Eco-friendly plant based extracts for the management of *Diaporthe* sp.

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***Abstract*- Stem canker and leaf spot are the common diseases of Sunflower plant caused by *Diaporthe* sp. Nowadays, management of this disease is done by chemical (synthetic) fungicide which is very harmful for environment. This is the reason why various scientists research for the development of natural fungicides. The present study was carried out to investigate the natural fungicidal potential of various parts (leaves, stem-bark, seeds, and fruits) of *Syzygium cumini* against the tested fungal pathogen. Aqueous extracts of leaves, stem-bark, seeds, and fruits of *S. cumini* were prepared and were evaluated for their fungicidal potential *in-vitro*. Data were analyzed by Tukey HSD test at 5% level of significance. Aqueous extracts showed variable degree of fungicidal potential. In general, aqueous fruit extracts exhibited the highest fungicidal effect against mycelial growth of the tested fungal pathogen followed by seed and leaves. In contrast, our study indicates that stem-bark extracts promotes the mycelial growth of test fungi. Among the various extracts, fruit extracts showed the best fungicidal potential against *Diaporthe* sp.**

***Index Terms*- Syzygium cumini, fungicidal potential, Diaporthe sp.**

I. INTRODUCTION

India is one of the nation blessed with a rich inheritance of traditional medical systems and rich biodiversity to balance the herbal needs of the treatment managed by these traditional medical systems. The Indian Systems of Medicine are Ayurveda, Siddha and Unani, which use herbs and minerals in the formulations. India has 15 agro-climatic zones, 47000 plant species of which 15000 are reported to have medicinal properties varying degrees. The World Health Organization (1980) has also recommended the evaluation of the effectiveness of plants in conditions where there is lack of safe synthetic drugs (Warrier et al., 1996).

Diaporthe species are saprobes, endophytes, and plant pathogens (Webber & Gibbs, 1984; Boddy & Griffith, 1989; Udayanga et al., 2011). *Diaporthe* sp. are responsible for diseases on a wide range of plants hosts, some of which are economically important worldwide, causing many severe diseases like twig canker, bud and shoot blight, dieback, wood decay and fruit rot of almond (Adaskaveg et al., 1999; Diogo et al., 2010; Gramaje et al., 2012); canker, shoot dieback, bud and shoot blight of peach (Ogawa et al., 1995; Farr et al., 1999; Thomidis & Michailides, 2009); cankers and shoot blight of apple (Roberts, 1913; Smit et al., 1996; Abreo et al., 2012); dieback and canker of pear and plum (Nakatani et al., 1984; Ogawa et al., 1995; Uddin et al., 1998). The genus *Diaporthe* was introduced by Nitschke (1870). It is placed in the family of Diaporthaceae, order Diaporthales, in the class Sordariomycetes (Maharachchikumbura et al., 2015, 2016). For many years, species in *Diaporthe* were introduced largely on the basis of host association, which resulted in a proliferation of species names. However, it is now recognized that species are not host-specific and a single species can be found on more than one host (Rehner & Uecker, 1994).

Syzygium cumini L., a well known medicinal plant, commonly known as "Jambolin" that belongs to the Myrtaceae family is a large evergreen tree indigenous to the Indian subcontinent. The trees grow up to a height of 50 ft and have large canopy. The leaves are elliptic to broadly oblong, smooth, glossy, leathery, and fibrous in nature. The young bark is pale brown in colour, while the mature bark is darkish brown and scaly. Each fruit is round or oblong or ellipsoid, 1/2 to 2 inch long with a centrally placed large seed. The process of fruit development takes about two months during which a lot of changes in the proximate composition and in the phytochemical constituents occur.

Most parts of the *Syzygium cumini* such as leaves, stem-bark, seed, and fruits have been used in

traditional medicines. The leaves have been extensively used for the treatment of diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy and dermopathy (Warrier et al., 1996). Plant stem-bark is astringent, sweet, refrigerant, stomachic, carminative, diuretic, digestive, antihelminthic, constipating, and antibacterial (Bhandary et al., 1995) and seeds are used to cure diabetes, pharyngitis, spleenopathy and ringworm infection.

Syzygium cumini is a rich source of tannins, flavonoids, essential oils, anthocyanins and other phenolic constituents (Vernin et al., 1991; De Lima et al. 1998). Therefore, it is also used extensively in Ayurveda, Homoeopathy and the traditional Indian system of medicines. The present research work has been undertaken to manage the various diseases caused by *Diaporthe* sp. in vitro through the bio-fungicides using aqueous leaf, stem-bark, seed and fruits extracts of *Syzygium cumini*.

II. MATERIALS AND METHODS

Plant material

Leaves, and stem-bark of *S. cumini* were collected from the trees, growing at Jiwaji University, Gwalior, and the seeds were separated from the fruits which were obtained from local market of Gwalior, Madhya Pradesh, India. To avoid contamination and for effective extraction, collected parts were washed with running tap water and sterilized with 70% alcohol, followed by 0.01% mercuric chloride (HgCl2), and further washed with sterilized distilled water. All Plant parts (Leaves, stem-bark, seeds, and fruits) were dried in hot-air oven at 60 °C and homogenized to fine powder. Powdered sample was stored in airtight container at room temperature for further study.

Test organism

Diaporthe sp. was isolated from the infected leaves of *S. cumini* by pour plate technique, using Potato Dextrose Agar media (PDA) supplemented with chloramphenicol. The pure culture was further sub cultured on PDA (Potato Dextrose Agar) at 26 ±1 °C for 7days.

