e-ISSN: 2320-0189
p-ISSN: 2347-2308

Research and Reviews: Journal of Botanical Sciences

Population Variance and Diversity of Endophytic Fungi in Soybean (*Glycine max* (L) Merril)/

JM Dalal*, and NS Kulkarni.

Microbiology Research Laboratory, Department of Microbiology, R. A. Mahavidyalaya, Washim (MS), India 444 505.

Research Article

Received: 19/08/2014
Revised : 12/09/2014
Accepted: 15/09/2014

*For Correspondence

Microbiology Research Laboratory, Department of Microbiology, R. A. Mahavidyalaya, Washim (MS), India 444 505.

Keywords: Endophytic fungi, Population variance, Soybean (*Glycine max* (L) Merril).

ABSTRACT

Present investigation was carried out to study variation in endophytic fungal population colonizing soybean. Endophytic population was assessed at different growth stages of soybean (C.V. JS-335) viz., vegetative and reproductive stages. A 182 (28.88 %) isolates were obtained from vegetative growth stages (V1-V5) and 448 (71.11 %) from reproductive growth stages. As plant grows the endophytic population increases progressively however, at the onset of reproductive stages viz., R1-R8 the endophytic population starts to decrease and attains a stable value at R8 stage. The maximum endophytic colonization was recorded found at V5 stage (25.00%) and R3 stages (37.03%) at which the plant attains maturity. Endophytic fungal isolates belonged to 07 genera viz; *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Fusarium, Penicillium* and *Rhizoctonia.*Our investigation provides a valuable insight in understanding of endophytic microorganisms in their unique ecological habitat.

INTRODUCTION

Plant-microbial interactions have been a premier area of research interest. These interactions may range from beneficial to harmful. Microbial endophytes are typically defined as plant associated microbes that colonize living internal tissues of plants without causing any visible symptoms or immediate over-negative effects and can be isolated from surface disinfected plant tissue [1,2]. Virtually all plants are hosts to endophytic microorganisms and endophytes may usually be fungi, bacteria and actinomycetes[3]. These microorganisms include both commensal species, which have no direct effect on the host plant, and mutualistic symbionts[4]. Thus, endophytic colonization improves the ecological adaptability of the host. Hence endophytes may be regarded as a true companion of host.

Intimate associations between endophytes and host plants can be formed without harming the plant and they have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens and/or various environmental stresses[5]. Endophytes have been shown to confer fitness benefits to host plants including tolerance to herbivory, heat, salt, disease, and drought, and increased below and aboveground biomass. Thus, endophytic colonization improves the ecological adaptability of the host. The unique ecological niche has made endophytic bacteria as attractive and potentially promising tool for agricultural applications especially, for those bacteria having commercial features such as plant growth promotion and activation of plant defense mechanisms [2]. Several bacterial endophytes have been reported as potential biocontrol agents that may improve and promote plant health[6].

Various reports indicate that endophytes exist in a variety of tissue types within numerous plant species, suggesting a ubiquitous existence in most plants[7]. The endophytic population may originate from the usual soil community and a great diversity has been reported in diverse plant community [8]. The study

e-ISSN: 2320-0189
p-ISSN: 2347-2308

of the structure of endophytic microbial populations, their distribution, interaction and functions within their host is important for understanding their ecological role[9].

Analysis of the structure of microbial populations has practical importance; the results can be used to assess the fate of released strains and their impact on resident microbial communities [10]. Hence, the present investigation was carried out to study variation in distribution and diversity of endophytic bacterial population colonizing soybean.

