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Isolation and *In Vitro* Screening of Endophytes against Wilt Pathogen in Pomegranate and Guava

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Abstract

Use of beneficial endophytic microbes is one of the best alternative methods due to its eco-friendly and cost-effective approach in sustainable farming. The objective of the study was to isolate the endophytes from healthy pomegranate and guava roots from different locations of Karnataka and screen them for their efficacy against wilt pathogens of pomegranate (*Ceratocystis fimbriata*) and guava (*Fusaruim oxysporum*). Among various endophytic isolates from pomegranate and guava, five bacterial and four fungal endophytes showed antagonistic effect on both wilt pathogens *in vitro*. Per cent inhibition of *C. fimbriata* by bacterial endophytes varied from 61.89 to 72.33 per cent with maximum inhibition by PREB-10 (72.33 %) followed by GREB-13 (71.83 %). Similarly, per cent inhibition of *F. oxysporum* varied from 42.07 to 62.09 per cent with maximum inhibition by PREB-10 (62.09 %) followed by GREB-13 (57.69 %). The per cent inhibition of *C. fimbriata* (CFB) by fungal endophytes ranged from 61.84 to 75.52 per cent with maximum by PREF-11 (75.52 %) followed by GREF-2 (73.61%). The per cent inhibition of *F. oxysporum* (GW-10) ranged from 47.92 to 61.42 per cent with maximum inhibition by GREF-2 (61.42 %) followed by GREF-3 (56.06 %). The effective endophytes *viz*., PREB-10, GREB-13, PREF-11 and GREF-3 were identified as *Bacillus subtilis*, *Paenibacillus polymyxa*, *Trichoderma atroviride* and *Fusarium solani,* respectively.

Keywords : *Ceratocystis fimbriata,* Pomegranate, Endophyte, Guava, *Fusaruim oxysporum*

Pomegranate (*Punica granatum*) and guava (*Psidium guajava*) suffer from diseases caused by fungus, bacteria and nematodes. Among them, fungal pathogens cause devastating diseases and result in yield losses in both crops. The soil borne disease, wilt in pomegranate and guava, is caused by *Ceratocytsis fimbriata* and *Fusarium oxysporum,* respectively. The application of agrochemicals for management of soil borne diseases is practically uneconomical. Besides, the development of pesticidal resistance and residual toxicity are major hurdle for disease management (Gerhardson**,** 2002; Sahu & Brahmaprakash, 2018 and Sonyal *et al*., 2015). Interesting alternative strategy for effective control of soil borne pathogens is the use of biological control agents.

Soil inhabitants potential antagonistic microorganisms which are helpful in reducing the pathogen population through various mode of actions such as competition for food and space (Martin, 1971), mycoparasitism, antibiosis, production of plant growth promoting compounds and production of enzymes (Janisiewicz *et al*., 2000). In recent years several microbes especially endophytes with potential bio-control properties have gained prominence. Plants host diverse group of microbe's *viz*., symbionts, epiphytes and endophytes. Endophytes are microorganisms (fungi or bacteria) that colonize the host tissues and establish a relationship where both partners get a benefit from their interactions (Reiter and Sessitsch, 2006). Some of endophytic fungi and bacteria enter into the plant by colonization and they do not harm them by establishing symbiotic, mutualistic, commensalistic and trophobiotic relationships (Nair and Padmavathy, 2014). Both endophytic bacteria and fungi co-exist in single host and they are acclimatized in host and produce the diverse range of natural products which could be consistent and successful source as drugs.

Therefore, endophytic microbes gain more importance in pharmaceutical, agrochemical and other industries for benefit of mankind.

Endophytes are known to produce some antibiotics, anti-cancer, anti-oxidant and secondary metabolites besides their role in suppressing the antagonistic microbes, plant growth promotion activities and inducing defence mechanism in plant against pathogens. Therefore, an attempt was made to study antagonistic activity of endophytic microorganisms isolated from roots of pomegranate and guava for management of wilt disease.

