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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

AN FTIR STUDY OF *PSEUDOMONAS AERUGINOSA* AVSCE-1 (KM236234) ANTAGONISM AGAINST *FUSARIUM OXYSPORUM* (MTCC10270) - INTERPRETATION OF FTIR DATA IN THE 4000-1000 CM^{-1} REGION

Tulasibai Vadithe, Narmada Sanneboyina, Amrutha V Audipudi*

Department of Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

**Corresponding author: audipudiamrita@gmail.com*

ABSTRACT

Fourier transform infrared (FTIR) spectroscopy may be a rapid, reliable, sensitive, and an economical technique, which is employed as an efficient tool for microorganism identification and FTIR spectrum obtained for any identified compound gives the information on the unique fingerprint. During this study, an effort was made to interpret FTIR spectral data in differentiating the ability of antagonism of endophytic bacteria against *Fusarium oxysporium var. lycopersii,* a phytopathogen of tomato. Antagonism of chilli endophytic bacteria *Pseudomonas aeruginosa* AVSCE-1 (FR3) against *Fusarium oxysporum*-MTCC10270 (FO) were studied by dual culture method. FR3 showed significant antagonism against the growth of FO with inhibition of 62.5%. FTIR spectra of ethyl acetate extracts of FO, FR3 and FO+FR3 were analyzed. In FTIR analysis, C-O and C-C stretch of chitin at 1073.35 cm^{-1}, C-N stretch of Amide II at 1232.97 cm^{-1}, C-O stretch of Amide III at 1300 cm^{-1} and C=O stretch of lipid at 1650-1800 cm^{-1} of FO revealed the presence of pathogenic compounds. N-H stretch of peptides at 3394.21 cm^{-1}, C-O and C-C stretch of chitin at 1073.35 cm^{-1}, C-H stretch of alkenes at 1449.66 cm^{-1}, C-O and C-C stretch of chitin at 1073.35 cm^{-1} and absence of peaks at 1500 to 2500 cm^{-1}, in FO+FR3 and C-H stretch at 2900.83 cm^{-1}, C=O stretch at 1732.72 cm^{-1}, C-H stretch of unsaturated ester at 1448.73 cm^{-1} and C-O stretch of alkyl ether at 1097.97 cm^{-1} and absence C-O and C-C stretch of chitin at 1073.35 cm^{-1}.

Keywords: Antagonism,Endophyticbacteria, Fourier transmission, Infrared, IR Fingerprint, *Fusarium oxysporum var. lycopersii*.

1. INTRODUCTION

The endophyte-plant interaction is one among the smallest amount studied biochemical systems in nature. Endophytes include fungi, bacteria and actinomycetes that are transiently symptomless and inconspicuous that resides within the tissues beneath the epidermal cell layers, either intercellularly or intracellularly, including phloem and xylem [1]. Plants serve as host to one or more endophytic microorganisms without causing any injury and disease to them [2, 3]. Endophytic bacteria colonize the host plant without showing any negative effect on the host [4, 5]. There is ample evidence that many endophytic bacteria have beneficial effects on plants [6]. Like PGPR, endophytes also influence the growth of plant directly by producing plant growth promoting traits such as IAA, Phosphate solubilization, siderophore production, ammonia production, nitrogen fixation antagonism against phytopathogens and indirectly by induced systemic resistance (ISR) and are commercially developed as biofertilizers [7, 8] and made as an alternative source to the chemicals by producing biocontrol strains mediated by its secondary metabolites [9, 10]. Microorganisms with phytopathogenic antagonism act as Biocontrol agents (BCA). Most of the biocontrol agents have not fulfilled their initial promise because of poor competence. The failure of BCA being attributed the difficulties in long-term culture. It would obviate the need for selecting bacterial types with high levels of competence and successful seed or root bacterization treatments before or at planting. The intimate relationship between endophytic bacteria and their hosts made the endophytes as natural candidates for selection as biocontrol agents with high level of competition [11, 12]. Antagonism of endophytic bacteria against *Clavibacter michiganensis subsp. Sepedonicum* which cause rot on tomato was reported [12]. *Pseudomonas chlororaphis, P. fluorescens, P. graminis, P. putida, P. tolaasii* and *P. veronii* have been reported as phytopathogenic bacterial antagonism against pathogenic bacteria [11, 13]. Some bacteria reported to produced cyanogens and inhibited fungal growth. *Bacillus*

sp. were considered as potential-biocontrol agents due to their high spore production ability, resistance and ability to survive desiccation, heat, ultraviolet (UV) irradiation and organic solvents [14]. *Pseudomonas aeruginosa* AVSCE-1 were endophytes reported to inhibit the growth of *Colletotrichum* [15, 7].