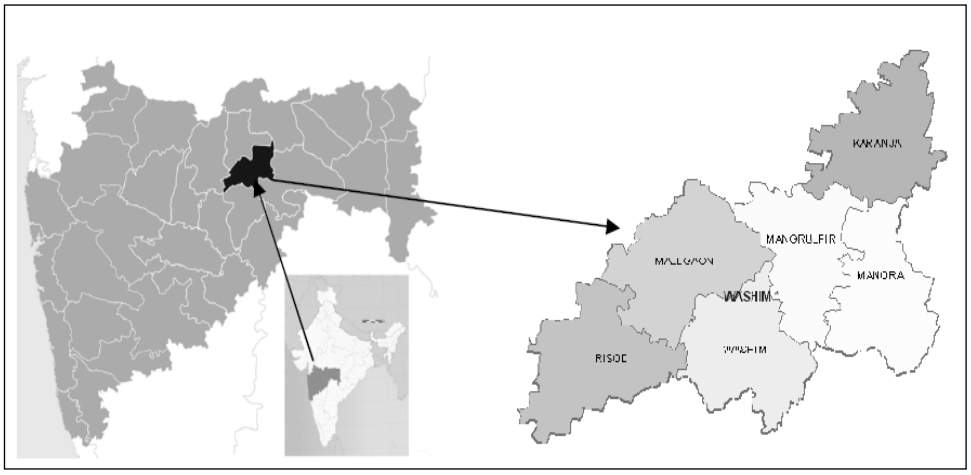
MATERIALS AND METHODS

Isolation of indigenous endophytic microorganisms in soybean

Study area

Washim (Latiude: 20° 05'58.90" N, Longitude: 7° 08'1.82" E, Altiude: 60M MSL) is situated in central Vidarbha region of Maharashtra state of India. The climate of this region is hot moist semi arid and the soil type is deep clayey to shalow black soil (Figure 1).

Figure 1: Study area



Sample collection

Healthy plants of soybean were screened from the 6 different locations of Washim district viz., Washim, Mangrulpir, Manora, Karanja, Risod and Malegaon. From each location, samples were collected randomly from 3 different sub-locations. From each sub-location 3-5 healthy plants were selected randomly. The growth stages of soybean were identified as specified by McWilliams[8] Sample represent of each growth stage viz., vegetative (V1-V5) and reproductive (R1-R8) were collected. The plants were uprooted, sealed into plastic bags and labeled. All samples were processed immediately after collection.

Surface sterilization

The collected plants were washed under tap water to remove soil and further separated into plant parts viz., root, stem and leaf. All root, stem and leaf samples were washed twice in distilled water then surface sterilized[2,3]. Surface sterilization was carried out by immersion for 1 minute in 70% (v/v) ethanol, in 0.1% $HgCl_2$ upto 3 minutes out for roots and nodules, whereas, upto 5 minutes for leaves and stems respectively. The tissue was then washed ten times using sterile distilled water.

Sterility checks after surface sterilization were carried out by monitoring separately the section impressions and rinse wash water for the presence or absence of microbial growth on selective medium viz, for bacteria on nutrient agar for 6 days at 30°C, for actinomycetes on glycerol yeast extract agar for 6 days at 30°C and for fungi on potato dextrose agar for 7 days at 30°C [2, 3]. The absence of growth was taken into consideration as positive test for surface sterilization.

e-ISSN: 2320-0189
p-ISSN: 2347-2308

Identification of endophytic fungi

After surface sterilization, the samples were cut into 5-7 mm pieces with sterile blade and forcep and aseptically transferred to plates containing Potato Dextrose Agar (PDA) supplemented with streptomycin (100 mg/mL)[3]. Four pieces of surface sterilized plant material (root, stem and leaves) were plated per plate and the experiment was replicated three times. To monitor the growth of slow growing and low sporulating fungi half of the plates were incubated at 25 ± 2 °C and other half at 37 °C [3]. The plates were monitored daily for up to 30 days for fungi growing from the cut end of segments. Fungal isolates were identified on the basis of conventional cultural and morphological characteristics followed by comparing with the available standard literature[11,12,13,14,15,16] and further purified and maintained on PDA at 4°C. Per cent colonization frequency was calculated as number of fragments colonized by fungi in relation to the total number of fragments[3,18]. The pathogenecity test was conducted for all the isolated strains of endophytic fungi and the pathogens were ignored [17].