Material and Methods

Collection of Pathogenic Isolates

Pathogen cultures maintained at Division of crop protection, ICAR-IIHR, Bengaluru were used in the present study. The cultures were identified as *C. fimbriata* and *F. oxysporum* by observing the morphological, cultural, spore and mycelial characteristics.

Pathogenicity Test

Pathogenicity test was carried out on three-month-old highly susceptible seedlings of pomegranate (cv. Bhagwa) and Guava (cv. Allahabad Safed). Nursery was raised in earthen pots (30 × 45 cm) containing potting mixture consisting soils and cowdung at 2:1:1 ratio. The potting mixture was sterilized by using four per cent formaldehyde solution and covered with a polythene sheet for 10 days. It was then kept open for two days with intermittent raking to remove the traces of formaldehyde fumes. This potting mixture was used for raising the nursery and for growing pomegranate and guava seedlings in earthen pots. The inoculum of pathogenic organism *Ceratocytsis fimbriata* for pomegranate and *Fusaruim oxysporum* for guava was prepared by growing them separately in potato dextrose broth (PDB) for 10 days at 30 °C. The pathogens were inoculated to root region of soil at 50 ml / plant having the concentration of 1×10^6 spores/ml (Fig. 1). The same method was replicated thrice with inoculation on other two plants under glasshouse condition. Plants treated with distilled water served as control. The inoculated plants were kept in glass house for further observation. After the development typical symptoms of the disease in the artificially inoculated plants, the disease samples were collected and the organism was re-isolated on potato dextrose agar medium thus confirming the Koch's postulates to establish the pathogen and proving pathogenicity and same pathogens were used for further studies.

Collection and Isolation of Endophytes

Healthy pomegranate and guava root samples were collected from in and around wilt affected plants at farmer's field in different districts of Karnataka. Random sampling was done carefully by cutting the roots of pomegranate and guava. The root samples were collected separately in sterilized polythene bags

Treated Control Treated Control

Fig 1. Pathogenicity test of pomegranate and guava wilt with respective pathogen isolates

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and brought to laboratory and are stored at refrigerator in sterile condition and theses samples were used to isolate both fungal and bacterial endophytes within 6-8 h of collection (Table 1).

TABLE 1

Bacterial and fungal endophytic isolates from root of pomegranate and guava

Endophytes	Crop	Variety	Location
Bacterial endophytes			
GREB-5	Guava	Taiwan Pink	Koppal
GREB-13	Guava	Allahabad safed	GKVK
PREB-4	Pomegranate	Bhagwa	IIHR
PREB-10	Pomegranate	Bhagwa	Chitradurga
GWB	Guava	Allahabad safed	Bellary
Fungal endophytes			
PREF-11	Pomegranate	Bhagwa	GKVK
PREF-21	Pomegranate	Bhagwa	Bellary
GREF-2	Guava	Allahabad safed	Kalatama-nahalli
GREF-3	Guava	Allahabad safed	Dodda-byalkere

GREB- Guava root endophytic bacteria; PREB- Pomegranate root endophytic bacteria; PREF- Pomegranate root endophytic fungus; GREF- Guava root endophytic fungus

Bacterial Endophytes

Collected samples were subjected for isolation of endophytes from internal tissues of different varieties of guava and pomegranate plant roots. Then samples were thoroughly washed in running tap water to remove the adhering soil, then surface sterilized with two per cent sodium hypochlorite for 10 min followed by 70 per cent alcohol for a min and rinsed five times in sterile distilled water. Surface sterility checks were carried out for each sample to monitor the efficiency of the disinfection procedure. For this, 0.1 ml of the last wash was transferred to 9.9 ml SOC broth (super optimal broth with catabolites repression that was comprised of 0.5 per cent yeast extract, two per cent tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$ and 20 mM glucose) incubated at 28 °C on a shaker or spread onto SOC plate for a sterility check. The root tissue samples (1 g) were ground aseptically in phosphate buffer saline (PBS) (g^{-l}) NaCl 8, KCl 0.2, Na_2HPO_4 1.44 and KH_2PO_4 0.24, pH 7.4) and were centrifuged (60 g) at 4 °C for a min. The supernatant was serially diluted up to 10^{-5}, pour plated on SOC medium and incubated at 28 °C for 48 - 72 h. The population of the bacteria in the tissue samples was expressed as colony forming units per g of tissue (CFU^{-g} tissue). The individual bacterial colonies from plate were selected and sub-cultured on SOC medium and stored at -80 °C in 30 per cent sterile glycerol for further studies.

