

# RESEARCH ARTICLE

# Exploration of bacterial endophytes in cucumber (Cucumis sativus L.)

**Priyanka, S<sup>1</sup>, Krishnamoorthy, A, S<sup>1\*</sup>, Latha, P<sup>1</sup>. and Kalaiselvi, T<sup>2</sup>** <sup>1</sup>Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641003

<sup>2</sup>Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore – 641003

# ABSTRACT

In an attempt to explore the potentials of the endophytic bacterial population in cucumber plants, thirty different isolates have been made and tested for its efficacy against damping off the pathogen, Pythium aphanidermatum (Edson) using dual culture technique. The result revealed the potentials of Pseudomonas aeurginosa (MPP EB 4 and MPP EB 3) and Achromobacter denitrificans (MPP EB 8). The isolates MPP EB 3, MPP EB 4 and MPP EB 8 showed the maximum inhibition of 36.7, 40.0 and 32.2 per cent over control, respectively. The convoction of animicrobial compound isolated from selected isolates of MPP EB 3, MPP EB 4 and MPP EB 8 were tested Received : 4<sup>th</sup> June, 2019 against the pathogen by using agar well diffusion technique, which showed Revised : 12<sup>th</sup> June, 2019 inhibition of 25.2, 43.2 and 13.7 per cent over control, respectively. GCMS Accepted : 13th June, 2019 analysis for the secondary metabolites of P.aeurginosa deciphered an array of compounds, which are known to have antifungal property. Achromobacter denitrificans (MPP EB 8) could not inhibit the pathogen, but enhanced the plant growth up to 57.9 per cent, when compared to control. In addition to suppressing the pathogen, isolate MPP EB4 and MPP EB 3 could increase the growth of cucumber seedlings up to 31.6 and 17.0 per cent over control, respectively. Hence, MPP EB 4 (P.aeuriginosa) could be exploited as endophytic bioinoculant with adequate biosafety measures.

**Keywords:** *Cucumber, endophytes, Pseudomonas aeuriginosa, Achromobacter denitrificans, biocontrol and plant growth promotion.* 

# INTRODUCTION

Cucumber (*Cucumbis sataivus*. *L*) (family: *Cucurbitaceae*) is the most important vegetable crop, which is originated from South Asia. China is the leading producer across the globe (about 59 million tonnes annually). India also plays an important role in exporting cucumber at large scale. The area under cucumber and gherkin cultivation in India was reported to be around 26,088 ha with an approximate production of 1.61 lakh tonnes of fruits during 2017 (FAOSTAT, 2017). The cucumber crop is being affected by damping off caused by *Pythium aphanidermatum*, which remarkably reduces the income of farmers.

Farmers have been resorting to monoculturing of cucumber resulting in increased effect of soil borne plant pathogens such as *Pythium* and *Fusarium* (Al-Sa'di *et al.*, 2007 and Jin *et al.*, 2019). Different species of *Pythium* were reported to cause damping off symptoms and resulted in 75% of mortality to the young seedlings (Al-Sa'di *et al.*, 2011b; Al-Balushi *et al.*, 2018).

Several methods are available for managing

damping off disease in crop plants that include chemical, physical and biological in nature. Repeated use of chemicals may cause health hazards, environmental issues and also the development of resistance in pathogens. The use of beneficial organism and potential biocontrol agents like *Pseudomonas fluorescens, Trichoderma harzianum* and *Penicillium stipitatum* served as the best alternative to the chemical method of disease management (Kloepper, 1999; Al-Sadi *et al.*, 2015b; Halo *et al.*, 2018).

Endophytic organisms are present in the endosphere region of a selected niche in their host plant. They provide resistance to the host plant against various biotic and abiotic stresses mediated through antimicrobial compounds, siderophores, competing for nutrient and space inside the host. Hallmann, (1997) reported that endophytes are micro organisms, which resides inside the plant cell or vascular system without producing any external symptoms. Endophytic bacteria is the potential biological control agent, which is isolated from the surface of disinfected parts of healthy plants. The presence of endophytes in vascular tissues could be effective against Fusarium oxysporum and Verticillium spp (Hallmann et al. 1997). There was less competition among a diverse population of endophytes than ectophytes present in the rhizosphere (Kloepper, 1999). Many of the endophytes like Bacillus (Lee et al., 2017), Pseudomonas (Priyanka et al., 2017) and Streptomyces (Lu et al., 2016) acted against plant pathogens and also improved the plant growth. In recent years, researchers have focused on the bioactivities of an endophytic microorganism to be used as biocontrol agents and biofertilizer as an alternative strategy for chemical control (Akbaba and ozaktan, 2018). With this background, the present investigation on potential endophytic bacteria of cucumber against damping off pathogen and elucidating their multiple actions against the pathogen was carried out.

