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Screening of Fungal Endopohytes against Soil-borne Fungal Pathogens in Tomato

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A B S T R A C T

A total of 66 fungal endophytes were isolated from apparently healthy tomato plant parts *viz.*, root, stem and leaf tissues and evaluated against soil- borne pathogens *viz.*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani* by dual culture method to test antifungal activity of tomato fungal endophytes. In dual culture method, among the 66 fungal isolates, irrespective of isolates from root, stem and leaf, some (RFHHO-7, RFHKM-9, RFDHE-10, RFBBA-23, SFDOF-11, SFDDE-12, LFDHO-3 and LFDLA-9) have shown maximum mycelial inhibition of three pathogens. Isolates RFBBE-19 and RFDUN-22 were fast growing isolates and were more effective against *F. solani* and *R. solani* as compared to *S. rolfsii*. Isolate LFDKA-20 though showed the minimum inhibition against all pathogens as compared to other effective isolates but it also showed the clear inhibition zone which may be due to the production of antimicrobial compounds from the endophyte. From the results of present study it is concluded that the possible role of growth inhibition by the endophytes was attributed to the production of diffusible and volatile metabolites, lytic enzymes and by competition with pathogens.

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 (Dias, 2012). 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 (Anon., 2017). 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 (Mandal *et al.,* 2017), *R. solani* causes up to 30 per cent (Muriungi *et al.,* 2014) and *F. oxysporum* f.sp. *lycopersici* causes 10 -90 per cent in tomato (Kumar and Sharma, 2015). Some of these pathogens are particularly challenging because they often survive in soil for many years (Steven, 2003). 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 (Kumar and Sharma, 2015). 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 (Pawle and Singh, 2014). With this view in present study an attempt was made to isolate fungal endophytes and evaluated them under *in vitro* condition by dual culture. A total of 66 fungal endophytes were isolated and evaluated against *S. rolfsii, R. solani and F. solani by* dual culture method to test antifungal activity of fungal endophytes.

Materials and Methods

Isolation of bacterial endophytes

A survey was conducted during 2016-2017 to isolate fungal 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, stems and leaf samples collected were washed in running tap water to remove soil dirt and debris and cut into 1 cm sections. After this, surface sterilization was done with 70 per cent ethanol for a minute followed by 1 per cent sodium hypochlorite for 3 minutes. Subsequently the sections were rinsed with sterile distilled water and placed on 9 cm Petri plates containing potato dextrose agar (PDA) medium amended with streptomycin (250 mg/l) to slow down the bacterial growth. Sterilized tissue segments were pressed onto the surface of PDA medium to check the efficacy of surface sterilization procedure and to confirm endophytic isolations only from internal tissues of the plant segments. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective in removing the surface fungi (Schulz *et al.,* 1993). All plates were incubated at 25 ± 1 °C and observed for fungal growth at 3 days interval for a duration upto 7 - 10 days. Fungi growing out from the plant tissues were transferred on to fresh PDA medium. After purifying the isolates for several times, final pure cultures were transferred on to PDA slants and stored in refrigerator at 4°C for further studies.

Antifungal activity of fungal endophytes by dual culture metho

Dual culture techniqueeas adopted for antagonistic activity of isolated endophytes against *S. rolfsii*, *R. solani* and *F. solani* on PDA plates (Deepa and Sally, 2015). In dual culture technique twenty ml of sterilized and cooled PDA was poured into sterilized Petri plates. Fungal endophytes were evaluated by inoculating the pathogen at one side of Petri plate and the fungal endophyte inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used with three replications.

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 (1947).

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

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

Results and Discussion

A total 66 fungal (26 from root, 15 from stem and 26 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 66 fungal endophytes were evaluated against *S. rolfsii, R. solani* and *F. solani* by dual culture technique to test antifungal activity of the endophytes.

