي

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الخلاصة

تم الحصول على احدى وثمانين عزلة بكتيرية عزلت من ثلاثة وخمسين عينة من ترب جذور نباتات مختلفة . تم التحري عن قابلية جميع العزلات على انتاج مادة مضادة للفطر *Fusarium oxysporum*. اظهرت ثلاثة عزلات فعالية مضادة للفطربمناطق تثبيط تراوحت بين5, 0 – 5, 2 سم . أظهرت النتائج ان عزلتين (Bd1 و Bd2) تعود للنوع *Brevundimonas diminuta* اما العزلة الثالثة(Pf1) فتعود الى النوع *Pseudomonas fluorecensce* . تم استخدام عزلة (Bd1) *B. diminuta* في هذه الدراسة والمعزولة

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من نبات الفجل ، والتي تمتلك أعلى فعالية تثبيط ضد الفطر *F.Oxysporum*.تمت معاملة شتلات الطماطة *Lycopersicon esculentum.* var.*commune* ببكتريا (Bd1) *B.diminuta* بتركيزين (10^{12} و10^{14} خلية مكونة للمستعمرة/ مل) ومن ثم زرعت داخل بيت زجاجي في ترب مضاف اليها سبورات الفطر بواقع (10^{6} سبور/ غرام تربة) وذلك قبل يوم من زراعتها. تم قياس مؤشرات النمو للنباتات بعد مرور ستين يوماً ، كما استخدم تقنية Fast Liquid Chromatography (FLC) للتحري عن الهرمونات قي اوراق نبات الطماطة. أظهرت نتائج التحليل الإحصائي ان للتداخل بين البكتريا والفطر (FB^{14}) زيادة معنوية ملحوظة في المساحة الورقية ومحتوى الكلوروفيل وارتفاع النبات والوزن الطري والجاف بالمقارنة مع نباتات السيطرة المعاملة بالفطر فقط (FC)، وقد تبين ان القيم ازدادت بزيادة تركيز البكتريا. وقد أظهرت النتائج فروقات معنوية في تراكيز الهرمونات لكل المعاملات، اذ أظهرت القراءات إزدياد نسب الاوكسينات والجبرليك اسد (مجموعة السايتوكاينينات) و (Kinetin وZeatin وBenzyl adenine) في النباتات المصابة بالفطر والمعاملة بالتركيز العالي للبكتريا (FB^{14}) بالمقارنة مع نباتات السيطرة والمعاملة بالفطر فقط(FC)، كما اظهرت النتائج ان هورمون ABA الذي يحفز المقاومة في النبات ضد الاصابة بالفطريات قد ازداد بشكل ملحوظ في النباتات المصابة بالفطر والمعاملة بالتركيز العالي للبكتريا (FB14) بالمقارنة مع نباتات السيطرة والمعاملة بالفطر فقط(FC) .

Introduction

Fungal phytopathogens pose serious problems worldwide in the cultivation of economically important plants [1]. Different methods have been used to control plant pathogens, being the most used cultural practices, resistant cultivars, chemical and biological control. Biological control is a natural and specific way to control pathogens and enhance crop yield by growth promoting attributes of environment friendly microorganisms [2]. To increase crop yields, it is necessary to apply agrochemicals, which have several negative side effects [3]. Chemical fungicides are extensively used in current agriculture. However, excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide. Since pathogen damage potentially causes large yield losses, the use of plant growth-promoting rhizobacteria(PGPR) with antifungal properties is an attractive alternative to the use of such xenobiotic compounds [4].The bacteria *Brevundimonasdiminuta*is considered as an effective PGPR as it possess a number of traits useful for their establishment and proliferation in soil [5].Microbial antagonist strains capable of producing both nonvolatile compounds and volatile compounds (VOCs), which exhibit strong inhibitory activity against plant pathogens, have received much attention [6,7].The aim of this research was to identify alternative PGPR for plant protection against*F. oxysporum* , which are less dependent on chemicals and environmentally friendly.

Materials and methods

SamplesCollection

Fifty-three samples of rhizospheres soils of different plants from Baghdad city,were collected under aseptic condition then transported to the laboratory until use.

Isolation of bacteria

1. One gm of each soil sample was added to 9 ml of D.W and mixed to homogenize and serial dilutions were prepared for each sample.
2. About 0.1 ml of each dilution was spreaded on MacConkey agar plates and incubated at 30°C for 2 days.
3. The bacterial colonies were purified by subculturing on nutrient agar plates until pure culture was obtained.

