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Effect of fungal endophytes of soybean (Cv. JS-335) on growth parameters and charcoal rot disease incidence of soybean

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Abstract
Use of chemicals for growth enhancement and disease control in plants has resulted in hazardous influences to the environment and human health. Therefore, less harmful methods should be implemented and the possibility of using microbes for this purpose has been investigated. Endophytic fungal assemblages have been known to enhance plant growth and decrease disease incidence in some crops including soybean and thus can be used as an alternative to chemicals. A total seven effective fungal endophytes *in vitro*, which were evaluated to assess their efficacy against *M. phaseolina*, by sick soil method, in polybags, under screen house condition, they also influenced on growth parameter of soybean (Cv. JS-335), thereby improving seed germination, root length, shoot length and seedling vigour index (SVI) in soybean. In the present study, the results were obtained on per cent mortality (pre-, post emergence and average) and reductions, over untreated control. Fungal endophytes *viz*., *Paecilomyces lilacinus, Aspergillus niger, Fusarium oxysporum* and *Penicillium* sp. were found most effective in reducing the mortality (PRESR, POESM and average) over untreated control, against *M. phaseolina* in soybean.

Keywords: Seed germination, Root length, Shoot length, Seedling vigour index (SVI)

Introduction

Soybean (*Glycine max* (L.) Merril) is one of the most important leguminous and extensively used as oilseed crop, grown in India. Among many soybean growing countries, India ranks fifth in respect of production and the major states growing soybean are *viz*., Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh and Karnataka. However, the production and productivity of soybean has been reducing day by day, for which diseases caused by fungi, bacteria, viruses and phytoplasma's, etc. are responsible.

Among varied fungal diseases infecting soybean, root rot / charcoal rot / stem rot caused by *Macrophomina phaseolina*, is one of the most devastating fungal disease, affecting the crop at all stages of crop growth but generally, it infects at post flowering stage. This disease of soybean has been reported to cause 70 per cent yield losses in India (Kumar *et al*., 2019) [11]. The overall average root rot incidence of soybean was 26.16 per cent during *kharif*, 2017-18 in the Marathwada region of Maharashtra state (Agale *et al*., 2018) [2]. When temperature is between 28^0 C- 32^0 C, the disease cycle for *M. phaseolina* begins with germination of microsclerotia. Usually, symptoms in adult plants are brown and red lesions on roots and stem and produces dark mycelia and black microsclerotia. The stem shows longitudinal dark lesions and plant becomes defoliated and wilted.

Nearly all plants that existing in the World were found to be related with endophytes. The term "endophyte" is derived from the Greek word "Endon" means within and "Phyte" means plant. This term was first used by Anton De Bary in 1866. Endophytes are the microbe that is residing in plant tissues without causing harmful effects to the host plants. They are everywhere in most plant species, especially in field grown plants and found in mainly all vascular plant species examined and includes fungi, bacteria and actinomycetes. Under natural conditions, endophytes promote plant growth by using various mechanisms. These include phosphate solubilization activity, indole acetic acid (IAA) production and therefore the production of a siderophore. A kind of other beneficial effects on growth of the plants are attributed to endophytes and that includes, the stomatal regulation, osmotic adjustment, modification of root morphology, enhanced intake of minerals and changes of nitrogen accumulation and also metabolism (Compant *et al*., 2005) [5]. Endophytes have the capacity to produce antibiotics, that act as antifungal, antibacterial, antiviral and insecticidal properties, which strongly inhibit growth of other microorganisms, including plant pathogens. So, endophytic fungal assemblages have been known to increase plant growth and reduce disease incidence in some crops including soybean and thus, can be used as an alternative to chemicals. Therefore, this study was subjected to the endophytic fungal communities associated with soybean crop with a view to examine the possibility of using them for plant growth enhancement and management of charcoal rot disease incidence.

