Plant Archives Vol. 24, No. 2, 2024 pp. 382-386 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.053

ANTAGONISTIC ACTIVITY OF SELECTED ENDOPHYTES AGAINST DRY-ROOT ROT PATHOGEN (*FUSARIUM SOLANI*) IN ACID LIME

G. Razia Sulthana Begum[1]*, B.G. Rajulu[2], T. Rajasekharam[3], Ch. Ruth[1] and B. Tanuja Priya[4]

[1]Department of Plant Pathology, College of Horticulture, Anantharajupeta, Y.S.R. Kadapa Dist., Andhra Pradesh, India.

[2]Department of Plant Pathology, K.V.K., Periyavaram, Nellore Dist., Andhra Pradesh, India.

[3]Department of Plant Pathology, Citrus Research Station, Tirupathi, Chittoor Dist., Andhra Pradesh, India.

[4]Department of Horticulture, Horticultural Research Station, Lam, Guntur, Andhra Pradesh, India.

*Corresponding author E-mail : rsb37725@gmail.com

(Date of Receiving-12-04-2024; Date of Acceptance-01-07-2024)

ABSTRACT

Endophytes are the microorganisms found in the living tissues of various plant parts (roots, fruits, stem, seed, leaf *etc*). Endophytes as bio-agents are well acknowledged as a step towards plant disease management. In the current investigation, a total of six fungal (EFA 1-6) and eight bacterial (EBA 1-8) endophytes were isolated from the roots of healthy acid lime plants. All the selected endophytes were assessed for their antagonistic effect against dry-root rot causing pathogen *Fusarium solani* in acid lime. The results revealed that EFA 4 *Aspergillus fumigatus* (66.92%) and EBA 7 *Pseudomonas aeruginosa* (63.42%) exhibited antagonistic activity on *Fusarium solani* under *in vitro* conditions. The acid lime seedlings were treated with the effective endophytic isolates and the existing bioagents under greenhouse conditions and transplanted in pathogen-infested sick soil. The seedlings treated with the bioagent *Trichoderma reesei* and the isolate EBA 7 exhibited a disease incidence of 13.33 and 16.67 per cent, respectively, followed by *Pseudomonas fluorescens* (20.00%) and *Trichoderma viride* (23.33 %). The findings imply that the endophyte EBA 7 *Pseudomonas aeruginosa* might be used as a bioagent in the management of plant diseases.

Key words : Antagonistic activity, Endophytes, Bio-agents, Dry root rot, Acid lime.

Introduction

Nowadays control of plant pathogens has been accomplished largely with the use of chemicals. Chemicals have provided a means of reducing plant disease. Over time this has proved to have negative side effects such as the development of resistance by pathogens, high cost, and negative effects on beneficial microorganisms as well as environmental, soil and water pollution. So, the focus has been shifted towards safer non-chemical methods for controlling diseases. Adopting endophytic bio-agents as an alternative to chemicals helps in promoting safer food and environment which will be beneficial for farmers. Many endophytes were isolated from the roots of healthy acid lime plants and were tested against the dry-root rot pathogen *Fusarium solani*.

Materials and Methods

Isolation of endophytes from the roots of healthy acid lime plants

Endophytic antagonistic microbes were isolated from roots of healthy plants within 48 hrs. of collection from the surveyed areas. For the isolating endophytes, 2 g of healthy root samples were weighed and exposed to two per cent sodium hypochlorite for 5 min followed by washing in three changes of sterile water and blot dried. The surface sterilized healthy roots of acid lime were triturated with 8 ml of sterile Potassium phosphate buffer (PB 0.1M, PH -7.0) using a sterile mortar and pestle. The triturate was serially diluted in sterile water blanks up to 10^{-7}. From the final buffer wash, one ml was pipetted out into a sterile Petri plate containing particular growth

media. A Petri plate inoculated with the sterile phosphate buffer alone serves as a sterility check. Tenfold serial dilution of obtained triturate was prepared. The serial dilution was prepared up to 10^{-4} for fungal isolation and up to 10^{-6} for the isolation of bacteria. The inoculated petri plates were incubated at room temperatures 25±2°C for at least 72 h. Among all the colonies obtained on dilution plates, a total of six fungal endophytic microbes and eight bacterial endophytic microbes were selected.

