IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DIVERSITY OF ENDOPHYTIC FUNGI ISOLATED FROM THE LEAVES, MIDRIB AND SEEDS OF PSIDIUM GUAJAVA. L.

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*Abstract: Psidium guajava L.*is an evergreen shrub or small tree cultivated for its edible fruits belongs to the family Myrtaceae were exploited for endophytic fungi which are the possible main source of bioactive secondary metabolites. About 60 segments from eight plants of *Psidium guajava* collected from four different locations of Chikkamagaluru , Karnataka during the year 2019 . A total of 31 endophytes were isolated from the seed, leaf and midrib on PDA and Czapek – Dox Agar media. Among 31 species 21 endophytes were isolated from leaf, 14 species from seeds and seven fungal species from the midrib region. *Aspergillus, Cladosporium , Pencillium and Phoma* are the dominating genera isolated Collected samples of different regions showed variation in the occurrence of endophytic fungi may be due to fluctuation of physicochemical factor and nutritional status of the plant in the study area.

Key words*: Psidium guajava*, PDA , Czapek -Dox agar, endophytic fungi, Diversity.

I. INTRODUCTION

Endophytic fungi are asymptomatic symbionts living within the ground tissue of flowering plants not affected by surface sterilization (Srimathi. *et.al.* 2011). De Bary (1866) first defined all organisms that colonize internal part tissues as endophyte. The study of endophytic fungal diversity is very important in plant biology to understand for the improvement of plant fitness. Generally endophytic fungi were widely distributed in all plant domains and produces valuable secondary metabolites which are utilized for medical, agricultural and industrial uses. Plants having an ethno botanical history or used by local indigenous people for any therapeutic purpose will provide the best opportunities to isolate novel endophytic fungi and to make use of them to produce novel bioactive products (Prabukumar *et.al* 2015). *Psidium guajava L.* (Guava) is one of the common tropical plant characterized by leathery opposite leaves and grown abundantly for sweet juicy fruits , native to Mexico, Central America and northern South America. It is popular due to its nourishing values and taste. It is rich in vitamin C, A, Beta- carotene, B_1, B_2, B_3, B_5 etc, fat, protein and minerals. It contains high content of organic and inorganic compounds like secondary metabolites e.g. antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds (Naseer. *et.al.* 2018). So ***Psidium guajava L.*** was chosen for the isolation of endophytic fungi from leaf lamina, mid rib, and seeds of four different locations of Chikkamagaluru, Karnataka..

II.Materials and method

2.1.Study area

We have selected Uppali, Karkipete, Housing Board and Heremagaluru of chikkamagaluru district as study area and are situated roughly in the south western part of Karnataka state. The district is situated in between $12^{0,}$ 54',42'',and 13^0 53' 53'' north latitude and between 75^0 04' 46'' and 76^0 21' 50'' east longitude. The average rainfall of 1925 mm. humidity is about minimum of 42% and maximum is 86% and temperature is about 20^0-36^0 C.

2.2.Collection of samples and Isolation of Endophytic Fungi

Mature and healthy part of fruit and leaf samples of *Psidium guajava* L. were collected in selected regions of Chikkamagaluru, Karnataka. from January to December 2019 in sterilized condition. for the isolation of endophytic fungi. Seeds are taken out from the collected fruit samples with sterilized knife. From the Leaf samples midrib and lamina was cut in to small fragments (0.5-1cm). All these samples were washed with running water then surface sterilized by immersing it in to 70% ethanol for 5 seconds followed by 4% sodium hypochlorite for 90 seconds then rinsed with distilled water for 10 seconds. (Dobranic *et. al.*, 1995) Excess water is removed by sterile filter paper. For the isolation of endophytic fungi PDA and Czapek -Dox agar media were used and leaf lamina, midrib and seeds were inoculated into the petri plates containing medium. To each Petri plates about 5 seeds are placed while one seed at the center and 4 seed are arranged at the periphery of the plate, in the same manner both leaf and midribs are inoculated in the sterilized condition, then petriplates were sealed and incubated it for 3 -7 days at room temperature (25 ± 2^0).

