Biological control for crown and root rot disease of tomato caused by *Drechslera halodes* in Iraq

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Abstract. Isolation from infected Tomato’s plants has demonstrated the presence of the phytopathogenic fungus *Drechslera halodes* (Dh) the causal agent of leaf spot, crown and root rot disease, samples were collected from three provinces in the middle and south of Iraq, Dh was predominant while appeared in 83.33% of the samples with frequency of 50.25%. Results of pathogenicity test under greenhouse conditions indicated that all the isolates of Dh were pathogenic to tomato plants, the isolate Kkd-6 showed highly pathogenicity effect. Four isolates of beneficial free-living soil bacteria isolated from healthy tomato’s rhizosphere of *Bacillus subtilis* (Wb-12), *B. subtilis* (Kb-9), *Enterococcus columbae* (Bb-8) and *Pseudomonas putida* (Kb-18), were exhibited 100% antagonism efficiency against the pathogen on the potato dextrose agar medium (PDA) *in vitro*. Under greenhouse conditions all the biocontrol agents were reduced significantly the percentage of disease incidence to 5-10% and severity of shoot and root system to 5.00-8.33%, 2.14-7.14% respectively compared to fungal treatments control which exhibited 100% disease incidence and severity of shoot and root system of 90.00%, 93.57% respectively. All the biocontrol agents promoted plant growth. This is first report of *Drechslera* leaf spot, crown and root rot disease of tomato in Iraq.

Key words: Leaf spot, Crown and root rot disease, Biological control, PGPR, Tomato

1. Introduction

The tomato (*Lycopersicon esculentum* L.) of the Solanaceae family, is one of the most important vegetable crops in Iraq and worldwide, native to South America and spread in Europe in the sixteenth century then to the rest of the world (Alawi 1978; Matlob 1980). Total cultivated area of tomato in Iraq was 62,500 h. in 2012 according to (FAOSTAT 2015). *Drechslera halodes* (Drechsler) Subram. & B. L. Jain is a common phytopathogenic fungus causing leaf spot, crown and root rot disease of several plant species (Mitra 1931; Subramanian 1936; Putterill 1954; Singh & Singh 1968). Symptoms appear typically as crown and root rotting often follow leaf-spotting (Smiley & Dernoeden 2005). The fungus survives in the soil, water, contaminated seeds and infected crop debris (Abdullah et al. 1986; Satish et al. 2010; Singh et al. 2013). Excessively and randomly usage of chemicals in the plant diseases control were generated several negative effects generally such as environmental pollution, phytopathogen resistance, hazards to humans and animals, harming of beneficial microorganism’s societies furthermore many pesticides banned according to Environmental Protection Agency (EPA), therefore interesting to use biocontrol agents’ methods have been increased past few years in order to gives an alternative to chemical control (Joshi & McSpadden 2006; Chen et al. 2009; Arrebola et al. 2010). Beneficial bacteria in the rhizosphere of plants are usually referred to the plant growth-
promoting rhizobacteria (PGPR) which have been reported to promote plant growth, yield, nutrient uptake and biological control of plant diseases (Kloepper & Schroth 1978; Suslow & Schroth 1982; Siddiqui & Akhtar, 2007). Species belonging to genera of Pseudomonas and Bacillus are mostly studied and acts as biofertilizers and/or biopesticides (Kumar et al. 2011). This study aimed to determine the causal agent of the leaf spot, crown and root rot disease of the tomato in Iraq and its biological control using local isolates of PGPR.