Isolation and identification of dry-root rot pathogen

The root samples from infected plants were collected for the isolation of the pathogen. A small portion of diseased tissue along with a portion of adjacent healthy tissue was cut into small bits (3 to 5 mm in length) and then surface sterilized with 2% sodium hypochlorite solution for three minutes followed by three rinses in distilled water. The sterilized root bits were transferred onto Petri plates containing potato dextrose agar (PDA). The inoculated Petri plates were incubated at 25 ± 2°C for five to six days. The pathogen pure culture is maintained by single hyphal tip culture.

Based on the morphological characters, *viz*., colony growth, colour, size and shape of the conidia, the pathogen was identified as *Fusarium solani* and the further identification of the pathogen up to species level was confirmed at National Centre for Fungal Taxonomy (NCFT), New Delhi as *Fusarium solani* (NCFT, 9624.19).

***In vitro* evaluation of antagonistic endophytic fungi and bacteria against dry-root rot pathogen**

The endophytic fungal isolates were assessed for their antagonistic effect by the dual culture method (Skidmore and Dickson, 1976). Fungal endophytes were evaluated for their efficacy through the dual culture technique. The bacterial endophytic isolates were assessed for their antagonistic effect by the dual culture method (Utkhede and Rahe, 1983). The per cent inhibition of the pathogen was tabulated using the formula given below by Vincent (1947).

$$PI = \frac{C - T}{C} \times 100$$

PI = Percent inhibition, C = Growth of the pathogen in control (mm), T = Growth of the pathogen in dual culture (mm). Based on the percent inhibition of the mycelial growth of the pathogen, the efficient antagonists were selected for further studies.

In planta* assay of antagonists against *Fusarium solani

Based on the *in vitro* studies, the efficient ones were selected. The *in planta* screening tests of the effective endophytes and existing bioagents (*Trichoderma viride*, *Trichoderma reesei* and *Pseudomonas fluorescens*) as antagonists against *Fusarium solani* were conducted under greenhouse conditions.

Preparation of pathogen inoculum

For preparing the pathogen inoculum, *Fusarium solani* was mass multiplied in polypropylene packets containing sorghum grains, which were formerly autoclaved at 121°C for an hour for 3 consecutive days. After cooling, the packets were inoculated with 7-10mycelial disks of a week-old culture of *Fusarium solani* under aseptic conditions. The inoculated packets were incubated at 25°C for 3 weeks. The pathogen colonized sorghum grains were transferred to paper bags for drying and were ground. Then the ground pathogen inoculum was used to infest potting soil @ 15 g/kg and the infested soil was transferred into polybags, watered properly and kept under a greenhouse for 3 weeks to allow the pathogen to proliferate and make the soil sick.

Preparation of spore/cell suspensions of considered antagonists

For preparing the spore/cell suspensions of the considered antagonists, the pure cultures of fungal and bacterial antagonist isolates were maintained at 25°C on sterilized potato dextrose and nutrient broths, in a shaking incubator at 150 rev/min for 5 days and 2 days, respectively. This allows the spores of fungi and bacterial isolates to disperse evenly in the broth.

For preparing fungal spore suspensions, the broths of individual fungal isolates were mixed thoroughly for a few minutes. The spore concentration was adjusted to 5×10^{-5} cfu/ml. The broths of individual bacterial isolates were mixed thoroughly for preparing bacterial suspensions. The cell concentration was adjusted to 5×10^{-7} cfu/ml.