# **MATERIALS AND METHODS**

# Isolation of pathogen

Cucumber seedlings showing damping off symptoms were collected from Annur, Coimbatore district (geographical coordinates: 11.2°N, 77.1°E and 411.2 MSL). The pathogen was isolated from infected root portions by following tissue segment method (Rangaswamy, 1972). Infected roots were cut into small pieces, surface sterilized using 1% sodium hypochlorite (w/v) for one min. The root bits were rinsed thrice in sterile distilled water for 1 min and placed in sterile Petri plate containing 20ml of Potato Dextrose Agar (PDA) medium. The plates were incubated at  $28\pm2$ °C. The pure culture was obtained by single hyphal tip method and it was transferred to PDA slant and stored at -20°C.

# Pathogenicity test

*Pythium* isolate was tested for pathogenicity in cucumber seedlings (Al-sadi *et al.*, 2012). The soil mixture was sterilized twice and seeds were sown in the pots filled with sterilized soil mixture. After the seedling emergence, the treatment pots were inoculated with 3 days old culture of *Pythium* along with agar disc at the collar region and incubated for the occurrence of symptoms and control was maintained without agar disc. After the appearance of the symptom, the pathogen was reisolated from the infected portion to prove Koch's postulate and compared with the original culture.

# Morphological characterization of the pathogen

The pathogen was identified up to genus level based on morphological character described (Van Der Plaats-Niterink, 1981 and Dick, 1990). A loopful of actively growing mycelium was taken on the clean glass slide containing water droplet and observed under the image analyzer. The mycelium, asexual (sporangia) and sexual (antheridium and oogonium) were visualized under the microscope and their size was measured using the technology available in the image analyzer.

# Molecular characterization of the pathogen

Genomic DNA was extracted from the damping off pathogen by using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Lee & Taylor, 1990). Genomic DNA was subjected to the PCR amplification of the Internal Transcribed Spacer (ITS) using the primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). PCR reactions were carried out on a thermal cycler (Eppendorf), and consisted of initially denaturing at 95°C for 10 min followed by 40 cycles at 95°C for 1 min, 60°C for 30 s, 72°C for 1 min and final extension by 72°C for 10 min. The PCR products were analyzed on 1.2% gel electrophoresis at 110 V for 1h. The gel visualized under gel documentation (UVITEC, Cambridge, UK). The size of the PCR products was determined by the 1Kb molecular marker. The amplified product was subjected to sequencing.

# Isolation of endophytes

Internal tissues of root, stem, leaves and petioles from the healthy cucumber plant were used for endophytes isolation. The plant parts were surface sterilized using 1 per cent sodium hypochlorite for 10 min and washed 70% ethanol for 1min and rinsed with sterile water for 5 times in order to remove the surface contaminants. The last wash was plated on Tryptic Soya Agar (TSA), which served as control and incubated at 28±2°C. The samples were macerated with saline phosphate buffer (pH 8.0) and filtered through a muslin cloth. The supernatant solution was serially diluted up to 10<sup>-6</sup> and last three dilutions were poured on a different medium (Nutrient Agar (NA), Ken knight (KKA), PDA and TSA) and incubated at 28±2°C. Growth of endophytic bacteria observed as a single colony was purified and it was transferred to 20% glycerol in Eppendorf tubes and stored at -70°C for further use.

# In vitro screening of endophytic bacteria against Pythium aphanidermatum (streak method)

The bacterial endophytes isolated from healthy cucumber plant were used for screening the antifungal activity against the pathogen, *P.aphanidermatum*. Antagonistic activities of endophytic bacterial isolates were tested by Dual culture technique using PDA medium (Dennies and Webster, 1971). Mycelial disc of the pathogen was placed at one end of the Petri plate and the endophytic bacteria were streaked 1.0 cm away from the periphery of the plates at the opposite side. Simultaneously control plate was maintained without a bacterial antagonist. The plates were incubated at room temperature. The radial mycelial growth of the pathogen was measured (mm) and per cent inhibition(PI) was calculated

Per cent Inhibition (PI) = 
$$\frac{C-T}{C} \times 100$$

Where.