Fungal Endophytes

The same samples were subjected for isolation of fungal endophytes. Then sample were cut into small pieces (2 mm) by a sterilized blade under aseptic conditions. Each sample was surface sterilized by 70 per cent ethanol for one min and after that immersed the plant parts in sodium hypochlorite (NaOCl) solution for 30 sec to one min. The samples were rinsed in sterile distilled water for one min and then allowed to surface dry on filter paper. After proper drying, 4 pieces of plant parts were inoculated in PDA plate supplemented with anti-biotic (streptomycin sulphate) and incubated at 28 ± 10°C for five to seven days. Pure colonies of fungi were transferred on PDA plate. The fungal strains in the pure culture were preserved on potato dextrose agar (PDA) slant at 4 °C further studies.

Screening of Isolated Endophytes for Antagonism against *C. fimbriata* (CFB) and *F. oxysporum* (GW-10)

The isolated representative fungal and bacterial endophytes were tested for their antagonistic reaction against *C. fimbriata* and *F. oxysporum* by adopting simultaneous antagonism method. Two-day-old bacterial culture, 6-day-old fungal cultures were used for *in vitro* evaluation. PDA was used that favored the growth of both antagonists and the pathogen.

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In Vitro Evaluation of Endophytic Bacteria against *C. fimbriata* (CFB) and *F. oxysporum* (GW-10)

A total of 20 each pomegranate and guava bacterial endophytes obtained from different locations were screened against both pathogens by dual culture technique. For the preliminary evaluation one ml of spore suspension was made from both the pathogens isolated from pomegranate and guava, respectively was taken from 10-day-old culture and diluted on mild cool PDA before pouring the plate and after few min 20 ml of medium was poured. Different bacterial cultures were inoculated on one side of petri dish 2 cm away from periphery of as a streak. Plates were incubated at 28 ± 2 ºC for 5-6 days and observed for inhibition of pathogen. The organism that showed antagonistic reactions were selected for further studies.

In Vitro Evaluation of Endophytic Fungus against *C. fimbriata* (CFB) and *F. oxysporum* (GW-10)

Forty-five endophytes isolated from pomegranate and guava were screened against both test pathogens. In the initial screening, one mycelium disc of the pathogen culture was kept in the center of petri-plate and four different isolated endophytes were simultaneously placed at four corners of the plates at equidistant points. These plates were incubated at 28 ± 2 ºC for 8-10 days. The isolates exhibiting antagonism were selected for further studies.

Dual Culture

In vitro screening was carried to test the antagonistic effect of both selected bacterial and fungal endophytes against the wilt pathogens by dual culture assay on the PDA medium. The pure fungal endophytes and both pathogens were cultured on PDA medium in petri-plates for one week prior to setting up the experiment. From the actively growing margins of fungal endophytes and the test pathogens, mycelium discs of 0.5 cm were inoculated in the periphery of the opposite direction of the single petri-plate containing solidified PDA medium. The bacterial endophytes were streaked on these plates on 3rd day after inoculation of the test pathogens. Five replications for bacterial endophytes and four replications for fungal endophytes were maintained with one control by maintaining only pathogen of each and endophyte, respectively. They were incubated at 28 ± 2 ºC. Observations were taken till the full growth in the control Petri-plates. The colony diameters of both the endophyte and the pathogen were measured in both directions and the average was recorded. Per cent inhibition of growth of the wilt pathogens was calculated by using the formula given below by Vincent (1927). Data were analysed statistically by using Completely Randomized Design (CRD) as depicted in Table 3.

