Iraqi Journal of Market Research and Consumer Protection
(2026) 18(1): 87-101.
DOI:
Ahmed& et al.,

المجلة العراقية لبحوث السوق
وحماية المستهلك

THE EFFICACY OF SOME ENDOPHYTES BACTERIA ISOLATED FROM TOMATO PLANTS TO SUPPRESS FUNGUS *Alternaria solani* IN LABORATORY CONDITIONS

Abdul jabbar S. Ahmed[1*], Neran Salem Aljarah[2], Nazar R.Merzah[3]

[1]Assistant Lecturer, Department of Plant Protection, College of Agricultural Engineering Sconces, University of Baghdad, Iraq. abduljabar@coagri.uobaghdad.edu.iq
[2]Assistant Professor PhD., Department of Plant Protection, College of Agricultural Engineering Sconces, University of Baghdad, Iraq. neran.aljarah@coagri.uobaghdad.edu.iq.
[3]Lecturer PhD, Plant Protection directorate, Ministry of Agriculture, Baghdad, Iraq. Nazar.rashid2013@gmail.com

Received 2/ 4/ 2024, Accepted 10/ 9/ 2024, Published 31/ 3/ 2026

ABSTRACT

Under laboratory conditions, the study assessed the efficacy of certain endophytic bacteria isolated from healthy tomato plants in suppressing the pathogenic fungus *Alternaria solani*, which causes early blight in laboratory conditions. The study was carried out in the Fungal Diseases Laboratory, Department of Plant Protection, Ministry of Agriculture for the 2022 season. 20 bacterial isolates were obtained in several fields in Baghdad Governorate. the pathogenicity assay on tomato plants grown in pots revealed that 50% of the endophytic bacterial isolates showed pathological symptoms when tested individually on aplant, such as yellow spots, necrosis, wilting and death of certain plant leaves or the whole plant. In laboratory conditions, the results showed that 40% of the endophyte bacterial non pathogenic isolates caused a significant reduction in the growth rate of the pathogen on Potato Dextrose Agar (PDA). *A.solani* growth was most significantly inhibited by the isolate B9, which achieved 81.11%. Molecular diagnostics of bacterial isolation (B9) using the Bacterial ribosomal genes (16S rRNA) showed that it is the bacterium *Alcaligenes faecalis*.

Keywords: early blight, *A.solani* ,Biological control ,endophytic bacteria.

[*]**This article is taken from the doctoral dissertation of the first researcher.**

اختبار فعالية بعض البكتريا من نوع endophytes المعزولة من نباتات طماطة في تثبيط الفطر *Alternaria solani* في ظروف المختبر

عبدالجبار صالح احمد [1] ، نيران سالم الجراح [2] ، نزار راشد مرزة [3]

[1] مدرس مساعد ، قسم وقاية النبات ، كلية علوم الهندسة الزراعية، جامعة بغداد ، بغداد، العراق. abduljabar@coagri.uobaghdad.edu.iq

[2] الاستاذ المساعد الدكتور،قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق.neran.aljarah@coagri.uobaghdad.edu.iq

[3] المدرس الدكتور، دائرة وقاية المزروعات، وزارة الزراعة، بغداد، العراق .Nazar.rashid2013@gmail.com

الخلاصة

اجريت الدراسة في مختبر الامراض الفطرية –دائرة وقاية المزروعات – وزارة الزراعة للموسم 2022 لاختبار فعالية بعض العزلات البكتيرية الاندوفايت المعزولة من نباتات طماطة سليمة في تثبيط الفطر الممرض *Alternaria solani* مسبب مرض اللفحة المبكرة في ظروف المختبر.تم الحصول على 20 عزلة بكتيرية اندوفايت في عدة حقول في محافظة بغداد.اظهرت نتائج اختبارات الامراضية على نباتات الطماطة في الاصص ان 50% من عزلات البكتريا كانت ممرضة اذ اظهرت اعراضا مرضية عند اختبارها بشكل منفرد على النبات، تدرجت من بقع صفراء او متنخرة الى ذبول او موت جزء من النبات او النبات باكمله. بينت نتائج اختبار فعالية العزلات البكتيرية غير الممرضة في تثبيط الفطر الممرض على الوسط الزرعي(البطاطا والدكستروز والاكار) ان 40% من عزلات البكتريا سببت خفضا معنويا في نسبة معدل اقطار مستعمرة الفطر الممرض مقارنة بمعاملة السيطرة اذ بلغ فيها معدل الاقطار 6 سم .سببت العزلة B9 اعلى معدل للنسبة المئوية لتثبيط نمو مستعمرة الفطر *A. solani* اذ بلغت 81.11% .اظهر التشخيص الجزيئي للعزلة البكتيرية (B9) باستعمال جين الريبوسوم البكتيري(16SrRNA) انها البكتريا *Alcaligenes faecalis* .

الكلمات المفتاحية: اللفحة المبكرة ،الفطر *A.solani* ، المكافحة الحيوية ، البكتريا الاندوفايت.

INTRODUCTION

Tomatoes are the most important and most consumed vegetable crops after potatoes (**Ahmed *et al*., 2023)**. As they are consumed fresh or cooked and are considered fruits of nutritional value because they contain a group of vitamins such as A, B and C, as well as some amino acids such as thymine and thymin and contain mineral elements such as iron, calcium and phosphorus (**Al-Sandooq &Al-Fattah, 2015; Al-Juboori & Alwan 2015, Hatem *et al*.; 2020, Abdul- Karim, 2021**).Early blight caused by the fungus *Alternaria solani* is one of the most important diseases of the Solanaceae family and highlights its importance on the tomato crop, as it affects the quantity and quality of production in different countries of the world **(Abd Al-Tamimi *et al*., 2020; Alaa-El-den 2022; Panthee *et al*., 2024**) and losses in the field in fruits after harvest are estimated at 50-86% **(Parvin *et al*., 2021**).The disease affects the crop in open and covered cultivation conditions, as well as the damage caused after harvesting during transportation or storage, and these damages increase if the storage conditions are poor. The severity of the disease under open cultivation varies from season to season depending on the appropriate environmental conditions of the pathogen, as the latter has a direct relationship with the rainy season and relative humidity, especially the frequency of rainfall and the dew drops at night and their alternation with dry climate conditions during the day, While covered agriculture is one of the ideal environments for this disease, as some preventive measures and treatments have not been taken that would reduce the seriousness of this disease and reduce its