Materials and Methods

Isolation and identification of endophytic fungi

All the sterilized / disinfected segments of each plant parts (stem, roots and leaves) were placed on Potato dextrose agar (PDA) medium supplemented with streptocycline (50µg/ml) to inhibit bacterial growth. Plates were sealed with parafilm to prevent desiccation of the medium and incubated in BOD incubator at 27^0 C for 6 to 7 days. The fungal growth was continuously observed. As soon as growth was observed, the hyphal tips were transferred to fresh PDA medium to enhance typical sporulation for better identification. Pure cultures were preserved on PDA slant maintained at 80^0 C with proper tags.

Cultures on PDA media were evaluated according to their morphology, mycelium colour, colony appearance and structure, shape of conidiophore and conidia (shape, color, etc.) and characters of conidiogenous cells were observed using a stereo-binocular microscope with 5X, 10X, 40X and 100X objective lenses for magnification. Also, lactophenol or lactophenol blue stains were used to study the characteristics of spores (Barnett and Hunter, 1998; Sutton, 1980) [4, 20]. Also, authoritative monographs and other taxonomic papers analogous to certain genera and species of endophytes were referred for identification of isolated endophytic fungi. Therefore, isolates were identified on the basis of morpho-cultural and microscopic characteristics and for isolates, those would not identified at Department of Plant Pathology, College of Agriculture, Latur, that were identified and confirmed by Division of Mycology, I.T.C.C., IARI, New Delhi. The identified fungal isolates were used for further studies.

Evaluation of fungal endophytes (pot culture)

Seven effective fungal endophytes were thus selected based on results of *in vitro* experiment and used for further studies. The effective fungal endophytes were tested against charcoal rot disease causing, *M. phaseolina*, in pot culture experiment, under screenhouse conditions. Sand: maize medium was used for mass multiplication of *M. phaseolina*. Sand: maize medium (3 part partially broken grain + one-part sand + distilled water to moisten the medium) was prepared, filled into polypropylene bags (9 × 12 cm) and autoclaved at 20 lbs pressure for 30 min, for two consecutive days. After autoclaving cooled at room temperature, then sand: maize medium in the bags were inoculated with 8 - 10 mycelial discs (5 mm dia.) obtained from 6 to 7 days old pure culture of *M. phaseolina* and inoculated at room temperature, for 14 to15 days. Black colored nursery polybags (size 20×30 cm) filled with the autoclaved potting mixture of soil : sand (3:1) was inoculated (each @ 50g / Kg mixture) with the test pathogen (*M. phaseolina*) mass multiplied (sand : maize medium) culture, watered lightly and incubated in screenhouse for two weeks, so as to proliferate the pathogen and made the potting mixture sick. After 14 to 15 days of incubation, these polybags were sown with soybean seeds, which were sterilized using (2 - 3% sodium hypochlorite solution, for 5 min.), soaked in the potato dextrose broth of mass multiplied with each effective endophytic fungi for 10 to 12 hours. Then, such seeds were sown (10 seeds / bag), watered regularly and maintained in the screenhouse. In the control treatment, seeds submersion was performed using sterilized distilled water was maintained as untreated control.

Experimental details

Design : C.R.D (Completely Randomised Design)

Replications : Four

Treatments : Eight

Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Aspergillus niger*	T_5	*Paecilomyces lilacinus*
T_2	*Curvularia lunata*	T_6	*Penicillium* sp.
T_3	*Nigrospora sphaerica*	T_7	*Phomopsis* sp. 2
T_4	*Fusarium oxysporum*	T_8	Control (untreated)

Observations on seed germination, pre-emergence seed rot (PRESR) and post-emergence seedling mortality (POESM) were recorded, respectively at seven days and thirty days after sowing and average mortality was calculated. Per cent seed germination, PRESR, POESM and average mortality were enumerated by following formulae.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{\% Pre-emergence mortality} = \frac{\text{No. of seeds un-germinated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{\% Post-emergence mortality} = \frac{\text{No. seedlings died}}{\text{Total no. of seedlings emerged}} \times 100$$

% Av. mortality = % PRESR + % POESM / 2

Further, per cent disease control / reduction over untreated control was calculated by applying following formula

$$\text{\% Disease control} = \frac{C - T}{C} \times 100$$

Where, C = per cent average mortality (pre- and post emergence) in treatment polybags.