Obtained fungi were identified on the basis of colony characteristics and microscopic examinations by using relevant literature (Watanabe. 2010. Nagamani. *et.al*, 2005).

Study area

Results

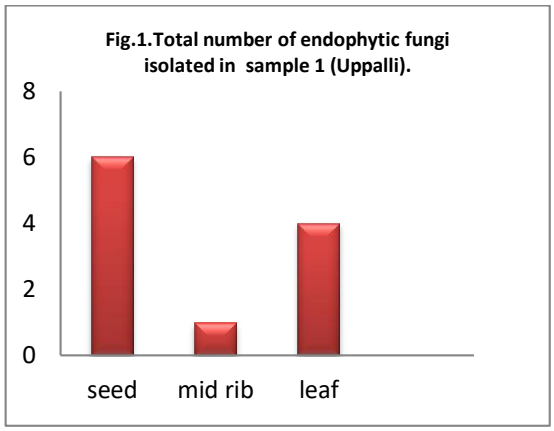
The plant parts were collected from four different location of Chikkamagaluru region. About 60 segments (20 segments of leaf , 20 segments of midrib, 20 segments of seeds) of *Psidium gujava* were used for isolation of endophytic fungi. A total of 31 fungal species were isolated from seeds, leaves and midrib from four samples (Table.1). Among thirty one species *Aspergillus, Cladosporium , Pencillium and Phoma w*ere the dominating genera were found to be predominant in all most all the samples.
Among four samples we have isolated highest endophytic fungi from sample one (Uppalli region) with 16 species, followed by sample two (Housing board) with 14 fungal species, sample three (Karkipete) with nine fungal species and least fungi are isolated from sample four (Heremagaluru) with seven fungal species (Fig. 1- 4).
From the seeds of all four samples we have isolated 14 endophytic fungal species, seven from the Mid rib and 21 endophytic fungal species were isolated from the Leaf samples (Fig.5).