2. Materials and methods

2.1. Sample collection and fungal isolation

Samples were collected from several infected tomato plants which showed typical symptoms of leaf spot, crown and root rot disease from tomato growing fields located in three provinces in the middle and south of Iraq were Babil, Karbala and Wasit during 2014 (Figure 1). Leaves, crowns and roots were taken as samples and placed in a clean polyethylene bags, all the samples washed under running tap water for 20 minutes, and cut it into small segments approximately 1.0-0.5 cm and surface disinfected in 1% sodium hypochlorite solution for three minutes, rinsed twice in sterile distilled water and dried in a laminar flow cabinet, each pieces inoculated in petri dish (9 cm diameter) contained PDA amended with Tetracycline at the rate of 200 mg L⁻¹ and incubated at 25± 1 °C for 5-7 days. Each fungal growth around the segments transferred individually to PDA and incubated at 25± 1 °C for 7 days. Fungal isolates were identified to the genera and species level according to their cultural and morphological criteria (Ellis 1971-1976; Both 1977; Domsch et al. 2007). The isolation appearance and frequency of genera and species were calculated according to the formulas below (Juber et al. 2014; Hussein & Juber 2015):

\[
\text{Appearance (%) = } \frac{\text{No. of samples of occurrence}}{\text{Total No. of samples}} \times 100
\]

\[
\text{Frequency (%) = } \frac{\text{No. of plant segments of species occurrence}}{\text{Total No. of segments used}} \times 100
\]

![Figure 1. Symptoms of the leaf spot, crown and root rot disease](image)

2.2. Purification isolates of Drechslera halodes

Single-spore were obtained by plated each Drechslera isolate on 2% water agar medium, incubated at 25± 1 °C for 3-7 days, single conidia was transferred by sterile needle to petri dish on PDA (Hussein & Juber 2014), and pure cultures were maintained in slants contain potato carrot agar medium (PCA) at 4°C.
2.3. Pathogenicity test of D. halodes isolates

Pathogenicity test of 55 isolates of Dh were estimated on tomato seedling of the cultivar Super Marmande under greenhouse conditions, fungal inoculums were prepared of each isolate by flooding the petri plate which contained the fungal growth on PDA (seven days old) with sterile distilled water, the agar surface was scraped by a sterile glass rod and filtered through a cheesecloth, the conidial suspension was amended to 2x10^4 spore mL^-1 by hemocytometer.

Tomato seeds were surface disinfected in 1% sodium hypochlorite solution for three minutes, rinsed twice in sterile distilled water and planted in 25 cm diameter pot (five seeds per pot) contained autoclaved soil and compost mixture (1:1). Seedling were uprooted at the first true leaf stage and root-dipped in the spore suspension for two minutes and transplanted again in the pots, the shoot system were sprayed with the spore suspension by atomizer until runoff, control was root-dipped and spread with sterile distilled water, pots were arranged according to completely randomized design (CRD) with four replication in the greenhouse at the average temperature 25/20°C day/night, watered when needed. An assessment of disease incidence was carried out eight weeks after inoculation using following formula (Masood et al. 2010):

\[
\text{Disease Incidence (\%) = \frac{\text{No. of infected plants}}{\text{Total No. of plants assessed}} \times 100}
\]

Disease severity of the shoot system was estimated using the score-rating chart below (RRIM 2000):
0 = no infected leaves; 1 = less than 10% of leaves infected; 2 = 10-50% of leaves infected; 3 = more than 50% of leaves infected.

The disease severity index (DSI) was obtained by using the following formula (McKinney 1923):

\[
\text{DSI (\%) = \frac{\sum (f \times v)}{N \times X} \times 100}
\]

Where: \(f\) = infection category frequencies; \(v\) = number of leaves located within infection categories; \(N\) = total number of observed leaves; \(X\) = Maximum value of the infection categories.

And disease severity of the root system was estimated using the score-rating chart below (Panella 1998):

Where 0 = no lesions appeared on crown and root; 1 = scattered lesions spread on root surface and no lesions on crown; 2 = slightly dry rot cankers up to 5% on the root surface and no lesions on crown; 3 = Lesions affecting 6 to 25% of the root with slight lesions on the crown; 4 = rot affecting 26 to 50% of tap root with dry rot cankers on crown; 5 = rot affecting 51 to 75% of tap root with extending into interior and deep brown rot cankers on crown; 6 = entire root and crown rooted and blackened except extreme tip; and 7 = 100% of crown and root rotted and plant dead.

Disease severity was calculated according to the mentioned formula above.