Fusarium oxysporum has several specialized forms infecting a variety of plants with various diseases of many symptoms such as vascular wilt, yellows, corm rot, root rot, and damping-off [16, 17]. Infected plants may wilt and die soon after the appearance of symptoms at the seedling stage itself. On older plants, symptoms are generally more apparent in the period between blossoming and fruit maturation [17]. Early detection of phytopathogens is critical since it enables precise and effective tracing and targeting of treatment or prevention [18]. This could save enormous financial losses [16].

Biological control of *Fusarium oxysporum* to caused Fusarium wilt diseases of tomato plant, has become potential as an alternative disease management strategy [17, 18]. Antagonist organisms including nonpathogenic rhizobacteria have successfully reduced the incidence of Fusarium wilt in many crops in field trials and greenhouse [18-21]. Use of antagonist organism agents, such as plant growth promoting rhizobacteria (PGPR), can be a convenient method in control of disease [22]. Plant growth promoting rhizobacteria (PGPR), such as *Bacillus*, *Enterobacter* and *Pseudomonas* strains, are the major root colonizers [23, 24] and can encourage plant defenses [25] and reported for their execution such as production of antibiotics, plant growth development by Indole acetic acid production (IAA), competition for nutrition and space, siderophore cyanide hydrogen, inactivation of pathogen's enzymes such as Protease, inducing resistance and enhancement of root through various types of mechanisms [26].

Fourier transform infrared (FTIR) spectroscopy is considered to be a rapid, reliable, sensitive, and a cost-effective technique, which could be used as an efficient tool for microorganism identification. Since bio-molecules, such as lipids, carbohydrates, and nucleic acids, have their own unique 'vibrational' fingerprints and characteristic functional groups, which correspond to specific infrared light frequencies, FTIR spectrum obtained for any compound gives the information on the unique 'fingerprint'. Infrared spectroscopy's unique advantages are simplicity, rapidity, and sensitivity [27]. In addition, much information already exists on the spectral bands obtained from FTIR spectra of living cells, adding to the promise of the method as a valuable tool for pathogen detection. FTIR-ATR spectroscopy has been successfully used to detect and identify fungi samples on the levels of genus, species, and isolates. With this background information, the present investigation aimed to interpret FTIR spectral data in differentiating the ability of antagonism of endophytic bacterial against *Fusarium oxysporum var. lycopersii* a phytopathogen of tomato, for the first time.

2. MATERIAL AND METHODS

2.1. Microbial strains

FO (Fusarium oxysporum-MTCC 1027) strain was collected from MTCC Chandigarh, India. Endophytic bacterial strains FR3 (*Pseudomonas aeruginosa* AVSCE-1) were taken from corresponding author's microbial cultures of Department of Microbiology, Acharya Nagarjuna University, Guntur, A.P., India.

2.2. Screening of Fungal Antagonism

Antifungal activities were assayed by dual culture method [28]. Bacterial isolate was streaked on Potato Dextrose Agar (PDA) medium at a distance of 3cm opposite to pathogenic fungi which was inoculated at the center of the medium. Antifungal activity was measured after 4 to 7 days of incubation at room temperature. The value of inhibition was measured using the formula: [7]

Value of inhibition=1-a/bX100%

a: distance between fungi in the center of petri dish to endophytic isolate, b: distance between fungi in the center of Petri dish to blank are without endophyte

2.3. Co Cultivation of phytopathogen and endo-phytic bacteria

FO and FR3 were co-cultivated as FO+FR3. FO and FR3 were cultivated separately as control. Three groups were grown in optimized medium (NAM supplemented with 0.5% Peptone, 0.5% Beef extract, 0.4% Sodium chloride and 0.5% Glucose) at pH 7.0 and 35°C for 96 h on a rotary shaker at 2000 rpm for four days.