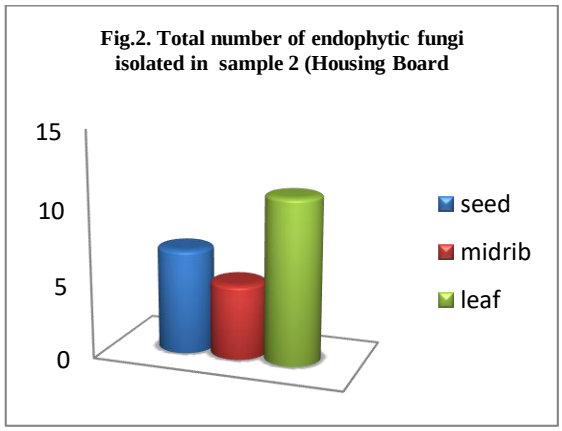
Disscussions

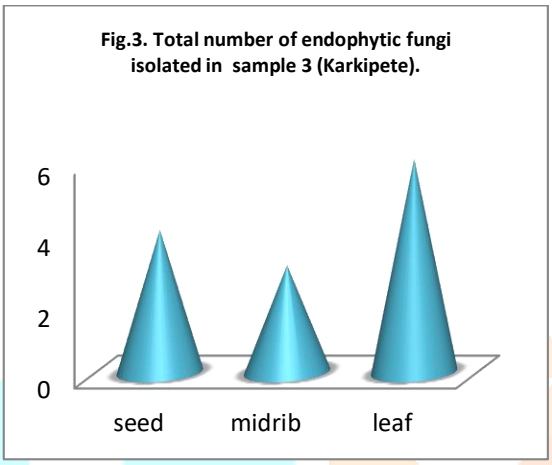
Aspergillus niger isolated as dominant species and found in all four samples of leaf, mid rib and seed except in mid rib of sample two. These fungi are also found commonly as plant pathogens and they might have evolved to endophytic life style due to loss of virulence (Freeman and Rodrigues 1993 and Kumanand Tayung 2012).
Aspergillus have been isolated as endophytes from many plant species with some of them displaying biological activity (Chakravarthi *et al*. 2008; Kour *et al*. 2008; Deng *et al*. 2009). Leaf shows more number of endophytic fungi because of broad lamina (Arnold *et al*. 2000) as it is a main source of food and also rich in essential oil (Yang *et.al*.2007)
In the mid rib region we found very less number of endophytic fungi because of continuous flow of water and nutrients spores also moves along with it and water leads to proper adsorption to the tissues of mid rib. Plant tissues, specially leaves and stems are excellent reservoirs for endophytic fungi (Petrini, 1991; Bokhary *et al.*, 2000).
With respect to number of endophytic fungi seeds stand next to the leaf and are the main source of transformation spore to next generation.
Few fungi showed tissues specificity. *Bartalinia robillardoides* isolated from leaf only and it is high heat resistant verity reported by Suryanarayanan (2012). *Nigrospora oryza* mainly grown on the leaf lamina of the medicinal plants reported on the leaf of *Taxus globosa* and *Tripterygium wilfordii* respectively (Prabukumar *et.al* 2015). The *Pestolopsis sps., Pestolotia sps.* isolated from the leaf and are commonly isolated as endophytic fungi (Brown *et.al* 1998, suryanarayanan *et. al* 2000). *Myrothecium species*, *Thielavia terricola* and *Torula herbarum*, are usually found in leaf and it is also present in the leaf of *Nerium oleander* L.(Vasundhara *et al.*, 2019) The *Thielavia terricola* also shows their presence as endophyte in leaf of *Rauwolfia tetraphylla* L (Alurappa *et.al* 2018). Four different endophytic *pencillium* species were isolated and many metabolites with cytotoxic and antibacterial effects have been isolated from this genus (Rukachaisirikul *et al*. 2007, Ge *et al*. 2008).
Two species of *Phoma* were isolated from the seed as an endophytic fungi helps in the Profuse growth of root hairs. The endophytic fungi show their effect on plants, right from the germination of seeds (Rai *et al*. 2014b). These endophytic fungi help to degrade cuticle cellulose during seed germination and thereby available carbon for growing seedling, which improves seed germination, vigor and establishment (Waqas *et.al*., 2012). The endophytic fungi secrete plant growth hormones such as cytokinins, auxins and gibberellins which promote seed germination and growth in crop plants (Bhagobaty and Joshi 2009). The endophytic *Phoma* species can be used as an efficient biofertilizer. (Kedar *et.al* 2014). *Curvularia trifolii* another endopytic fungus isolated from leaf and seed sample. Many *Curvularia* species shows endophytic in nature in that *C. trifolii* is one of them (Herrmann LW. 2019) and many curvularia species produce particular secondary metabolites helps in the increasing the efficiency of medicinal value of the plant (Kaaniche F. 2019). *Alternaria citri* pathogenic fungus usually causes black rot in Lemon isolated as endophyte from stem and leaf may be due to the presence of the nitrogen containing metabolites and it acts as growth promoter (Li Si Zhou *et. al* 2017, Jingfeng Lou, *et.al*.2013). *Pithomyces sacchari Oidodendron flavum Ascochyta graminicola, Phythium sulcatum, Pyrenochaeta terrestris,* shows very less growth and may be due to the seasonal variation in the environmental condition and are not showed any tissue host growth specificity (Suradkar *et. al*, 2015).