$$I = (R1\text{-}R2) \div R1 \times 100$$

Where, I = Per cent inhibition

R1 = Colony diameter (cm) of pathogen in control

R2 = Colony diameter (cm) of pathogen in treatment

Molecular Identification of Selected Fungal and Bacterial Endophytes

Genomic DNA was isolated from the fungal mycelial mass grown in PDB for 10 days by using the method as described by Vainio *et al.* (1998). *ITS1* and *ITS4* primers were used to amplify the different regions (18 S and 28 S rRNA genes) of the internal transcribed spacer region. The amplified product of approximately 600bp was sequenced and obtained data was analysed by blasting the sequences in NCBI Gen Bank. The fungal species was identified by looking at the nearest match or homology. Similarly, identification of endophytic bacteria inoculated in nutrient broth for 24 h in rotatory shaker carried out by amplifying 16s rRNA using universal primers at 1500bp (Srinivasa *et al*., 2012).

Results and Discussion

Isolation of Endophyte Population from Collected Samples

The endophytic microorganisms were isolated from roots samples of pomegranate and guava which were collected from different locations of Karnataka. The

endophytes isolated from samples collected from different locations varied significantly among them. From the isolated microbial population, the predominant isolates of microorganisms consisting of 20 each bacterial endophytes from pomegranate and guava, 30 and 15 fungal endophytes were selected from pomegranate and guava and preliminary *in vitro* screening was carried out.

Molecular Identification of Endophytes

Based on the results of molecular identification, endophytic bacteria were tentatively identified as *Bacillus subtilis* (GREB-5), *Bacillus subtilis* (PREB-10), *Paenibacillus polymyxa* (GREB-13), *Lysinibacillus* spp. (PREB-4) and *Bacillus paralicheniformis* (GWB). The endophytic fungi were tentatively identified as *Trichoderma atroviridae* (PREF-11), *Chaetomium* spp. (PREF-21), *Fusarium solani* (GREF-2) and *Talaromyces* spp. (GREF-3).

TABLE 2

Efficacy of bacterial endophytes against *C. fimbriata* (CFB) and *F. oxysporum* (GW-10) under dual culture

Isolate	Genera	Per Cent Inhibition*	
		CFB	GW-10
GREB-5	*Bacillus subtilis*	61.89 (51.86)	43.44 (41.21)
GREB-13	*Paenibacillus polymyxa*	71.83 (57.92)	57.69 (49.40)
PREB-4	*Lysinibacillus* spp.	62.02 (51.94)	51.30 (45.72)
PREB-10	*Bacillus subtilis*	72.33 (58.24)	62.09 (51.98)
GWB	*Bacillus paralicheniformis*	65.18 (53.81)	42.07 (40.42)
Control	-	100 (90.00)	100 (90.00)
SE(m)		0.273	0.239
C.D. @ 1%		0.801	0.701

*Mean five replications, Figures in parenthesis are arcsine transformed

***In Vitro* Evaluation of Endophytic Bacterial against *Ceratocystis fimbriata* (CFB) and *Fusarium oxysporum* (GW-10)**

Among each 20 bacterial endophytes isolated from pomegranate and guava screened, three isolates from guava and two isolates from pomegranate showed antagonistic reaction against respective pathogens (Table 2). The per cent inhibition of *C. fimbriata* varied from 61.89 to 72.33 per cent. The maximum percent inhibition of *C. fimbriata* was exhibited by PREB-10 (72.33 %) followed by GREB-13 (71.83 %) and GWB (65.18 %) and the lowest inhibition was exhibited by GREB-5 (61.89 %). The per cent inhibition of *F.oxysporum* was varied from 42.07 to 62.09 per cent. The maximum per cent inhibition of *F. oxysporum* was exhibited by PREB-10 (62.09 %) followed by GREB-13 (57.69 %) and PRE-4 (51.30%) and the lowest inhibition was by GWB (42.07 %).