C is the growth of test pathogen (mm) in the absence of the antagonist

T is the growth of test pathogen (mm) in the presence of the antagonist

#### Molecular characterization of endophytes

The endophytic bacteria were inoculated in NA broth for 24 h from which genomic DNA was isolated by lysis method (He et al., 2011). The isolated DNA was subjected to PCR amplification using 16s forward and reverse primers, 16sF (5' AAGTCGTAACAAGGTAG 3') and 16sR (5' GACCATATATAACCCCCAAG3') (Kumar et al., 2002), and the PCR product was sequenced (Agrigenome Pvt. Ltd, Kerela, India) and subjected blast analysis. The compared sequences were submitted in the Gene bank database for identification of the organism.

# Testing of antifungal biomolecules from potential endophytic bacteria against P.aphanidermatum

#### a. Extraction of antifungal biomolecules

Antimicrobial metabolites were extracted from the potential endophytes possessing maximum antifungal activity against P.aphanidermatum. The endophytes were inoculated in NA broth and incubated in a rotary shaker for 3-4 days. After incubation, the culture filtrate was centrifuged at 6000 rpm for 10 min, sequentially extracted with ethyl acetate and chloroform at 150rpm for 24h. Finally, condensed using rotary evaporator. The crude extract was dried, dissolved in methanol and tested against Pythium aphanidermatum.

# b. Testing antifungal biomolecules against P.aphanidermatum

Antimicrobial activity of secondary metabolites extracted using each solvent was evaluated by agar well diffusion method (Stoke and Ridgway, 1980). After solidification of the PDA medium, wells (9 mm diameter) were made on all four sides of each plate using sterile cork borer, leaving 1 cm space from the periphery. Later 50µl of the crude bacterial metabolite was transferred into each well. Five days old mycelial disc of the pathogen (5 mm diameter) was placed at the centre of each plate and incubated at 28±2°C for seven days. The radial growth of mycelium (mm) and zone of inhibition (mm) were recorded after 7 days of incubation. Based on the observation, per cent inhibition over control was calculated. In order to assess the minimum inhibitory 106 | 4-6 | 408

concentration, the ethyl acetate fraction of MPP EB 4 was tested at different concentrations (0.1%, 0.5%, 1.0% and 1.5%) by agar well technique.

# c. GCMS analysis of secondary metabolites

A secondary metabolite of potential endophyte (P.aeuriginosa) was analyzed for identifying the compounds present in it through Gas chromatography coupled mass spectrometry (GC-MS) (GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited). The instrument was set in the following order, 1. injector port temperature set as 250°C, 2. the ion source temperature was 200°C, 3. oven temperature was programmed as 110 °C for 2 min, 200 °C @ 10 °C/min, up to 280 °C @ 5 °C/min. The split ratio was set as 1:50 and the splitless mode of injector were used. The DB-35 MS nonpolar column with the dimension of 0.25 mm OD  $\times$  0.25  $\mu$ m ID  $\times$ 30 meters length was used. Helium gas was used as a carrier gas with a constant flow at the rate of one ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200°C and <40 motor vacuum pressure. The ionization energy of -70eV. The MS was also having inbuilt pre-filter, which reduce the neutral particles. The data system had two inbuild library such as NIST4 and WILEY9. Those compounds with spectral fit values either equal to or greater than 700 considered as positive identification. By comparing the data obtained through the spectrum with MS data library, the compounds present in the sample were identified.