The healthy acid lime seedlings of six months old were surface sterilized and washed thoroughly with distilled water. The seedlings were root dipped in the antagonist's spore/cell suspensions for 30 minutes and were planted in the pathogen-infected sick soil in polybags @ 5 plants per bag. The treatment polybags were maintained under greenhouse conditions for symptom development. The treatments included were as below (Table 1). The percent disease incidence was recorded at monthly intervals up to 3 months of planting.

$$\text{Percent disease incidence} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Table 1 : *In planta* assay treatment details.

Treatments
T_1: *Aspergillus fumigatus* (EFA4)
T_2: *Pseudomonas aeruginosa* (EBA7)
T_3: *Trichoderma viride*
T_4: *Trichoderma reesei* (TCT 10)
T_5: *Pseudomonas fluorescens*
T_6: Pathogen-infested control (only pathogen)
T_7: Healthy control (no pathogen and no antagonist)

Results and Discussion

A total of six fungal and eight bacterial endophytic microbes were chosen from all the colonies formed on dilution plates.

In vitro evaluation of antagonistic endophytic fungi and bacteria against dry-root rot pathogen

All the six endophytic fungal isolates were assessed for their antagonistic activity over the *Fusarium solani* causing dry-root rot pathogen by dual culture method (Skidmore and Dickson, 1976) in terms of per cent inhibition over the control. The experimental findings demonstrated that all the six endophytic antagonistic fungi inhibited the growth of the pathogen at varying levels in a range of 45.68 to 66.92 per cent (Fig. 1). Among them, EFA 4 had shown the highest mycelial (66.92%) inhibition of pathogen followed by EFA 1 (54.49%) and EFA 2 (52.26%), whereas the isolate EFA 5 had shown the lowest inhibition of 45.68% against *Fusarium solani*. Among all the treatments EFA 4 had shown the fastest mycelial growing capacity compared to the pathogen, they had overgrown and locked the growth of the pathogen. Hence EFA 4 had shown strong inhibitory effect on pathogen (Table 2).

Similarly, Al-badi *et al*. (2020) carried out *in vitro* dual culture assay of five endophytic fungi isolated from Shirazi Thyme against the mycelial growth of *Monosporascus cannonballus*. The endophytes *Nigrospora sphaerica* E1, *Polycephalomyces sinensis* E8, *Polycephalomyces sinensis* E10, *Nigrospora sphaerica* E6 and *Subramaniula cristata* E7 have shown 81.7%, 80.6%, 75.8%, 66.1% and 38.7%, respectively. *Aspergillus terreus* has shown antagonistic activity by shrinking the hyphae of *Pythium aphanidermatum* (Halo *et al*., 2018). Several different *Aspergillus* species like *Aspergillus niger*, *A. nidulans*, *A. flavus*, *A. fusispora*, *A. fumigatus* and *A. terreus* var. *flococcus* were proved to be antagonistic against soil-borne fungi *Pythium* spp. (Al-Shibli *et al*., 2019 and Halo *et al*., 2018).

All the eight endophytic bacterial isolates were assessed for their antagonistic activity over the *Fusarium*

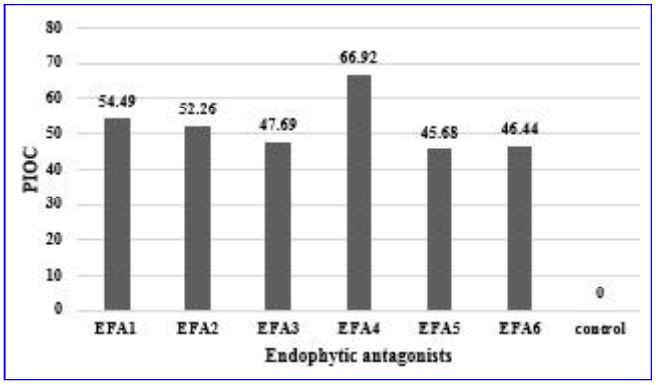

Fig. 1 : Graphical representation of the inhibition percentage of *Fusarium solani* by the fungal endophytes.