severity, the high humidity inside the greenhouse causes condensation of water vapor and then falls over the parts of the vegetative system of the plant, which leads to the occurrence of disease, especially on the old lower leaves, which are more sensitive than the rest of the other parts of the plant, causing severe infection of leaves and full down so the Fruits are exposed to direct sunlight, which causes blight on the fruits and thus the lack of production and low marketing value of the crop (**El-Nagar, *et al*., 2020; Adss, *et al*., 2021, Fatehpuria & Sasode, 2022; Ansari *et al*., 2023**).The disease cycle can be repeated more than once during the growing season because it takes only 5-7 days, and plants that are more stressful due to lack of fertilization, fruiting season or nematode attack are more sensitive to this disease.Many methods were used to control this disease and reduce the economic losses. The chemical pesticides were used to control the disease, but the over use of pesticides has many problems, especially on humans health and to the environment, in addition to the strains become more virulent and fungicides resistance (**Roy *et al*., 2019; Riaz *et al*., 2021**). Therefore, other eco-friendly techniques like the plant oil extract of Cloves, Datura, Neem, and other plants resulted in a 75–85% suppression of fungal mycelium and conidia of early blight pathogen *A.solani* **(Sahu *et al*., 2014; Nivedha *et al*., 2019;Mugao *et al*., 2021)** .Additionally the plant breeders also used resistant varieties to stop the spread of early blight disease and reduce its epidemic, and this strategy was mostly successful **(Ajeel, 2015; Kaushal *et al*.,2020**). The biological control was and still is one of the promising and sustainable solutions in the fight against various pathogens, which gave excellent results in combating many plant diseases and reducing their spread over the past years, as well as increasing awareness among producers and consumers towards organic products, all of this led to an increase in conviction of the importance of adopting this method of control (**Demir *et al*., 2023; Montoya-Martínez *et al*., 2024**).The rhizospheric area is one of the most important areas from which many microorganisms such as bacteria and fungi have been isolated, and many studies have shown the high effectiveness of the bacteria in this area, which are known as plant growth promoting bacteria (PGPR) in inhibiting many pathogens, including the fungus *A.solani* (**Attia *et al*., 2020; Shrestha *et al*., 2022, AL-Aamel; AL-Maliky,2023**).Studies have confirmed that soil organisms have begun to decrease in number, and this has prompted many researchers to search for new habitats in which microorganisms exist, including the organisms that inhabit the internal tissues of plants with no pathological symptoms, which are known as endophytes. The endophytes microorganisms have an important role in the health of host plant by promoting growth as well as resistance to environmental stress conditions. They are an important source of biologically active plants such as antibiotics, anti-oxidants and growth regulators (**Ancheeva *et al*.,2020; Singh *et al*., 2020; Siraj *et al*.,2023**) Studies have also reported the success of some endophytic bacteria in combating fungal and bacterial pathogens (**Maulani, *et al*.,2019; Murtado *et al*., 2020; Prasad *et al*., 2024**).Due to the high incidence of early blight in most tomato growing areas in Baghdad province, including fields and greenhouses, as well as the significant economic losses caused by this disease, the study's proposal was to isolate endophytic bacteria from healthy tomato plants and evaluate their effectiveness in inhibiting the pathogenic fungus *A.solani* in laboratory conditions.

MATERIAL AND METHODS

Sample collection, isolation and purification of endophytic bacteria:

Healthy tomato plants, free of any disease symptoms, were collected from tomato fields in Baghdad Governorate/ Iraq (AL-Taji, AL-Tarmiya, AL-Yousufiyah, Abu Gharib, AL-Radwaniya and AL-Jadriyah). The location, date of the collection and the variety of tomato cultivated were indicated on the polyethylene bags containing the tomato plant samples. the samples were transferred to the Fungal Diseases Laboratory- Department of Plant Protection - Baghdad - Ministry of Agriculture / Iraq. Plants were washed well with tap water to get rid of impurities and dust, then cut and separated into (roots, stems, leaves and fruits), cut the plant parts into small pieces (0.5 cm). the plant parts were sterilized triple sterilization sequentially (first with ethanol 96% for one minute, then transferred to sodium hypo chlorate 3% for three minutes and finally put in ethanol 96% for 30 seconds). **(Rebotiloe *et al*.,2018; Bahmani *et al*.,2021**). To get rid of microorganisms on the surface of the plant pieces. After that, the pieces were twice cleaned with sterile distilled water to get rid of any remaining sterilized material, and the extra water was disposed of by placing them on sterile filters. The plant pieces were placed in Petri dishes filled with ready-made Nutrient Agar (NA) (28 g/L; Hi Media India), which had been sterilized by autoclave for 15 minutes at 121°C and 1.5 kg/cm^2. Four pieces of plants/ petri dish and three replicates for each sample were made. The dishes were incubated at a temperature of 27 ± 2 ° C with daily monitoring until bacterial growths appeared around the plant pieces. A portion of the bacterial growth tip was taken and replanted in other Petri dishes containing NA medium to obtain pure bacterial colonies. Using the four-way streak method, the bacterial isolates were purified using the single colony technique. The inoculating loop was flame-sterilized and then allowed to cool. a small portion of the pure bacterial colony was pick up. The four sections of the nutrient agar Petri plate are streaked one after the other in a sequential manner. The loop was sterilized after each streaking over the surface of NA media to ensure obtaining individual bacterial colonies for each isolate. three dishes (replicates) of each bacterial culture were made. The dishes were incubated at a temperature of 27 ± 2° C with daily monitoring. Single bacterial colonies were selected and inoculated a new Petri dishes containing culture medium (NA) **(Doolotkeldieva *et al*., 2016; Axler-DiPerte,2017**).