# Assay of in vitro biocontrol efficacy by seed germination method (Roll towel method)

Cucumber seeds were surface sterilized using 70% ethanol and 1% sodium hypochlorite, rinsed twice with sterilized distilled water. The endophytic bacterial broth of 72h old culture was prepared and mixed with 1% glycerol (10ml), 1% tween 20 (10ml) and 1% Polyvinyl Pyrrolidone (PVP) (10g) kept in a shaker for 5 min to ensure uniform mixing. The cfu were assessed by making serial dilution technique. The liquid formulation comprising  $2 \times 10^{10}$  cfu/ml was used. The surface sterilized seeds were treated with liquid formulation by soaking it for 12hrs and air-dried overnight. Roll towel method was adopted to assess the efficacy of biocontrol agent. Root length and shoot length of the individual seedlings were measured and the germination percentage was observed. The vigour index was calculated by using the formula suggested by Abdul baki and Anderson (1973).

Vigour index = (Mean length + shoot length) × Germination per cent germinated seed

total seeds tested × 100 Germination per cent

#### Statistical analysis

All the experiments were analyzed independently.

The treatment means were compared by Duncan's Multiple Range-Test (DMRT) (Gomez *et al.*, 1984). All the data were analyzed statistically by AGRESS and interpreted.

# **RESULTS AND DISCUSSION**

# Isolation and identification of the pathogen

Damping off pathogen (*P.aphanidermatum*) was isolated from infected cucumber plant showing symptoms of yellowing, wilting and thinning of the crown region (Hatami *et al.*, 2019).

# Table 1. Effect of an antimicrobial crude fractionof bacterial endophytes againstP.aphanidermatum

Treatments	Mycelial growth* (mm)		Per	cent Inhibition
	Ethyl acetate	chloroform	Ethyl acetate	Chloroform
MPP EB 4	51.10ª	60.60ª	43.20ª	32.67 ª
	(45.63)	(51.12)	(41.09)	(34.86)
MPP EB 3	67.30 <sup>b</sup>	78.00 <sup>b</sup>	25.22 <sup>b</sup>	13.33 <sup>b</sup>
	(55.12)	(62.03)	(30.14)	(21.41)
MPP EB 8	77.60 <sup>c</sup>	88.00°	13.77°	2.22°
	(61.75)	(69.73)	(26.88)	(8.57)
Control	90.00 <sup>d</sup>	90.00°	0.00 <sup>d</sup>	0.00°
	(71.56)	(71.56)	(0.81)	(0.81)

During pathogenicity study, the seedling produced water-soaked lesions at the crown region, wilting and collapse. The cultural character of *Pythium* on the PDA medium showed white, cottony and fluffy aerial growth. Upon microscopic observation, mycelium was aseptate, hyaline ( $6.812\mu$ m) with lobed sporangia and produced terminal oogonia, which has

# Table 2. Minimum Inhibitory Concentration (MIC)assay for P.aeuriginosa (MPP EB 4)metabolites against P.aphanidermatumusing agar diffusion method

Concentrations	Mycelial	Per cent	
(Per cent)	growth*(mm)	inhibition	
0.1	78.00 <sup>b</sup>	13.33 <sup>b</sup>	
	(62.03)	(21.41)	
0.5	76.00 <sup>b</sup>	15.00 <sup>b</sup>	
	(60.67)	(23.22)	
1.0	54.0ª	40.00 ª	
	(47.29)	(39.23)	
1.5	52.67ª	<b>41.47</b> <sup>a</sup>	
Control	(46.53) 90.00°	(40.08) 0.00 °	
	(71.56)	(0.74)	

\*Mean of three replication Note: Data in parentheses are arc sine transformed values

Means followed by a common letter are not significantly different at 5% level by DMRT at  $P \le 0.05$ 

smoothly walled, globose in structure, antheridia were intercalary and broadly sac with 2-3 oogonium

and produced aplerotic oospore (22.854µm) (figure 1). Morphological characters of the isolate was identical to those of *Pythium aphanidermatum* (Edson) as reported by Alaei, (2013). The mycelial and sporangial characters were similar to the result obtained by Al-Sheikh, (2010), *P.aphanidermatum* that infected who observed similarly in case of wheat plant. Approximately 830bp amplicon size of PCR product was obtained. The sequence showed 99% homology with the sequence of *P. aphanidermatum* in the NCBI database. The sequence was deposited in the NCBI database and published with the accession no. MK758062.