RESULTS AND DISCUSSION

Variation of endophytic fungal population

Initially, endophytic fungal population was found to increase with plant development (Table 1). However, at onset of reproductive stages viz., R1-R8 the endophytic population started to decrease. Thus, as plant senescenced a decline in endophytic population was observed.

Table 1: Endophytic fungal population at different growth stages in soybean

Growth stages		Total number of isolates	Colonization rate (%)
Vegetative	V1	16	7.40
	V2	26	12.03
	V3	37	17.12
	V4	49	22.68
	V5	54	25.00
	TOTAL	182	
Reproductive	R1	60	27.77
	R2	68	31.48
	R3	80	37.03
	R4	73	33.79
	R5	66	30.55
	R6	48	22.22
	R7	33	15.27
	R8	20	9.25
	TOTAL	448	

The maximum endophytic fungi were isolated for vegetative stage at V5 (54) with colonization rate (CR) of 25 % followed by V4 (49/CR= 22.68 %) and for reproductive stages at R3 (80/CR= 37.03 %) followed by R4 (73/CR= 33.79 %). The endophytic fungal isolates obtained at vegetative growth stages V1, V2 and V3 were 16 (CR= 7.40 %), 26 (CR= 12.03 %) and 37 (CR= 17.12 %) whereas at reproductive growth stages R1, R2, R5 R6 R7 and R8 were 60 (CR= 27.77 %), 68 (CR= 31.48 %), 66 (CR= 30.55 %), 48 (CR= 22.22 %), 33 (CR= 15.27 %) and 20 (CR= 9.25 %).

Significant variations in the populations of both indigenous and introduced endophytes have been reported. These variations are attributed to plant source, plant age, tissue type, time of sampling, and environment [19]. Soil type, to a large extent, determines the endophytic population[20]. Factors such as sampling site, tissue specificity, tissue age or associated vegetation can influence the composition of endophytic communities[21].

Among these, geographical variation is recognized as one of the most important. Taxa isolated from the same plant species tend to vary according to the location of each individual. Age and phytosanitary status of the host plant can also exert some effect on the composition of endophytic community. Endophytic population is influenced by different factors that include the host developmental stages environmental conditions and geographical location of the experimental site. Moreover, genotype or cultivar of plant also affects significantly the endophytic population[6].

Our findings are in congruence with previous reports. The population size of endophytic bacteria in agronomic plants including soybean varied between 0-6.0 $\log_{10}$ CFU/sample tissue. Endophytic bacterial population is affected by the growth stages of plant. At reproductive maturity (R1-R3) population is maximum as compared to other growth stages[19]. Significant differences in bacterial population densities are found to be influenced by soybean growth phase and type of tissue sampled[6].

Present investigation also correlates to the reports of Prakamhang[22] endophytic bacteria in cultivated rice were found in greatest density in roots. Generally, bacterial populations are larger in roots and decrease in the stems and leaves [19]. However, there were no significant differences between mean counts of actinomycetes from *A. fragrantissima* from different sites within Saint Katherine [23]. Irrespective of the growth stage of rice and type of soil, the majority of endophytic bacteria were recovered from roots ($2 \times 10^5 - 3 \times 10^6$ CFU/g fresh weight) as compared to other parts of rice plants. While the endophytic bacterial population density in stem samples was $4 \times 10^3 - 10^5$ CFU/g fresh weight, the lowest population density was present in leaf tissue with $1 \times 10^3 - 2.7 \times 10^4$ CFU/g fresh weight[22].

A decline in endophytic population was observed as plant aged. The decrease in endophytic population from vegetative to reproductive stages could have been due to unavailability of essential nutrients during maturation and senescence of plants[6,7]. As plants mature all the nutritional requirements for microorganisms are optimum and a stable endophytic population is obtained. Thus, there appear to be coincidence of plant maturity and endophytic population.