Pathogenicity testing of Endophytic bacterial isolates on tomato seedlings in pots condition:

Nutrient Broth (NB) was prepared. Pour 10 ml of the NB into glass tubes (20 ml) and autoclaved then inoculated with pure bacterial isolates using Sterile loop and incubated at 27 ± 2°C for 48 hours. A series of dilution (10^{-1}-10^{-6}) were prepared from each bacterial cultures. Three replicates/dilution were made. To estimate the number of bacterial cells, one ml from the first dilution was put in the Petri dish using micropipette and 20 ml of sterilized NA medium at 45° C was poured in each dish with continuous stirring to ensure the homogeneity of the bacterial cells with the NA medium. The process was repeated for other dilution, the dishes

were incubated at 27 ± 2°C for 24 hours. The average number of bacterial colony/ml was calculated according to the equation (average number of colonies× inverted dilution). **(Ahmed & Alani, 2013**).

Plastic pots (size 2 kg), were sterilized by ethanol 70%. The mixture of peat moss and soil was moistened, then sterilized in an autoclave twice, with 24 hours between each time , to ensure that the mixture is sterilized well, and distributed in the pots. The pots were planted with 60 days old tomato plants of the Rawea variety, produced locally by the Department of Horticulture / Ministry of Agriculture –Iraqi, and left 15 days with watering when needed with sterile water. Each pot was planted with one plant. The plant's leaves were injured using a sterile needle. Each plant was sprayed with five ml of bacterial inoculum at a concentration of 10^3 CFU/ml in each pot, with three plants (replicates) for each bacterial isolate. Three plants were sprayed with distilled water as a control. the plants were covered with sterile polyethylene bags for three days to maintain moisture for bacterial cells. after 5-7 days of inoculation, the results were recorded, the appearance or absence of any disease symptoms appearing on the plants (**Al-khafagi,2019**).

The effectiveness of endophytic bacterial isolates against the pathogenic fungus *A.solani* on the culture medium in laboratory :

The medium, Potato Dextrose agar (PDA), was prepared (Hi Media-India) and autoclaved as previously explained. The sterile media was poured in Petri dishes 20 ml/dish. An amount of 0.1 ml of bacterial inoculum was added to each dish and was spread using a sterile glass bacterial diffuser (all non-pathogenic endophytic bacterial isolates were used,24 hours old, at concentrations of 10^3 colony-forming units/ml, prepared as in the previous paragraph). Then, the plates were inoculated at their center with a 5 mm diameter disk of the pathogenic culture *A.solani* (the pathogenic isolate A.s5 was identified phenotypically and molecularly by the first researcher in a previous study), which was taken from the edge of the fungal culture.Three replicates were made for each endophytic bacteria, and three dishes were inoculated with the *A. solani* on PDA medium only as control **(Matlob & Juber,2012**). .The dishes were incubated at a temperature of 25 ± 2 ºc and the results were recorded after seven days to calculate the percentage of inhibition using the equation (**Jamel et *al*.,2023):**

$$\%inhibition = \frac{\text{diameter of the pathogen in the control} - \text{diameter of the pathogen in the Treatment}}{\text{diameter of the pathogen in the control}} \times 100$$

The results were analyzed statistically using CRD using Genstat 2007.

RESULTS AND DISCUSSION

Sample collection, isolation and purification of endophyteic bacteria:

Twenty isolates of endophytic bacteria were isolated from all healthy tomato samples collected in this study according to regions, tomato varieties, and the plant part from which the endophytic bacteria were isolated on the basis of phenotypic differences, the colonies were purified and then replanted using the single-colony technique, and codes were given from B1 to B20 as shown in (Table.1).No endophyte bacteria were obtained from Al- Taaji and Al-Tarmiya fields.

Table (1): Isolates of Endophytic bacteria isolated from healthy tomato plants from different regions in Baghdad Governorate / Iraq for the season 2022.

Isolation number	Samples place	Part of plant	Tomato variey
B1	Abi gharib	Stem	24s Local
B2	Alyusfiya	fruit	futon Holland Imported
B3	Alyusfiya	stem	futon Imported
B4	Alyusfiya	Stem	futon Holland Imported
B5	Abi gharib	leaves	25s Local
B6	Abi gharib	Rot	25s Local
B7	Alyusfiya	Stem	futon Holland Imported
B8	Abi gharib	leaves	24s Local
B9	Alradwaniyah	Stem	Local shahd
B10	Alradwaniyah	Rot	Local Rawea
B11	Abi gharib	Rot	24s Local
B12	Alyusfiya	Rot	futon Holland Imported
B13	Abi gharib	Stem	25s Local
B14	Abi gharib	leaves	25s Local
B15	Abi gharib	Rot	25s Local
B16	Abi gharib	Rot	futon Holland Imported
B17	Aljadriya	fruit	Local shahd
B18	Aljadriya	Stem	Local shahd
B19	Aljadriya	leaves	Local shahd
B20	Aljadriya	Rot	Local shahd

Pathogenicity testing of Endophyte bacterial isolates on tomato seedlings in pots condition:

The results showed that the bacterial isolates B1, B3, B6, B10, B11, B13, B17, B18, B19 and B20 were pathogenic to the tomato plants, as they showed pathological symptoms (Table 2. and Figure 1.) graded from yellow spots and dead necrotic spots to wilting the whole plant and death in some cases after 5-7 days of spraying the plants with bacterial suspension, While the isolates B2, B4, B5, B7, B8, B9, B12, B14, B15, and B16 did not differ from the control treatment (spraying the plants with sterile distilled water only), as no disease symptoms appeared on the tomato seedlings that were treated with it. Some bacterial isolates showed disease symptoms despite being isolated from healthy tomato plants. This may be attributed to

several reasons. The first is that these bacteria may be pathogenic to the plant, but no disease symptoms appeared on it because the amount of bacterial inoculum (number of bacterial cells) present with the healthy plant was not sufficient to cause disease in plants due to the balance between all types of bacteria in the plant host, but when grown in a laboratory with appropriate environmental conditions and the absence of other competing organisms, it led to the appearance of clear disease symptoms on tomato plants treated with the bacteria (**Omer *et al.*, 2021; Ibanez *et al.*, 2023**).

