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In vitro Screening of Bacterial Endophytes Against Soilborne Fungal Pathogens of Tomato by Volatile Method

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ABSTRACT

A total of 45 bacterial endophytes were isolated from apparently healthy tomato plant parts viz., root, stem and leaf tissues and evaluated against soil- borne pathogens viz., Sclerotium rolfsii and Rhizoctonia solani by volatile method to test antifungal activity of volatile metabolites of the tomato bacterial endophytes. Among the 22 root endophytic isolates RBDNA-4, RBDLA-5 and RBDDE-14 showed the maximum inhibition against S. rolfsii to the tune of 89.63, 88.52 and 87.41 per cent, respectively. Out of 12 stem endophytic isolates SBDKA-7 and SBDOF-6 showed the maximum inhibition against S. rolfsii to the tune of 89.26 and 88.52 per cent, respectively and isolate SBHKA-2 showed the maximum inhibition against R. solani to the tune of 66.67 per cent followed by SBDOF-6 (54.81%). Out of 11 endophytic leaf isolates, LBHRA-5 showed maximum inhibition against S. rolfsii to the tune of 81.96 per cent followed by LBDHO-2 (23.53 %) and against R. solani only one isolate LBHRA-5 showed the maximum inhibition to the tune of 55.44 per cent and the remaining all isolates did not show inhibition. Results clearly indicated that bacterial endophytes were more effective in inhibiting the growth of S. rolfsii than the growth of R. solani. Root and stem isolates were more effective than leaf isolates against both pathogens in volatile method.

Key words: *Bacterial endophytes, Sclerotium rolfsii, Rhizoctonia solani, Volatile method*

INTRODUCTION

Among the vegetables tomato is the second most consumed and widely grown vegetable in the world after potato. Tomato is popular fresh and in many processed forms (e.g., ketchup, canned whole or in pieces, puree, sauce, soup and juice). The ripe fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food[3]. At present, the total tomato production in India is about 19.70 million tonnes from 0.808 million ha area with productivity of 24.4 tonnes per hectare. In Karnataka, tomato occupies 63.73 thousand ha with a production of 2138.13 thousand metric tonnes having productivity of 33.55 tonnes per hectare[1].

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Among the pathogens that affect the tomato crop, soil-borne fungal pathogens, including species belonged to *Sclerotium*, *Fusarium*, *Pythium*, *Rhizoctonia*, and *Verticillium* genera causing the root rot or damping- off and wilt which affect the quality with yield reduction. *S. rolfsii* reported yield loss up to 30 per cent[5], *R. solani* causes up to 30 per cent[7] and *F. oxysporum* f.sp. *lycopersici* causes 10 -90 per cent in tomato[4]. Some of these pathogens are particularly challenging because they often survive in soil for many years[10]. To manage such diseases, farmers presently use different fungicides formulations at least for 8-10 times in one growing season which has resulted in several undesirable effects like pesticide pollution, fungicide resistance, elimination of beneficial fauna, environmental pollution and human health hazards[4]. So integrated disease management where biological control is one practice is becoming key consideration for soil- borne diseases. Use of endophytes as biocontrol agent may open up new area of research in plant protection in the recent decades under various agro-climatic situations. Endophytes are plant associated microorganisms that live inside plant tissues without causing any harm to plants. The interest in endophytic research has increased, as they colonize the internal tissues of their host plants and improve plant tolerance to various abiotic stress factors and can protect plants from various pathogenic microbes[9]. With this view in present study an attempt was made to isolate bacterial endophytes and evaluated them under *in vitro* condition by volatile method. A total of 45 bacterial endophytes were isolated and evaluated against *S. rolfsii* and *R. solani* by volatile method to test antifungal activity of volatile metabolites of the endophytes.

MATERIAL AND METHODS

Isolation of bacterial endophytes

A survey was conducted during 2016-2017 to isolate bacterial endophytes in tomato. Apparently healthy leaves, stems and root samples from tomato crop were collected from fields in Belagavi, Dharwad and Haveri districts of northern Karnataka. Roots, stem, leaves were washed in running tap water to remove dirt and split into longitudinal sections. After this, surface sterilization was done with ethanol (70 %) for a minute followed by sodium hypochlorite (1 %) for 3 minutes. Subsequently the sections were rinsed with sterile distilled water. Then the sections were rinsed with 0.02M potassium phosphate buffer 3 times (0.1ml aliquot was taken and transferred to 9.9 ml of nutrient broth which served as sterility check). One gram of plant parts were macerated with 9 ml of potassium phosphate buffer in pestle and mortar and serial dilution was made up to 10^{-3} dilution. Dilutions of 10^{-2} and 10^{-3} were plated on Nutrient Agar (NA), King's B and HiCrome Bacillus agar media. The plates were incubated at 28 ± 2°C for 48-72 h for observing colonies developed on them and isolated colonies were picked up and streaked again on fresh nutrient agar plates and incubated. Final pure cultures were transferred on NA slants and stored for further studies in refrigerator at 4 °C.