Screening for antifungal substance producing bacteria[8]

All bacterial isolates were tested for antifungal substance production as following:

1. Nutrient broth tubes (5ml) were inoculated with activated bacterial suspensions and incubated at 30 °C for 24hrs.
2. Discs of sterilized filter paper NO.1(5mm) were saturated with bacterial suspensions (O.D = 0.3 at 600nm).
3. Plates of MEA pH 6, were inoculated with 10^{6} fungal spores/ml of *F.oxysporum* by swabbing, the plates were leaved for 10 min.

4. The saturated filter paper discs were placed on the surface of the MEA plates and incubated at 30 °C for 7 days.
5. The diameters of inhibition zones around the discs were measured.

Identification of bacterial isolate:

Bacterialwas identification by used VITEK 2 compact device (Biomerienx USA) in Ibn Balady hospital laboratories, Ministry of health.This device contains 47-biochemical tests.

Greenhouse experiment

The field trials were conducted in the Greenhouse at the nursery field Universityof Baghdad, Baghdad, Iraq. Soil samples were collected and air dried,and examined for different physical-chemical parameters before used.Table-1. The pots used in the experiment were filled with soil and pitmous (1:1) at 2 Kg/pot. The soil was mixed with 10^6spores/gm one day before seedling planting.

Table 1- Physical and chemical properties of greenhouse soil

Parameters *	Value
pH**	7.4
EC	1.1 ds/m
Na^+	5.817 meq/L
K^+	0.410 meq/L
Mg^{++}	6.00 meq/L
Ca^{++}	11.00 meq/L
Cl^-	1.00 meq/L
HCO_3^-	3.00 meq/L
Soil isolate (mg/g soil) :	
Sand	320 g/kg
Silt	430 g/kg
Clay	250 g/kg
Soil texture	Loam
Field capacity (%)	31

*Each value was an average of 4 replicates.
** In saturated paste extract at 25°C.

Seedling of tomato cultivar *Lycopersiconesculentum* .var. *commune* were brought from Iraqi nursery and identified by the specialists University of Baghdad Herbarium in the Department of Biology, College of Science, University of Baghdad. The seedling inoculation according to the treatment plan. Two seedlings were planted in each plot at 16/10/2014. The pots were arranged randomly and each treatment was replicated thrice according to completely randomized design.

The seedlings were infected with the selected bacterial isolate by a standard root-dip inoculation method [9] ,two numbers of bacteria were used 10^{12} and 10^{14} , Table-2. Seedling root was incubated in the bacterial suspension for 5 minutes and individually planted in the pots. Pots were irrigated with tap water every day. Weeds were eliminated by hand. The growth parameters were recorded after 60 days.

Table 2- Numbers of microorganisms used in greenhouse experiment

Treatment	symbol	Number of Microorganisms	Number of repeats
Control Plant	C	-----	3
Control Fungus	CF	10^6 spore/ml	3
Control Bacteria 10^{12}	CB^{12}	10^{12} cell/ml	3
Control Bacteria 10^{14}	CB^{14}	10^{14} cell/ml	3
Bacteria 10^{12} + Fungus 10^6	FB^{12}	10^{12} cell/ml + 10^6 spore/ml	3
Bacteria 10^{14} + Fungus 10^6	FB^{14}	10^{14} cell/ml + 10^6 spore/ml	3

Agronomic traits:

Leaf area (cm^2)

Leaf area of three repeats of leaves from each treatment was measured; graph papers were used to estimate the leaf area of plants leaves (three leaves for each treatment).

Plant height (cm)

Plant height of three repeats of plants from each treatment was measured from plant base to the tip of main stem spike excluding awns, and then the mean was recorded.

Determination of chlorophyll content [10]

Chlorophyll content was estimated in three repeats of plant leaves from each treatment by using Minolta SPAD 502.

Biomass fresh weight

To determine the total fresh weight, three repeats from each treatment at flowering stage recorded and expressed in grams per plant, then the mean was recorded.

Biomass dry weight

Three repeats of plants from each treatment at flowering stage were over dried at 65°C a constant weight. The dry biomass was recorded and expressed in grams per plant, and then the mean was recorded.

