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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 05

*Res. Jr. of Agril. Sci.* (2022) 13: 1660–1666

CARAS

Res. Jr. of Agril. Sci. (Sep-Oct 2022)
13(5): 1660–1666

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

*Full Length Research Article*

# Development of Resistance against *Cercospora punicae* and *Ceratocystis fimbriata* by Endophytic Bacterial Consortium Introduced into *Solanum lycopersicum* Seedlings

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Received: 25 Jul 2022 | Revised accepted: 07 Oct 2022 | Published online: 31 Oct 2022


## A B S T R A C T

With the limited availability of cultivable land, it is important that we improve the yield of the crops, but this can be hindered by the attack of pests and pathogens. Even though chemical pesticides and herbicides are used for the prevention and reduction of the attack of pests and pathogens, these chemicals are harmful to the environment as well as to the consumers of the crop. It is also harmful to the soil microflora and soil health. Hence, it is important to adopt environmentally friendly ways that can help in providing the plant with protection against pests and pathogens. One of the most common, efficient, and environmentally friendly ways is the introduction of endophytes into the crop plant. In the present study, the seedlings of *Solanum lycopersicum* were treated with six bacterial endophytes isolated from *Curcuma longa*. They were further infected with two fungal pathogens *Cercospora puniceae* and *Ceratocystis fimbriata.* The plant growth parameters were recorded after every 5 days for 45 days. The percentage of infection, disease incidence, and biocontrol efficacy were calculated. The seedlings treated with endophytic bacterial consortium had an ability to withstand the fungal pathogens and survive when compared to the seedlings grown in control.

***Key words*:** Endophytes, *Cercospora puniceae, Ceratocystis fimbriata, Curcuma longa, Solanum lycopersicum,* Biocontrol efficacy

The use of agrochemicals in excess amounts, even though help control pests and pathogens, has been a major reason for environmental pollution. Most of them are carcinogenic and are also harmful to non-target organisms. It is important to control the pests and disease-causing organisms to increase the yield of the plants. This has led to the use of biological control as an alternative method to agrochemicals to control pests and pathogens [1]. Biocontrol mostly involves the use of living organisms or their metabolic by-products to control pests and pathogens. The use of endophytes as a biocontrol is more beneficial as the organism colonises the same ecological niche as that of the plant pathogen, preventing their growth [2-3]. The endophytes either act directly (parasitism, antibiosis, competition) or indirectly (induction of plant defense) on the plant pathogen, thus triggering biocontrol effects [4].

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The endophytes have the ability to produce novel secondary metabolites and a wide range of enzymes that act as effective biocontrol agents against pests and pathogens, thus having a potential application in agriculture [5]. The endophytic microbes protect their host plant from the attack of pests and pathogens, usually by the mechanism of antagonism [6-7]. They also have the ability to produce secondary metabolites which have antimicrobial activities [8]. Endophytes have the ability to act as biocontrol of phytopathogens and also produce enzymes that can help in the sustainability of the agro-industry. This ability of endophytes to produce a wide range of secondary metabolites, (majorly VOCs) characterized by remarkable pesticidal, bactericidal, fungicidal, antinematicidal, herbicidal, and algicidal properties can be exploited to use for the production of biocontrol agents to minimize the use of chemical pesticides and can act as an integral part of IPM [9]. The property of the endophytes to enhance growth in host plants and to increase resistance against pests and pathogens allows them to be termed biocontrol agents and can be used in agriculture in place of chemicals. In the present study, the endophytes isolated from the rhizomes and leaves of *Curcuma longa* were introduced into tomato (*Solanum lycopersicum*) seedlings, and their ability to improve plant growth and resistance against two selected plant fungal pathogens were tested. The presence of endophytes inside the plant tissue was identified using confocal microscopy.

## MATERIALS AND METHODS

*Biocontrol efficacy of endophytic bacterial isolates on the seedlings of S. lycopersicum*

*Seedling treatment under greenhouse conditions*

Healthy seedlings from the nursery were treated with the endophytic bacterial consortium (*Kocuria rocea*, *Brevibacterium casei*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus velezensis*, *Actinobacterium JS14 strain mixed in PBS*) by dipping the seedlings in 48 h cultures (approximately 3.5 9 $X10^{10}$ CFU/ mL) for 20 min. The treated seedlings were planted in a standard pot mixture and were again inoculated with 48-h culture (10 mL/plant) of respective bacterial endophytic isolates. A week after transplanting, the seedlings were challenge-inoculated with 5 ml of *Cercospora punicae* and *Ceratocystis fimbriata* (approximately 1.2 $9X10^{6}$ CFU /mL) around the root zone. Two sets (3 replicates with 5 plants each and 3 replicates with 10 plants each) were maintained for each fungal pathogen [10].

*Plant growth parameters*

The values for root length, shoot length, total height, fresh weight, the total number of leaves, and infected leaves were recorded for 45 days. The data were analyzed statistically using ANOVA.