# Antagonistic effect of endophytic bacteria against P. aphanidermatum

The internal tissues of healthy cucumber plants yielded thirty endophytic bacterial isolates. The isolated bacterial endophytes were tested against *P. aphanidermatum* by dual culture technique (Dennies and Webster, 1971). Among the 30 endophytes, three isolates showed the maximum inhibition of mycelial growth *P. aphanidermatum*. The endophytic isolates MPP EB 3, MPP EB 4 and MPP EB 8 significantly inhibited the growth of *P. aphanidermatum* 36.7, 40.0 and 32.2 per cent, respectively (figure 2 & 3). These potentially endophytic bacterial isolates were used for metabolite extraction.

Similar to the present study, Bamon *et al.*, (2018) isolated six bacterial endophytes and tested against *Pythium* sp. causing ginger rot and among them four isolates viz., *Alcaligenes* sp. (GE-1), and *Bacillus* spp (GE-3,4,5) / viz., showed mycelial inhibition more than 70%, when tested by dual culture technique.

# Molecular characterization of endophytes

The molecular characterization of endophytic isolate MPP EB 4 and MPP EB 3 showed the amplicon size of approximately 501bp, while MPP EB 8 showed 240bp amplicon size and they were compared with NCBI database. MPP EB 4 and MPP EB 3 showed the maximum similarity with *Pseudomonas aeruginosa* and MPP EB 8 showed similarity with *Achromobacter denitrificans*.

# Antimicrobial metabolites of endophytes against P.aphanidermatum

Ethyl acetate and chloroform crude fractions of endophytic bacterial isolates MPP EB3, MPP EB4 and MPP EB 8 were tested against the *P.aphanidermatum*. The ethyl acetate MPP EB 4 showed maximum inhibition of 43.2 per cent, over control. Among the four different concentrations, ethyl acetate extract tested 1.0% and 1.5% showed higher inhibition of 40.56 and 41.67 per cent respectively. The least inhibition was observed at

Retenti Time (RT)	on	Compounds	Local area (per cent)	Chemical formula	Structure	Molecular weight (g/mol)
12.68		Pyrrolo[1,2-a]pyrazine-1,4- dione, hexahydro-	9.82	$\underline{C}_{\underline{7}}\underline{H}_{\underline{10}}\underline{N}_{\underline{2}}\underline{O}_{\underline{2}}$	" L L	154.169
19.36		2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	11.05	$C_{12}H_{22}N_2O_2$		226.31
15.69		Dasycarpidan-1-methanol, acetate (ester)	5.72	$C_{20}H_{26}N_2O_2$		326.43
26.74		9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl) oxy]propyl ester, (Z,Z,Z)	3.47	$C_{27}H_{52}O_4Si_2$	Harrow. Ala and and and a	496.879
22.91		Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'- (phenylmethyl)-, (5'à)	1.16	$C_{33}H_{35}N_5O_5$		581.00
25.66		Lycopene	1.25	<u>C<sub>40</sub>H<sub>56</sub></u>		536.888
29.24		.psi.,.psiCarotene, 1,1',2,2'-tetrahydro-1,1'- dimethoxy-	0.77	$C_{42}H_{64}O_{2}$	h Jahang Kang	600.00
	13.98	Actinomycin C2	0.24	<u>C<sub>63</sub>H<sub>88</sub>N<sub>12</sub>O<sub>16</sub></u>		1269.46

# Table 3. Profile of P.aeruginosa (MPP EB 4) secondary metabolites using GCMS

the concentration of 0.1per cent followed by 0.3 per cent.



Figure 1. Morphological features of *P.aphanidermatum* 

a) Aseptate mycelium, b) Antheridium with oogonium c) Oospore d&e) lobed sporangia

However, the experiment revealed that the higher concentration of crude extract of bacterial metabolites showed higher inhibition of mycelium growth (figure 4 & 5, table 1 & 2). In a similar study, Robert *et al.* (2007) reported that the bacterial cells and cell-free culture filtrate of *Serratia marcescens* was found to be effective in controlling the damping off of cucumber caused by *P.ultimum*.

The bacterial endophytes isolated from cucumber were screened for their antagonistic activity against *Phytophthora capsici*. Among them, *Pseudomonas stutzeri*, *Bacillus subtilis*, *Stenotrophomonas maltophilia* and *Bacillus amyloliquefaciens* inhibited the mycelial growth *P.capsici* and also improved seed germination and plant growth (Islam *et al.*, 2018). Secondary metabolite produced from *Bacillus* 





atrophaeus showed inhibitory effect on Verticillium dahlia to the extent of 75.5 per cent in the medicinal plant (Mohanad *et al.*, 2018). Endophytes had the ability to synthesis secondary metabolites with higher antimicrobial activity against the plant pathogens (Saikkonen *et al.*, 2004).