2.4. Extraction of crude

After 96 hours of incubation (4day), the culture was harvested and centrifuged at 10,000 rpm for 20 min at 4°C and supernatant was collected. An equal volume of ethyl acetate was added to the collected supernatant and vigorously shaken for 30-40 min. The organic layer was fractionated with a separating funnel. The extraction was repeated twice with equal volume of ethyl acetate and collected organic layer. The crude was processed for further screening tests.

2.5. FTIR Spectroscopy

Fourier transform infrared (FTIR) analysis was performed on Thermo Nicolet Nexus 670 spectrometer (Thermo Scientific, USA) at 4000-400 cm^{-1} wavenumber regions with scan resolution of 4 cm^{-1}. KBr was used as beam splitter and DTGS KB as detector.

3. RESULTS AND DISCUSSION

The present research work encompasses antagonism of FR3 against FO and FTIR spectral study of ethyl acetate extracts of FO, FR3, FO+FR3 understand antagonism of FR3 against FO at metabolite level.

3.1. Antagonism of FR3 against FO

Pseudomonas aeruginosa AVSCE-1(FR3) potential endophytic isolates with significant antagonism against *Colletotrichum* sp along with IAA production, phosphate solubilization, ammonia, siderophore production and nitrogen fixation [7]. In present study, FR3 showed significant antagonism against *Fusarium oxysporum*-MTCC 1027 (FO) in dual plate culture. Antagonism of chilliendophytic bacteria *Pseudomonas aeruginosa* AVSCE-1 (FR3) against *Fusarium oxysporum*-MTCC10270 (FO) were studied and FR3 showed significant antagonism against the growth of FO with inhibition of 62.5%.

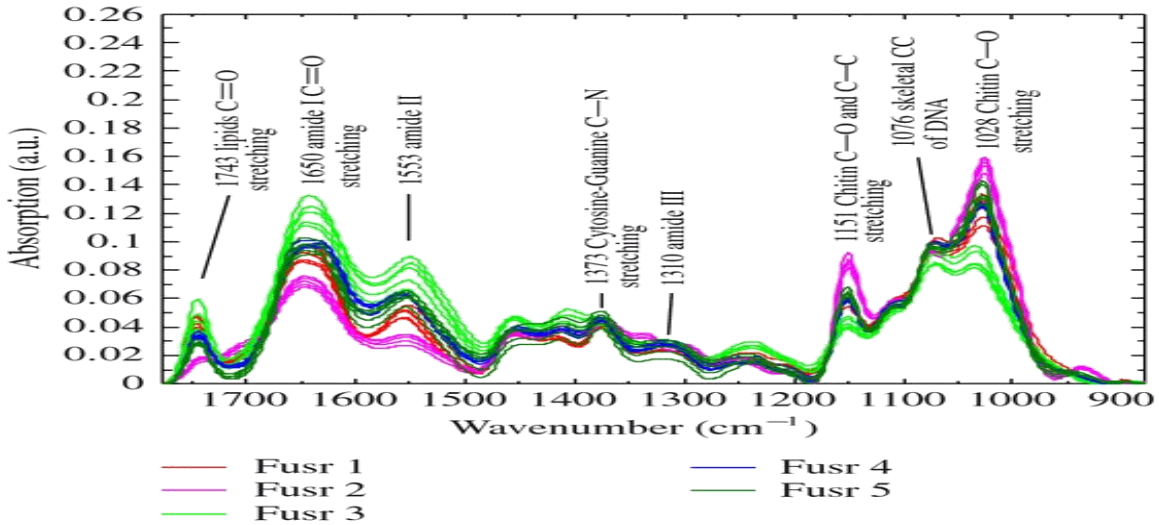
3.2. FTIR spectral Analysis and interpretation

Fourier transform infrared (FTIR) spectroscopy is considered to be a rapid, reliable, sensitive, and a cost-effective technique, which could be used as an efficient tool for microorganism identification and FTIR spectrum obtained for any compound gives the information on the unique 'fingerprint. In this study, an attempt was made to interpret FTIR spectral data in differentiating the ability of antagonism of endophytic bacterial against *Fusarium oxysporum var. lycopersii,* a phytopathogen of tomato.