The treated plants were monitored for disease development for 45 days and the disease index was rated using the following scale: DI 0: no wilting; DI 1: 1-25% wilt symptom; DI 2: 26-50% wilt symptom; DI 3: 51-75% wilt symptom; DI 4: 76-100% wilt symptom or dead [11]. The disease incidence and biocontrol efficacy were estimated accordingly [12].

$$\text{Disease incidence (\%)} = \frac{\text{Disease index} \times \text{Number of diseased plants in this index}}{\text{Total number of plants investigated} \times \text{The highest disease index}} \times 100$$

$$\text{Biocontrol efficacy (\%)} = \frac{\text{DI of control} - \text{DI of antagonist-treated group}}{\text{Disease incidence of control}} \times 100$$

*Confocal imaging of the roots of the crop plant*

The roots of tomato (*Solanum lycopersicum*) and seedlings were collected after the 30th day of observation, surface sterilised with 4% (w/v) sodium hypochlorite solution for 1 minute, and washed twice with distilled water. Thin sections were cut and were transferred to trichloroacetic acid (0.15% (wt/vol) trichloroacetic acid in 4:1 (vol/vol) ethanol/chloroform) fixation solution. The sections were stained using Ethidium bromide (5 µl, EtBr 1.25 mg/mL), incubated at room temperature for 10 minutes, and transferred to a glass slide to be examined using an Advanced Spectral Confocal Microscope [13].

## RESULTS AND DISCUSSION

*Plant growth parameters of the crop plants infected with the fungal pathogens and bacterial consortium*

The seedlings were grown in accordance with the procedure mentioned above, and the plant growth parameters indicating the growth of the plant were observed. The shoot length, root length, total height, and weight were calculated for day 0, day 5, day 10, day 15, day 20, day 25, day 30 and day 45. The infection with *Cercospora punicae* were seen mostly the leaves and *Ceratocystis fimbriata* infected the stems causing stem lesions and the infected leaves developed black spots. The leaves of the infected plants developed light brown spots, and the stems developed dark elliptic spots on them. The stems of *Ceratocystis fimbriata* and infected plants in control started to become flat, thin, and depressed after the 15th day. The plants in control wilted and died by the 25th day whereas, the seedlings in the test had the ability to survive and showed a lesser percentage of infection compared to control.

Table 1 Average shoot length of the tomato seedlings infected with *Cercospora punicae*

| Day | Average shoot height | |
|---|---|---|
| | Control ((mm) | Test (mm) |
| 0 | 1.73±0.77 | 1.88±0.05 |
| 5 | 1.67±0.76 | 2.06±0.55 |
| 10 | 1.72±0.69 | 2.05±0.52 |
| 15 | 1.72±0.60 | 2.10±0.86 |
| 20 | 1.72±0.72 | 2.20±0.48 |
| 25 | 1.77±0.62 | 2.35±0.50 |
| 30 | 1.77±0.45 | 2.40±0.96 |
| 45 | 1.77±0.50 | 3.37±1.12 |

Fig 1 Infected leaf and stem of the tomato seedlings

Table 2 Average shoot length of tomato seedling infected with *Ceratocystis fimbriata*

| Day | Average shoot height | |
|---|---|---|
| | Control ((mm) | Test (mm) |
| 0 | 1.80±0.50 | 1.65±0.61 |
| 5 | 1.80±0.50 | 1.75±0.61 |
| 10 | 1.80±0.50 | 1.85±0.69 |
| 15 | 1.75±0.60 | 1.90±0.70 |
| 20 | 1.60±0.45 | 2.00±0.75 |
| 25 | 1.60±0.45 | 2.05±0.78 |
| 30 | 1.60±0.40 | 2.20±0.78 |
| 45 | 1.60±0.40 | 2.8±0.87 |

The shoot length was recorded after every 5-day interval till the 45 days. It was observed that the shoot length of the plants treated with the bacterial endophytes were more compared to that of the control. There was a significant difference in the height of the plant. The plants in control started to wilt, reducing the height of the plant. The average shoot length of seedlings infected with *Cercospora punicae* was 1.73±0.77 mm in control and 1.88±0.05mm in Test on the 0th day and 1.77±0.50mm in control and 3.37±1.12mm in Test on the 45th day. In seedlings infected with *Ceratocystis fimbriata*, the mean shoot height was 1.80±0.50mm in control and 1.65±0.61mm in Test in tomato, and 1.60±0.40mm in control, and 2.8±0.87mm in Test in tomato; on the 45th day. The root length was recorded by uprooting the plants carefully after every 5 days. The root length of seedlings infected with C. *punicae was* 6.13±0.27mm in control 5.86±0.17mm in test