Antifungal activity of bacterial endophytes by volatile method or sealed plate method

The sealed plate technique was performed for all bacterial endophytes against *R. solani* and *S. rolfsii* to test antifungal activity of volatile metabolites of the tomato endophytes[8]. For this test, twenty ml of sterilized and cooled PDA was poured into sterilized Petriplates and inoculated with endophyte. A second PDA Petri plate was challenged with pathogens plug only. Both half plates were wrapped together with parafilm to seal in the volatile compounds in such a way that endophye at bottom plate and pathogen at top plate. For control plates, pathogen-challenged half plate (upper plate) over a half one containing PDA (bottom plate) only. After required period of incubation *i.e.* after growth of colony in control plate reached 90 mm diameter, the radial growth of pathogen in treated plate was measured. Per cent inhibition over control was calculated using above said formula given by Vincent[11]. After required period of incubation *i.e.,* after growth of colony in control plate reached 90 mm

diameter, the radial growth of pathogen in treated plate was measured. Per cent inhibition over control was worked out according to formula given by Vincent[11].

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

Where, I = Per cent inhibition, C = Radial growth in control (mm), T = Radial growth in treatment (mm)

RESULTS AND DISCUSSION

A total 45 bacterial (22 from root, 12 from stem and 11 from leaf) endophytic isolates were obtained from different parts of healthy tomato samples, which were collected from 30 locations in three districts of northern Karnataka. A total of 45 bacterial endophytes were evaluated against *S. rolfsii* and *R. solani* by volatile method to test antifungal activity of volatile metabolites of the endophytes.

A total of 22 bacterial root endophytes were evaluated against two pathogens by volatile method and results are presented in Table1. Results clearly indicated that root endophytes significantly inhibited the mycelial growth of both pathogens. Efficacy of root endophytes against both pathogens ranged from 0.00 to 89.63 per cent. This was the highest range of inhibition by any kind of isolates studied in this investigation. Isolates RBDNA-4, RBDLA-5 and RBDDE-14 showed the maximum inhibition against *S. rolfsii* to the tune of 89.63, 88.52 and 87.41 per cent, respectively and these were on par with each other followed by RBHNI-2 (83.70 %). Isolates RBHKA- 3, RBDHE-6, RBDCH-16 and RBDNU-17 showed no any inhibition against *S. rolfsii*. Isolates RBDNA-4 and RBDDE-14 showed the maximum inhibition against *R. solani* to the tune of 56.30 and 54.81 per cent, respectively which were on par with each other followed by RBBBE-20 (47.04%) and the isolates RBHNI-2, RBHCH-7, RBDCH-16, RBDNU-17, RBBHU-21 and RBBSA-22 showed no any inhibition.

A total of 12 bacterial stem endophytes were evaluated against two pathogens by volatile method and results are presented in Table2. Results clearly indicated that stem endophytes significantly inhibited the mycelial growth of both pathogens. Efficacy of stem endophytes against both pathogens ranged from 0.00 to 89.26 per cent. Isolates SBDKA-7 and SBDOF-6 showed the maximum inhibition against *S. rolfsii* to the tune of 89.26 and 88.52 per cent, respectively and these were on par with each other followed by SBHKA-2 (82.96%) and SBHHO-3 (82.22%) and these were also on par with each other. Isolates SBHNI- 1, SBHCH-4 and SBBSA-12 did not show any inhibition. Isolate SBHKA-2 showed the maximum inhibition against *R. solani* to the tune of 66.67 per cent followed by SBDOF-6 (54.81%) and the isolates SBHNI-1, SBHCH-4, SBHBK-5, SBDKA-7, SBDCH-8, SBDVA-10, SBBSA-11 and SBBSA-12 did not show any inhibition.