The second reason may be due to the presence of relative resistance in the plant variety from which it was isolated, such that no disease symptoms appear on the plant, whereas when the local variety was inoculated individually with endophyte bacteria, which appear to be sensitive to these bacteria, distinctive disease symptoms appeared on it because it does not have resistance genes to protect it from Pathogens (**Aslam *et al.*,2017; Lalramhlimi *et al.*, 2023**). In addition, the environmental conditions in the fields from which the endophytic bacteria were isolated may not have been appropriate and ideal for causing diseases on the plants grown in them, and when the appropriate environmental conditions were provided in this test, such as heat, light, and humidity, as a result of watering the plant well, spraying it with a bacterial suspension, and then covering the plants with sterile polyethylene bags. All of these conditions helped the endophytic bacteria to cause disease symptoms on the tomato plants they treated (**Abrahamian *et al.*, 2021**).

Table (2): Pathogenicity test of endophytic bacterial isolates on healthy tomato plants.

Tretment	Symptoms disease appear	Symptoms type
Control (D.W +H.P.)*	-	-
B1	+	Necrosis spot+ wilt some branch
B2	-	-
B3	+	Wilt + Death the plant
B4	-	-
B5	-	-
B6	+	Yellow sopt + wilt the plant
B7	-	-
B8	-	-
B9	-	-
B10	+	Necrosis + Blight some leaves
B11	+	Wilt some branch of plant
B12	-	-
B13	+	Yellow and Necrosis the leaves.
B14	-	-
B15	-	-
B16	-	-
B17	+	Wilt all plant
B18	+	Blight and Death some leaves
B19	+	Wilt and Death the plant
B20	+	Necrosis spot+ wilt some branch

Transactions were carried out on seedlings of tomato varieties of a local Rawea 60 days old in pots. * The control plants were spry with D.W= Distell Water , H.P. = Healthy Plant.

Based on the results of this test, all pathogenic bacterial isolates that caused pathological symptoms of tomato seedlings were excluded.

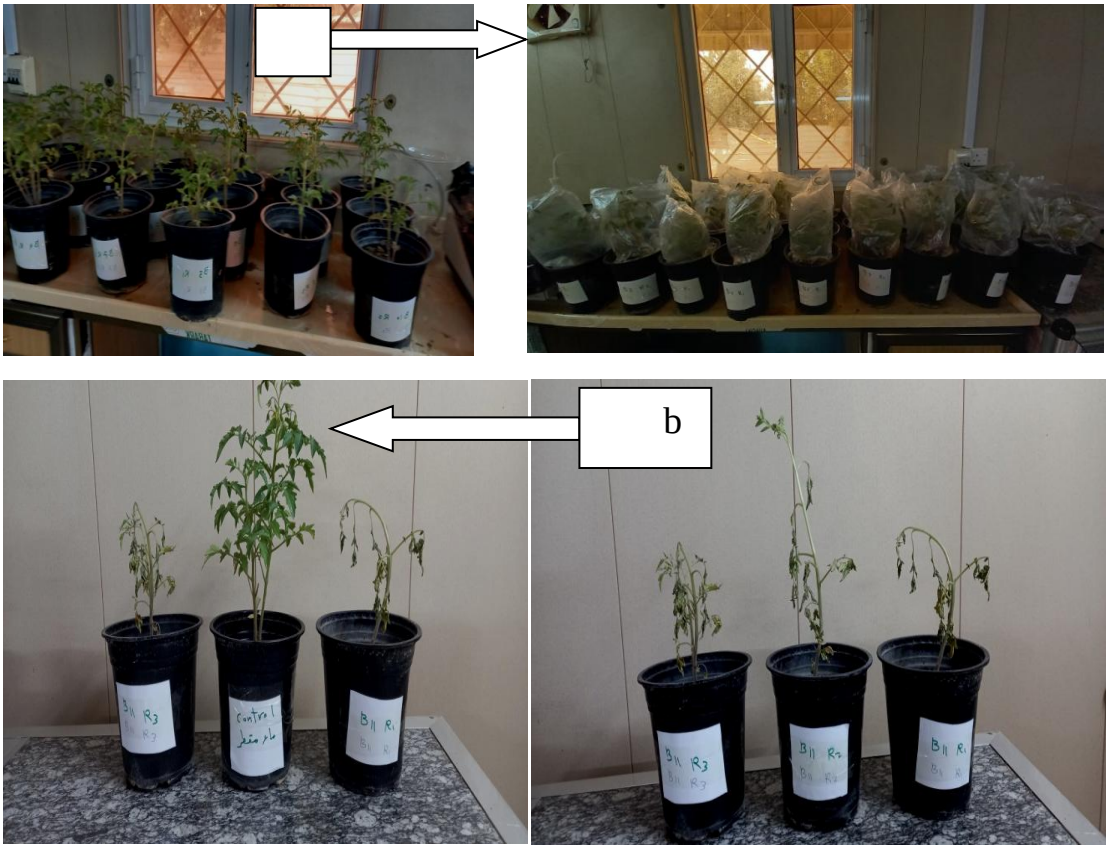

Figure (1): a =An experiment of pathogenetic testing of endophyte bacterial isolates in pots

b = Some pathological symptoms caused by pathogenic bacterial isolates on tomato plants.