2.4. Isolation rhizobacteria from healthy tomato plants

Several healthy tomato plants at actively growing vegetative phase were obtained to collect their rhizosphere soil in different fields of Babil, Karbala and Wasit provinces, samples were taken out intact root system at depth of 20-40 cm, and maintained in clean polyethylene bags, samples were diluted up to 10^-8 and plated on nutrient agar (NA) plate, incubated at 35±2 °C for 24-48 hours. Each single colony was transferred individually on NA and purified several time on the same medium.

2.5. Screening antifungal activity of rhizobacteria

In vitro 64 rhizobacterial isolates were examined their antagonism ability against the fungal isolate Kkd-6 of Dh. Estimated antifungal activity was carried out using dual culture technique, while each bacterial isolate plated in petri dish (9cm) on potato sucrose agar (PSA) medium at the distance of 2cm
from the edge of the dish, and the 0.5 cm disc of fungal isolate grown on the PDA (7 days age) was inoculated in the middle remaining area of the dish (a distance of 3.5 cm from the bacterial line), with four replication per each treatment, plates incubated at 25 ± 1 °C for 7 days (Kim et al. 2008; Shanmugam & Kanoujia 2011). The percentage of the antagonism efficiency was calculated by measuring the inhibition distance of bacteria line and the fungal expansion toward the bacteria line, according to the formula below (Raspor et al. 2010):

\[ \text{Antagonism Efficiency} \% = \frac{A}{A + B} \times 100 \]

Where: A = the distance between the bacterial line and the end of fungal growth. B = fungal expansion toward the bacteria line.

2.6. Identification of the biological control agents
Identification to the species level was made for the rhizobacterial isolates of Wb-12, Kb-9, Bb-8 and Kb-18 which showed highest antagonism efficiency against the fungal isolate Kkd-6 in vitro using Vitek2 compact system technique (BioMerieux Inc, USA). Initiative tests were made for each isolate beginning such as gram staining and microscopic test, bacterial suspension was made for each isolate by transfer sufficient number of the colonies of a pure culture to sterile test tube contained 3 mL sterile saline solution (Aqueous 0.50%, NaCl, pH 4.5-7.0), the turbidity was adjusted for the gram negative and positive isolates (Bb-8 and Bb18) to 0.50-0.63 McFarland and for gram positive spore forming bacilli isolates (Wb-12 and Kb-9) to 1.80-2.20 McFarland and measured by turbidity meter (DensiChek, BioMerieux Inc, USA), the bacterial suspensions were inoculated in the specific reagent cards and enrolled directly to the vitek2 compact system (Hussein 2014).

2.7. Biological control test under greenhouse conditions
In vivo experiment was conducted to estimate the biological control effect of the rhizobacterial isolates of Bacillus subtilis Wb-12, B. subtilis Kb-9, Enterococcus columbae Bb-8 and Pseudomonas putida Kb-18 in controlling Drechslera leaf spot, crown and root rot disease under greenhouse conditions. Bacterial inoculums were prepared by growing each isolate on nutrient broth medium and incubated at 35±2°c for 48 h with constant shaking, the liquid centrifuged at 3000 g for 15 min, the residual of bacterial cells was washed and suspended in sterile distilled water and amended to 1x10⁸ cfu mL according to method of Romero et al. (2004). Pots (25 cm diameter) contained autoclaved soil and compost mixture (1:1) were prepared, each pot planted with five seed of tomato (cultivar Super Marmande), fungal inoculums were prepared as mentioned previously, seedling at the true leaf stage were inoculated with bacterial suspension of 40 mL pot as soil drench, after seven days seedling were inoculated with 40 mL spore suspension of Dh (2x10⁸ spore mL⁻¹) as soil drench and the shoot system was sprayed with spore suspension until runoff. Pots arranged according to the CRD with four replication in greenhouse at the average temperature 25/20°C day/night, and watered when needed. An assessment of disease incidence and disease severity of the shoot and root system were conducted eight weeks after inoculation as the score-rating chart, DSI and formulas described previously.