Figure 3. Dual culture: Bacterial endophytes Vs *P.aphanidermatum* a) MPP EB 4, b) MPP EB 3, c) MPP EB 8 and d) Control

# GCMS analysis of ethyl acetate fraction

GCMS analysis of ethyl acetate fraction of *P.aeuriginosa* (MPP EB 4) indicated the presence of different compounds such as Pyrrolo [1,2-a]

pyrazine-1,4-dione, hexahydro-, 2,5-Piperazinedione, 3,6-bis(2-methyl propyl)-, Dasycarpidan-1-methanol, acetate (ester), 9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z),



Figure 4. Inhibitory effect of biomolecules of bacterial endophytes against P.aphanidermatum a) MPP EB 4, b) MPP EB 3, c) MPP EB 8 and d) Control

Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'à), Lycopene, and psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy. The highest peak was recorded for 2,5-Piperazinedione, 3,6-bis(2-methyl propyl)- followed by Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro- with the retention time of 12.68 and 19.36, respectively. Raut *et al.*, (2018) reported that *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* isolated from rhizosphere region showed an antagonist effect against bacterial blight pathogen of cotton when tested with the diffusible secondary metabolites. GCMS



Figure 5. Minimum Inhibitory Concentration (MIC) assay for *P.aeuriginosa* (MPP EB 4) metabolite against *P.aphanidermatum* a) 0.1% b) 0.5% c) 1.0% d) 1.5% and control

analysis of metabolite find the presence of Pyrrolo[1,2-a] pyrimidine-2,6-dione, hexahydro-, 2,5-Piperazinedione, 3,6-bis(2-methyl propyl)- and Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)- 2-butynyl]-. The results obtained in the present study was also in corroboration with the earlier reports, where endophytes produced antimicrobial compounds with antagonistic activity against plant pathogens (table 3 & figure 6).



Figure 6. Chromatogram of P.aeruginosa (MPP EB 4) metabolites

#### Assay of in vitro biocontrol efficacy

In this in vitro assay, MPP EB 8 showed maximum germination of 88 per cent followed by MPP EB 4 (60.67 per cent) and minimum germination by MPP EB 3. Vigour index of the three isolates, MPP EB 8, MPP EB 4 and MPP EB 3 was found to be 2830.41,

1744.52 and 1437.72 (as mentioned in table 4). Among the three isolates, MPP EB 8 had higher plant growth promoting activity, but had only minimum antagonistic property. But the isolate *P.aeuriginosa* (MPP EB 4) had both the antagonistic and growth promotion activities.

Treatments	Root length*(cm)	Shoot length*(cm)	Germination* Per cent	Vigour index
MPP EB4	18.62 <sup>b</sup>	9.73 <sup>b</sup>	60.76 <sup>b</sup> (51.21)	1744.52 <sup>b</sup>
			56.47 <sup>b</sup>	
MPP EB3	16.93 <sup>b</sup>	8.53 <sup>b</sup>	(48.71)	1437.72 bc
			88.23ª	2830.41ª
MPP EB8	<b>21.67</b> ª	10.41ª	(69.93)	
			54.88 <sup>b</sup>	1193.09 <sup>d</sup>
Control	13.44°	8.3 <sup>b</sup>	(47.80)	
*Mean of three replication				

Table 4. Plant growth promotion effec	t of bacterial endophytes on cucumber
---------------------------------------	---------------------------------------

Note: Data in parentheses are arc sine transformed values

Means followed by a common letter are not significantly different at 5% level by DMRT at  $\mathsf{P} \leq 0.05$ 

# CONCLUSION

This research has been focused to exploit endophytes as biocontrol agents in order to avoid the hazardous effect of fungicides in the environment. Many endophytes had successfully emerged for the management of a wide range of plant pathogens and they reported to produce several secondary metabolites. The outcome of the present investigation led to identifying *P.aeruginosa* an endophyte possesing the maximum inhibitory potential against *P.aphanidermatum* and also showed growth promotion activity. Hence, *P.aeuriginosa* (*MPP EB4*) could be an ideal bioinoculant if employed with adequate biosafety precautions.