The endophytic population in plants is highly variable and occasionally transient[24]. The observed differences in endophytic population in our study is supported by other observation where endophytic bacterial population densitites are found to be influenced by soybean growth phase and tissue sampled [6] and pointed that as plant ages the number of endophytic fungal isolates decreases [3]. Initial increase in endophytic actinomycetal population was recorded in cucumber roots and the population densities were found to be 4.56 $\log_{10}$ CFU g^{-1} fresh root weight[25].

Isolation and identification of endophytic fungi

A total of 630 endophytic fungal isolates were isolated; 182 (28.88 %) from vegetative growth stages and 448 (71.11 %) from reproductive growth stages (Table 2) with colonization rate of 22.43 %. Based upon the plant parts, 279 (44.28 %) were isolated from roots, 185 (29.36 %) from leaves and 166 (26.34 %) from stems. It was found that the number of isolates obtained from vegetative stages is larger than that of reproductive stages. As a primary site of entry, the roots showed maximum population, followed by leaves and stems respectively.

Table 2: Distribution of endophytic fungal isolates at different growth stages of soybean

Growth stage		Total number of endophytic fungal isolates			Total no. of isolates	Total
		Plant source				
		Root	Stem	Leaf		
Vegetative	V1	7	4	5	16	182
	V2	13	7	6	26	
	V3	19	10	8	37	
	V4	26	12	11	49	
	V5	26	15	16	54	
Reproductive	R1	28	17	18	60	448
	R2	31	18	20	68	
	R3	36	21	23	80	
	R4	32	20	21	73	
	R5	27	17	22	66	
	R6	20	12	16	48	
	R7	12	09	12	33	
	R8	02	04	07	20	
Total		279	166	185		630

During course of present investigation from a total culturable population 20 distinct fungal isolate were isolated (Table 3) that were frequently and commonly recorded during the different growth stages of

soybean. Isolates were obtained from 07 genera viz; *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Fusarium, Penicillium* and *Rhizoctonia.* Of the 20 isolates, *Alternaria* (04)*, Aspergillus* (03)*, Cladosporium* (02)*, Colletotrichum* (02)*, Fusarium* (04)*, Penicillium* (03) and *Rhizoctonia* (03) were obtained. These isolates were selected and maintained for further studies.

Fusarium (20 %) and *Alternaria* (20 %) were found to be predominant endophytic fungi present in soybean. Whereas, *Aspergillus,Penicillium,* and *Rhizoctonia* were moderately distributed and their predominance was at par 15 %. However, *Cladosporium* and *Colletotrichum* were not so frequent and were at par 10 %.

The diversity of endophytic fungi has been explored in various plant host. Our findings on the diversity of endophytic fungi correlate with different studies. the diversity of fungi associated to varieties of grapevines (*Vitis vinifera* L.) cultivated in Canary Islands (Spain) and in Azores (Portugal) was investigated and isolated were identified as *Hortaea werneckii* (Horta) Nishimura and Miyaji, *Alternaria alternata* (Fr.) Keissl., *A. tenuissima* (Nees) Wiltshire*, Aspergillus* spp.*, Pestalotiopsis* spp.*, Botryosphaeria parva* Pennycook & Samuels, *B. lutea* A.J.L. Phillips, *Chaetomium globosum* Kunze ex Fr., *Gibberella intricans* Wollenw and *Penicillium* spp. Wollenw and *Penicillium* spp [21].