Infrared absorption spectra revealed that a peak at 1076 cm^{-1}arises mainly from nucleic acid vibrations and carbohydrate. Amide I at 1650 cm^{-1} and amide II at 1553 cm^{-1} are dominant in this region. Due to the C=O vibration a typical lipid band is formed at 1743 cm^{-1}. Other important bands are the chitin C-O and glycogen and C-C stretching vibrations at 1151 and 1028 cm^{-1}, respectively and the spectra are dominated by water absorption bands which were excluded as a part of the analysis procedure in the higher wavenumber region. The lipids CH_2 absorption peaks at 2849, 2917 and 3008 cm^{-1} appear in the higher wave number region [27]. Similar to the above reference, FTIR spectra of FO also showed peaks as shown in fig.1.

Table 1: Fungal antagonism of FR3 against Fusarium Oxysporum

Endophytic bacterial combinations	Zone of inhibition (mm) of Fusarium oxysporium (FO)			
	Diameter(cm)	Area($A=\pi r^2$)	Circumference ($2\pi r$)	% inhibition
FO (Control)	4	12.56	12.56	
FR3	1.25	4.90	7.85	62.5



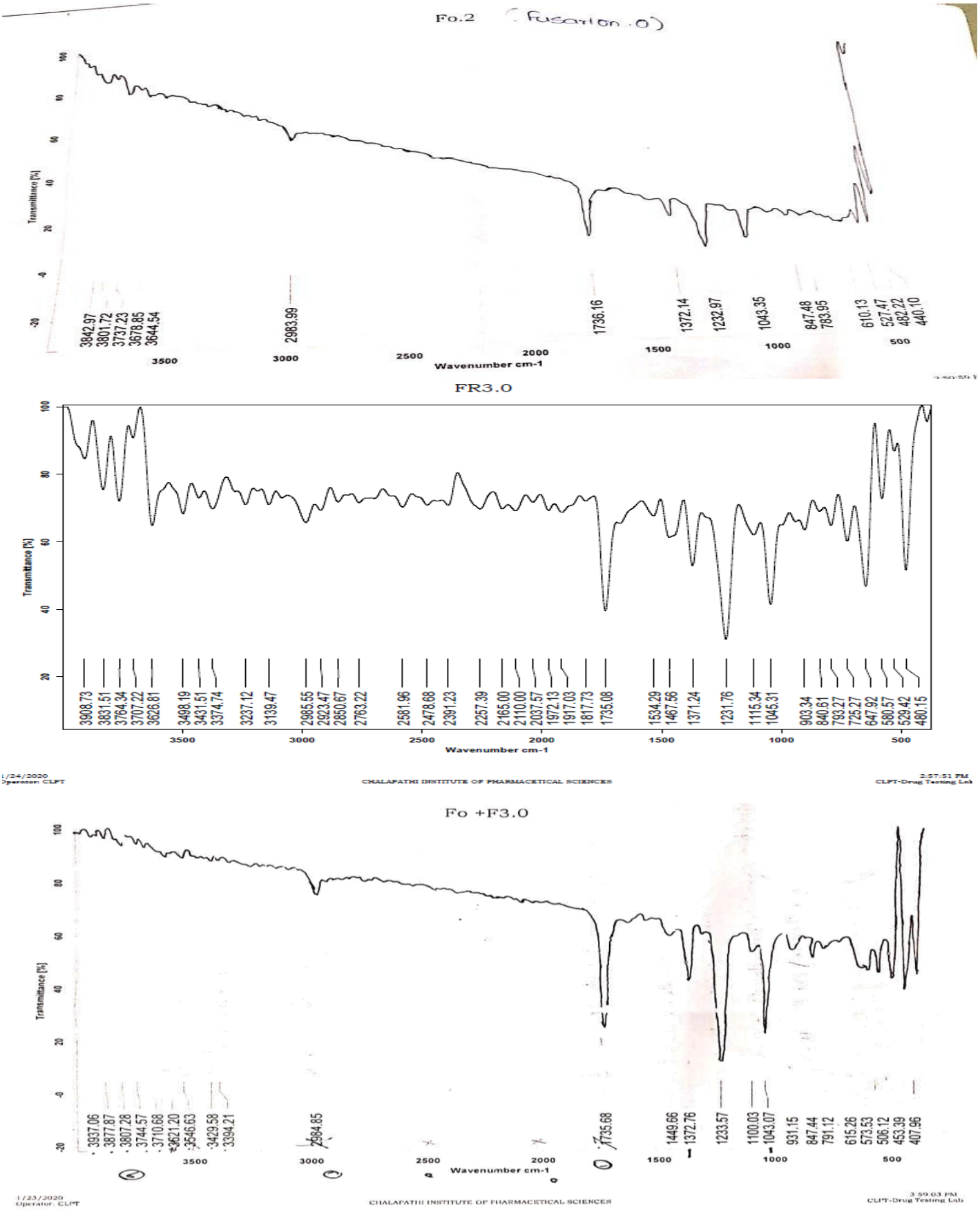


Fig. 1: FTIR spectral analysis and interpretation of FO, FR3 and FO+FR3

FTIR spectra of ethyl acetate extracts FO, FR3 and FO+FR3 were analyzed (Table 2). In FTIR analysis, C-O and C-C stretch of chitin at 1073.35 cm^{-1}, C-N stretch of Amide II at 1232.97 cm^{-1}, C-O stretch of Amide III at 1300 $cm^{-1}$and C=O stretch of lipid at 1650-1800 $cm^{-1}$of FO revealed the presence of pathogenic compounds. N-H stretch of peptides at 3394.21 cm^{-1}, C-O and C-C stretch of chitin at 1073.35 cm^{-1}, C-H stretch of alkenes at 1449.66 cm^{-1}, C-O and C-C stretch of chitin at 1073.35 cm^{-1}. In spectra of FO+FR3 showed absence of peaks at 1500 to 2500 cm^{-1}, showed C-H stretch at 2900.83 cm^{-1}C=O stretch at 1732.72 cm^{-1}, C-H stretch of unsaturated ester at 1448.73 cm^{-1} and C-O stretch of alkyl ether at 1097.97 cm^{-1} and absence C-O and C-C stretch of chitin at 1073.35 cm^{-1}.