tomato seedlings and 5.68±0.27mm in control and 7.56±0.17 mm in test in tomato seedlings on the 45th day. The total height was 5.26±0.38mm control and 2.27±0.40mm Test in tomato seedlings on the 0th day. 2.18±0.39mm in control and 2.98±0.18mm test in tomato seedling on the 45th day. The fresh weight of the seedlings infected with *Cercospora punicae* were 0.17±0.40mg in control 0.22±0.12 mg in test in tomato seedlings on 0th day and 0.25±0.41mg in control 0.56±0.12mg test from tomato seedlings on 45th day. In seedlings infected with *Ceratocystis fimbriata*, the root length was 1.23±0.07 mm in control 0.80±0.07 mm in test in tomato seedling on the 0th day and 0.92±0.08 mm in control 0.87±0.17 mm in test in tomato seedling on the 45th day. The total height was 2.21±0.09 mm in control 2.27±0.05 mm in test in tomato seedlings on the 0th day;1.88±0.11 mm in control 2.95±0.07 mm in test in tomato seedlings on the 45th day. The fresh weight was 0.15±0.07mg in control 0.21±0.08mg test in tomato seedlings on the 0th day and 0.17±0.17 mg in control 0.52±0.17 mg in test in tomato seedlings on the 45th day. The height, dry weight, the number of leaves per plant, and levels of phytochrome increased in beets inoculated with endophytic bacteria when compared to the uninoculated control [14]. In an experiment conducted to study the effect of bacterial endophyte in enhancing the growth and nitrogen fixation in corn plants where the corn seedlings were inoculated with strains of endophytic bacteria *Paenibacillus polymyxa* strain P2b-2R and P2b-2Rgfp, it was recorded that the length and the biomass of the inoculated corn seedlings were enhanced by 68% and 67% respectively and were also able to fix more nitrogen. The results stated that the long-term association of the endophytes with the corn seedlings helps enhance the growth of the plant when compared to the uninoculated seedlings [15]. The application of live bacterial biofertilizers (endophytic *Pseudomonas fluorescens* and bacterial consortium) enhanced the drop yield and growth. An increase in the crop height, stem, leaf and pod biomass of *Brassica napus* was recorded both in greenhouse and field conditions [16]. Endospore forming endophytic bacteria isolated from *Amaranthus spp*. was assessed for their plant growth promoting activities, the inoculation of the plants with *Bacillus amyloliquefaciens* improved the fresh shoot weight of the plant to up t0 35% [17]. The endophytic bacteria isolated from the maize plants showed plant growth promoting activities and antagonistic bacterial activities against phytopathogen. The seedling inoculated with the endophytic bacteria showed increased growth, and increased shoot and root formation when compared to uninoculated seedlings [18].

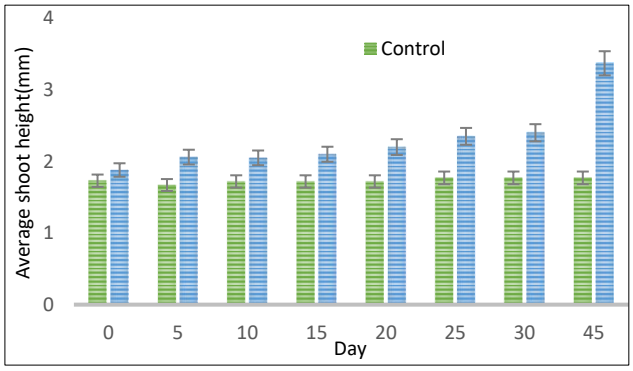



Fig 2 Average shoot length of the tomato seedlings infected with *Cercospora punicae*

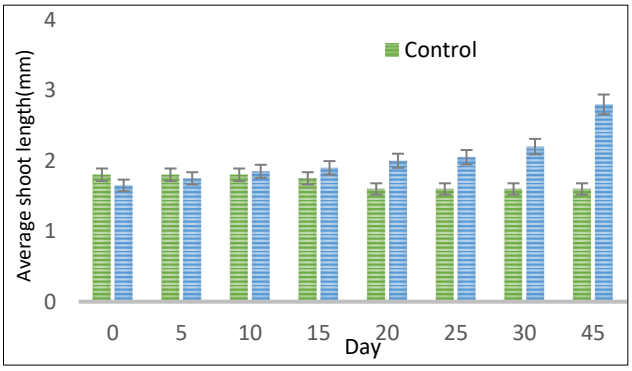



Fig 3 Average shoot length of tomato seedling infected with *Ceratocystis fimbriata*

Table 3 Average root length, total heigh and fresh weight of tomato seedlings infected with *Cercospora punicae*

| | Control | | Test | |
|---|---|---|---|---|
| | Day 0 | Day 45 | Day 0 | Day45 |
| Root length | 6.13±0.27 | 5.68±0.27 | 5.86±0.17 | 7.56±0.17 |
| Total height | 5.26±0.38 | 2.18±0.39 | 2.27±0.40 | 2.98±0.18 |
| Fresh weight | 0.17±0.40 | 0.25±0.41 | 0.22±0.12 | 0.56±0.12 |

Table 4 Average root length, total heigh and fresh weight of tomato seedlings infected with *Ceratocystis fimbriata*

| | Control | | Test | |
|---|---|---|---|---|
| | Day 0 | Day 45 | Day 0 | Day45 |
| Root length | 1.23±0.07 | 0.92±0.08 | 0.80±0.07 | 0.87±0.17 |
| Total height | 2.21±0.09 | 1.88±0.11 | 2.27±0.05 | 2.95±0.07 |
| Fresh weight | 0.15±0.07 | 0.17±0.17 | 0.21±0.08 | 0.52±0.17 |