Table 3: Morphological and cultural characteristic of soybean fungal isolates

Isolate	Growth	Front view	Reverse view	Texture	Mycelium septation	Conidial morphology	Possible species
JDF1	Slow	Olivaceous black	Black	Cottony	Septate	Ovoid Sepate	*Alternaria sp.*
JDF2	Moderate	Grayesih	Black	Cottony	Septate	Ovate Septate	*Alternaria sp.*
JDF3	Moderate	Black	Black	Cottony	Septate	Ellipsoidal Septate	*Alternaria sp.*
JDF4	Moderate	Grayeish	Black	Cottony	Septate	Ovate Septate	*Alternaria sp.*
JDF5	Slow	Gray	Colorless	Cottony	Septate	Ellipsoidal	*Aspergillus sp.*
JDF6	Slow	Deep green	Colorless	Velvety	Septate	Ellipsoidal	*Aspergillus sp.*
JDF7	Good	Blushgreen	Colorless	Velvety	Septate	Globose	*Aspergillus sp.*
JDF8	Moderate	Olivaceous green	Black	Velvety	Septate	Elliptical	*Cladosporium sp.*
JDF9	Moderate	Olivaceous black	Black	Velvety	Septate	Cylindrical	*Cladosporium sp.*
JDF10	Good	Gray	Pale gray	Velvety	Septate	Fusiform	*Colletotrichum sp.*
JDF11	Good	Black	Black	Thick	Septate	Falcate	*Colletotrichum sp.*
JDF12	Good	Cream white	Cream	Wooly	Septate	Macroconidia: Slightly curved, Septate	*Fusarium sp.*
JDF13	Moderate	White	Colorless	Wooly	Septate	Microconidia: Sickle shaped, Septate Macroconidia: Slightly curved, Septate	*Fusarium sp.*
JDF14	Good	White with lavender tinged	Colorless	Cottony	Septate	Microconidia: Oval Macroconidia: Straight, Septate	*Fusarium sp.*
JDF15	Moderate	Faint Pink	Redish	Cottony	Septate	Microconidia: Sickle shaped, Septate Macroconidia: Slightly curved, Non-Septate	*Fusarium sp.*
JDF16	Moderate	Dark green	Pale yellow	Powdery	Septate	Spherical	*Penicillium sp.*
JDF17	Moderate	Dark green	Yellowish cream	Powdery	Septate	Ellipsoidal	*Penicillium sp.*
JDF18	Moderate	Green color with yellowish border	Reddish	Powdery	Septate	Round	*Penicillium sp.*
JDF19	Moderate	Brown	Brown	Cottony	Septate	Moniloid	*Rhizoctonia sp.*
JDF20	Moderate	White	Pale yellow	Cottony	Septate	Moniloid	*Rhizoctonia sp.*

The endophytic fungal community of grape (*Vitis labrusca* cv. Niagara Rosada) leaves collected from Salesopolis, SP, Brazil included *Aporospora terricola*, *Aureobasidium pullulans*, *Bjerkandera adusta*, *Colletotrichum boninense*, *C. gloeosporioides*, *Diaporthe helianthi*, *D. phaseolorum*, *Epicoccum nigrum*,

Flavodon flavus, *Fusarium subglutinans*, *F. sacchari*, *Guignardia mangiferae*, *Lenzites elegans*, *Paraphaeosphaeria pilleata*, *Phanerochaete sordida*, *Phyllosticta* sp, *Pleurotus nebrodensis*, *Preussia africana*, *Tinctoporellus epiniltinus*, and *Xylaria berteri* [26].

Abdel-Mottal *et al.,* (2010) isolated 44 strains of. Endophytic fungi from the medicinal plant *Hyoscyamus muticus* L included *Alternaria alternata, Aspergillus fumigatus, Drechslera hawaiiensis, Fusarium solani, Penicillium citrinum, Neoscytalidium dimidiatum, Thyrostromella myriana* and *Ulocladium chartarum* as well as 6 strains of non-pathogenic fungi viz., *Alternaria alternata, Cladosporium cladosporioides, Cladorrhinum foecundissimum, Curvularia clavata, Penicillium janthinellum* and *Ulocladium chartarum* [27].