Separation of plant growth regulator hormone

Separation of some plant hormones was made by Fast liquid chromatography (FLC).Five hormones were separated for six treatments (C, CF, CB12, CB14, FB12, FB14) .The apparatus type was Shimatzu LC 10-Japan , the procedure was applied by using column type C-18(50mm×2.1mm), 3μm particle size, mobile phase which consisted of a mixture of solvents: solvent (A) acetonitrile, solvent (B) 1% acetic acid methanol in 1mM tetrabutylamonium phosphate + 400 μl triethylamine(pH 3.0) using linear gradient from 0-100% B in 5minutes , flow rate 1ml/min, detection Uv-Vis at 280nm ,20 μl of the sample was injected , 5μg of standard was used for each hormone.. The area under a peak is used for calculating the concentration of a sample as the following formula:

Concentration of sample (μg/ml) = [[Area of the sample/Area of the standard]] × Standard Conc. × Dilution factor

Extraction procedure

Growth regulator hormones were extracted according to the procedure reported by Unyayar*et al.*(1996)[11], which were modified according to FLC separation which briefly described as follow :

1. The sample 10 mg were weighted and crushed into fine paste using clean mortar and pestle by adding 60ml combined extract , the extract contain methanol : ammonia : chloroform solution in ratio 12 : 5 :3 V/V/V.
2. The combined extract filtered and the filtrate centrifuged at 6000 rpm for 15 min.
3. Combined extract filtrate was treated with 25ml deionized water, the chloroform phase was discarded.
4. The water methanol phase was evaporated to dryness in rotary evaporator at 30°C , and re-dissolved in known volume of the mobile phase.
5. The water phase was adjusted the extract to pH 2.5.
6. 20 μl were injected to HPLC system.

Field experiment and statistical analysis

Randomized Complete Block Design (R.C.B.D.) was used as an field experimental design. Data were analyzed by using statistical analysis system- SAS (Y.2001) to study the effect of different factors on the diameters of inhibition zones. Least significant difference (LSD) was used to compare the significant difference between means at $P \leq 0.05$.

Results and Discussion

Isolation of bacteria

Eighty one bacterial isolates were obtained from 53 soil samples, after culturing on MacConkey agar .This medium is selective for gram negative bacteria and differentiate between lactose fermenter and non lactose fermenter [12].

The result showed that 51 isolates were non lactose fermenter which appeared as pale colonies and 30 isolates were lactose fermenter which appeared as pink colonies.

Screening for antifungal substance producing bacteria

Eighty one bacterial isolates were screened for antifungal effect against *Fusariumoxysporum* , the results showed that only three isolates gave antifungal activity with inhibition zone ranged between (0.5-2.5 cm) , and the isolate isolated from (*Raphanussativus*) gave the highest inhibition zone (2.5 cm).Table-3.

Table 3- Diameters of inhibition zone of*F.oxysporum* by the bacterial isolates.

Isolate number	Diameter of inhibition zone (cm)
1	2.5
2	1.8
3	2.0

Identification of bacterial isolates by VITEK

All the isolates which gave inhibition zones were identified by VITEK2 compact device. The results showed that two from the three isolates were *B.diminuta*(Bd1) and (Bd2) which isolated from (*Raphanussativus*) and (*Triticumaestivum*) respectively , and one isolate was *Pseudomonas fluorescence* (Pf1) isolated from (*Anethumgraveolens*) .

The influence of Bacteria and Fungus on some characteristics growth:
leaf area(cm^2), total chlorophyll content (SPAD) and plant height (cm).

Recent efforts have focused on effective bio-control method for the controlling of plant diseases. Results in Table-4 shows that mean of leaf area, total Chlorophyll and plant height were significantly affected by using the bacterial treatments.The result recorded that the highest leaf area, total chlorophyll content and plant height were 99.3 cm^2,41.55 SPAD and 58.50 cm ; by using B^{14} treatment, respectively. While at control treatment, they reduced to 53.2 cm^2, 32.12 SPAD and 37.08 cm, respectively. Also, the result exhibited that the mean of fungi treatment was decreased the leaf area, leaf total Chlorophyll and plant height. But this decreasing was not significant with other treatments.