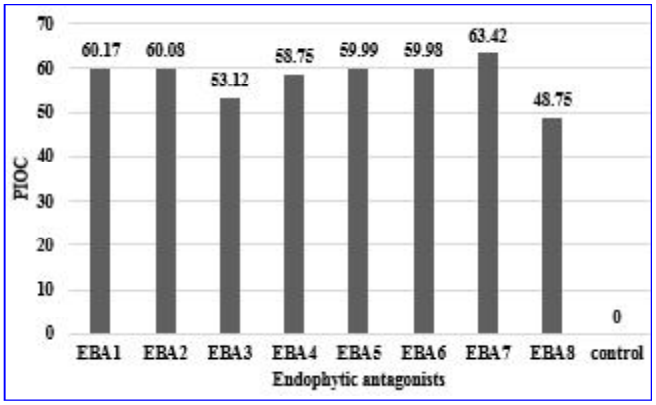

Fig. 2 : Graphical representation of the inhibition percentage of *Fusarium solani* by the bacterial endophytes.

Table 2 : *In vitro* antagonistic activity of Endophytic Fungal Antagonists (EFA 1-6) against *Fusarium solani*.

S. no.	Endophytic antagonists	Radial growth of pathogen (mm)*	Per-cent inhibition over control (PIOC%)
1.	EFA 1	40.56	54.49 (47.58)
2.	EFA 2	42.55	52.26 (46.30)
3.	EFA 3	46.62	47.69 (43.68)
4.	EFA 4	29.48	66.92 (54.89)
5.	EFA 5	48.41	45.68 (42.52)
6.	EFA 6	47.73	46.44 (42.96)
7.	Control	89.13	0 (0.00)
	SE(m) ±	0.17	
	C.D. at 5%	0.53	

*Mean of three replications
Figures in parenthesis are arc sin transformed values.

solani causing dry-root rot by dual culture method (Utkhede and Rahe, 1983) in terms of per cent inhibition over the control. The experimental findings demonstrated that the per cent inhibition of the growth of the pathogen by the eight endophytic bacteria was in the range of 48.75 to 63.42 per cent (Fig. 2). Among them, EBA 7 (63.42%)

Table 3 : *In vitro* antagonistic activity of Endophytic Bacterial Antagonists (EBA 1-8) against *Fusarium solani*.

S. no.	Endophytic antagonists	Radial growth of pathogen (mm)*	Per-cent inhibition over control (PIOC%)
1.	EBA 1	35.29	60.17 (50.87)
2.	EBA 2	35.37	60.08 (50.82)
3.	EBA 3	41.54	53.12 (46.79)
4.	EBA 4	36.55	58.75 (50.04)
5.	EBA 5	35.45	59.99 (50.76)
6.	EBA 6	35.46	59.98 (50.76)
7.	EBA 7	32.41	63.42 (52.78)
8.	EBA 8	45.41	48.75 (44.28)
9.	Control	88.61	0 (0.00)
	SE(m)±	0.07	
	C.D. at 5%	0.21	

*Mean of three replications
Figures in parenthesis are arc sin transformed values.

Table 4 : *In planta* assay of effective endophytes and existing bio-agents against *Fusarium solani*.

Treatments	Average seedling height (cm)*	Percent disease incidence (%)* in 3rd month
T_1: *Aspergillus fumigatus* (EFA4)	28.13	36.67 (37.22)
T_2: *Pseudomonas aeruginosa* (EBA7)	40.30	16.67 (23.86)
T_3: *Trichoderma viride*	37.23	23.33 (28.78)
T_4: *Trichoderma reesei* (TCT 10)	42.50	13.33 (21.14)
T_5: *Pseudomonas fluorescens*	38.73	20.00 (26.57)
T_6: Pathogen-infested control (only pathogen)	20.33	66.67 (54.78)
T_7: Healthy control (no pathogen and no antagonist)	45.43	0.00 (0.00)
SE(m)±	0.24	1.29
C.D. at 5%	0.76	3.95

*Mean of three replications
Figures in parenthesis are arc sin transformed values.

has shown the highest antagonism capacity over the pathogen, followed by EBA 1 (60.17%) and EBA 2 (60.08%), whereas, the lowest inhibition of per cent was recorded by EBA 8 (48.75%) against *Fusarium solani* with a defined inhibition zone (Table 3).