Evaluation of the effectiveness of some endophytic bacterial isolates against the pathogen *A.solani* on the culture medium in the laboratory:

The results are shown in (Table 3. and Figure 2.) Most of the Endophytic bacterial isolates (B2, B4, B5, B9, B12, B14, B15 and B16) were effective in inhibiting the growth of *A. solani*, where the average percentage inhibition ranged between 41.11 and 81.11% with significant differences to the control treatment (pathogen only). The lowest percentage rate of inhibition was recorded with isolates B8, B7 and B5, as it reached 41.11, 48.89 and 57.78%, respectively, with the highest rate of the colony diameter of the fungus colony was recorded as

it reached 3.53, 3.06 and 2.35 cm compared to the measurement of the colony of the pathogen in the control treatment, which amounted to 6.00 cm. While the highest percentage of inhibition was recorded with isolates B9, B2 and B14, reaching 81.11, 78.33 and 77.78%, in which the average diameter of the *A. solani* colony reached 1.13, 1.30 and 1.33 cm, respectively, The three isolates B2, B9 and B14 were molecular diagnosed in another study of the researchers based on the 16SrRNA gene and the results showed that these isolates belong to the bacterium *Alcaligenes faecalis* and were registered at the National Center for Biotechnology Information NCBI under the accession numbers PP217985.1, PP217986.1 and PP2179888.1 respectively.

The inhibitory effectiveness of these isolates may be attributed to one of the following reasons. The first is the production of some compounds that have an inhibitory effect on plant pathogens in the cultural medium, such as alkaloids, steroids, acids, fats, peptides, phenols, flavonoids, and terpenes. (**Priyashantha *et al*.,2023**). It has been known that Endophytic bacteria are an important source of secondary metabolites such as HCN, Salicylic acid, Siderophore and IAA, which are known for their many different uses, for example in the agricultural field and as antibiotics , as well as their use as biological control agents against plant-pathogenic fungi (**Latha, *et al*., 2019; Rana *et al*.,2020, Basit *et al*., 2021; Eid *et al*., 2021).** Indicated that the suppression of the pathogen may be due to competition for space or for the nutrients it needs, as the endophyte bacteria are known to quickly invade their planthost in the field and exploit the nutrients needed by the pathogens for ease of freshness, which leads to their contribution and sometimes their death. These characteristics, carried by the Endophytic bacteria individually or in combination, may explain their high antagonist activityin vitro against *A.solani*.

These results are consistent with several studies that have confirmed the high efficacy of endophytic bacteria isolated from tomato plants in inhibiting and controlling many plant pathogenic fungi, including *A.solani* under laboratory or field conditions **(Maulani *et al*., 2019 ; Boyno *et al*., 2020, Murtado *et al*., 2020 ; Gorai *et al*., 2021).**

Table (3): Effectiveness of Endophytic bacteria Isolated from Tomato Plants in Inhibition of *Alternaria solani* on PDA in Laboratory Conditions.

Symbol of Tretment	Tretment	Radial growth (cm)	Percentage of inhibition
T1	Control =pathogen only	6	0.00
T2	B2+Pathogen	1.30	78.33
T3	B4+Pathogen	2.33	61.11
T4	B5+Pathogen	2.53	57.78
T5	B7+Pathogen	3.06	48.89
T6	B8+Pathogen	3.53	41.11
T7	B9+Pathogen	1.13	81.11
T8	B12+Pathogen	1.96	67.22
T9	B14+Pathogen	1.33	77.78
T10	B15+Pathogen	1.83	69.44
T11	B16+Pathogen	1.73	71.11
L.S.D $_{0.05}$	-	0.22	3.80

Each number in the table represents the rate of three replicates. The data were recorded seven days after inoculation the tomato plant (Rawea Variety) with the pathogenic fungus *A.solani* in petri-dishes (Ø9).