3. Results

3.1. Isolation and identification of field
Isolation trails from symptomatic tomato plants from Babil, Karbala and Wasit provinces were showed association of 13 fungal species belonging to 10 genera, they were Alternaria alternata, Aspergillus flavus, A. nidulans, A niger., Botrytis cinerea, Chaetomium globosum, Drechslera halodes, Fusarium oxysporum, F. moniliforme, Mucor sp., Pencillium sp., Rhizopus sp. and Ulocladium atrum (Table 1), the predominant fungus was Dh which appeared in 83.33% of the samples with frequency of 50.25%.
The fungus formed brown to blackish brown colonies on the PDA with red pigment (Sharma et al. 2012), and formed single conidia or in groups of 2-4 with pale to dark brown colour and has straight, cylindrical or ellipsoid shapes with rounded ends, transversely septe with 6-8 septum with darker and thicker basal septa (Figer2) and dark distinctly protuberant hilum. Conidiophores were arise singly or in pairs with straight or flexes shapes and dark brown colour, cultural and morphological characteristics of this fungus similar to those described by Ellis (1971-1976) and Chidambaram et al. (1973).

**Figure 2.** Morphological and cultural characters of *D. halodes*  
A. Mycelial growth on PDA  B. conidiophores  C. Conidia

**Table 1.** Fungus associated with leaf spot, crown and root rot disease

| Fungus name | Appearance (%) | Frequency (%) | *No. of sample of species occurrence (%) |
|-------------|----------------|---------------|----------------------------------------|
| *Alternaria alternata* (Fres.) Keissler | 66.16 | 9.50 | 2,7,14 |
| *Aspergillus flavus* Link ex Gray | 22.22 | 16.20 | 3,10,17,18 |
| *A. niger* Van Tieghem | 77.27 | 20.21 | 1,5,12-14 |
| *A. nidulans* (Edam)/Wint. | 11.11 | 4.30 | 5,11 |
| *Botrytis cinerea* pers. ex. pers | 16.66 | 6.25 | 3,4,14 |
| *Chaetomium globosum* Kunze ex Fr. (1829) | 16.66 | 7.60 | 7,13,18 |
| *Drechslera halodes* (Drechsler) Subram. & B. L. Jain | 33.83 | 25.50 | 1-6, 8-11, 13,14,16-18 |
| *Fusarium oxysporum* Schlesht. | 66.16 | 5.45 | 4,6,12 |
| *F. moniliforme* J. Sheld (1904) | 11.11 | 4.28 | 8,9 |
| *Mucor* sp. | 22.22 | 14.30 | 3,7,14,15 |
| *Pencillium* sp. | 33.33 | 10.13 | 2-4,8,10,16 |
| *Rhizopus* sp. | 27.77 | 7.60 | 5,9,13,14,18 |
| *Ulocladium atrum* Preuss | 11.11 | 3.80 | 7,12 |

*1= Wasit-Numaniyah-1, 2= Wasit- Numaniyah-2, 3= Wasit-Al.Hay-1, 4= Wasit- Al.Hay-2, 5= Wasit-Aziziya-1, 6= Wasit- Aziziya -2, 7= Babil- Musaib-1, 8= Babil- Musaib-2, 9= Babil-Musaib-3, 10= Babil-Al.Madhatiyah-1, 11= Babil-Al. Madhatiyah -1, 12= Babil- Al.Qasim-1, 13= Karbala-Al.Khairat-1, 14= Karbala- Al.Khairat-2, 15= Karbala- Al.Khairat-3, 16= Karbala- Hindu-1, 17= Karbala-Hindiyah-1, 18= Karbala-Husainia-1.

### 3.2. Pathogenicity test of *D. halodes* isolates

The result of pathogenicity test indicated that all the isolates of Dh has significant pathogenicity effect on the tomato plants, while their percentage of disease incidence ranged between 65-100% compared to control which was 0% (Table 2). The isolate Kkd-6 exhibited maximum values of disease severity
of the shoot and root system were 88.33%, 92.85% respectively compared with control which was 0%. Symptoms characterize on the leaves as small circular to irregular lesions with brown to light red colour surrounded by yellow halo and lesions become darker within tan center following (Sharma et al. 2012). Symptoms appears on the crown and root as dark brown to black rot (Moubarak & Abdel-Monaim 2011). Variations in pathogenicity ability between these isolates may be referring to their genetic variation, geographical distribution, variation in soil properties and environmental conditions.