The percentage of infection in seedlings infected with *Cercospora punicae* were 1.24±0.07% in control, 0.91±0.01% in Test on 0th day; 96.06±3.22% in control, 26.58±2.59% in Test on the 45th day in tomato seedlings, in seedlings infected with *Ceratocystis fimbriata* the percentage infection was 1.32±0.04% in control and 0.37±0.10% in test on 0th day 96.54±3.01% in control, 27.55±2.12% in Test on the 45th day in tomato seedlings. While assessing the ability of 11 endophytic bacteria isolated from the tissues of potatoes to act as a biological control against bacterial wilt disease, all 11 endophytic strains were able to reduce the percentage of infection from 73% to 45% and also helped in increasing the plant growth [19]. The ability of the endophytic bacterium *Bacillus amyloliquefaciens* to develop resistance against the pathogen *Streptomyces griseoplanus* (*Streptacidiphilus griseoplanus*) which causes potato scab was assessed. The inoculation of the plants with the bacterial strain was seen to reduce the percentage of infection for up to 61.1% when compared to chemical bactericides and uninoculated plants [20].

*ANOVA test*: was conducted to understand the relationship between the presence of the endophytic bacterial inside the plant and the difference in rate of infection in plants.

*Null hypothesis*: There is no effect of the endophytic bacterial isolates on the rate of infection in plants.

*Alternate hypothesis*: The rate of infection is less when endophytic bacterial isolates are inoculated in to the crop plant.

It was noted that the F value of the samples were higher than the F crit value, and the p value was lesser than the alpha.

The F value for tomato seedlings infected with *C. punicae* was 11.5 which was higher than the F crit value 2.17 and the P value (5.81E-09) was far lesser than the value of alpha (0.05).

11.52>2.17 (F>Fcrit)
0.0001<0.05(P<α)

For tomato infected with *C. fimbriata* the F value obtained was 21.7 which was higher than the F crit value 2.17 and the P value (7.55E-14) was far lesser than the value of alpha (0.05)
21.7>2.17 (F>Fcrit)
0.00001<0.05(P<α)

Table 5 Percentage infection in tomato seedlings

| Day | Percentage (%) | Percentage (%) | Percentage (%) | Percentage (%) |
|---|---|---|---|---|
| 0 | 1.24±0.07 | 0.91±0.01 | 1.32±0.04 | 0.24±0.02 |
| 5 | 25.46±1.42 | 7.70±0.04 | 23.55±0.13 | 6.53±0.26 |
| 10 | 40.27±1.86 | 14.23±0.09 | 40.90±0.95 | 15.12±0.95 |
| 15 | 50.60±2.75 | 16.17±0.09 | 51.58±1.56 | 17.25±0.95 |
| 20 | 62.31±3.03 | 18.65±1.62 | 66.18±1.86 | 19.42±0.95 |
| 25 | 69.83±3.51 | 20.51±1.83 | 72.97±2.11 | 20.03±1.58 |
| 30 | 79.04±3.83 | 21.34±2.09 | 80.64±2.96 | 24.44±2.12 |
| 45 | 96.06±3.22 | 26.58±2.59 | 96.54±3.01 | 27.55±2.12 |

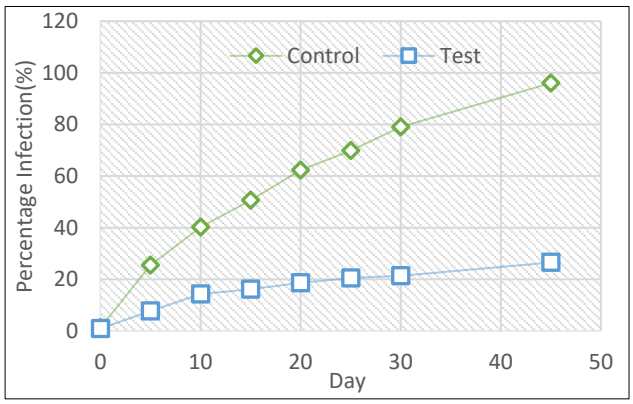



Fig 4 Percentage infection in tomato seedlings infected with *Cercospora punicae*

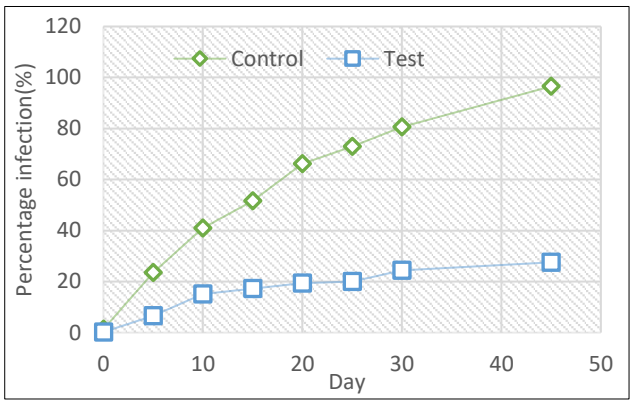



Fig 5 Percentage infection in tomato seedlings infected with *Ceratocystis fimbriata*