Likewise, Uzair *et al*. (2018) isolated four *Pseudomonas* strains (SP19, SP22, PS24 and SP25), the strain PS24 strain exhibited biocontrol activity against plant pathogenic fungi *Fusarium oxysporum* and many others. Luu *et al*. (2021) isolated nine bacterial endophytes from the weed *Echinoclova colonum*. Five of them inhibited the growth of *Alternaria alternata* isolated from Pitaya. The strain EC80 has shown strong inhibition and the other strains EC79, EC83, EC90 and EC97 recorded weak inhibition against the pathogen. The combination of EC79 and EC80 highly reduced the mycelial growth of the pathogen.

The effective endophytic antagonists were further characterized at the molecular level. The fungal and bacterial antagonists, EFA 4 and EBA 7 were identified as *Aspergillus fumigatus* and *Pseudomonas aeruginosa*, respectively.

In planta assay of antagonists against *Fusarium solani*

The effect of potential antagonists on root rot incidence caused by *Fusarium solani*on acid lime seedlings varied greatly. The disease incidence of antagonist-treated plants was considerably lower than that of plants treated with pathogen alone. The Survival rates were greater in antagonist-treated plants than in pathogen-treated plants. The treatments T_2 and T_4 comprising *Pseudomonas aeruginosa* (EBA 7) and *Trichoderma reesei* (TCT 10) exhibited a disease incidence of 13.33 and 16.67 per cent with an average plant height of 42.50 cm and 40.30 cm, respectively and were found most effective. Both the antagonists were on par with each other. The treatments T_3 and T_5 with the existing bio-agents had recorded a disease incidence of 23.33% and 20.00% with a plant height of 37.23 cm and 38.73 cm, respectively. A percent disease incidence of 36.67% was recorded by *Aspergillus fumigatus* (EFA4). The pathogen-infested control plants recorded a per cent inhibition of 66.67% with a least average plant height of 20.33 cm (Table 4).

Our results indicated that the antagonist EBA 7 was as effective as the current bioagent *Trichoderma reesei* in suppressing *Fusarium solani* in both *in vitro* and *in planta* experiments. In contrast, the antagonist EFA 4 was promising under *in vitro* conditions but was less effective under *in planta* experiments when compared to the other antagonists.

Srivastava *et al*. (2010) studied the efficacy of chemicals, bioagents and plant extracts against guava wilt pathogen *Fusarium oxysporum* f. sp. *psidii*. In their studies, they reported that *Trichoderma* species was effective in controlling the pathogen followed by

Plate 1 : Inhibitory effect of Endophytic Fungal Antagonists (EFA 1-6) against *Fusarium solani*. Left to right – 1) EFA 1, 2) EFA 2, 3) EFA 3, 4) EFA 4, 5) EFA 5, 6) EFA 6, Control.

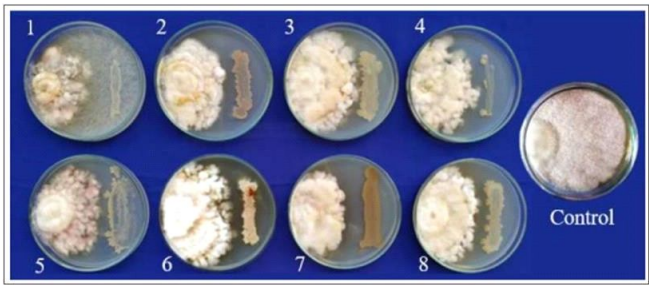

Plate 2 : Inhibitory effect of Endophytic Bacterial Antagonists (EBA 1-8) against *Fusarium solani*. Left to right – 1) EBA 1, 2) EBA 2, 3) EBA 3, 4) EBA 4, 5) EBA 5, 6) EBA 6, 7) EBA 7, 8) EBA 8, Control.