# ACKNOWLEDGMENT

The authors would like to thank funding support from UGC-SAP DRS1 and DST - FIST scheme for the research work.

# REFERENCES

- Abdul Baki, A. A. and Anderson, J.D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop science*. 13, pp: 630-633
- Alaei, H., and Rostami, F. 2013. Identification of cucumber damping-off based on morphological and molecular characterizations in rafsanjan. *Iran. J. Plant Path.* 48(4):177-182
- Al-Sheikh, H. 2010. To pathogenic species of Pythium *: P.aphanidermatum and P.diclinum* from a wheat field. *Saudi Journal of biological science*. 17: 347–352
- Akbaba. M., and Ozaktan. H. 2018. Biocontrol of angular leaf spot disease and colonization of cucumber (*Cucumbis sativus* L.) by endophytic bacteria. *Egyptian journal of biological pest control*, 28:14
- Al-Sa'di, M., Drenth, A, Deadman, M. L, de Cock, A.W, and Aitken, E. A. B. 2007. Molecular characterization and pathogenicity of *Pythium* species associated with damping-off in greenhouse cucumber (*Cucumis sativus* L.) in Oman. *Plant Pathology*. 56:140–149
- Al-Sadi A.M., F.A. Al-Said, M.L. Deadman, A. Drenth and E.A.B. Aitken. 2010b. Can silicon improve defense responses of cucumber seedlings to Pythiuminduced damping-off disease? Acta Horticulturae 871, 441–444.
- Al-sadi, A. M., Al-Ghaithi, A. G., Al-Balushi, Z. M. and Al-Jabri, A. H. 2012. Analysis of diversity in *Pythium aphanidermatum* populations from a single greenhouse reveals phenotypic and genotypic changes over 2006 to 2011. *Plant Dis.* 96, 852–858.
- Al-Sadi, A. M., Al-Masoodi, R. S., Al-Ismaili, M. and Al-Mahmooli, I. H. 2015a. Population structure and development of resistance to hymexazol among *Fusarium solani* populations from date palm, citrus and cucumber. *J. Phytopathol.* 163:947– 955.

- Bamon, M., Majumder, D, Thakuria, D and Rajesh, T. 2018. *In vitro* efficacy of bacterial endophytes against *Pythium* sp. causing soft rot of ginger in Meghalaya, *Int.J.Curr.Microbiol.App.*Sci. 7(8): 367-374
- Dennies, C and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma. Trans. Br, mycol*, Soc. 57 (I), 25-39
- Dick, MW. 1990. Keys to Pythium. Reading College of estate Management. p. 64
- Gomez, K. A. and Gomez, A. A. 1984. Statistical procedure for Agricultural Research. John wiley and sons, New York.
- Hallmann, J., Quadt-Hallmann, A, Mahaffee, W.F, and KI~epper, J.W. 1997. Bacterial endophytes in agricultural crops, *Canadian journal of microbiology*. Vol 43.
- Halo, B.A., Al-Yahyai, R.A, and Al-Sadi, A.M. (2018). *Aspergillus terreus* inhibits growth and induces morphological abnormalities in *Pythium aphanidermatum* and suppresses *Pythium*-induced damping-off of cucumber. *Front. Microbiol.* 9:95.
- He, F. 2011. E. coli genomic DNA extraction. Bio-Protocol Bio.101:e97.
- Islam, S., Akanda, M. A., Prova, A., Islam, T. M., and Hossain, M, M. 2018. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on Plant Growth Promotion and disease suppression. *Frontiers of Microbiology*. 6:1360
- Ji, SN., Paul, N.C, Deng, J.X, Kim, Y.S, Yun, B, Yu, S.H. 2013. Biocontrol activity of *Bacillus amyloliquefaciens* CNU114001 against fungal plant diseases. *Mycobiology* 41: 234–242
- Jin, X., Wang, J, Li D, Wu, F, and Zhou, X. 2019. Rotations with indian mustard and wild rocket suppressed cucumber *Fusarium* wilt disease and changed rhizosphere bacterial communities. *Microbiology*. 7:57
- Kloeppe, J. W., Rodriguez-Kabana, R., Zehnder, A. W., Murphy, J. F., Sikora, E. and Fernandez, C. 1999. Plant root-bacterial interactions in biological control of soil borne diseases and potential extension to systemic and foliar diseases. *Australas. Plant Pathol.* 28:21-26.
- Kumar, N.R., Rai, A.B, and Rai, M. 2008. Research note export of cucumber and gherkin from India: Performance, Destinations, Competitiveness and Determinants. *Agricultural Economics Research*. 21:130-138
- Lee, S. B. & Taylor, J. W. Innis M.A., Gelfand, D.H., Sninsky, J.J., White T.J. 1990. Isolation of DNA from fungal mycelia and single spores. PCR Protocols: a Guide to Methods and Applications. Academic Press, *San Diego*, pp. 282-287.
- Lee, T., Park, D, Kim, K, Lim, S.M, Yu, N.H, Kim. S, Kim H.Y, Jung, K.S, Jang, J.Y, Park, J.C, Ham, H, Lee, S, Hong, S.K, Kim, J.C. 2017. Characterization of *Bacillus amyloliquefaciens* DA12 showing potent antifungal activity against mycotoxigenic *Fusarium* species. *Plant Pathol J.* 33:499–507