Results in Table-4 indicate that all the interaction between bacteria and fungus treatments significantly affected leaf area, total chlorophyll content and plant height. The highest values (102.5cm^2, 41.87SPAD and 62.67cm) were recorded by FB^{14}, respectively. While the lowest values (35.8 cm^2,31.13 SPAD and 33.67 cm) were recorded by control treatment with fungus, respectively. However, at higher bacterial levelapplication , differential incensed in aforementioned agricultural traits had been observed among the*F. oxysporum* treatment . Maximum values were observed in FB^{14} followed by FB^{12} treatment.

Fresh and dry biomass weight (g):

In this study fresh and dry biomass characters were greatly increased by all bacterial treatments in comparison to the control (zero bacteria) with significant differences in most cases. The values correspondingly increased by increased bacteria dose in comparison with control.
According to the result in Table-5. Average biomass fresh and dry weights were significantly increased up to 80.7 and 45.3g by application of B^{14} bacteria, while control treatment significantly reduced them to 17.5 and 8.3 g, respectively.

Also, the means of Biomass fresh and dry weight were significantly decreased to 38.1 and 23.7g for control treatment, respectively .While they raised to 64.3and 37.7g with fungus treatment, respectively. Interaction between the bacteria and fungus significantly impact biomass fresh and dry weight. The minimum weights were 13.3 and 4.2g recorded in control treatment, while maximum weights were 110.3 and 50.3g recorded in FB^{14} treatment.

Table 4-The influence of Bacteria and Fungus onleaf area(cm^2), total chlorophyll content (SPAD) and plant height (cm).

Leaf area (cm^2)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	Control	B12	B14	Mean
Control	70.7	64.0	96.2	76.9
F.oxysporum	35.8	91.5	102.5	76.6
Mean	53.2	77.8	99.3	76.8
L.S.D	F = 16.22	B = 19.87	F×B = 28.09	
Leaf chlorophyll content (SPAD)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	Control	B12	B14	Mean
Control	33.10	39.10	41.23	38.02
F.oxysporum	31.13	40.27	41.87	37.54
Mean	32.12	39.68	41.55	37.78
L.S.D	F = 4.32	B = 5.29	F×B = 7.48	
Plant height (cm)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	Control	B12	B14	Mean
Control	40.50	52.30	54.33	49.04
F.oxysporum	33.67	56.33	62.67	50.89
Mean	37.08	54.32	58.50	49.97
L.S.D	F = 4.43	B = 5.43	F×B = 7.68	

Table 5- The influence of Bacteria and Fungus onfresh and dry biomass weight (g)

Biomass fresh weight(g)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	Control	B12	B14	Mean
Control	13.3	50.0	51.0	38.1
F.oxysporum	21.7	61.0	110.3	64.3
Mean	17.5	55.5	80.7	51.2
L.S.D	F = 9.08	B = 11.12	F×B = 15.73	
Biomass dry weight(g)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	Control	B12	B14	Mean
Control	4.2	26.7	40.3	23.7
F.oxysporum	12.4	50.3	50.3	37.7
Mean	8.3	38.5	45.3	30.7
L.S.D	F = 4.46	B = 5.46	F×B = 7.72	

The influence of the Bacteria and Fungus on some plant growth regulators

Rhizosphere is a rich habitat of micro-organisms and should be explored for obtaining potential PGPR, which can be useful in developing bio-inoculants for enhancement of growth and yield of plants.*B.diminuta*(Bd1) inoculations improved plant agronomic traits and yield.

The result indicated that soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Six plant growth regulators (Indole Acetic Acid, Gibberellic Acid, Zetain, Kinten ,Benzyladenine andAbscisic acid) were determined using the Fast Liquid Chromatographic Apparatus ,the result shown in Table-6.Table-6 showed significantly impact on PGPR means for the bacterial treatments on the tomato plant growth . The FB^{14} treatment means were recorded the highest vales(13.72,8.82,13.77,16.40 and 28.67) for Indole Acetic Acid, Gibberellic Acid, Zetain, Kinten and Benzyladenine , comparing with control treatment lost values for Indole Acetic Acid, Gibberellic Acid, Zetain (5.67,6.01 and 2.68) and for the Kinten and Benzyladenine (5.58 and 11.83) in FB^{12} treatments ,respectively. Beside, all control means treatment

value were higher than fungus means except in Indole Acetic Acid. The data of the interaction between the bacteria and fungus presented in Table-6 revealed a significant differences between the treatments. The highest Indole Acetic Acid value was for FB[14] 22.06 , while for Gibberellic Acid and kinetin values were for the CB[14]treatments 9.64 and 23.75 and CB[12] treatment for Zetain 15.28 ,while the control treatment was the highest for Benzyladenine 47.57. Also , the results of field experiment showed that the lowest value for Indole Acetic Acid , Gibberellic Acid and kinetin were 2.62,5.79 and 2.68 for the control treatments. besids, for Benzyladenine was CF treatment 10.50 and CB[12]for Kintein 3.56 . PGPR have been reported to improve plant growth either through direct stimulation by the synthesis of phytohormones [13] or by decreasing the effect of pathogens [14,15].