Aspergillus niger. Likewise, Singh *et al*. (2010) concerning their pot culture trials reported that the *Pseudomonas aeruginosa* PN1 strain was effective in controlling *Macrophomina phaseolina* thereby increasing plant growth and biomass. Al-Ghafri *et al.* (2020) reported two strains of *P. aeruginosa* ISO1 and ISO2 hadshown their antagonistic ability against *Fusarium solani* and *Pythium aphanidermatum in vitro* tests.

Conclusion

The study revealed that the endophytic fungal and bacterial antagonists EFA 4 and EBA 7 have shown antagonistic action against the root-rot pathogen under *in vitro* conditions and were promising. Under *in planta* conditions, only EBA 7 was promising and was on par with already existing bioagents in controlling *Fusarium solani* causing dry root rot in acid lime. Further studies are required to grasp how these antagonists protect plants at the cellular level. This may further find application in optimizing biocontrol programs in agriculture.

Compliance with ethical standards

Conflict of interest: the authors declare that they have no conflict of interest.

Acknowledgment

I want to express my gratitude to all the authors from the department of pathology and pomology for providing the lab facilities and facilitating the successful completion of my work.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Authors contribution

All authors contributed equally in data acquisition, analysis and interpretation and also in revising the article.

References

Al-badi, R.S., Karunasinghe T.G., Al-sadi A.M., Al-mahmooli I.H. and Velazhahan R. (2020). *In vitro* antagonistic activity of endophytic fungi isolated from Shirazi Thyme (*Zataria multiflora* Boiss.) against *Monosporascus cannonballus. Polish J. Microbiol*., **69(3)**, 379-383.

Al-Ghafri, H.M., Velazhahan R., Shahid M.S. and Al-Sadi A.M. (2020). Antagonistic activity of *Pseudomonas aeruginosa* from compost against *Pythium aphanidermatum* and *Fusarium solani. Biocontrol Sci. Technol.*, **30(7)**, 642-658.

Al-Shibli, H., Dobretsov S., Al-Nabhani A., Maharachchikumbura S.S.N., Rethinasamy V. and Al-Sadi A.M. (2019). *Aspergillus terreus* obtained from mangrove exhibits antagonistic activities against *Pythium aphanidermatum*- induced damping-off of cucumber, **7**, e7884.

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.*, **1(9)**, 95.

Luu, T.A., Phi Q.T., Nguyen T.T.H., Dinh M.V., Pham B.N. and Do Q.T.(2021). Antagonistic activity of endophytic bacteria isolated from weed plant against stem end rot pathogen of pitaya in Vietnam. *Egyp. J. Biolog. Pest Control*, **31**, 14.

Singh, N., Kumar S., Bajpai V.K., Dubey R.C., Maheshwari D.K. and Kang S.C. (2010). Biological control of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promotory activity in chir-pine. *Crop Protection*, **29(10)**, 1142-1147.

Skidmore, A.M. and Dickson C.H. (1976). Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans. Brit. Mycolog. Soc*., **66**, 57-64.

Srivastava, S., Singh V.P., Kumar R., Srivastava M., Sinha A. and Simon S. (2010). *In vitro* evaluation of Carbendazim 50% WP, antagonists and botanicals against *Fusarium oxysporum*f. sp. *psidii* associated with Rhizosphere soil of Guava. *Asian J. Plant Pathol*., 1819-1541.

Utkhede, R.S. and Rahe J.E. (1983). Interactions of antagonists and pathogen in biological control of onion white rot. *Phytopathology*, **73**, 890-893.

Uzair, B., Kausar R., Bano S.A., Fatima S., Badshah M., Habiba U. and Fasim F. (2018). Isolation and molecular characterization of a model antagonistic *Pseudomonas aeruginosa* Divulging *in vitro* plant growth promoting characteristics. *Biomed Res. Int.*, **2018**, 1-7.

Vincent, J.M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, **150**, 850-856.