A total of 11 bacterial leaf endophytes were evaluated against two pathogens by volatile method and results are presented in Table 3. Isolate LBHRA-5 showed maximum inhibition against *S. rolfsii* to the tune of 81.96 per cent followed by LBDHO-2 (23.53%) and isolates LBDNU-8, LBBBS-10 and LBBBE-11 showed no any inhibition. Isolate LBHRA-5 showed the maximum inhibition against *R. solani* to the tune of 55.44 per cent and the remaining all isolates did not show inhibition. From the above results it is indicated that bacterial endophytes were more effective in inhibiting the growth of *S. rolfsii* than the growth of *R. solani*. Root and stem isolates were more effective than leaf isolates against both pathogens in volatile method. The results are in agreement with the work of Nandhini *et al.*[8], they have used volatile technique and reported antagonistic activity of bacterial endophytes against *Fusarium oxysporum* f.sp. *lycopersici* in tomato. Mousa and Raizada[6] reviewed diverse classes of secondary metabolites, focusing on antimicrobial

compounds, synthesized by endophytes including terpenoids, alkaloids, phenylpropanoids, aliphatic compounds, polyketides and peptides from the interdisciplinary perspectives of biochemistry, genetics, fungal biology, host plant biology, human and plant pathology. Many endophytes produce secondary metabolites such as auxin, gibberellin *etc.* that help in growth and development of the host plant. Some of these compounds are antibiotics having antifungal, antibacterial and insecticidal properties, which may inhibit the growth of plant pathogens. Cosoveanu *et al.*[2] used volatile technique and evaluated antagonistic activity of banana fungal endophytes against *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f.sp. cubense, *F. moniliforme* , *Alternaria alternata*, *Geotrichum* sp., *Phoma* sp. and *Cladosporium* sp. and results revealed that *F. moniliforme* and *F. oxysporum* to be the most susceptible to the inhibitory effects of the volatile compounds of various endophytes.

Table1. *In vitro* evaluation of bacterial root endophytes against *Sclerotium rolfsii* and *Rhizoctonia solani* by volatile method

Isolate	Per cent inhibition	
	S. rolfsii	***R. solani***
RBDHO -1	47.04 (43.28)*	20.00 (26.55)
RBHNI-2	83.70 (66.16)	0.00 (0.00)
RBHKA-3	0.00 (0.00)	2.96 (9.91)
RBDNA-4	88.52 (70.17)	56.30 (48.60)
RBDLA-5	89.63 (71.19)	38.89 (38.56)
RBDHE-6	0.00 (0.00)	4.07 (11.64)
RBHCH-7	58.52 (49.88)	0.00 (0.00)
RBHKM-8	21.85 (27.86)	4.07 (11.64)
RBHRA-9	61.48 (51.62)	21.85 (27.86)
RBHBA-10	64.07 (53.15)	6.30 (14.53)
RBHBK-11	32.96 (35.02)	32.59 (34.80)
RBHMU -12	28.52 (32.26)	2.59 (9.26)
RBDOF-13	59.63 (50.53)	2.96 (9.91)
RBDDE-14	87.41 (69.19)	54.81 (47.74)
RBDCH-15	44.44 (41.79)	44.81 (42.01)
RBDCH-16	0.00 (0.00)	0.00 (0.00)
RBDNU-17	0.00 (0.00)	0.00 (0.00)
RBDUN-18	59.26 (50.32)	6.30 (14.53)
RBBBA-19	10.37 (18.78)	15.56 (23.22)
RBBBE-20	54.07 (47.32)	47.04 (43.28)
RBBHU-21	12.59 (20.78)	0.00 (0.00)
RBBSA-22	51.85 (46.04)	0.00 (0.00)
S.Em. ±	0.27	0.52
C.D.at 1%	1.01	1.96

* Arcsine transformed values

Table 2. *In vitro* evaluation of bacterial stem endophytes against *Sclerotium rolfsii* and *Rhizoctonia* solani by volatile method

Isolate	Per cent inhibition over control	
	S. rolfsii	***R. solani***
SBHNI-1	0.00 (0.00)*	0.00 (0.00)
SBHKA-2	82.96 (65.60)	66.67 (54.71)
SBHHO-3	82.22 (65.04)	2.59 (9.26)
SBHCH-4	0.00 (0.00)	0.00 (0.00)
SBHBK-5	33.33 (35.25)	0.00 (0.00)
SBDOF-6	88.52 (70.17)	54.81 (47.74)
SBDKA-7	89.26 (70.84)	0.00 (0.00)
SBDCH-8	73.70 ((59.13)	0.00 (0.00)
SBDVA-9	79.63 (63.15)	22.22 (28.11)
SBDVA-10	60.37 (50.96)	0.00 (0.00)
SBBSA-11	77.41 (61.60)	0.00 (0.00)
SBBSA-12	0.00 (0.00)	0.00 (0.00)
S.Em. ±	0.33	0.24
C.D. at 1%	1.28	0.92