Table 2: FTIR Spectral Data Analysis of FO, FR3 and FO+FR3

S. No	Peak range	FO	FO+FR3	FR3
1.		3842.97	3621.20	…….
2.		3801.72	3546.63	…….
3.	3000-4000	3737.23	3429.58	…….
4.		3678.85	3394.21	…….
5.		3644.54		…….
1.		2983.99	2984.85	…….
2.		……..	……..	…….
3.		__……._	_……..	……..
4.	2000-3000	…….	……..	……..
5.		……	…….	……..
6.		…….	……..	…….
7.		……..	……..	……..
1.		1736.16	#1449.66	…….
2.		1372.14	*1372.76	……..
3.		*1300	*1233.57	……..
4.	1000-2000	*1232.97	……..	AmideII
5.		*1073.35	……..	……..
6.		……..	……..	AmideIII
7.		……..	……..	……..
8.		……..	…….	……..

*# Exclusive, * Present in both*

4. SUMMARY AND CONCLUSION

FTIR spectra of ethyl acetate extracts FO, FR3, and FO+FR3 were analyzed. In FTIR analysis, C-O and C-C stretch of chitin at 1073.35 cm^{-1},C-N stretch of Amide II at 1232.97 cm^{-1},C-O stretch of Amide III at 1300 $cm^{-1}$and C=O stretch of lipid at 1650-1800 $cm^{-1}$of FO revealed the presence of pathogenic compounds. N-H stretch of peptides at 3394.21 cm^{-1},C-O and C-C stretch of chitin at 1073.35 cm^{-1}, C-H stretch of alkenes at 1449.66 cm^{-1}, C-O and C-C stretch of chitin at 1073.35 cm^{-1} and absence of peaks at 1500 to 2500cm^{-1}, in FO+FR3 evidenced that FR3 elicited chitin degrading compounds in presence of FO.

5. ACKNOWLEDGEMENT

First Author is thankful to Ministry of Tribal Affairs No: 201718-NFST-AND-01167 and Corresponding Author is thankful to UGC New Delhi for financial assistance to UGC-MRPF. No. 40-132-2011 (SR) and central instrumentation centre of Acharya Nagarjuna University for laboratory facility.

Conflict of interest

Authors declared no conflict of interest and also declared that all authors have equal contribution.