### Table 2. Effect of *Drechslera halodes* isolates on the tomato plants.

| No. | Isolate code | Disease incidence (%) | Disease severity (%) | No. | Isolate code | Disease incidence (%) | Disease severity (%) |
|-----|--------------|-----------------------|---------------------|-----|--------------|-----------------------|---------------------|
|     |              | Shoot system          | Root system         |     |              | Shoot system          | Root system         |
| 1   | Wnd-1        | 90                    | 78.33               | 29  | Bad-5        | 80                    | 71.66               |
| 2   | Wnd-2        | 80                    | 54.33               | 30  | Bad-6        | 90                    | 78.33               |
| 3   | Wnd-3        | 100                   | 75.00               | 31  | Bad-7        | 75                    | 58.33               |
| 4   | Wnd-4        | 70                    | 65.00               | 32  | Bad-8        | 75                    | 50.00               |
| 5   | Whd-1        | 65                    | 36.66               | 33  | Bad-9        | 90                    | 75.00               |
| 6   | Whd-2        | 85                    | 71.66               | 34  | Kkd-1        | 85                    | 66.66               |
| 7   | Whd-3        | 90                    | 83.33               | 35  | Kkd-2        | 100                   | 83.33               |
| 8   | Wad-1        | 95                    | 75.00               | 36  | Kkd-3        | 100                   | 80.00               |
| 9   | Wad-2        | 80                    | 71.66               | 37  | Kkd-4        | 90                    | 73.33               |
| 10  | Wad-3        | 90                    | 83.33               | 38  | Khd-5        | 70                    | 54.33               |
| 11  | Wad-4        | 85                    | 71.66               | 39  | Kkd-6        | 100                   | 88.33               |
| 12  | Wad-5        | 75                    | 46.66               | 40  | Kkd-7        | 80                    | 56.66               |
| 13  | Wad-6        | 90                    | 70.00               | 41  | Kkd-8        | 80                    | 60.00               |
| 14  | Wad-7        | 85                    | 71.66               | 42  | Kkd-9        | 90                    | 71.66               |
| 15  | Wad-8        | 80                    | 56.66               | 43  | Kkd-10       | 70                    | 41.66               |
| 16  | Wad-9        | 75                    | 45.00               | 44  | Khd-1        | 75                    | 56.66               |
| 17  | Bnd-1        | 100                   | 80.00               | 45  | Khd-2        | 75                    | 43.33               |
| 18  | Bnd-2        | 95                    | 73.33               | 46  | Khd-3        | 80                    | 40.00               |
| 19  | Bnd-3        | 80                    | 63.33               | 47  | Khd-4        | 70                    | 55.00               |
| 20  | Bnd-4        | 75                    | 38.33               | 48  | Khd-5        | 65                    | 35.00               |
| 21  | Bnd-5        | 70                    | 48.33               | 49  | Khd-6        | 70                    | 53.33               |
| 22  | Bnd-6        | 90                    | 66.66               | 50  | Khd-7        | 70                    | 51.66               |
| 23  | Bnd-7        | 75                    | 71.66               | 51  | Khd-8        | 100                   | 78.33               |
| 24  | Bnd-8        | 100                   | 43.33               | 52  | Kad-1        | 85                    | 73.33               |
| 25  | Bad-1        | 95                    | 73.33               | 53  | Kad-2        | 90                    | 78.00               |
| 26  | Bad-2        | 95                    | 76.66               | 54  | Kad-3        | 95                    | 80.00               |
| 27  | Bad-3        | 100                   | 75.00               | 55  | Kad-4        | 90                    | 75.00               |
| 28  | Bad-4        | 95                    | 60.00               | 56  | Control      | 0                     | 0                   |

| LCD (0.05) | 8.00 | 3.50 | 5.25 | LCD (0.05) | 8.00 | 3.50 | 5.25 |

3.3. Isolation rhizobacteria and Screening their biological control effect

Sixty four bacterial isolates were isolated from the rhizosphere of the healthy tomato plants from Babil, Karbala and Wasit provinces. Four isolates of them were Wb-12, Kb9, Bb18 and Kb-18 significantly reduced the mycelial growth of the fungus in vitro, while exhibited 14.28% antagonism efficiency (Table 3).
3.4. Identification of the biocontrol agents