***In Vitro* Evaluation of Endophytic Fungal Endophytes against *C. fimbriata* (CFB) and *F. oxysporum* (GW-10)**

Out of 30 and 15 selected fungal endophytes isolated from pomegranate and guava tested, each two isolates from pomegranate and guava showed antagonistic property against both pathogens and results were represented (Table 3). It is found that the per cent inhibition of *C. fimbriata* (CFB) was ranged from 61.84 to 75.52. The maximum percent inhibition of *C. fimbriata* was exhibited by PREF-11 (75.52 %) followed by GREF-2 (73.61 %) and the other isolates GREF-3 and PREF-21 showed antagonistic activity of 71.99 and 61.84 per cent inhibition. The per cent inhibition of *F. oxysporum* (GW-10) was ranged from 47.92 to 61.42 per cent with maximum inhibition by GREF-2 (61.42 %) followed by GREF-3 (56.06 %).

Plants are in continuous associations with microbes and microbes interact with plants in positive, neutral ways and enhance the host survival under harsh environmental situations. Most of the microbes act as symbionts in the host plants. Till now it has been reported that at least one or more endophytes reside in every three lakh plant species that exist on the earth. But only six to seven per cent of the endophytes

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TABLE 3

Efficacy of fungal endophytes against *C. fimbriata* (CFB) and *F. oxysporum* (GW-10) under dual culture

Isolate	Genera	Per Cent Inhibition*	
		CFB	GW-10
PREF-11	Trichoderma atroviridae	75.52 (60.32)	47.92 (43.793)
PREF-21	Chaetomium spp.	61.84 (51.83)	53.25 (46.848)
GREF-2	Fusarium solani	73.61 (59.06)	61.42 (51.582)
GREF-3	Talaromyces spp.	71.99 (58.02)	56.06 (48.46)
Control		100 (90.00)	100 (90.00)
SE(m)		0.222	0.254
C.D. @ 1%		0.677	0.772

*Mean four replications, Figures in parenthes is are arc sine transformed

existence has been known (Gupta *et al*., 2019). There are some endophytes which are commonly present in all plant parts, including roots (Chen *et al*., 2019), root nodules (Martinez-Hidalgo *et al*., 2015), seeds (Gond *et al*., 2015) and stems (Chung *et al*., 2015). Generally, microbes enter into plant tissues through natural openings like stomata, lenticels, wounds, germinating radicles, etc. The major sites of colonization are the intercellular spaces of the epidermal and cortical regions and lysed plant cells (Gupta *et al*., 2019). Use of beneficial microorganisms such as endophytes has been gaining much attention due to its eco-friendly and cost-effective approach. *In vitro* studies are useful for identifying effective endophytes as biocontrol agents and understanding the mechanisms by which they inhibit the pathogens (Mejia *et al*., 2008). In this study endophytic isolates were obtained and screened for *in vitro* inhibitory effect on wilt pathogens in pomegranate and guava plants. Of the 20 bacterial endophytes isolated, each from pomegranate and guava and screened, three isolates from guava and two isolates from pome granate showed antagonism ranging from 61.89 to 72.33 per cent inhibition against test pathogen with maximum being observed with bacterial endophyte PREB-10. Inhibition of *F. oxysporum* ranged from 42.07 to 62.09 per cent with maximum inhibition by GREB-13 (57.69 %). Recently the endophytic strains of *B. subtilis* have been used as bio-control agents for diseases caused by fungi and bacteria (Zeriouh *et al*., 2011). *B. subtilis* also demon-strated *in vitro* antagonistic activity against various species of *Fusarium* and the component responsible for the antifungal activity was identified as fengycin, a lipopeptide commonly produced by *B. subtilis* (Rebib *et al*., 2012). Similarly, several researchers have reported the use of endophytic bacteria for controlling many soil borne pathogens such as *Sclerotium rolfsii*, *Colletotrichum capsici*, *Pythium* sp., *Verticillium dahlia* and *Fusarium oxysporum* (Nandhini *et al.,* 2012). *In vitro* evaluation of endophytic bacteria *i.e., Bacillus subtilis* and *Pseudomonas fluorescens* against root and pod rot diseases of peanut caused by *Aspergillus niger* and *Fusarium oxysporum* showed inhibition to pathogens (Ziedan, 2006).