A total of 26 fungal root endophytes were evaluated against three pathogens by dual culture technique and results are presented in Table 1. The maximum mycelial inhibition against *S. rolfsii* was observed by the root endophytic isolates RFBBA-23 (70.59 %) and RFHKM-9 (69.02 %), which were on par with each other. The isolate RFHAR-6 showed the least mycelial inhibition (29.80 %). Isolate RFHKM-9 showed the maximum mycelial inhibition of 60.39 per cent against *R. solani* which was followed by RFBBA- 23 (54.51 %) and RFDHE-10 (51.76). The isolates RFHAR-5, RFDHE-11 and RFBSA-25 did not show any inhibition against *R. solani*. Isolates RFHHO-7 and RFBBA-23 showed maximum mycelial inhibition of 79.22 and 77.65 per cent, respectively against *F. solani* and these were on par with each other. The isolate RFBHU-26 showed the least inhibition (41.96) as compared to other isolates. Overall the isolates RFHHO- 7, RFHKM-9, RFDHE-10 and RFBBA-23 were effective against all the three pathogens and isolates were less effective against *R. solani* as compared to other two pathogens.

A total of 15 fungal stem endophytes were evaluated against three pathogens by dual culture technique and results are presented in Table 2. The maximum mycelial inhibition of *S. rolfsii* was showed by the stem endophytic isolate SFDOF-11 (69.02 %) followed by SFDDE-12 (64.31 %) and isolate SFBHU-15 showed the least mycelial inhibition (23.14 %). Isolate SFDDE-12 showed the maximum mycelial inhibition of 61.96 per cent against *R. solani* followed by SFDDE-12 (54.90 %) and isolates SFDUN-13 and SFBBE-14 did not show any inhibition against *R. solani*. Isolate SFDOF-11 showed the maximum mycelial inhibition of 69.41 per cent against *F. solani* followed by SFDDE-12 (65.10 %) and isolate SFBHU-15 (38.82 %) showed the least mycelial inhibition as compared to other isolates. Overall the isolates SFDOF-11 and SFDDE-12 were effective against all the three pathogens.

A total of 26 fungal leaf endophytes were evaluated against three pathogens by dual culture technique and results are presented in Table 3. The maximum mycelial inhibition of *S. rolfsii* was shown by the leaf endophytic isolate LFDLA-9 (65.10 %) followed by LFDHO-3 (64.31 %) and isolate LFHHO-7 showed the least mycelial inhibition (18.04 %). The maximum mycelial inhibition of *R. solani* was shown by the isolates LFDHO-3 (52.22 %), LFDLA-9 (51.48), LFHCH-13 (50.37), LFHCH-12 (49.63 %) and LFHMU-16 (49.63 %), these were on par with each other and isolate LFHHO-7 did not show any inhibition of *R. solani*.

Table.1 *In vitro* evaluation of fungal root endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani* by dual culture method