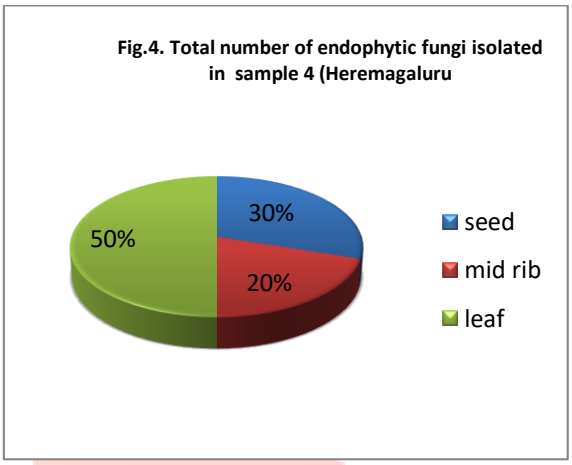
Table .1. List of Endophytic fungi isolated from the seed, Midrib and Leaf of *Psidium guajava L.* (Seed-S, Leaf-L, Mid rib-M)

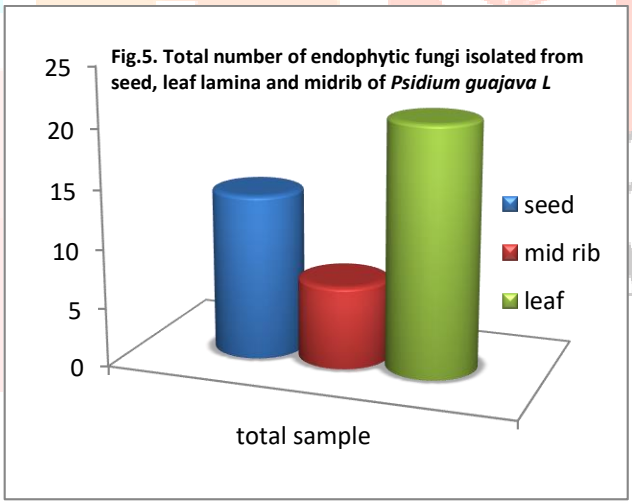
Sl. No	Species name	Sample-1			Sample 2			Sample-3			Sample-4		
		S	M	L	S	M	L	S	M	L	S	M	L
1.	*Alternaria citri*	-	-	-	+	-	-	-	-	+	-	-	-
2.	*Ascochytagraminicola*	-	-	-	-	-	-	-	-	-	-	-	+
3.	*Aspergillus awamori*	-	-	-	-	+	+	-	-	-	-	-	-
4.	*Aspergillus candidus*	-	-	-	-	-	+	-	-	-	-	-	-
5.	*Aspergillus flavipes*	-	-	+	-	-	-	-	-	-	-	-	-
6.	*Aspergillus flavus*	-	-	-	-	-	-	-	-	-	+	-	-
7.	*Aspergillus glauca*	-	-	-	-	-	-	-	+	-	-	-	-
8.	*Aspergillus kanagwansensis*	+	-	+	-	-	-	-	-	-	-	-	-
9.	*Aspergillus niger*	+	-	+	+	+	+	+	+	+	+	+	+
10.	*Aspergillus niveus*	-	-	-	-	-	-	-	-	+	-	-	+
11.	*Aspergillus sulphurus*	-	-	-	-	-	-	+	-	-	+	-	-
12.	*Basidiomycetes*	-	-	-	+	-	-	-	-	-	-	-	-
13.	*Batalinia robillardoides*	-	-	-	-	-	+	-	-	-	-	-	-
14.	*Cladosporium cladosporioides*	-	+	+	-	+	+	+	+	+	-	+	+
15.	*Cladosporium oxysporum*	+	-	-	+	-	-	-	+	-	-	-	-
16.	*Curvularia trifoli*	-	-	-	-	-	+	-	-	-	+	-	+
17.	*Myrothecium sps.*	-	-	-	-	-	-	-	-	-	-	-	+
18.	*Nigrospora oryzae*	-	-	-	-	-	-	-	-	+	-	-	+
19.	*Oidodendron flavum*	-	-	-	-	-	-	-	-	-	-	+	-
20.	*Pencillium adamentzi*	-	-	-	-	-	-	-	-	-	-	-	+
21.	*Pencillium atramentosum*	-	-	-	-	+	+	-	-	-	-	-	-
22.	*Pencillium aurantiogrisecum*	+	-	-	-	-	+	-	-	-	-	-	-
23.	*Pestolopsis sps.*	-	-	-	-	-	+	-	-	-	-	-	-
24.	*Pestolotia sps.*	-	-	-	-	-	+	-	-	-	-	-	-
25.	*Phoma eupyrena*	-	-	-	+	+	-	-	-	-	-	-	-
26.	*Phoma sps.*	-	-	-	-	-	-	-	-	-	+	-	-
27.	*Phythium sulcatum*	-	-	-	-	-	+	-	-	-	-	-	-
28.	*Pithomyces sacchari*	+	-	-	-	-	-	-	-	-	-	-	-
29.	*Pyrenochaeta terrestris*	-	-	-	-	-	-	-	-	-	-	-	+
30.	*Terula herbarum*	-	-	-	-	-	-	-	-	+	-	+	+
31.	*Thielavia tericola*	-	-	-	+	+	-	-	-	-	-	-	-