In the screening of 45 fungal endophytes isolated from pomegranate and guava and screened, two isolates from guava and two isolates pomegranate fungal endophytes exhibited antagonism of *C. fimbriata* (CFB) with 61.84 to 75.52 per cent inhibition and 75.52 per cent inhibition was observed by PREF-11 followed by GREF-2 (73.61 %). Similarly, the per cent inhibition of *F. oxysporum* was ranged from 47.92 to 61.42 per cent with maximum antagonism by GREF-2 (61.42%) followed by GREF-3 (56.06%). Many workers have reported the antagonistic ability of endophytic fungi against different pathogens. Haiyan *et al.* (2005) isolated 130 endophytic fungi from Chinese medicinal plants and evaluated, among these isolates, only 30 per cent exhibited antagonistic activity against soil borne pathogens. Similarly, Abro *et al*. (2019) isolated 30 endophytic fungal species from various plants and studied *in vitro* dual culture assay against *F. oxysporum* f. sp. *cucumerinum*, causing wilt in cucumber and all the endophytic fungal isolates were highly capable of inhibiting the mycelial growth of pathogen with inhibition of 66 per cent as compared to control treatments. (Yuan *et al*., 2017) also reported that endophytic *Pencillium*

simplicissimum CEF-818 isolated from cotton showed *in vitro* antagonism of *Verticillium dahliae* in cotton. Kim *et al.* (2007) observed that endophytic fungi isolated from vegetable plants showed *in vitro* antagonism against *Pythium multimum*, *P. infestans* and *P. capsici*.

It is necessary for the utilization of endophytic microorganisms and their novel bioactive metabolites to be explored for their potential exploitation in sustainable way of agriculture farming. The results from *in vitro* assays suggest that endophytes isolated from pomegranate and guava plants have the potential role in controlling soil borne pathogens causing wilt disease and thereby increasing plant growth. Hence, microbial inoculants capable of inducing systemic resistance in host plants and providing more resistance against pathogenic infection can be used in sustainable agriculture.

REFERENCES

ABRO, M. A., SUN, X., LI, X., JATOI, G. H. AND GUO, L. D., 2019, Bio control potential of fungal endophytes against *Fusarium oxysporum* f. sp. *cucumerinum* causing wilt in cucumber. *The Plant Pathol. J.*, **35** (6) : 598.

CHEN, L., SHI, H., HENG, J., WANG, D. AND BIAN, K., 2019, Antimicrobial, plant growth-promoting and genomic properties of the peanut endophyte *Bacillus velezensis* LDO2. *Microbiol. Res.*, **218** : 41 - 48.

CHUNG, E. J., HOSSAIN, M. T., KHAN, A., KIM, K. H., JEON, C. O., CHUNG, Y. R., 2015, *Bacillus ory-zicola* sp. nov., an endophytic bacterium isolated from the roots of rice with antimicrobial, plant growth promoting and systemic resistance inducing activities in rice. *Plant Pathol. J.*, **31** (2) : 152.

GERHARDSON, B., 2002, Biological substitutes for pesticides. *Trends in Biotechnol.*, **20** : 338 - 43.

GOND, S. K., BERGEN, M. S., TORRES, M. S. AND WHITE JR, J. F., 2015, Endophytic *Bacillus* spp. produces anti fungal lipopeptides and induces host defence gene expression in maize. *Microbiol. Res.*, **172** : 79 - 87.

GUPTA, A., VERMA, H., SINGH, P. P., SINGH, P., SINGH, M., MISHRA, V. AND KUMAR, A., 2019, Rhizome endophytes: roles and applications in sustainable agriculture. In: Verma, S., White, Jr., J. (Eds.), Seed Endophytes. Springer, Heidelberg, pp. : 405 - 421.

HAIYAN, L. I., QING, C., ZHANG, Y., ZHAO, Z., 2005, Screening for endophytic fungi with anti-tumour and antifungal activities from Chinese medicinal plants. *World J. Microbiol. Biotechnol.*, **21** : 1515 - 1519.

JANISIEWICZ, N., FRAZIER, K., JUDIA, H. AND SUMATHY, V., 2000, Isolation and characterization of *Bacillus subtilis* isolated from soil. *Afric. J. Biotec.*, **17** (49) : 36 - 44.