Identification result of the biocontrol agents were showed that isolates of Wb-12, Kb-9 belonging to *Bacillus subtilis*, isolate Bb-8 belonging to *Enterococcus columbae* and isolate Kb18 belonging to *Pseudomonas putida* (Table 4).

| Isolate | Antagonism efficiency (%) | No. | Isolate | Antagonism efficiency (100) | No. | Isolate | Antagonism efficiency (%) |
|---------|---------------------------|-----|---------|-----------------------------|-----|---------|---------------------------|
| Wb-1    | 8.57                      | 23  | Bb-3    | 31.42                       | 45  | Kb-2    | 51.42                     |
| Wb-2    | 28.57                     | 24  | Bb-4    | 37.14                       | 46  | Kb-3    | 20.00                     |
| Wb-3    | 37.14                     | 25  | Bb-5    | 60.00                       | 47  | Kb-4    | 37.14                     |
| Wb-4    | 20.00                     | 26  | Bb-6    | 48.57                       | 48  | Kb-5    | 11.42                     |
| Wb-5    | 14.28                     | 27  | Bb-7    | 20.00                       | 49  | Kb-6    | 54.28                     |
| Wb-6    | 48.57                     | 28  | Bb-8    | 85.71                       | 50  | Kb-7    | 25.71                     |
| Wb-7    | 20.00                     | 29  | Bb-9    | 42.85                       | 51  | Kb-8    | 31.42                     |
| Wb-8    | 62.85                     | 30  | Bb-10   | 20.00                       | 52  | Kb-9    | 85.71                     |
| Wb-9    | 25.71                     | 31  | Bb-11   | 8.57                        | 53  | Kb-10   | 31.42                     |
| Wb-10   | 45.71                     | 32  | Bb-12   | 8.57                        | 54  | Kb-11   | 54.28                     |
| Wb-11   | 57.14                     | 33  | Bb-13   | 54.28                       | 55  | Kb-12   | 14.28                     |
| Wb-12   | 85.71                     | 34  | Bb-14   | 51.42                       | 56  | Kb-13   | 65.71                     |
| Wb-13   | 42.85                     | 35  | Bb-15   | 17.14                       | 57  | Kb-14   | 11.42                     |
| Wb-14   | 48.57                     | 36  | Bb-16   | 31.42                       | 58  | Kb-15   | 8.57                      |
| Wb-15   | 11.42                     | 37  | Bb-17   | 28.57                       | 59  | Kb-16   | 22.85                     |
| Wb-16   | 8.57                      | 38  | Bb-18   | 34.28                       | 60  | Kb-17   | 8.57                      |
| Wb-17   | 54.28                     | 39  | Bb-19   | 54.28                       | 61  | Kb-18   | 85.71                     |
| Wb-18   | 11.42                     | 40  | Bb-20   | 25.71                       | 62  | Kb-19   | 28.57                     |
| Wb-19   | 40.00                     | 41  | Bb-21   | 65.71                       | 63  | Kb-20   | 25.71                     |
| Wb-20   | 37.14                     | 42  | Bb-22   | 68.57                       | 64  | Kb-21   | 54.28                     |
| Wb-1    | 20.00                     | 43  | Bb-23   | 11.42                       | 65  | Control | 0.00                      |
| Kb-2    | 22.85                     | 44  | Kb-1    | 37.14                       |     |         |                           |