T = per cent average mortality (pre- and post emergence) in untreated control polybags.

Observations on root length and shoot length (cm) were noted at 30 days after sowing. For the purpose, five soybean seedlings / treatment / replication were uprooted gently, washed under gentle flow of tap water and air dried. Root length was measured from collar region to tip of the tap / main root and shoot length from collar region to tip of the seedling / plant. Also, seedling vigour index (SVI) was calculated by following formula.

SVI = per cent seed germination x (Root length + shoot length in cm).

Results and Discussion

Isolation and identification

A total of 14 endophytic fungi isolated from soybean plant samples (leaves, stems and roots), five isolates from leaves, five isolates from stems and four isolates from roots were obtained. Amongst them, isolated from the leaves were *Curvularia lunata*, *Cladosporium cladosporioides*, *Nigrospora sphaerica*, *Penicillium* sp. and *Paecilomyces lilacinus* and from stems were *Alternaria alternata*, *Phomopsis* sp. 1, *Rhizoctonia* sp., *Phomopsis* sp. 2 and *Macrophomina phaseolina*. From roots were *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus* sp. and *Chaetomium* sp., respectively.

Isolated fungal strains such as *Aspergillus niger*, *Aspergillus* sp., *Fusarium oxysporum*, *Chaetomium* sp., *Curvularia lunata*, *Cladosporium cladosporioides*, *Penicillium* sp. and *Alternaria alternata*, were identified at Department of Plant Pathology, College of Agriculture, Latur by observing morpho-cultural and microscopic characteristics such as colony appearance, mycelium color and structure, shape of conidia and conidiophore (color, shape, etc.) and characters of conidiogenous cells using stereo-binocular microscope. Also its authoritative monographs and other taxonomic papers relating to particular genera as well as species of endophytes were referred for identification of fungal endophytes. Fungal isolates such as *Nigrospora sphaerica*, *Paecilomyces lilacinus*, *Phomopsis* sp. 1, *Rhizoctonia* sp., *Phomopsis* sp. 2 and *Macrophomina phaseolina* were identified and confirmed by Division of Mycology, I.T.C.C., IARI, New Delhi. The identified fungal isolates were used for further studies.

These results were in conformity with several earlier workers (Sutton, 1980; Miller and Roy, 1982; Piemental *et al*., 2006; Seifert *et al*., 2011; Anitha *et al*., 2013; Dalal *et al*., 2014) [20, 13, 14, 17, 3, 6].

Efficacy of endophytic fungi (pot culture)

A total seven effective fungal endophytes *in vitro* were evaluated as described earlier in materials and methodology to assess their efficacy against *M. phaseolina*, by sick soil method, in polybags, under screen house condition. The results were obtained for per cent mortality (pre-, post emergence and average) and reductions, over untreated control are presented in Table 1 and represented in PLATE 1 and fig. 1.

Pre-emergence seed rot (PRESR)

The results (PLATE 1, Table 1 and Fig. 1a) revealed that all of the test fungal endophytes significantly influenced pre-emergence seed rot (PRESR) which were ranged from 10.00 to 21.65 per cent, as against 40.00 per cent in untreated control. However, it was significantly least with *Paecilomyces lilacinus* (10.00 %), followed by *Aspergillus niger* (12.00 %), *Fusarium oxysporum* (15.00 %) and *Penicillium* sp. (16.00 %), respectively. Rest of the fungal endophytes resulted with comparatively maximum PRESR in the range of 20.00 to 21.65 per cent, but it was significantly highest in untreated control (40.00 %).

Post-emergence seedling mortality (POESM)

Similar trend as that of pre-emergence seed rot (PRESR) was observed in respect of post-emergence seedling mortality (POESM), which ranged from 12.40 to 25.00 per cent, as against 44.55 per cent in untreated control. However, it was significantly least with *Paecilomyces lilacinus* (12.40 %), followed by *Aspergillus niger* (15.65 %), *Fusarium oxysporum* (18.32 %) and *Penicillium* sp. (20.64 %), respectively. Rest of the fungal endophytes resulted with comparatively maximum POESM in the range of 24.18 to 25.00 per cent, but it was significantly highest in untreated control (44.55 %).