Table 6-The influence of the Bacteria and Fungus on some plant growth regulators

Indole Acetic Acid (µg/ml)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	control	B12	B14	Mean
Control	2.86	11.40	5.39	6.55
F.oxysporum	8.49	11.99	22.06	14.18
Mean	5.67	11.69	13.72	10.36
L.S.D	F = 0.85	B = 1.05	F×B = 1.48	
Gibberellic Acid (µg/ml)				
Bacteria / Fungus	***B.diminuta*(Bd1)**			
	Control	B12	B14	Mean
\\\Control	5.79	7.75	9.64	7.73
F.oxysporum	6.23	9.01	8.00	7.75
Mean	6.01	8.38	8.82	7.74
L.S.D	F = 0.85	B = 1.05	F×B = 1.48	
Abscisic acid (µg/ml)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	control	B12	B14	Mean
Control	5.97	10.38	11.46	9.27
F.oxysporum	16.81	11.99	39.59	22.80
Mean	11.39	11.18	25.52	16.03
L.S.D	F = 1.8	B = 2.2	F×B = 3.6	
Kintein(µg/ml)				
Bacteria / Fungus	***B.diminuta*(Bd1)**			
	control	B12	B14	Mean
Control	8.62	7.60	23.75	13.32
F.oxysporum	8.65	3.56	9.05	7.09
Mean	8.64	5.58	16.40	10.21
L.S.D	F = 1.15	B = 1.40	F×B = 1.99	
Benzyladenine(µg/ml)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	control	B12	B14	Mean
Control	47.57	12.06	18.21	25.95
F.oxysporum	10.50	11.60	39.13	20.41
Mean	29.04	11.83	28.67	23.18
L.S.D	F = 1.73	B = 2.12	F×B 3.00	
Zetain(µg/ml)				
Bacteria / Fungus	***B. diminuta*(Bd1)**			
	control	B12	B14	Mean
Control	2.68	15.28	12.79	10.25
F.oxysporum	2.68	8.30	14.75	8.58
Mean	2.68	11.79	13.77	9.41
L.S.D	F = 0.85	B = 1.05	F×B = 1.48	

Sustainable agriculture, based on environmentally friendly methods, tends to use bacteria as tools that could by the way reduce the use of chemicals. The diversity and richness of the soil rhizosphere effect on plant growth. Our results indicate that all tested plant with the bacteria caused a significant reduction in the effect of *F. oxysporum* on tomato plants. This decrease was gradually increased by higher bacterial level application in the growth medium.

Attitalla*et.al.,*(2001)[16]reported that from four isolates of *Pseudomonas fluorescens*, one bacterial isolate, MF30 of *Pseudomonas fluorescens*, protected plants from *Fusarium* wilt, even though the fungal pathogen and not MF30 actually colonized the plant. Several mechanisms may have contributed to the suppression of *Fusarium*wilt, including systemic induced resistance.

In this study all growth characters were greatly increased by FB^{14} treatments in comparison to the CF treatment with significant differences in most cases. The values correspondingly increased by increased bacterialdose in combination with fungi.