6. REFERENCES

1. Petrini O. *In: Microbiology of the Phyllo 49 sphere. Fokkema JN, Heuvel Van-Den J. eds Cambridge University Press, Cambridge*, 1986: 175-187
2. Petrini O. *In: Andrews JH, Hirano SS (eds) Microbial Ecology of Leaves. Springer-Verlag, New York*, 1991; 179-197.
3. Wilson D. *Oikos*, 1995; **73**:274-276
4. Holliday P. *A Dictionary of Plant Pathology, Cambridge University Press, Cambridge.*,1989
5. Schulz B, Boyle C. *What are endophytes? Microbial Root Endophytes (Schulz BJE, Boyle CJC & Sieber TN, eds)* Springer-Verlag, Berlin, 2006;1-13.
6. Hallman J, Quadt-Hallman A, Mahaffee WF, Kloepper JW. *Canadian Journal of Micro- biology*, 1997; **43**: 895-914.
7. Sudhir A, Pradeep KN, Amrutha VA. *International Journal of Current Microbiology and Applied Science*, 2014; **3(2)**: 318-329.
8. Kim SY, Lee SY, Weon H-Y, Sang MK, Song J. *J. Biotechnol.,* 2017; **241**: 112-115.
9. Ongena M, Jacques I. *Trends in Microbiol*. 2008; **16(3)**: 115-125.
10. Pal KK, Tilak KV, Saxena AK, Dey R, Singh CS. *Microbiol Res.,* 2000; **155(3)**: 233-242.
11. Chen C, Banske EM, Musson G, Kloepper JW. In: *Improving Plant Productivity with Rhizosphere Bacteria,* Ryder MH, Stephens PM, Bowen GD. Eds., CSIRO, Adelaide, Australia, 1994; 191-193.
12. Van Buren AM, Andre C, Ishimaru CA. *Phytopathology,* 1993; **83**:1406.
13. Adhikari TB, Hansen JM, Gurung S, Bonman JM. *Plant Diseases*, 2011; **95(5):**582-588.

14. Romero RS. Filiio L, Vieira JR, Silva HSA, Baracat-pereira MC, Carvalho MG. *Journal of Phytopathology,* 2004; **153:**120-123.
15. Amrutha V, Sudhir A, Kumar N, Chowdappa P. *Annals of Biological Research.*, 2014; **5(5):**15-21
16. Agrios GN, *Plant Pathology*, Academic Press, New York, NY, USA, 3rd edition., 1988.
17. Tsror L, Hazanovsky M, Mordechi- Lebiush, S, Sivan S. *Plant Pathology*, 2001; **50(**4):477-482.
18. Doern GV, Vautour R, Gaudet M, Levy B. *Journal of* Clinical *Microbiology.*, 1994; **32(7)**: 1757-1762.
19. Alabouvette C, Schippers B, Lemanceau, P, Bakker, P. A. H. I. *In: Plant-Microbe Interactions and Biological Control. G. J. Boland and L. D. Kuykendall, eds. Marcel Dekker, New York.*, 1998: 15-36
20. Datnoff LE, Nemec S, Pernezny K. *Biol. Control.*, 1995; **5**:427-431.
21. Alabouvette C, Couteaudier Y. *In: Biological Control of Plant Diseases. E. C. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Plenum Press, New York*, 1992: 415-426
22. Alabouvette C, Lemanceau P, Steinberg C. *Pestic.Sci.*, 1993; **37**:365-373.
23. Larkin RP, Hopkins DL, Martin FN. *Phytopathology*, 1996; **86**:812-73 819.
24. Schmidt CS, Agostini F, Leifert C, Killham K, Mullins CE. *Phytopathology*, 2004; **94**:351-363.
25. Manikanda R, Saravanakumar D, Rajendran L, Raguchander T, Samiyappan R. *Biol. control.*, 2010; **54**:83-89.
26. Joseph B, Patra RR, Lawrence R. *International* Journal *of Plant Production*, 2007; **1(2)**:141-151.
27. Salman A, Pomerantz A, Tsror L, Lapidot I, Zwielly A, Moreh R, et al. *Analyst*, 2011; **136:** 988-995.
28. Kumar NR, Arasu VT, Gunasekaran P. *Curr. Sci.,* 2002; **82**:1463-1466.