Isolate	Per cent inhibition over control		
	S. rolfsii	***R. solani***	***F. solani***
RFDGP-1	41.96 (40.36)*	38.04 (38.06)	51.37 (45.77)
RFDHO-2	40.78 (39.67)	20.39 (26.83)	46.67 (43.07)
RFDHO-3	34.12 (35.73)	17.25 (24.53)	49.80 (44.87)
RFHNI-4	32.94 (35.01)	20.78 (27.11)	46.27 (42.85)
RFHAR-5	36.08 (36.90)	0.00 (0.00)	46.67 (43.07)
RFHKA-6	29.80 (33.07)	23.92 (29.27)	50.98 (45.54)
RFHHO-7	58.04 (49.61)	50.59 (45.77)	79.22 (62.85)
RFDLA-8	38.43 (38.29)	31.37 (34.05)	60.78 (51.21)
RFHKM-9	69.02 (56.17)	60.39 (50.98)	76.08 (60.69)
RFDHE-10	65.10 (53.77)	51.76 (45.99)	76.08 (60.69)
RFDHE-11	38.04 (38.06)	0.00 (0.00)	47.45 (43.52)
RFHBA-12	40.39 (39.44)	35.69 (36.67)	47.45 (43.52)
RFDOF-13	36.08 (36.90)	27.06 (31.33)	52.55 (46.44)
RFDOF-14	33.33 (35.25)	33.33 (35.25)	51.37 (45.77)
RFDDE-45	39.61 (38.98)	25.49 (30.31)	54.51 (47.57)
RFDCH-16	30.98 (33.80)	30.98 (33.81)	56.86 (48.92)
RFDNU-17	36.47 (37.13)	32.55 (34.77)	46.27 (42.85)
RFDVA-18	40.39 (39.44)	29.80 (33.07)	47.45 (43.52)
RFBBE-19	35.69 (36.66)	47.06 (43.30)	58.04 (49.61)
RFBBE-20	38.43 (38.29)	45.88 (42.62)	46.27 (42.85)
RFDUN-21	34.90 (36.20)	37.25 (37.60)	52.16 (46.22)
RFDUN-22	47.06 (43.30)	50.20 (45.09)	62.75 (52.36)
RFBBA-23	70.59 (57.13)	54.51 (47.57)	77.65 (61.76)
RFBBA-24	40.39 (39.44)	28.63 (32.33)	45.49 (42.40)
RFBSA-25	36.47 (37.14)	0.00 (0.00)	63.14 (52.60)
RFBHU-26	30.20 (33.31)	13.33 (21.41)	41.96 (40.36)
S.Em. ±	0.37	0.47	0.33
C.D. at 1%	1.40	1.76	1.24

* Arcsine transformed values

Table.2 *In vitro* evaluation of fungal stem endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani* by dual culture method

Isolate	Per cent inhibition over control		
	S. rolfsii	***R. solani***	***F. solani***
SFDHO-1	41.18 (39.90)*	41.96 (40.36)	56.86 (48.92)
SFHNI-2	35.69 (36.67)	38.43 (38.30)	50.20 (45.09)
SFHKA-3	38.43 (38.30)	36.47 (37.14)	53.33 (46.89)
SFDLA-4	45.88 (42.62)	29.02 (32.58)	46.27 (42.85)
SFDHE-5	36.86 (37.37)	30.98 (33.81)	47.45 (43.52)
SFDHE-6	26.67 (31.08)	45.88 (42.62)	46.27 (42.85)
SFHKM-7	32.94 (35.01)	43.92 (41.49)	47.84 (43.75)
SFHRA-8	29.80 (33.07)	46.27 (42.85)	52.55 (46.44)
SFHBK-9	29.02 (32.58)	24.71 (29.79)	40.00 (39.22)
SFHMU-10	35.69 (36.67)	41.18 (39.90)	46.67 (43.07)
SFDOF-11	69.02 (56.16)	54.90 (47.79)	69.41 (56.40)
SFDDE-12	64.31 (53.30)	61.96 (51.90)	65.10 (53.77)
SFDUN-13	49.02 (44.42)	0.00 (0.00)	44.31 (41.72)
SFBBE-14	32.16 (34.53)	0.00 (0.00)	46.67 (43.07)
SFBHU-15	23.14 (28.74)	8.24 (16.67)	38.82 (38.53)
S.Em. ±	0.28	0.20	0.29
C.D. at 1%	1.09	0.77	1.13

* Arcsine transformed values

Table.3 *In vitro* evaluation of fungal leaf endophytes against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani* by dual culture method