In Greenhouse experiment , Analyses of the interaction between the bacteria and fungi data indicated that the increasing percentage in leaf area , total chlorophyll and plant height under FB^{14} treatment were multiply up to 65 , 25.65 and 46.27% compared to CF treatment , respectively. Similar results were found with increasing percentage for fresh and dry weight, which grow upto 80.32 and 75.34% ,respectively. This result may be due to rhizobacteria PGPR ,that are known to employ one or more direct and indirect mechanisms of action to improve plant growth and health, although the major mode of action of many PGPRs is through increasing the availability of nutrients for the plant in the rhizosphere region [17]. On the other hand, may be because the mechanisms of biological control by which rhizobacteria can promote plant growth indirectly, by reducing the level of disease, include antibiosis, induction of systemic resistance and competition for nutrients and niches [18]. Kumar and Gera , (2014)[19] reported that , the potential of the multi-trait*Brevundimonas* MDB4 isolate on plant growth promotion was evaluated by using it as bioinoculant for Bt-cotton in pot experiments. A reference strain (HT-54) was also used as inoculant for comparison. Data obtained from thisexperiment showed stimulatory effects on plant height and dry weights of root and shoot after inoculation with MDB4 isolate and HT-54. the MDB4 strain was found to be more effective as compared to HT-54 in promoting plant growth which could be attributed to its nitrogen-fixing ability.

In our study, the FLC analysis for the hormones showed significant differences in hormone concentration between the treatments. The data indicated that the increasing percentage in Indole acetic acid , gibberellic acid with cytokinine group (Kintein ,Zatain and Benzyl adenine) under FB^{14}treatment were (61.49, 22.02 ,4.41 ,82.58 and 73.16%) compared with CF treatment , respectively . The Abscisic acid hormone which promote for plant resisting against fungi was increased by 158.1% in FB^{14} plants compared with CF plants .These factors and individuals coming together to provide optimal conditions for a healthy plants .

IAA biosynthesis has been correlated with stimulation of root proliferation by rhizosphere bacteria [20,21], which enhanced uptake of nutrients by the associated plants [22]. The effect of IAA has been found to depend on the concentration, that is, low concentrations of exogenous IAA can promote, whereas high concentrations can inhibit root growth [23].

Several kinds of bacteria produce IAA phytohormon . IAA produced by *Azospirillum* spp. can promote plant growth by stimulating root formation [24]. Seed bacterization of chickpea cultivar C235 with different *Pseudomonas* isolates showed stunting effect on the development of root and shoot at 5 and 10 days of seedling growth.

Salamone*et,al.*(2001)[25] documented that Cytokinins phytohormones can produced by plants and microorganisms. Beside, it can be expected that plant inoculation with bacterial species capable of producing cytokinins may increase the level of cytokinins in root tissues. In turn, this may have an impact on plant growth. ABA can be a positive or a negative signal depending on the necrotroph that is attacking the plant. Against the soil-borne fungus *F.oxysporum*, ABA acts as a negative regulator of defense through its antagonistic interaction with the JA/ET signaling pathway [26].

Interestingly, certain saprophytic and parasitic fungi, such as *Botrytis*, *Ceratocystis*, *Fusarium*, and *Rhizoctonia*, are also able to produce ABA [27].

Abscisic acid can positively regulate the resistance to some pathogens, such as *Alternariabrassicicola* and *Pythiumirregulare*, as ABA-deficient and-insensitive mutants (*abi1-1*, *abi2-1*, *abi4-1*, *aba1-6*, *aba2-12*, *aao3-2*, and *npq2-1*) were found to be more susceptible than wild-type plants to these

pathogens [28,29,30]. In Arabidopsis, ABA has been shown to be required for JA biosynthesis that is essential for resistance to *Pythiumirregulare*[28].

Many reports support the role of ABA in disease promotion during pathogenic infection of plants [31,32,33,34,35]. Treatment of potato plants with ABA before infection with *Phytophthorainfestans*or *Cladosporiumcucumerinum* suppresses the accumulation of phytoalexins and significantly decreases plants resistance [36].

Rezzonico*et al*. (1998)[37] showed that ABA also downregulates an antifungal β-1,3-glucanase (also known as PR2) in tobacco cell suspensions. Such a downregulation might lead to a reduction in disease resistance in tobacco afterABAtreatment; however, this has never been confirmed.

Therefore, the greenhouse experiments indicated that the treatment of tomato plants with *B. diminuta* resulted in a significant reduction in vascular wilt fungal disease which caused by *F. oxysporum* .

Funnilythe*B.diminuta*(Bd1)was able to produce extracellular antifungal substance against *F.oxysporuminin vitro* and *in vivo*.Also,*B.diminuta*(Bd1) can be used as biocontrol against the soil borne fungi infecting plants.Beside ,*B.diminuta* (Bd1) can be used as biofertilizer to promote plant growth by increasing the agronomic traits such as leaf area and plant height , also physiological parameters such as chlorophyll content , fresh and dry biomass weight.

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