Total 28 isolates of endophytic fungi from the leaves, and stems of *Sesbania grandiflora (L.) Pers* were isolated. Among of them, 24 strains were isolated from stem and 4 from leave [28]. Endophytic filamentous fungi isolated from the root of maize belonged to *Fusarium* sp. and *Acremonium* sp., *Phomosis* sp., *Paecilomyces* sp., *Phaeoacremonium* sp. and *Cladosporium* sp. *Beaveria bassiana, A. strictum, A. alternata, Phoma sp.* and *T.* [29].

Endophytic fungi were isolated from 22 species of medicinal plants in Taiwan [30]. Among 156 isolates of endophytic fungi, 67 isolates, belonging to 21 genera in 15 families, were from Lauraceae and 89 isolates, belonging to 27 genera in 16 families, were from Rutaceae. The most abundant genera were *Colletotrichum*, *Guignardia*, *Hypoxylon*, *Nigrospora*, *Phomopsis* and *Xylaria*, especially *Colletotrichum* spp. with 14 isolates and *Phomopsis* spp. with 15 isolates. In addition, species of *Chaetomium*, *Alternaria*, *Stemphylium*, *Coprinopsis* and *Cyanodermella* were dominant and host specific to Rutaceae, whereas species of *Podospora*, *Leptosphaeria* and *Pilidiella* were only observed in Lauraceae.

CONCLUSIONS

It appears from the present study that as soybean development progresses; endophytic population increased. However, at maturity, the high population density was observed and thereafter the population declined. Thus, endophytic population is influenced by developmental stages of soybean plant. Moreover, research on endophytic fungi offers an innovative and enlivening opportunity for the discovery of novel strains with biotechnological utility.

ACKNOWLEDGEMENTS

Authors are thankful to University Grants Commission, New Delhi, for providing the financial assistance under the scheme of major research project as per XIth plan.

REFERENCES

1. Wilson D. Endophyte - The Evolution of a term, and clarification of its use and Definition. Oikos; 1995;73(2):274-276.
2. Hung PQ, Annapurna K. Isolation and charecterization of endophytic bacteria in soybean (*Glycine* sp.*).* Omonrice. 2004;12:92-101.
3. Pimentel IC, Glienke-Blanco C, Gabardo J, Stuart RM and Azevedo JL. Identification and Colonization of Endophytic Fungi from Soybean (*Glycine max* (L.) Merril) under Different Environmental Conditions. Brazilian Arch Biol Technol. 2006;49(5): 705-711.
4. Procopio REL, Araujo WL, Maccheroni WJr. And Azevedo JL. Characterization of an endophytic bacterial community associated with *Eucalyptus* spp. Genet Mol Res. 2009;8:1408-1422.
5. Hasegawa S, Meguro A, Shimizu M, Nishimura T. and Kunoh H. Endophytic actinomycetes and their interactions with host plants. Actinomycetologica. 2006; 20(2):72–81.
6. Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol. 2004;6:1244-1251.
7. McInroy JA, Kloepper JW Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil. 1995;173:337-342.
8. McWilliams DA, Berglund DR, Endres GJ. Soybean, Growth and Management (A-1174), NDSU Extension Service. North Dakota State University Fargo, North Dakota 58105; 2004. Available: http://www.ag.ndsu.nodak.edu.