Conclusion

Over all work reveals that the endophytic fungi which are isolated from the collected samples plays an very important role in every step of the plant growth and also enhancing the medicinal property of plants. Maximum number of endophytic fungi isolated from the leaf lamina compare to the other part of the plant because of broad lamina, nutritive content and also rich in essential oil have potential biological control with antimicrobial activity and plants are commonly used to cure many diseases in Ayurveda indicates the production of secondary metabolites.

Reference

1. Alurappa R, Chowdappa S. (2018), "Antimicrobial activity and phytochemical analysis of endophytic fungal extracts isolated from ethno-pharmaceutical plant Rauwolfia tetraphylla L", Journal of Pure and Applied Microbiology. 1;12(1):317-2.
2. Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA (2000). "Are tropical fungal endophytes hyperdiverse?." Ecology letters. Jul;3(4):267-74.
3. Bhagobaty RK, Joshi SR (2009), "Promotion of seed germination of Green gram and Chick pea by *Penicillium verruculosum* RS7PF, a root endophytic fungus of *Potentilla fulgens* L". Adv Biotechnol 16–18
4. Bokhary, H.A., Al-Sohaibany, S., Al- Sadoon, Q.H., Parvez, S. (2000),"Fungi associated with Calotropis procera and Capparis spinosa leaves". J. King Saud. Univ., 12: 11 23
5. Brown K. B, Hyde K. D, and Guest D. I. (1998) "preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia." Fungal diversity 1:27-51
6. Chakravarthi BV, Das P, Surendranath K, Karande AA, Jayabaskaran C (2008) " Production of paclitaxel by Fusarium solani isolated from Taxus celebica". Journal of biosciences. 1;33(2):259-67.
7. De Bary. A. (1866), "Morphologie and physiologie der PrizeFlechten and Myxomyceten.Holfmeister's handbook of physiological Botany", Vol.2. Leipzing.
8. Deng B, Liu K, Ding X, Chen W. (2009), " Isolation and characterization of endophytic taxol-producing fungi from Taxus chinensis". Journal of Industrial Microbiology and Biotechnology. 1;36(9):1171.
9. Dobranic .J.K. JhonsonJ.A and Alikhan Q.R.(1995), "Isolation of endophytic fungi from eastem larch (Larix larcina) leaves from New Brunswick, Canada", Can.J.Microbial. 41;194-198.
10. Herrmann LW, Poitevin CG, Schuindt LC, De Almeida AB, Pimentel IC.(2019), " Diversity of fungal endophytes in leaves of Mentha piperita and Mentha Canadensis". Int J Botany Stud. ;4:44-9.
11. Kaaniche F, Hamed A, Abdel-Razek AS, Wibberg D, Abdissa N, El Euch IZ,. (2019)," Bioactive secondary metabolites from new endophytic fungus *Curvularia*. sp isolated from *Rauwolfia macrophylla*." PLoS ONE 14(6): e0217627.
12. Kedar A, Rathod D, Yadav A, Agarkar G, Rai M, (2014), ".Endophytic Phoma sp. isolated from medicinal plants promote the growth of Zea mays. Nusantara Bioscience..6(2).
13. Kour A Rehman S, Shawl AS, Andrabi R, Sudan P, Sultan P, Verma V, Qazi GN.(2008), " An endophytic Neurospora sp. from Nothapodytes foetida producing camptothecin". Applied biochemistry and microbiology;44(2):203-9.