* Arcsine transformed values

Table 3. *In vitro* evaluation of bacterial leaf endophytes against *Sclerotium rolfsii* and *Rhizoctonia solani* by volatile method

Isolate	Per cent inhibition over control	
	S. rolfsii	***R. solani***
LBDGP-1	5.10 (13.04)*	0.00 (0.00)
LBDHO-2	23.53 (29.01)	0.00 (0.00)
LBDNA-3	16.86 (24.24)	0.00 (0.00)
RBDNA-4	11.37 (19.70)	0.00 (0.00)
RBDLA-5	81.96 (64.84)	54.44 (47.53)
LBHBK-6	6.67 (14.96)	0.00 (0.00)
LBDDE-7	6.27 (14.50)	0.00 (0.00)
LBDNU-8	0.00 (0.00)	0.00 (0.00)
LBBBA-9	8.24 (16.67)	0.00 (0.00)
LBBBA-10	0.00 (0.00)	0.00 (0.00)
LBBBE-11	0.00 (0.00)	0.00 (0.00)
S.Em. ±	0.71	0.11
C.D. at 1%	2.8	0.44

* Arcsine transformed values

COCLUSION

Out of 45 bacterial endophytes, eight isolates (RBDNA-4, RBDLA-5, RBDDE-14, SBHKA-2, SBDOF-6, SBDVA-9, SBBSA-11 and LBDRA-5) showed maximum mycelial inhibition against all tested pathogens in volatile method. The extent of inhibition of two pathogens by bacterial endophytes in volatile method ranged from 0.00 to 89.63 per cent, respectively.

REFERENCES

1. Anonymous, *Ministry of Agriculture and Farmers Welfare*, Govt. of India (2017).
2. Cosoveanu, A., Elena, T., Cristina, G., Matias, R., Rhiannon, M. and Raimundo, C., Endophytic fungi isolated from *Musa acuminata* 'dwarf cavendish' and their activity against phytopathogenic fungi. *J. Agr. Biotechnol,* **1(1):** 35-43 (2016).
3. Dias, J. S., Nutritional quality and health benefits of vegetables: A review. *Food Nutrition Sci.,* **3:** 1354-1374 (2012).
4. Kumar, N. and Sharma, S., Fusarial wilt of *Solanum lycopersicum* L. (tomato) at Panchgaon. *Int. J. Curr. Microbiol. App. Sci.,* **4(11):** 253-260 (2015).
5. Mandal, A. K., Praveen, K. M., Subrata, D. and Arup, C., Effective management of major tomato diseases in the gangetic plains of eastern India through integrated approach. *Agri. Res. Tech.,* **10(5):** DOI: 10. 19080 (2017).
6. Mousa, W. and Raizada, N., The diversity of anti-microbial secondary metabolites produced by fungal endophytes: An interdisciplinary perspective. *Frontiers Microbiol*, **65(4):** 18-36 (2012).
7. Muriungi, S. J., Mutitu, E. W. and Muthomi, J. W., Efficacy of cultural methods in the control of *Rhizoctonia solani* strains causing tomato damping off in Kenya. *African J. Food Agri. Nutri. Develop*, **14(2):** 8776-8790 (2014).
8. Nandhini, S., Vaithiyanathan, S. and Subramanian, B., Endophytic bacteria from tomato and their efficacy against *Fusarium oxysporum* f.sp. *lycopersic*i, the wilt pathogen. *J. Biopest*., **5(2):** 178-185 (2012).
9. Pawle, G. and Singh, S. K., Antimicrobial, antioxidant activity and phytochemical analysis of an endophytic species of *Nigrospora* isolated from living fossil *Ginkgo biloba. Curr. Res. Environ. App. Myco.*, **4(1):** 1-9 (2014).
10. Steven, T. K., Commercial greenhouse vegetable handbook. *ANR publications*, California, pp, 1-13 (2003).
11. Vincent, J. M., Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **150:** 850-857 (1947).