9. Reiter B, Pfeifer U, Schwab H, Sessitsch A. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *Atroseptica.* Appl Environ Microbiol. 2002;68:2261–2268.
10. Hallmann J, Rodríguez-Kábana R, Kloepper JW. Nematode interactions with endophytic bacteria, in: A. Ogoshi K, Kobayashi Y, Homma F, Kodama N, Kondo, Akino S. (Eds.), Plant Growth-Promoting Rhizobacteria -Present Status and Future Prospects. Nakanishi Printing, Sapporo. Pp.243-245; 1997.
11. Raper KB and Fennell DI. The Genus *Aspergillus*. The Williams and Willkins Company, Baltimore; 1965.
12. Subramanian CV. Hyphomycetes, Published by ICAR, New Delhi; 1971.
13. Barron GL. The Genera of Hyphomycetes from soil, Rebert, E. Kriengner Publication; 1972.
14. Eillis MB. Dematiaceous Hypomycetes, CAB International. Published by Commonwealth Mycological Institute, Kew, Surrey, England; 1993.
15. Barnett HL and Hunter B. Illustrated Genera of Imperfect Fungi, IV edition. Published by APS Press St. Paul, Minnesota; 1998.
16. Gilman JC. A Manual of Soil Fungi, 2nd Indian Edition, Biotech Books Delhi; 2001.
17. Mahesha B. Investigations of multiple diseases resistance in soybean, Thesis submitted to the University of Agricultural Sciences, Dharwad in partial fulfillment of the requirements for the Degree of Master of Home Science in Plant Pathology, Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad - 580 005; 2006.
18. Gehlot P, Bohra NK and Purohit DK. Endophytic mycoflora of inner bark of *Prosopsis cineraria* – A key stone tree species of Indian desert. American-Eursian J Bot. 2008;1(1): 01-04.
19. Zinniel DK, Lambrecht NB, Harris ZF, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG and Vidaver AK. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol. 2002;68:2198–2208.
20. Conn VM. and Franco CMM. Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by Terminal Restriction Fragment Length Polymorphism. Appl Environ Microbiol 2004;70(11):6407-6413.
21. Garoe N, Cabrera R, Lisbel RBR, Evelyn DS, Cristina G, Andreea C and Nelida B. Endophytic fungi from *Vitis vinifera* L. isolated in Canary Islands and Azores as potential biocontrol agents of *Botrytis cinerea* Pers.:Fr. J Hort Forestr Biotechnol. 2012;16(1):1- 6.
22. Prakamhang J, Minamisawa K, Teamtaisong K, Boonkerd N, and Teaumroong N. The communities of endophytic diazotrophic bacteria in cultivated rice (*Oryza sativa* L.) App Soil Ecol. 2009;42:41–149.
23. El-Shatoury S., Omnia EK, Waleed EK and Ahmed D. Antimicrobial activities of actinomycetes inhabiting *achillea fragrantissima* (family: compositae). Egyptian Journal of Natural Toxins. 2009;6(2): 1-15.
24. Hardoim PR, van Overbeek LS, van Elsas JD. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 2008;16:463-471.
25. El-Tarabily KA, Nassar AH, Hardy GE and Sivasithamparam K. Plant growth promotion ssand biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J App Microbiol. 2009;106:13–26.
26. Brum MCP, Araujo WL, Maki CS and Azevedo JL. Endophytic fungi from *Vitis labrusca* L. (Niagara Rosada) and its potential for the biological control of *Fusarium oxysporum*. Genet Mol Res. 2012;11(4): 4187-4197.
27. Abdel-Motaal FF, Nassar MSM, El-Zayat SA, El-Sayed M. A. and Ito S. Antifungal activity of endophytic fungi isolated from egyptian henbane (*Hyoscyamus muticus* L.). Pak J Bot. 2010;42(4): 2883-2894.
28. Powthong P, Bajaree J, Acharawan T, and Pattra S. Evaluation of endophytic fungi extract for their antimicrobial activity from *Sesbania grandiflora (L.) Pers.* Int J Pharm Biomed Res. 2012;3(2), 132-136.
29. Orole OO and Adejumo TO. Activity of fungal endophytes against four maize wilt pathogens. African J Microbiol Res. 2009;3(12):969-973.
30. Ho M, Chung W, Huang H, Chung W and Chung W. Identification of endophytic fungi of medicinal herbs of lauraceae and rutaceae with antimicrobial property. Taiwania. 2012;57(3):229-241.