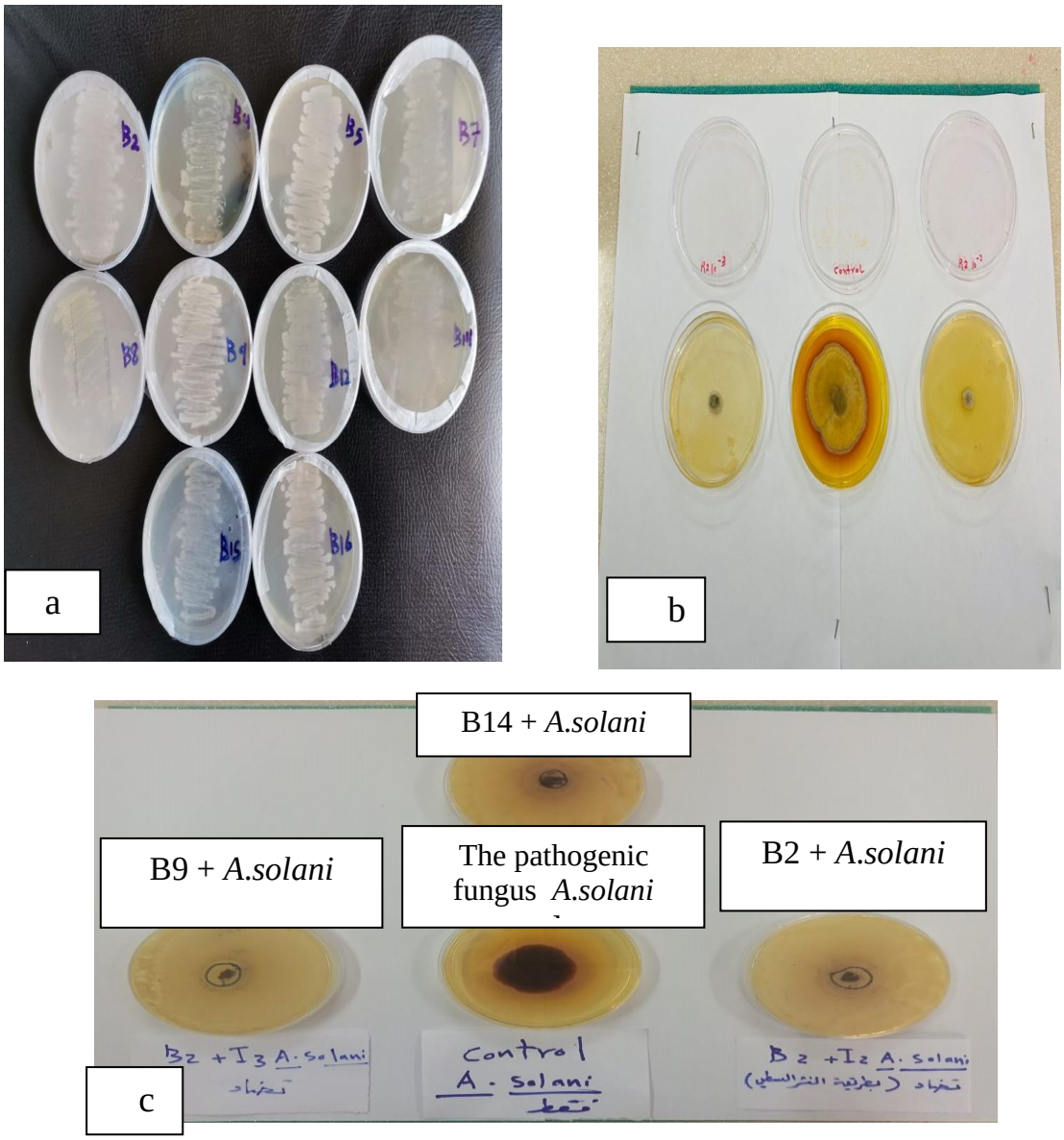

Figure (2): a = bacterial isolates used to inhibit the pathogenic fungus.
b = a picture showing the Percentage of inhibition.
c =The zone of inhibition of pathogenic fungus *A.solani* is on the culture medium in the presence of endophyte bacteria

CONCLUSION

Based on the results that appeared in this study, most of the non-pathogenic endophyte bacterial isolates isolated from healthy tomato plants led to inhibition of pathogenic fungus *A. solani* is the causative agent of early blight on tomatoes on the culture medium, and isolation B9 surpassed the rest of the isolates and led to the highest percentage rate of inhibition amounting to 81.11%. this isolation (B9) was diagnosed as the bacterium *Alcaligenes faecalis* molecularly.As a recommendation, these bacterial isolates can be used in the fight against early blight disease under field conditions, because biological control is a safe and long-term method, as well as endophyte bacteria work to help the plant withstand environmental stress conditions and improve the vegetative qualities of the plant

REFERENCES

1.Abdul-Karim, E.K., (2021). The efficiency of magnesium oxide, nano magnesium oxide and cinnamon alcoholic extract in controlling Fusarium oxysporum f. sp. lycopersici which causes Fusarium wilt on tomato. *International Journal of Agricultural and Statistical Sciences*, *17*,Supplement 1. pp.1611-1618.

2. Abd AL-Tamimi, Q.A., Hussein, H.Z. & Ali, A.M.,(2020). The efficacy test of nano chitosan and phylex in resistance early blight disease in tomato caused by *Alternaria Solani* fungus. *International Journal of Pharmaceutical Research,* 12(1).pp.2209-

3. Abrahamian, P., Sharma, A., Jones, J.B. & Vallad, G.E. (2021). Dynamics and spread of bacterial spot epidemics in tomato transplants grown for field production. *Plant Disease*, *105*(3), pp.566-575.

4. Adss, I.A., Amer, G., Bayoumy, S.R. & Eid, R., (2021). Effect of abscisic acid, salicylic acid, potassium silicate, and Trichoderma harzianum as biocontrol agent to induce the tomato resistance against early blight disease caused by Alternaria solani. *Alexandria Science Exchange Journal*, *42*(3), pp.773-788.

5. Ahmed, A.S. & Alani,R.A. (2013). Identification andBiocontrol of soft rot Bacteria Disease on onion underLAB. Condition.*Iraqi Journal of Agricultural Sciences*. 44(3):341-347.

6. Ahmed,A.S., Aljarah, N.S. & Merzah, N.R.,(2023).The Effect of Different Culture Media and Different Environmental Conditions on the Alternaria solani Sporulation. In *IOP Conference Series: Earth and Environmental Science* . 1262(3). p. 032055. IOP Publishing.

7. Ajeel,H K.(2015). *Epidemiology of early blight disease on tomato caused by the fungus Alternaria solani on tomato hybrids S-19 and S-10 grown locally in local agriculture*. Mse. College of Agriculture. University of Baghdad. Iraq.

8. Alaa- El-den H.R, A., Abd El-Wahab, G.M. & Masoud, S.A., (2022). Using Salicylic Acid ,Folic Acid and Mancozeb in controlling Tomato Early Blight Biotic Stress and Their Effect on Growth , Yield Fruit Quality and stress- Related Enzymes. *Iraqi Journal of Agricultural Sciences,* 53(6), pp.1548-1559.