KIM, H. Y., CHOI, G. J., LEE, H. B., LEE, S. W., LIM, H. K., JANG, K. S., SON, S. W., LEE, S. O., CHO, K. Y., SUNG, N. D. AND KIM, J. C., 2007, Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. *Lett. Appl. Microbiol.,* **44** : 332 - 337.

MARTIN, J. K., 1971, 14^{C} labelled material leached from the rhizosphere of plants supplied with 14^{CO2}. *Aust. J. Biol. Sci.*, **24** : 1131 - 1142.

MARTINEZ-HIDALGO, P., GARCIA, J. M. AND POZO, M. J., 2015, Induced systemic resistance against *Botrytis cinerea* by *Micromonospora* strains isolated from root nodules. *Front in Microbiol.*, **6** : 922.

MEJIA, L. C., ROJAS, E. I., MAYNARD, Z., VAN BAEL, S., ARNOLD, A. E., HEBBAR, P., SAMUELS, G. J., ROBBINS, N. AND HERRE, E. A., 2008, Endophytic fungi as bio-control agents of *Theobroma cacao* pathogens. *Biological Control.*, **46** (1) : 4 - 14.

NAIR, D. N. AND PADMAVATHY, S., 2014, Impact of endophytic microorganisms on plants, environment and humans. *The Sci. World. J.*, **44** : 12 - 15.

NANDHINI, S., SENDHILVEL, V. AND BABU, S., 2012, Endophytic bacteria from tomato and their efficacy against *Fusarium oxysporum* f. sp. *lycopersici*, the wilt pathogen. *Journal of Biopesticides*, **5** (2) : 178.

REBIB, H., HEDI, A., ROUSSET, M., BOUDABOUS, A., LIMAM, F. AND SADFI-ZOUAOUI, N., 2012, Biological control of fusarium foot rot of wheat using fengycin-producing *Bacillus subtilis* isolated from salty soil. *Afri. J. of Biotechnol.*, **11** (34) : 8464 - 8475.

REITER, B. AND SESSITSCH, A., 2006, Bacterial endophytes of the wildflower *Crocus albiflorus* analyzed by characterization of isolates and by a cultivation independent approach. *Can. J. Microbiol.*, **52** : 140 - 149.

SAHU, P. K. AND BRAHMAPRAKASH, G. P., 2018, Modified liquid dual culture methodology for screening bacterial endophytes against fungal pathogens. *Mysore J. Agric. Sci.*, **52** (2) : 234 - 240.

SONYAL, S., RAVICHANDRA, N. G., REDDY, B. M. R., 2015, Efficacy of bioagents on *Ceratocystis fimbriata* and *Meloidogyne incognita* wilt complex in pomegranate. *Mysore J. Agric. Sci.,* **49** (2) : 350 - 354.

SRINIVASA, C., SHARANAIAH, U. AND SHIVAMALLU, C., 2012, Molecular detection of plant pathogenic bacteria using polymerase chain reaction single-strand conformation polymorphism. *Acta Biochim Biophys Sin.*, **44** (3) : 217 - 223.

VAINIO, E. J., KORHONEN, K. AND HANTULA, J., 1998, Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers. *Mycological research.*, **102** (2) : 187 - 192.

VINCENT, J. M., 1927, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159** : 850.

YUAN, Y., FENG, H., WANG, L., LI, Z., SHI, Y., ZHAO, L., FENG, Z. AND ZHU, H., 2017, Potential of endophytic fungi isolated from cotton roots for biological control against verticillium wilt disease. *PLoS One*, **12** (1) : p.e0170557.

ZERIOUH, H., ROMERO, D., GARCIA-GUTIERREZ, L., CAZORLA, F. M., DE VICENTE, A. AND PEREZ-GARCIA, A., 2011, The Iturin-like lipopeptides are essential components in the biological control arsenal of *Bacillus subtilis* against bacterial diseases of cucurbits. *Mol. Plant-Microbe Interact.,* **24** : 1540 - 1552.

ZIEDAN, E. H. E., 2006, Manipulating endophytic bacteria for biological control to soil borne diseases of peanut. *J. Appl. Sci. Res.*, **2** (8) : 497-502.

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