Isolate	Per cent inhibition over control		
	S. rolfsii	***R. solani***	***F. solani***
LFDGP-1	44.71 (41.94)*	37.41 (37.69)	44.31 (41.72)
LFDGP-2	39.61 (38.99)	33.70 (35.47)	40.78 (39.67)
LFDHO-3	64.31 (53.30)	52.22 (46.26)	59.22 (50.29)
LFHNI-4	41.18 (39.90)	9.63 (18.07)	50.59 (45.32)
LFHAR-5	40.78 (39.67)	28.15 (32.03)	47.84 (43.75)
LFHHO-6	43.14 (41.04)	40.74 (39.65)	47.84 (43.75)
LFHHO-7	18.04 (25.12)	0.00 (0.00)	38.04 (38.06)
LFDNA-8	45.88 (42.62)	41.85 (40.29)	42.75 (40.81)
LFDLA -9	65.10 (53.77)	51.48 (45.83)	67.84 (55.43)
LFHKM-10	27.84 (31.84)	36.67 (37.25)	45.88 (42.62)
LFHKM-11	34.51 (35.96)	44.07 (41.58)	46.67 (43.07)
LFHCH-12	27.84 (31.84)	49.63 (44.77)	51.76 (45.99)
LFHCH-13	38.04 (38.06)	50.37 (45.19)	47.84 (43.75)
LFHBA-14	36.86 (37.37)	40.74 (39.65)	43.14 (41.04)
LFHMU -15	35.69 (36.67)	42.59 (40.72)	49.80 (44.87)
LFHMU-16	43.92 (41.49)	49.63 (44.77)	50.20 (45.09)
LFDOF-17	27.84 (31.84)	44.07 (41.58)	51.37 (45.77)
LFDDE-18	21.18 (27.39)	45.19 (42.22)	55.29 (48.02)
LFDKA-19	26.27 (30.82)	34.07 (35.70)	49.41 (44.64)
LFDKA-20	48.24 (43.97)	41.85 (40.29)	47.84 (43.75)
LFDNU-21	29.80 (33.07)	41.85 (40.29)	49.02 (44.42)
LFDUN-22	32.55 (34.77)	37.78 (37.91)	45.10 (42.17)
LFBHA-23	43.14 (41.04)	43.33 (41.15)	47.45 (43.52)
LFBHU-24	40.78 (39.67)	38.15 (38.13)	50.20 (45.09)
LFBSA-25	46.27 (42.85)	45.56 (42.43)	51.37 (45.77)
S.Em. ±	0.37	0.30	0.40
C.D. at 1%	1.39	1.12	1.51

* Arcsine transformed values

Isolate LFDLA-9 showed the maximum mycelial inhibition of 67.84 per cent against *F. solani* followed by LFDHO-3 (59.22 %) and the least mycelial inhibition was observed by LFDGP-2 (40.78 %). Overall the isolates LFDHO-3 and LFDLA-9 were effective against all the three pathogens. Though isolate LFDKA-20 showed less inhibition of all three pathogens in comparison with other isolates, it showed the clear inhibition zone against all the three pathogens. The findings of the present study are in agreement with Narayan *et al.*, (2012) and Ngatia *et al.*, (2015) who evaluated fungal endophytes against chilli pathogens (*Fusarium oxysporum*, *Phytophthora capsici* and *Colletotrichum acutatum)* and tomato pathogen (*P. infestans* causes late blight), respectively by employing dual culture method. Mousa and Raizada (2013) 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. The extent of inhibition of three pathogens by fungal endophytes in dual culture method ranged from 0.00 to 79.22 percent. From the results of present study it is concluded that the possible role of growth inhibition by the endophytes was attributed to the production of diffusible and volatile metabolites, lytic enzymes and by competition with pathogens.

It is concluded that among the 66 fungal isolates, irrespective of isolates from root, stem and leaf, some (RFHHO-7, RFHKM-9, RFDHE-10, RFBBA-23, SFDOF-11, SFDDE-12, LFDHO-3 and LFDLA-9) have shown maximum mycelial inhibition of three pathogens. Isolates RFBBE-19 and RFDUN-22 were fast growing isolates and were more effective against *F. solani* and *R. solani* as compared to *S. rolfsii*. Isolate LFDKA-20 though showed the minimum inhibition against all pathogens as compared to other effective isolates but it also showed the clear inhibition zone which may be due to the production of antimicrobial compounds from the endophyte. From the results of present study it is concluded that the possible role of growth inhibition by the endophytes was attributed to the production of diffusible and volatile metabolites, lytic enzymes and by competition with pathogens.

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