Table 1: Effect of fungal endophytes on mortality caused by *M. phaseolina* in soybean

Tr. No.	Treatments	Seed Ger-mination* (%)	Rot/ mortality (%)*		Av. Mort. (%)	(%) Reduction*		Av. Redn. (%)
			PRESR	POESM		PRESR	POESM	
T_1	*Aspergillus niger*	88.00 (69.73)	12.00 (20.26)	15.65 (23.30)	13.82 (21.82)	70.00 (56.78)	64.87 (53.65)	67.43 (55.20)
T_2	*Curvularia lunata*	79.66 (63.19)	20.34 (26.80)	24.18 (29.45)	22.26 (28.15)	49.15 (44.51)	45.72 (42.54)	47.43 (43.52)
T_3	*Nigrospora sphaerica*	78.35 (62.27)	21.65 (27.72)	24.89 (29.92)	23.27 (28.84)	45.87 (42.63)	44.13 (41.62)	45.00 (42.13)
T_4	*Fusarium oxysporum*	85.00 (67.21)	15.00 (22.78)	18.32 (25.34)	16.66 (24.08)	62.50 (52.23)	58.87 (50.10)	60.68 (51.16)
T_5	*Paeciliomyces lilacinus*	90.00 (71.56)	10.00 (18.43)	12.40 (20.61)	11.20 (19.55)	75.00 (60.00)	72.16 (58.15)	73.58 (59.06)
T_6	*Penicillium* sp.	84.00 (66.42)	16.00 (23.57)	20.64 (27.02)	18.32 (25.34)	60.00 (50.76)	53.67 (47.10)	56.83 (48.92)
T_7	*Phomopsis* sp. 2	80.00 (63.43)	20.00 (26.56)	25.00 (30.00)	22.50 (28.31)	50.00 (45.00)	43.88 (41.48)	46.94 (43.24)
T_8	Control	60.00 (50.76)	40.00 (39.23)	44.55 (41.87)	42.27 (40.55)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE ±	0.84	0.79	0.81	-	0.74	0.64	-
	CD (P=0.01)	2.46	2.34	2.39	-	2.18	1.89	-

Average mortality influenced by the test fungal endophytes was ranged from 11.20 to 23.27 per cent. However, it was significantly least with *Paecilomyces lilacinus* (11.20 %), followed by *Aspergillus niger* (13.82 %), *Fusarium oxysporum* (16.66 %) and *Penicillium* sp. (18.32 %). Rest of the test fungal endophytes recorded average mortality in the range of 22.26 to 23.27 per cent, but it was significantly highest in untreated control (42.27 %).

Reduction in mortality (pre-, post- and average mortality)

All of the fungal endophytes tested (Table 1 and Fig. 1b) were found effective in reducing pre-emergence, post-emergence and average mortality, over untreated control. However, the most effective fungal antagonistic endophyte found was *Paecilomyces lilacinus*, with significantly highest reduction in PRESR (75.00 %), POESM (72.16 %) and average mortality reduction (73.58 %). This was followed by *Aspergillus niger* (70.00 %, 64.87 % and 67.43 %, respectively), *Fusarium* *oxysporum* (62.50 %, 58.87 % and 60.68 %, respectively) and *Penicillium* sp. (60.00 %, 53.67 % and 56.83 %, respectively). Rest of the test fungal endophytes recorded reductions of PRESR in the range of 45.87 to 50.00 %, POESM in the range of 43.88 to 45.72 % and average mortality in the range of 45.00 to 47.43 per cent.

In the present study, fungal endophytes *viz*., *Paecilomyces lilacinus*, *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium* sp. were found most effective in reducing the mortality (PRESR, POESM and average) over untreated control, against *M. phaseolina* in soybean.