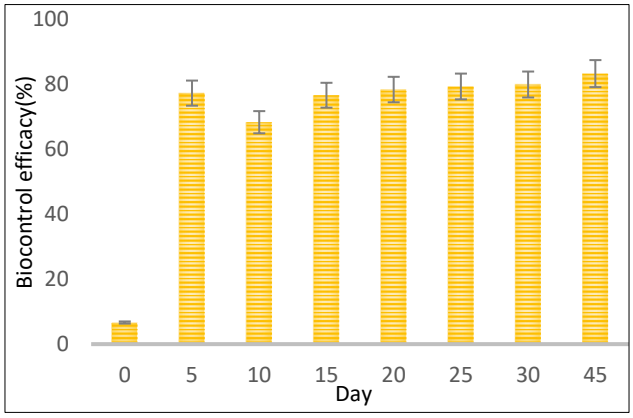



Fig 6 Biocontrol efficacy of endophytic bacteria in tomato seedlings infected with *Cercospora punicae*

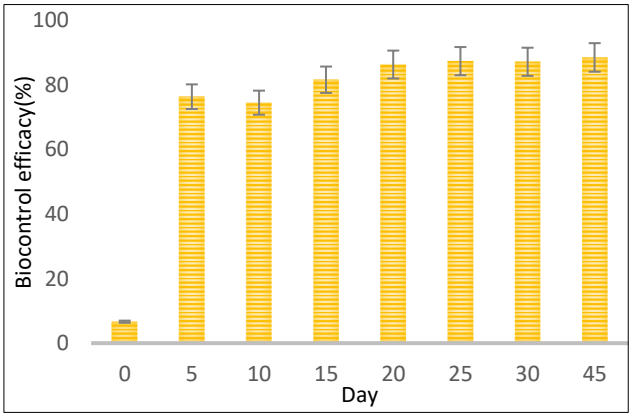



Fig 7 Biocontrol efficacy of endophytic bacteria in tomato seedlings infected with *Ceratocystis fimbriata*

Table 6 Percentage disease incidence and biocontrol efficacy in tomato seedling infected with *Cercospora punicae*

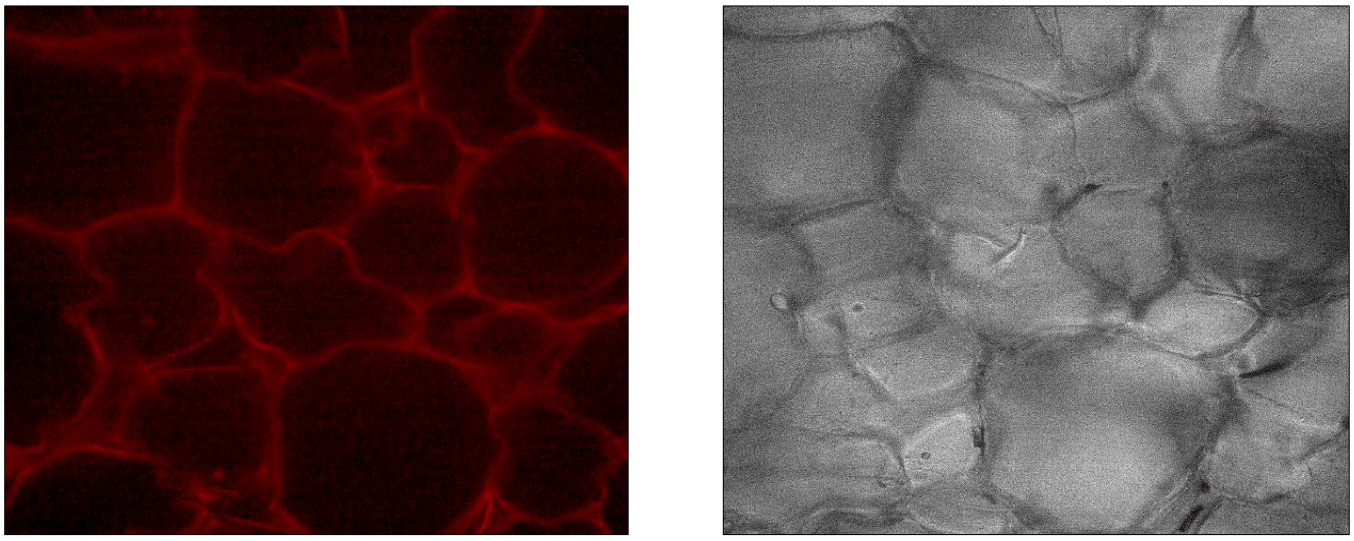
| Day | Disease incidence (%) | | Biocontrol efficacy (%) |
|---|---|---|---|
| | Control | Test | |
| 0 | 0.31±0.05 | 0.22±0.03 | 6.65±1.21 |
| 5 | 10.45±1.55 | 2.13±0.31 | 77.25±1.26 |
| 10 | 22.79±3.38 | 4.05±0.59 | 68.31±1.35 |
| 15 | 31.21±4.65 | 4.61±0.69 | 76.63±1.43 |
| 20 | 46.34±6.90 | 2.12±0.31 | 78.35±1.56 |
| 25 | 56.73±8.45 | 6.15±0.91 | 79.32±1.76 |
| 30 | 71.04±10.59 | 8.48±1.26 | 79.94±1.82 |
| 45 | 95.72±14.27 | 10.71±1.59 | 83.25±2.01 |