9. AL-Aamel, A. N. A., & AL-Maliky, B. S. A. (2023). Efficacy of Pseudomonas fluorescens and iron chelated fe-eddha against *fusarium oxysporum f. sp*. the causal agent of root rot and wilt disease on pepper:*Iraqi Journal of Market Research and Consumer Protection*, *15*(1), 81-91.
10. Al-Juboori, A.W.A. & Alwan, K.A. (2015). Testing and Diagnosis of Genetic Variability in Tomato Affected by Electric and SodiumAzide under Salt Stress Conditions 1-Vegetative and yield characteristics. *Iraqi Journal of Agricultural Sciences, 46*(5).802-812.
11. Al-khafagi, A.M. (2019). Pathogenesis and Toxicity of *Alternaria solani* Apathogen of Early Blight on Tomato and its Control.phd. Thesis. college of Agriculture & Forestry Plant protection University of Mosul.Iraq.
12. Al-Sandooq, D.L. & Al-Fattah, F.A., (2015). Effect of plant physiology age on induced systemic resistance in tomato agent root knot nematodes by chemical inducers. *Iraqi Journal of Agricultural Sciences*, *46*(2).236-245.
13.Ansari, M., Ahmed, S., Khan, M.T., Hamad, N.A., Ali, H.M., Abbasi, A., Mubeen, I., Intisar, A., Hasan, M.E. & Jasim, I.K. (2023). Evaluation of In Vitro and In Vivo Antifungal Activity of Green Synthesized Silver Nanoparticles against Early Blight in Tomato. *Horticulturae*, *9*(3),369-376.
14. Attia, M.S., El-Sayyad, G.S., Abd Elkodous, M. & El-Batal, A.I., (2020). The effective antagonistic potential of plant growth-promoting rhizobacteria against Alternaria solani-causing early blight disease in tomato.plant. *Journal of Scientia.Horticulturae*,10(2),266-279.
15. Ancheeva, E., Daletos, G. & Proksch, P. (2020). Bioactive secondary metabolites from endophytic fungi. *Current medicinal chemistry*, *27*(11),1836-1854.
16. Aslam, M.N., Mukhtar, T., Hussain, M.A. & Raheel, M., (2017). Assessment of resistance to bacterial wilt incited by Ralstonia solanacearum in tomato germplasm. *Journal of Plant Diseases and Protection*, *124*,(4) 585-590.
17. Axler-DiPerte, G.L., (2017). Modeling and visualizing bacterial colony purification without the use of bacteria or laboratory equipment. *Journal of Microbiology & Biology Education*, *18*(2), pp.10-18.
18. Bahmani, K., Hasanzadeh, N., Harighi, B. & Marefat, A., (2021). Isolation and identification of endophytic bacteria from potato tissues and their effects as biological control agents against bacterial wilt. *Physiological and Molecular Plant Pathology*, *116*(2) 14-21.
19. Basit, A., Shah, S.T., Ullah, I., Ullah, I. & Mohamed, H.I., (2021). Microbial bioactive compounds produced by endophytes (bacteria and fungi) and their uses in plant health. *Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management*,22(3).285-318.
20. Boyno, G., Demir, S. & Akköprü, A., (2020). The effect of some endophytic bacteria against *Alternaria solani* (Ell. & G. Martin) Sor. In tomato *Uluslararasi Tarim ve Yaban Hayati Bilimleri Dergisi*, *6*(3) 469-477.

21. Demir, S., Durak, E.D., Güneş, H., Boyno, G., Mulet, J.M., Rezaee Danesh, Y. & Porcel, R., (2023). Biological control of three fungal diseases in strawberry (Fragaria× ananassa) with arbuscular mycorrhizal fungi.*Journal of Agronomy*, *13*(9),.2439-2448.
22. Doolotkeldieva,T., Bobusheva, S. & Suleymankisi, A., (2016). Biological cotrol of Erwinia carotovora ssp. carotovora by Streptomyces species. *Advances in Microbiology*, *6*(02).104 -112.
23. Eid, A.M., Fouda, A., Abdel-Rahman, M.A., Salem, S.S., Elsaied, A., Oelmüller, R., Hijri, M., Bhowmik, A., Elkelish, A. & Hassan, S.E.D. (2021). Harnessing bacterial endophytes for promotion of plant growth and biotechnological applications: an overview. *Plants*, *10*(5).935-947.
24. El-Nagar, A., Elzaawely, A.A., Taha, N.A. & Nehela, Y. (2020). The antifungal activity of gallic acid and its derivatives against Alternaria solani, the causal agent of tomato early blight. *Journal of Agronomy*, *10*(9), 1402-1409.
25. Fatehpuria, P.K. & Sasode, R.S., (2022). Scenario of Early Blight and Leaf Curl of Tomato in Gwalior Districts of Madhya Pradesh. *Indian Journal of Ecology*, *49*(2) 549-552.
26. Gorai, P.S., Ghosh, R., Konra, S. & Mandal, N.C., (2021). Biological control of early blight disease of potato caused by Alternaria alternata EBP3 by an endophytic bacterial strain Bacillus velezensis SEB1. *Biological Control*, *156*(4) 1045-1052.
27. Hatem, M.W., Shukri, H.M., Rasheed, K.A., Nawar, M.H., Hasan, S.M. & Adnan, R., (2020). The Effect of Magnetically Treated Water Against Fusarium Wilt Disease in Tomato Caused by the Fungus Fusarium oxysporum and Its Effect on Production Under Fertilized Farming Conditions. *Plant Archives*, *20*(1),.533-538.
28. Ibanez, A., Garrido-Chamorro, S. & Barreiro, C., (2023). Microorganisms and Climate Change: A Not so Invisible Effect. *Microbiology Research*, *14*(3) pp.918-947.
29. Jamel, D. S., Abdul-Razzak, A. K., & Taha, A. S. (2023). Antifungal activity of garlig and neem extracts against cobweb mold dactylium and green rot diseases in *Agaricus bisporus*: *Iraqi Journal of Market Research and Consumer Protection*, *15*(2), 60-73.
30. Kaushal, A., Sadashiva, A.T., Ravishankar, K.V., Singh, T.H., Prasanna, H.C., Rai, A.K. & Jatav, V.K., (2020). A rapid disease resistance breeding in tomato (Solanum lycopersicum L.). *Accelerated Plant Breeding. Journal of Vegetable Crops*,.2(4)17-55.
31. Lalramhlimi, B., Pramanik, S., Chattopadhyay, S.B., Bhattacharjee, T., Maurya, P.K., Mandal, A.K., Chatterjee, S., Chattopadhyay, A. & Hazra, P., (2023). Response of bacterial biovars to inheritance pattern of bacterial wilt disease in tomato. *International Journal of Vegetable Science*, *29*(5),425-443.
32. Latha, P., Karthikeyan, M. & Rajeswari, E. (2019). Endophytic bacteria: prospects and applications for the plant disease management. *Plant Health Under Biotic Stre. Journal of Microbial Interactions*.4(3)1-50.
33. Matlob, A. A. H., & Juber K. S. (2012). Isolation and Identification of The Causal Agent of Charcoal Rot Disease on Bean and Evaluation of some Biocontrol Agents under Laboratory Conditions. *Iraqi Journal of Agricultural Sciences*. 43(2) (Special Issue) 34-43