Similarly, antifungal effects of various fungal endophytes against a number of seed or soilborne plant pathogens or diseases, including charcoal / root / stem rots in many crops were reported earlier by several workers. Lahlali and Hijri (2010) [12] reported effectiveness of endophytic fungi *Trichoderma atroviride* against *Rhizoctonia solani*, in potato. Deepa and Sally (2015) [8] evaluated and reported *in vivo* efficacy of seventeen endophytic fungi isolated from tomato against bacterial wilt (*R. solanacearum*) under pot culture. Urooj *et al*. (2018) [8] reported the effect of endophytic *Penicillium* species on root rotting fungi on sunflower in earthen pots in screenhouse and reported that, *P. lividum* alone showed no infection of *M. phaseolina* on sunflower roots. Zuhria *et al*. (2018) [23] evaluated *in vivo* efficacy of 15 species of endophytic fungi from soybean against *S. rolfsii* and reported that, *Trichoderma* sp. showed lowest disease incidence (21.25%). Priyadarshini *et al*. (2018) reported the effectiveness of endophytic fungi such as *Trichoderma* sp.1 and *Chaetomium* sp. of rice against *Bipolaris oryzae*. Sudarma *et al*. (2019) [19] also evaluated *in vivo* efficacy six exophytic and endophytic fungi isolated from sugar apple against *Lasiodiplodia theobromae* and reported highest inhibition ability in endophytic fungi of leaves 4 (*Aspergillus* sp.) and fruit exophytic 5 (*A. niger*) with 0% infection. Wati *et al*. (2019) [22] evaluated *in vivo* efficacy of five endophytic fungi isolated from *Artemisia annua* L. against *R. solani* and reported the highest inhibition ability in *Phoma* sp.

Plate 1: Efficacy of fungal endophytes against *M. phaseolina*, causing charcoal rot of soybean (Cv. JS-335)

Effect of fungal endophytes on growth parameters

Results (Table 2) revealed that, all of the fungal endophytes tested for the management of *M. phaseolina* (polybag culture) also influenced on growth parameters of soybean (Cv. JS-335), thereby improving seed germination, root length, shoot length and seedling vigour index (SVI) in soybean.

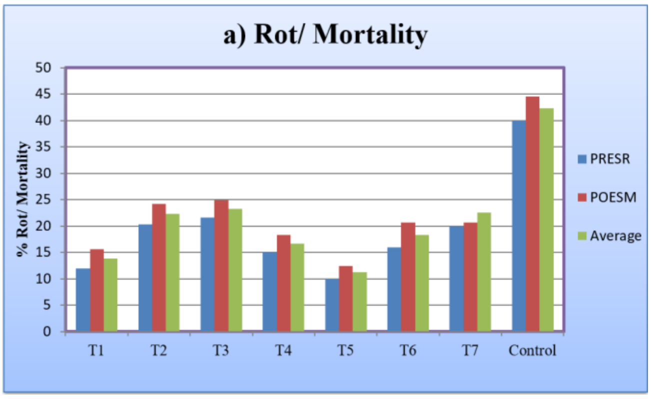
However, the *Paecilomyces lilacinus* resulted with significantly highest seed germination (90.00 %), root length (12.59 cm), shoot length (23.12 cm) and SVI (3213.9). This was followed by *Aspergillus niger* (88 %, 12.35 cm, 22.49 cm and 3065.92, respectively), *Fusarium oxysporum* (85 %, 11.54 cm, 21.56 cm and 2813.5, respectively) and *Penicillium* sp. (84 %, 11.90 cm, 19.28 cm and 2619.12, respectively). Rest of the fungal endophytes, were also found effective in improving plant growth parameters of soybean. Whereas, untreated control showed significantly minimum seed germination (60.00%), root length (08.45 cm), shoot length (14.49 cm) and (SVI) (1376.4).

Similar results regarding effect of fungal endophytes on growth parameters in many crops were reported earlier by several workers. (Senthilkumar *et al*., 2009; Urooj *et al*., 2018; Priyadarshani *et al*., 2018; Abro *et al*., 2019) [18, 8, 16, 1].