The disease incidence helps in understanding the percentage of diseased plants or the percentage of diseased parts (leaves, stems, leaves) in a population under consideration. The disease incidence and the biocontrol efficacy of control and test varied from day 0 to day 45. There was an increase in the disease incidence in both sets, the seedlings in the test showed little disease incidence when compared to control and they wilted and died by the 45$^{th}$ day. The incidence of disease in seedlings infected with *C. punicae* was 0.3±0.05% in control and 0.2±0.03% in test in on the 0$^{th}$ day and 95.7±14.27% in control and 10.7±1.59% in test in on the 45$^{th}$ day. In seedlings infected with *C. fimbriata*, the incidence of disease was 5.35±0.87% in control and 0.00±0.0% in test on the 0$^{th}$ day and 97.75±2.30% in control and 13.45±0.99% in test in on the 45$^{th}$ day. Endophytic bacteria isolated from wild pistachio trees were able to inhibit and reduce the incidence of diseases of the plant pathogens *Pseudomonas syringae pv. syringae* Pss20 and *Pseudomonas tolaasii* under *in vitro* conditions. An endophytic bacterium showed maximum inhibition against both the pathogens [21]. While assessing the antagonistic activity of

the endophytic bacteria isolated from peanut seeds, in pot studies, the inoculation of two different strains of the same endophytic bacteria was able to reduce the disease incidence and severity of stem rot ($P < 0.05$) in the inoculated plants when compared to uninoculated plants. The filtrate was also able to inhibit the formation of the sclerotia and reduced its germination [22]. The biocontrol efficacy of the endophytic bacterial consortium against *Cercospora punicae* was 6.65±1.21% on the 0$^{th}$ day and 83.25±2.01% on the 45$^{th}$ day and against *Ceratocystis fimbriata* the biocontrol efficacy was 6.65±0.99 on the 0$^{th}$ day and 88.45±2.61% on the 45$^{th}$ day. The plants in test were able to survive the attack of the fungal plant pathogen and grow when compared to that of the plants in control. While understanding the ability of endophytic bacteria isolated from Amaranthus sp. to reduce the incidence of blight disease, the *Bacillus* sp. isolated from the leaf blight resistant wild species *Amaranthus viridis*, were able to supress the leaf blight disease to 41% in susceptible red variety Arun when compared to uninoculated plants. The inoculation of endophytes was more effective to the leaf blight disease when compared to fungicide [23]. *Bacillus velezensis* isolated from *Fraxinus hupehensis* were assessed for its ability to supress the growth of rice sheath blight pathogen, *Rhizoctonia solani*. The inoculation of the plants with the endophytic bacterial strain showed a biocontrol efficacy of about 61.5% and 74.6% in green house and field studies. The length of the lesion was reduced up to 71% detached inoculated leaf assay [24].

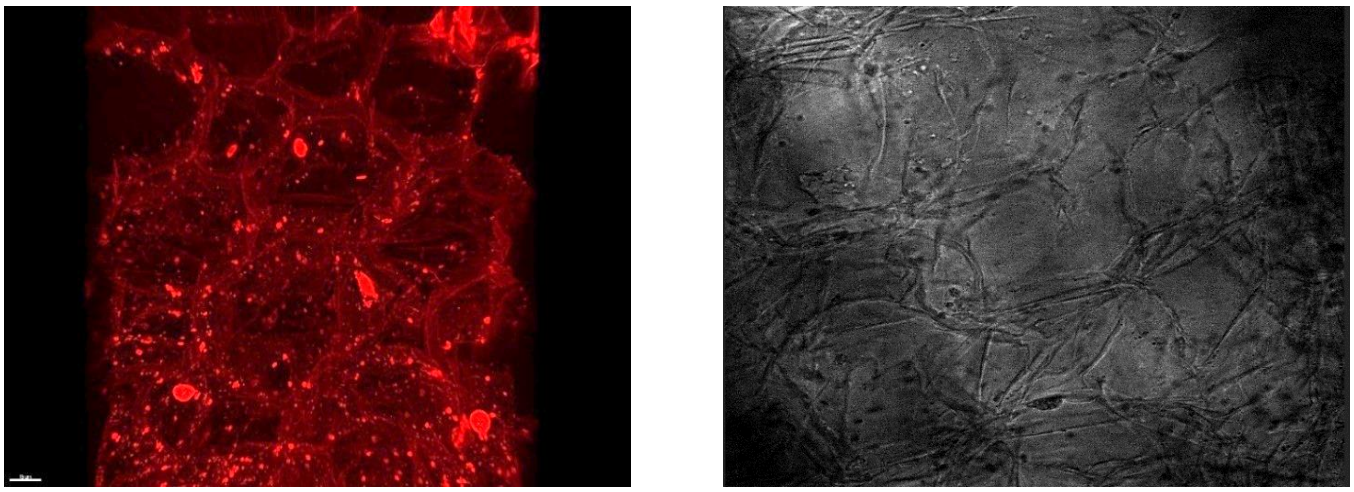
Table 7 Percentage disease incidence and biocontrol efficacy in tomato seedling infected with *Ceratocystis fimbriata*

| Day | Disease incidence (%) | | Biocontrol efficacy (%) |
|---|---|---|---|
| | Control | Test | |
| 0 | 5.35±0.87 | 0.00±0.00 | 6.65±0.99 |
| 5 | 14.72±0.99 | 2.55±0.06 | 76.34±0.99 |
| 10 | 38.33±0.98 | 6.55±0.09 | 74.45±1.02 |
| 15 | 50.15±1.15 | 8.56±0.14 | 81.55±1.02 |
| 20 | 82.85±1.36 | 10.35±0.16 | 86.25±1.23 |
| 25 | 88.95±1.74 | 11.02±0.27 | 87.32±1.52 |
| 30 | 91.73±1.95 | 12.03±0.56 | 87.13±1.86 |
| 45 | 97.75±2.30 | 13.45±0.99 | 88.45±2.61 |