- Lu, D., Ma, Z, Xu, X, and Yu, X. 2016. Isolation and identification of biocontrol agent *Streptomyces rimosus* M527 against *Fusarium oxysporum f. sp. cucumerinum. J Basic Microbiol.* 56:929–933
- Mohamad, A. A., <u>Li, L., Jin-Biao, Ma., Shaimaa,</u> <u>H., Xu, L., Guo, J. W., Rasulov, B. A., Liu, Y. H.,</u> <u>Hedlund</u>, B. P. and <u>Li</u>, W. J. 2018. Evaluation of the Antimicrobial Activity of Endophytic Bacterial Populations From Chinese Traditional Medicinal Plant Licorice and Characterization of the Bioactive Secondary Metabolites Produced by *Bacillus atrophaeus*Against *Verticillium dahlia F*. 2018; 9: 924.
- Priyanka, A.T., Kotasthane, A.S, Kosharia, A, Kushwah, R, Zaidi, N.W, and Singh, U.S. 2017. Crop specific plant growth promoting effects of ACCd enzyme and siderophore producing and cynogenic fluorescent *Pseudomonas. Biotech.* 7(1):27
- Ramette, A., Frapolli, M, Défago, G and Loccoz, Y. 2003. Phylogeny of HCN synthase-encoding hcnBC genes in biocontrol Fluorescent *pseudomonads* and its relationship with host plant species and HCN synthesis ability. Molecular Plant-Microbe Interactions. 16( 6):525–535

- Rangaswami, G. 1958. An agar block technique for isolating soil microorganisms with special reference to Pythiaceous fungi. Science and Culture. 24: 85.
- Raut, L.S. and Hamde, V. 2018. *in vitro* antagonism of resident rhizobacteria, *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* against the bacterial blight pathogen of bt cotton. *International Journal of Pharmacy and Biological Sciences*. 8: 2321-3272
- Roberts, D.P., Mckenna, L.F, Lakshman, D.K, Meyer, S.L.F, Kong, H, Souza, J.T, Lydon, J, Baker, C.J, Buyer, J.S and Chung, S. 2007. Suppression of damping-off of cucumber caused by *Pythium ultimum* with live cells and extracts of *Serratia marcescens* N4-5.
- Saikkonen, K., Piippa, W., Helander. M, and Faeth, H. S. 2004. Evolution of endophyte–plant symbioses. *Science direct*. 9:6
- Stoke, J.E. and Ridgway, G.L. (1980): Clinical Bacteriology. Edward, Arnold Ltd. London.
- Van Der Plaats-Niterink AJ. 1981. Monograph of the genus Pythium studies in mycology. Baarn: Central Bureau voor schimmel Cultures. p. 211–242.
- Zainab, M., Balushi, A, Agrama, H, Issa H. Mahmooli, A., Sajeewa, S.N, Maharachchikumbura, and Al-Sadi, M. 2018. Development of resistance to hymexazol among *Pythium* specie in greenhouses in Oman. *The American Phytopathological Society* 102:1