Table 2: Effect of fungal endophytes on growth parameters in soybean

Tr. No.	Treatments	Av. Mort. (%)	Seed Germination* (%)	Root length (cm)*	Shoot length (cm)*	SVI*
T_1	*Aspergillus niger*	13.82 (21.82)	88.00 (69.73)	12.35	22.49	3065.92
T_2	*Curvularia lunata*	22.26 (28.15)	79.66 (63.19)	10.50	21.20	2525.22
T_3	*Nigrospora sphaerica*	23.27 (28.84)	78.35 (62.27)	12.23	22.35	2709.34
T_4	*Fusarium oxysporum*	16.66 (24.08)	85.00 (67.21)	11.54	21.56	2813.5
T_5	*Paeciliomyces lilacinus*	11.20 (19.55)	90.00 (71.56)	12.59	23.12	3213.9
T_6	*Penicillium* sp.	18.32 (25.34)	84.00(66.42)	11.90	19.28	2619.12
T_7	*Phomopsis* sp.2	22.50 (28.31)	80.00 (63.43)	10.40	22.23	2610.4
T_8	Control	42.27 (40.55)	60.00 (50.76)	08.45	14.49	1376.4
	SE ±	-	0.84	0.55	0.72	-
	CD (P=0.01)	-	2.46	1.62	2.12	-

*- Mean of four replications, Figures in parenthesis are arc sine transformed values,
SVI: Seedling vigour index



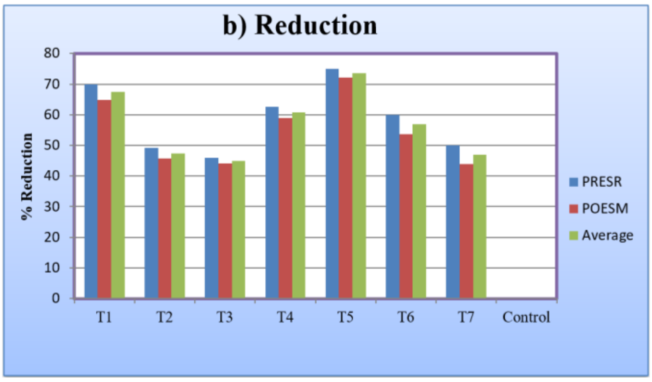


Fig 1: Effect of fungal endophytes on seed rot/ mortality (*M. phaseolina*) of soybean

Conclusion

Fungal endophytes were found effective to improve seed germination and growth parameters in polybag culture and significantly reduced pre-, post- emergence and average mortality, caused by *M. phaseolina* in soybean. However, *Paecilomyces lilacinus*, *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium* sp. were found most promising.