Control

Fig 7 Biocontrol efficacy of endophytic bacteria in tomato seedlings infected with *Ceratocystis fimbriata*



Test

Fig 8 Confocal images of the roots of tomato seedlings

*Confocal imaging of the leaves of the crop plant*

The leaves of the tomato and finger millet were analyzed by confocal microscopy. The plant samples collected from test which were inoculated with the bacterial endophytes showed internal colonization of the endophytic bacteria in between the plant cells. The dye used; EtBr coloured the cells red. The plant samples from control did not show any internal colonization of the endophytic bacteria. While analyzing the ability to use the endophytic bacteria isolated from *Curcuma longa* as a biocontrol agent against rhizome rot and leaf blight diseases,

two bacterial isolates *Bacillus cereus* RBacDOB-S24 and *Pseudomonas aeruginosa* Bac-DOB-E19 showed biocontrol activities against the pathogens and were imaged using confocal microscope. They were seen to colonize the internal tissue of the plant [25]. Two endophytic bacteria, *Pseudomonas protegens* and *Serratia plymuthica* isolated from the root nodules were analyzed for their ability to inhibit the growth of *Meloidogyne* spp., causing root knot disease. The endophytes inoculated in to the tomato plants colonized the internal tissues of the plants and were imaged using a confocal microscope. Both the endophytes were seen to colonise the roots and stem of tomato plants for a long period of time [26]. The inoculation of two bacterial endophytes *Azospirillum baldaniorum* (Sp245) and *Gluconacetobacter diazotrophicus* (LP343) inside two elephant grass genotypes were analyzed. Confocal microscopy showed the colonization of the two bacteria inside the plant tissue [27]. While examining the interaction of the green fluorescent protein-labelled bacterium *Herbaspirillum lusitanum*, strain P6-12, with *Triticum aestivum* L. and *Phaseolus vulgaris* L., the presence of the endophytes colonizing the peripheral cells of the root cap and internal plant tissue was imaged using a confocal microscope [28].

## CONCLUSION

The use of endophytes as biocontrol can act as a successful alternative in replacing the use of chemicals to prevent the attack of pests and pathogens. The seedlings treated with the endophytic consortium had an ability to withstand the disease-causing fungal pathogen and survive whereas the untreated seedlings showed all the symptoms of the disease and they wilted and died. In the confocal imaging, the treated seedlings were seen to have the endophytes inside their cells whereas the control did not have any endophytes in them, thus the presence of endophytes inside the internal tissues of the tomato seedlings helped the plant to survive the pathogen attack.