Preparation of plant part Extract and Evaluation of fungicidal potential

Ten gram (10gm) powdered sample of each part i.e. leaves, stem bark, seed and fruit of *S. cumini* was dissolved in 100 ml of PDA media. Media was sterilized at 121^0C for 15min. Total 20 ml PDA medium was poured into sterilized Petri dishes and allowed to solidify. Five replicates & one control (without extract) were maintained. 5 mm disc of 7 days old culture of test organism was inoculated. All Petri-plates were incubated at 26 ±1 °C for 8 days. Mycelial growth diameter of *Diaporthe* sp. was measured in mm at fourth, sixth and eighth day of incubation period and the fungicidal potential of the extracts in terms of percentage inhibition of mycelial growth were calculated (Deans and Svoboda, 1990).

III. STATISTICAL ANALYSIS

Statistical analysis was performed using Minitab software: version 16.0. The results are presented as the mean ± S.D. One-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to check the significance of the results with the level of significance set at $p<0.05$.

IV. RESULTS

Present study showed significant antifungal activity of aqueous extracts of various part of *S. cumini*, against *Diaporthe* sp. Antifungal activity of various parts and their potency was assessed by measuring the mycelial growth diameter of test fungi. The results of screening are encouraging as out of four parts extracts i.e. leaves, stem-bark, seeds and fruits, fruits extracts was found very much effective against the growth diameter of *Diaporthe* sp.

Maximum inhibition in growth diameter was observed in aqueous fruit extract (16.0±0.54 mm); whereas leaves and seed extracts showed least inhibition in growth diameter of test fungi. Stem-bark extract enhanced the growth diameter of test fungi in comparison to control i.e. aqueous stem-bark extract was not found effective in inhibiting the growth diameter of *Diaporthe* sp.

Table: Effect of aqueous extracts of leaves, stem-bark, seeds, and fruits of *S. cumini* on the mycelial growth diameter of *Diaporthe* sp.

S. N.	Plant parts	Mycelial growth in diameter (mm)					
		4th Day		6th Day		8th Day	
		Control	Treatment	Control	Treatment	Control	Treatment
1.	Leaves	30.0±0.00[a]	29.0±0.00[b]	50.0±0.54[a]	48.0±0.54[b]	68.0±0.54[a]	65.0±0.54[b]
2.	Stem-bark	30.0±0.44[a]	33.0±0.54[c]	50.0±0.54[a]	54.0±0.54[c]	68.0±0.54[a]	70.0±0.44[c]
3.	Seeds	30.0±0.44[a]	27.0±0.44[b]	50.0±0.54[a]	43.0±0.54[d]	68.0±0.54[a]	63.0±0.54[b]
4.	Fruits	30.0±0.44[a]	07.0±0.00[d]	50.0±0.54[a]	14.0±0.54[e]	68.0±0.54[a]	16.0±0.54[d]

Values are given as mean ±S.D. of five replicates

Means in columns (control and treatment) that do not share a superscript letter are significantly different (One way ANOVA at P <0.05 followed by Tukey HSD test).

V. DISCUSSION

The results obtained in vitro could be useful from the bio-fungicidal point of view. The aqueous extracts of leaves, stem-bark, seeds and fruits of *syzygium cumini* were evaluated for the antifungal properties against the *Diaporthe* sp. Among all aqueous extracts, fruits extract can be used in future field trial evaluation of the efficacy of control of Diaporthe sp. . In an agreement with the study of Alice et al., (2015), he isolated a hundred and eight endophytic fungal sp. and reported that the most frequent species associated with *Opuntia humifusa* were *Alternaria* sp., the endophytic communities , *Aureobasidium pullulans* and *Diaporthe* sp. may be a source of bioactive molecules, able to inhibit or control plant disease pathogens. Gupta and Bhadauria, (2012) also reported fungicidal activity of the aqueous extract of *S. cumini* against the *F. oxysporum* and *Alternaria alternata.* Starovic et al., (2017) . They also investigated in vitro antifungal activities of several essential oils against the phytopathogenic fungus *Phomopsis theicola*.

Phomopsis stem canker (*Diaporthe helianthi*) is a worldwide fungal disease which is responsible for high yield losses in sunflower crop in the main regions of production. Debaeke and Moinard (2010) observed the relative humidity within the canopy, the number of leaf infection events and the final proportion of stem lesions were positively related which clearly demonstrated the key role of crop management in the development of stem canker in sunflower. Stem-bark extract of *syzygium cumini* enhanced the growth diameter of *Diaporthe* sp. in comparison to control i.e. aqueous stem-bark extract was not found effective in inhibiting the growth diameter of *Diaporthe* sp. Similarly Bastos et al., (2017) reported that the *Diaporthe* sp. has the potential to produce molecules with herbicidal activity. This study showed that the leaves and seed extracts exhibited least inhibition in growth diameter of test fungi. An agreement of Chandrasekaran and Venkatesalu (2004) reported good antifungal activity from the aqueous leave extracts of *S. cumini* as compared to the ethanolic fractions against the *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, & *Aspergillus niger* and they also suggested that it may be due to the high concentration of some phytoconstituent soluble in aqueous extract than ethanolic. Several earlier investigations have already been reported antimicrobial activity from leaves of *S. cumini* and other plant parts (Ugbabe *et al*., 2010; Gupta et al., 2014; Gupta et al., 2015; Gupta et al., 2016).

VI. CONCLUSION

The main purpose of using plant part (leaves, stem-bark, seed and fruits of *S. cumini*) extracts was to study their fungicidal (antifungal) activity against the pathogen *(Diaporthe* sp.) used in the present study as eco-friendly means, as most of the plant part extracts are readily available, environmentally safe, less risky for developing resistance in pests and pest resurgence, has less adverse effect on plant growth, less harmful to seed viability and quality, and above all, less expensive. The results of the present study may be exploited for formulating integrated various disease managements by reducing the mycelial growth diameter of destructive pathogens, *Diaporthe* sp. from the infected plants.

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