14. Lou J, Fu L, Peng Y, Zhou L (2013), " Metabolites from Alternaria fungi and their bioactivities. Molecules" 18(5):5891-935.
15. Nagamani A, Kunwar IK, Manoharachary C.(2006) "Hand book of soil fungi. IK International pvt, ltd. New Delhi, 1-51
16. Petrini, O. (1991). "Fungal endophytes of tree leaves. In: Microbial Ecology of Leaves". pp. 179 197.
17. Prabukumar S, Rajkuberan C, Ravindran K, Sivaramakrishnan S., (2015), " Isolation and characterization of endophytic fungi from *Taxus globosa* and *Tripterygium wilfordii*, and their antibacterial, antioxidant and anticancer properties". Int J Pharm Pharm Sci.;7(11):316-21.
18. Rai M. Kedar A, Rathod D, Yadav A, Agarkar G,(2014), " Endophytic Phoma sp. isolated from medicinal plants promote the growth of Zea may"s. Nusantara Bioscience.;6(2).
19. Rodriguez RJ, Redman RS, Henson JM., (2005), " Symbiotic lifestyle expression by fungal endophytes and the adaptation of plants to stress: unraveling the complexities of intimacy". Mycology Series. 23:683.
20. Rukachaisirikul V, Phongpaichit S, Nikom J, Rungjindamai N, Sakayaroj J, Hutadilok-Towatana N, Kirtikara K.(2007), " Biological activities of extracts from endophytic fungi isolated from Garcinia plants" . FEMS Immunology & Medical Microbiology 1;51(3):517-25.
21. Srimathi Selvanathan Isaivani indrakumar and Muthumary Johnpaul. (2011), "Biodiversity of the endophytic fungi isolated from Calotropis Gigantea", (l)R.BR.Recent research in Science and Technology, 3 (4): 94-100.
22. Sumra Naseer, Shabbir Hussain, Naureen Naeem, Muhammad Pervaiz and Madiha Rahman. (2018), "The phytochemistry and medicinal value of Psidium guajava(Guava). Clinical phytoscience" volume 4 : 32..
23. Suradkar KP Khandekar SR, (2015), "Synthesis and Characterization of Cycloruthenation of Cycloruthenated Complexe".RESEARCH JOURNAL OF INDIA. 13..
24. Suryanarayanan, T.S and Kumarsen v (2000), "Endophytic fungi of some halophytes from an esturine mangrove forest". Mycological research 104:1465-1467
25. Suryanarayanan, T.S., Murali, T.S., Thirunavukkarasu, N., Govinda Rajulu M.B., Venkatesan, G., Sukumar R. (2012)." Endophytic fungal communities in woody perennials of three tropical forest types of the Western Ghats, southern India." Biodiverse. Conserv., 20(5): 913 928
26. Vasundhara M, Reddy MS, Kumar A,(2019) Secondary metabolites from endophytic fungi and their biological activities. New and future developments in microbial biotechnology and bioengineering", 1:237-58.
27. Waqas M, Khan AL, Hamayun M, Kamran M, Kang SM, Kim YH, Lee IJ., (2012) "Assessment of endophytic fungi cultural filtrate on soybean seed germination". African Journal of Biotechnology., 11(85):15135-43.
28. Yang X, Hsieh K, Liu J. Guajadial. (2007), "An Unusual Meroterpenoid from Guava Leaves Psidium guajava", Org Lett ;9:5135–8.
29. Watanabe, T. (2010). "*Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species".* CRC Press, pp. 7-381.