34. Maulani,B. I. G., Rasmi D .A. C & Zulkifli L.(2019). Isolation and characterization of endophytic bacteria from mangrove *Rhizophora mucronata* Lam. and antibacterial activity test against some pathogenic bacteria Department of Science Education. Faculty of Teacher Training and Education, Mataram . *Journal of Physics: Conference Series* 15(2)1402-1412.
35. Mugao, L.G., Gichimu, B.M., Muturi, P.W. & Njoroge, E.K., (2021). Essential oils as biocontrol agents of early and late blight diseases of tomato under greenhouse conditions. *International Journal of Agronomy, 2021*,44(12).1-10.
36. Murtado, A., Mubarik N. R & jahjoleksono A. T.(2020). Isolation and characterization endophytic bacteria as biological control of fungus *Colletotrichum* sp. on onion plants (*Allium cepa* L.) Department of Biology, *Faculty of Mathematics and Natural Sciences*. IOP Conf. Series: Earth and Environmental Science.45(4)457-463:012043
37. Montoya-Martínez, A.C., Ruiz, V.V., Chávez-Luzanía, R.A., Villa-Rodríguez, E.D. & de los Santos Villalobos, S.,(2024). *Biological control agents for mitigating plant diseases. In New Insights, Trends, and Challenges in the Development and Applications of Microbial Inoculants in Agriculture.* Academic.27-35. Press.
38. Nivedha, M.;E.B.benezar,K.kalpana & R.kumar.(2019). Invitro antifungal evaluation of various plant extracts against leaf Blight disease of jisminum grandi florum caused by *A.alternata* (Fr) Kessler. *Journal of Pharmasognosy and Phytochemistery* 8(3) :2143-2147.
39. Omer, A.K., Mohammed, R.R., Ameen, P.S.M., Abas, Z.A. & Ekici, K., (2021). Presence of biogenic amines in food and their public health implications: A review. *Journal of food protection*, *84*(9) 1539-1548.
40. Panthee, D.R., Pandey, A. & Paudel, R., (2024). Multiple Foliar Fungal Disease Management in Tomatoes:Acomprehensive Approach. *International Journal of Plant Biology*, *15*(1) 69-93.
41. Parvin, I., Mondal, C., Sultana, S.,Sultana,N. & Aminuzzaman, F.M.,(2021). Pathological Survey on Early Leaf Blight of Tomato and In Vitro Effect of Culture Media,Temperature and pH on Growth and Sporulation of *Alternaria solani*.*Open Access Library Journal*. *8*(3) pp.1-17.
42. Prasad, R.V.M., Sivakumar, K., Agnihotri, C., Agnihotri, S., Passari, A.K. & Singh, B.P., (2024). Fungal Endophytes and Their Role in Postharvest Disease Management: An Overview. *Endophytic Fungi: The Hidden Sustainable Jewels for the Pharmaceutical and Agricultural Industries Journal.*10(4).129-151.
43. Priyashantha, A.K.H., Karunarathna, S.C., Lu, L. & Tibpromma, S., (2023). Fungal Endophytes: An Alternative Biocontrol Agent against Phytopathogenic Fungi. *Encyclopedia*, *3*(2), 759-780.
44. Rana, K.L., Kour, D., Kaur, T., Devi, R., Yadav, A.N., Yadav, N., Dhaliwal, H.S. & Saxena, A.K., (2020). Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie Van Leeuwenhoek*, *113*(5).1075-1107.

45. Riaz, H.M., Chohan, S. & Abid, M., (2021). Occurrence of Tomato Eaely Blight Disease dand Associated Alternaria speices in Punjab, Pakistan. *JAPS: Journal of Animal & Plant Sciences*, *31*(5).465-472.
46. Rebotiloe, F.M., Eunice, U.J. & Mahloro, H.S.D. (2018). Isolation and identification of bacterial endophytes from Crinum macowanii Baker. *African Journal of Biotechnology*, *17*(33)1040-1047.
47. Roy, C.K., Akter, N., Sarkar, M.K., Pk, M.U., Begum,N., Zenat, E.A. & Jahan, M.A.,(2019). Control of early blight of tomato caused by and screening of tomato varieties against the pathogen. *The Open Microbiology Journal*, *13*(1).223-229.
48. Sahu, D.K.; C.P.Khave , & C.P.Patel .(2014). Eco friendly management of early blight of tomato using botanical plant extracts. *Journal of Industrial Pollution control* .30 (2) : 215- 218 .
49. Singh, S.P., Bhattacharya, A., Gupta, R., Mishra, A., Zaidi, F.A. & Srivastava, S., (2020). Endophytic Phytobiomes as Defense Elicitors: Current Insights and Future Prospects. *Phytobiomes: Current Insights and Future Vistas*,21(3)299-334.
50. Siraj, M.A., Medha, M.M., Nahar, A.U., Islam, M.A. & Seidel, V. (2023). *Mangrove endophytes and their natural metabolites: role in promoting plant health*. In *Microbial Endophytes and Plant Growth* Academic Press. 99-116.
51. Shrestha, A., Hada, M.S.S., Tuladhar, R., Manandhar, S., Ilyas, N., Al Tawaha, A.R.M. & Singh, A., (2022). Plant Growth-Promoting Rhizobacteria as Antifungal Antibiotics Producers. In *Antifungal Metabolites of Rhizobacteria for Sustainable Agriculture* . Cham: Springer International Publishing.83-102