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References

1. Abro MA, Sun X, Li X, Jatoi GH, Guo LD. Biocontrol potential of fungal endophytes against *Fusarium oxysporum* f. sp. *cucumerinum* causing wilt in cucumber. Pl. Pathol. J 2019;35(6):598-608.
2. Agale RC, Suryawanshi AP, Apet KT, Daunde AT. Prevalence of *Rhizoctonia bataticola*, inciting dry root rot of soybean in agro-climatic zones of Marathwada region of the Maharashtra State. J Pha. phytochem 2018;7(5):2562-2566.
3. Anitha D, Vijaya T, Pragathi D, Reddy NV, Mouli KC, Venkateswarulu N *et al*. Isolation and characterization of endophytic fungi from endemic medicinal plants of Tirumala hills. Int. J L. Sc. Bt. Pharm. Res 2013, 2(3).
4. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. *APS Press*: St. Paul. Minnesota, 1998).
5. Compant S, Reiter B, Sessitsch A, Nowak J, Clement C, Barka EA. Endophytic colonization of *Vitis vinifera* L. by plant growth promoting bacterium *Burkholderia* sp. strain PsJN. Appl. Environ. Microbiol 2005;71:1685-1693.
6. Dalal JM, Kulkarni NS, Bodhankar MG. Antagonistic and plant growth promoting potentials of indigenous endophytic fungi of soybean (*Glycine max*. (L.) Merril). Indian J Adv. Pl. Res 2014;1(7):9-16.
7. De Barry A. Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten, Hofmeister's Handbook of Physiological Botany, Leipzig, Germany, 1866, Vol. II.
8. Deepa J, Sally M. Evaluation of endophytic microbial consortium for the management of bacterial wilt of tomato caused by *Ralstonia solanacearum*. J Biol. Cont 2015;29(3):148-156.
9. Hanlin RT. Combined keys to illustrated genera of ascomycetes, Vols. I & II. 1st ed Saint Paul: APS Press, 1998.
10. Kulkarni NS, Dalal JM. Isolation and identification of endophytic microorganisms of soybean (*Glycine max* (L.) Merril). Bio. Pharma. J 2012;5(2):383-385.
11. Kumar V, Soni R, Jain L, Dash B, Goel R. Endophytic Fungi : Recent Advances in Identification and Explorations. In: B. P. Singh (ed.), Advances in Endophytic Fungal Research, Fungal Biology, Swizterland: Springer Int. Pub. AG. 2019, 267-281.
12. Lahlali, Rachid, Hijri Mohamed. Screening, identification and evaluation of potential bio-control fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. FEMS Microbiol. Lett., 2010;311:152-159.
13. Miller WA, Roy KW. Mycoflora of soybean leaves, pods and seeds in Mississipi. Canadian J Bot 1982;60:2716-2723.
14. Piemental IC, Glienke-Blanco C, Gabardo J, Makowiecky S, Azevedo L. Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.) Merril) under different environmental conditions. Brazil Arc. Bio. Tech. 2006;49(5):705-711.
15. Pieterse Z, Aveling TA, Jacobs A, Cowan DA. Seasonal variability in fungal endophytes from Aizoaceae plants in the Succulent Karoo biodiversity hotspot, South Africa J. Arid Environ, 2018 https://doi.org/10.1016/j.jaridenv.2018.05.004.
16. Priyadarshani CDN, Deshappriya N, Sandamal TGI. Effect of fungal endophytes of rice variety Ld 368 on growth and brown spot disease incidence of rice. J Soc. Tro. Pl. Res 2018;5(3):292-302.
17. Seifert KA, Morgan-Jones G, Gams W, Kendrick B. The genera of Hyphomycetes. 2nd ed Utrecht: CBS-KNAW Fungal Biodiversity Centre, 2011.
18. Senthilkumar M, Swarnalakshmi K, Govindasamy V, Lee YK, Annapurna K. Bio-control potential of soybean bacterial endophytes against charcoal rot fungus, *Rhizoctonia bataticola*. *Curr. Microbiol.* 2009;58:288-293.
19. Sudarma M, Ni WS, Darmiati NN. Exophytic and endophytic fungus that potential as bio-control agents on *Lasiodiplodia theobromae* caused fruit rot of sugar-apple. Int. J Curr. Microbiol. Appli. Sci. 2019;8(2):131-142.
20. Sutton BC. The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. 1st ed Kew: CMI, 1980.
21. Urooj F, Farhat H, Ali SA, Ahmed M, Sultana V, Shams ZI *et al*. Role of endophytic *Penicillium* species in suppressing the root rotting fungi of sunflower. Pakistan J Bot. 2018;50(4):1621-1628.
22. Wati, Murni Sinitiya, Hadiwiyono, Yunus Ahmad. Antagonism of endophytic fungi isolates *Artmisia annua* towards *Rhizoctonia solani*, causal agent of rice sheath blight. Int. J Innov. Engineer. Tech 2019;14(1):075-079.
23. Zuhria SA, Djauhari S, Muhibuddin A. Exploration and antagonistic test of endophytic fungi from soybean (*Glycine max* L. Merr) with different resistance *Sclerotium rolfsii*. J Exp. Life Sci 2018;6(2):101-105.