## LITERATURE CITED

1. Heydari A, Pessarakli M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Sciences* 10: 272-290.
2. Griffin MR. 2014. Biocontrol and bioremediation: Two areas of endophytic research which hold great promise. *In*: (Eds) Verma V, Gange A. Advances in Endophytic Research. Springer, New Delhi.
3. Xue QY, Chen Y, Li SM, Chen LF, Ding GC, Guo DW, JH Guo. 2009. Evaluation of the strains of acinetobacter and enterobacter as potential biocontrol agents against Ralstonia wilt of tomato. *Biol. Control* 48(3): 252-258.
4. Liarzi O, Ezra D. 2014. Endophyte-mediated biocontrol of herbaceous and non-herbaceous plants. *In*: (Eds) Verma V, Gange. Advances in Endophytic Research. Springer, New Delhi.
5. Falade AO, Adewole KE, Ekundayo TC. 2021. Aptitude of endophytic microbes for the production of novel biocontrol agents and industrial enzymes towards agro-industrial sustainability. *Beni-Suef Univ. Jr. Basic Appl. Science* 10: 61.
6. Xiang L, Gong S, Yang L, Hao J, Xue MF, Zeng FS, Zhang XJ, Shi WQ, Wang H, Yu D. 2015. Biocontrol potential of endophytic fungi in medicinal plants from Wuhari botanical garden in China. *Biological Control* 94: 47-55.
7. Liu X, Dru D, Ma Y. 2016. Potential of endophytes from medicinal plants for biocontrol and plant growth promotion. *Jr. Gen. Plant Pathology* 82: 165-173.
8. Chen M, Lin L, Zhang Y, Sun L, An Q. 2013. Genome sequence of Klebsiella oxytoca SA2, an endophytic nitrogen-fixing bacterium isolated from the pioneer grass *Psammochloa villosa*. *Genome Announc*. 1: e00601-13.
9. Alam MZ, Crump AR, Haque MM, Islam MS, Hossain E, Hasan SB, Hasan SB, Hossain MS. 2016. Effects of integrated pest management on pest damage and yield components in a rice agro-ecosystem in the Barisal region of Bangladesh. *Front Environ Science* 4: 22. https://doi.org/10.3389/fenvs.2016.00022
10. Rado R, Andrianarisoa B, Ravelomanantsoa S, Rakotoarimanga N, Rahetlah V, Fienena FR, Andriambeloson O. 2015. Biocontrol of potato wilt by selective rhizospheric and endophytic bacteria associated with potato plant. *African Journal of Food, Agriculture, Nutrition and Development* 15(1): 9762-9776.
11. Almoneafy AA, Xie GL, Tian WX, Xu LH, Zhang GQ, Ibrahim M. 2012. Characterization and evaluation of Bacillus isolates for their potential plant growth and biocontrol activities against tomato bacterial wilt. *African Journal of Biotechnology* 11(28): 7193-7201.
12. Ziccaa S, Palmira De-Bellisb, Mario M, Maria S, Pasquale S, Donato B, Angelo S. 2020. Antagonistic activity of olive endophytic bacteria and of *Bacillus spp*. strains against *Xylella fastidiosa*, *Microbiological Research* 236: 126467. doi: 10.1016/j.micres.2020.126467.
13. Someya T. 1995. Three-dimensional observation of soil bacteria in organic debris with a confocal laser scanning microscope. *Soil Microorganisms* 46: 61-69.
14. Shi Y, Lou K, Li C. 2009. Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biol. Fertil. Soils* 45: 645-653.
15. Puri A, Padda KP, Chanway CP. 2016. Evidence of nitrogen fixation and growth promotion in canola (*Brassica napus* L.) by an endophytic diazotroph *Paenibacillus polymyxa* P2b-2R. *Biology and Fertility of Soils* 52(1): 119-125.
16. Lally RD, Galbally P, Moreira AS, Spink J, Ryan D, Germaine KJ, Dowling DN. 2017. Application of endophytic *Pseudomonas fluorescens* and a bacterial consortium to Brassica napus can increase plant height and biomass under greenhouse and field conditions. *Frontiers in Plant Science* 8: 2193.
17. Yashaswini MS, Nysanth NS, Anith KN. 2021. Endospore-forming bacterial endophytes from *Amaranthus spp*. improve plant growth and suppress leaf blight (*Rhizoctonia solani* Kühn) disease of *Amaranthus tricolor* L. *Rhizosphere* 19: 100387. https://doi.org/10.1016/j.rhisph.2021.100387.
18. Pal G, Kumar P, Verma A, Verma SK. 2022. Seed inhabiting bacterial endophytes of maize promote the seedling establishment and provide protection against fungal disease. *Microbiological Research* 255: 126926.
19. 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: 101692. https://doi.org/10.1016/j.pmpp.2021.101692.

20. Cui L, Yang C, Wei L, Li T, Chen X. 2020. Isolation and identification of an endophytic bacteria *Bacillus velezensis* 8-4 exhibiting biocontrol activity against potato scab. *Biological Control* 141: 104156. https://doi.org/10.1016/j.biocontrol.2019.104156.
21. Etminani F, Harighi B. 2018. Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. *Plant Pathology Journal* 34(3): 208-217. doi: 10.5423/PPJ.OA.07.2017.0158.
22. Nagpal S, Sharma P, Sirari A, Gupta RK. 2020. Coordination of *Mesorhizobium sp*. and endophytic bacteria as elicitor of biocontrol against Fusarium wilt in chickpea. *European Journal of Plant Pathology* 158(1): 143-161.
23. Guo JH, Guo YH, Zhang LX, Qi HY, Fang ZD. 2001. Screening for biocontrol agents against cayenne pepper bacterial wilt. *Chi. Jr. Biol. Control* 17: 101-106.
24. Tong-wen Zheng, Lu Liu, Qian-wen Nie, Tom Hsiang, Zheng-xiang Sun, Yi Z. 2021. Isolation, identification and biocontrol mechanisms of endophytic bacterium D61-A from *Fraxinus hupehensis* against *Rhizoctonia solani*. *Biological Control* 158: 104621. https://doi.org/10.1016/j.biocontrol.2021.104621.
25. Vinayarani G, Prakash HS. 2018. Growth promoting rhizospheric and endophytic bacteria from *Curcuma longa* L. as biocontrol agents against rhizome rot and leaf blight diseases. *Plant Pathology Journal* 34(3): 218-235.
26. Zhao J, Wang S, Zhu X, Wang Y, Liu X, Duan Y, Chen L. 2021. Isolation and characterization of nodules endophytic bacteria *Pseudomonas protegens* Sneb1997 and Serratia plymuthica Sneb2001 for the biological control of root-knot nematode. *Applied Soil Ecology* 164: 103924.
27. Camelo A, Barreto CP, Vidal MS. 2021. Field response of two seed propagated elephant grass genotypes to diazotrophic bacterial inoculation and in situ confocal microscopy colonization analyses. *Symbiosis* 83: 41-53.
28. Velichko NS, Bagavova AR, Burygin GL, Baymiev AK, Pylaev TE, Fedonenko YP. 2022. In situ localization and penetration route of an endophytic bacteria into roots of wheat and the common bean. *Rhizosphere* 23: 100567.