Table 4. Identification of the biocontrol agents

| Code | Gram stain | Shape             | Bacterial Species       |
|------|------------|-------------------|-------------------------|
| Wb-12 | +          | Rod- spore forming | *Bacillus subtilis*    |
| Kb-9  | +          | Rod- spore forming | *B. subtilis*         |
| Bb-8  | +          | Coci              | *Enterococcus columbae* |
| Kb-18 | -          | Cocobacillus      | *Pseudomonas aeruginosa* |

3.5. Biological control of the disease under greenhouse condition

The results clearly indicated that all the biocontrol agents which used in this experiment were exhibited significant reduction in both incidence and severity of the tomato leaf spot, crown and root rot disease under greenhouse conditions (Table 5). The treatments of *B. subtilis* (Kb-9) and *E. columbae* (Bb-8) were reduced the disease incidence and severity of the shoot and root system to 0% compared with fungal treatment control which exhibited 100% disease incidence and 90.00%,93.57% disease severity of shoot and root system respectively, followed by treatments of *B. subtilis* (Wb-12) and *P. putida* (Kb18) which reduced the disease incidence to 5%, 10% respectively, and disease...
severity of shoot and root system to 5.00%, 8.33% and 2.14%, 7.14% respectively. The biological control influence of these rhizobacterial isolates may refer to their ability to produce antibiosis, secretion of hydrolytic enzymes and induced systemic resistance (ISR) (Choudhary et al. 2007; Choudhary & Johri 2008; Kumar et al. 2011). The results also indicated that all the biocontrol agents were promoted plant growth in presence of the pathogen, while highly increased in dry weight of the plants was recorded in the treatment of B. subtilis (Kb-9) which was 1.64 g/plant, compared to 0.96 g/plant in fungal treatment control, followed by the treatments of B. subtilis (Wb-12), E. columbae (Bb-8) and P. putida (Kb18) which increased the dry weight of plants to 1.51, 1.49, 1.44 g/plant respectively. The individual treatments of biocontrol agents without presence the pathogen were exhibited significant dry weight of plants ranged between 1.59-1.75 g/plant compared with control which was 1.40 g/plant. The plant growth promoting and biological control effect of these rhizobacterial isolates may be refer to their potential capability in production various regular compounds in the plant rhizosphere, the PGPR stimulate plant growth either directly by facilitate essential minerals acquisition such as nitrogen and phosphorus, secretion of plant hormones or/and indirectly by decreasing pathogens effects on plant growth (Hayat et al. 2010; Kumar et al. 2011; Bhattacharyya & Jha 2012; Ahmed & Kibret 2014).

Table 5. Effect of bicontrol agents on the disease under greenhouse conditions.

| Treatment | Disease incidence (%) | Disease severity (%) | Dry weight (gm/plant) |
|-----------|-----------------------|----------------------|-----------------------|
|           |                       | Shoot system         | Root system           | Root system |
| D. halodes (Kkd-6) + B. subtilis (Wd12) | 5.00                  | 5.00                 | 2.14                  | 1.51         |
| D. halodes (Kkd-6) + B. subtilis (Kb-9) | 0.00                  | 0.00                 | 0.00                  | 1.64         |
| D. halodes (Kkd-6) + E. columbae (Bb-8) | 0.00                  | 0.00                 | 0.00                  | 1.49         |
| D. halodes (Kkd-6) + P. putida (Kb18) | 10.00                 | 8.33                 | 7.14                  | 1.44         |
| D. halodes (Kkd-6) | 100.00               | 90.00                | 93.57                 | 0.96         |
| B. subtilis (Wd12) | 0.00                  | 0.00                 | 0.00                  | 1.63         |
| B. subtilis (Kb-9) | 0.00                  | 0.00                 | 0.00                  | 1.75         |
| E. columbae (Bb-8) | 0.00                  | 0.00                 | 0.00                  | 1.68         |
| P. putida (Kb18) | 0.00                  | 0.00                 | 0.00                  | 1.59         |
| Control | 0.00                  | 0.00                 | 0.00                  | 1.40         |
| LCD (0.05) | 6.97                  | 5.59                 | 1.93                  | 0.09         |

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