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In vitro antifungal activity of *Tamarindus indica* L. extract against pathogenic fungi

Satpute SB, Vanmare DJ

Department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Samarthnagar, Aurangabad, Maharashtra, India

Abstract
An improper use of agrochemicals especially fungicides which are more carcinogenic risk than insecticides and herbicides which gives rise to undesirable side effects. Natural fungicides were widely used in the perspective of sustainable methods of plant disease control because of their ecofriendly properties, being easily accessible and relatively less cost. The aim of the present work was to determine the *in vitro* antifungal activity of the aqueous leaf extracts of *Tamarindus indica* L**.**, collected from Shendra M.I.D.C. area Aurangabad (MS).Their antifungal properties were tested against the five pathogenic fungi- *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum sp.* and *Gibberella avenaceum* at different concentrations ranging from 10,20,.....100%. At 100% concentration, aqueous leaf extract of *Tamarindus indica* L. shows excellent inhibitory activity against *Alternaria citri* (87.59%), *Gibberella avenaceum* (51.67%), *Fusarium incarnatum* (42.11%). The application of plant extracts for disease management could be less expensive, easily available, non- polluting and ecofriendly.

Keywords: *tamarindus indica,* pathogen, *antifungal* activity, ecofriendly, *gibberella avenaceum*

1. Introduction
Fungal plant diseases represent an important cause of increased annual crop losses. More than 70% of all major crop diseases are caused by fungi (Agrios, 2005) [3]. Generally, the control of plant diseases and pests is well established with synthetic fungicides and other agricultural practices such as crop rotation inter-cropping and sanitation (Pretty, 2008) [24]. However, in the recent years the farmers all over the world have reported an efficacy decrease of the treatments with traditionally used fungicides to control early blight and other plant diseases (Fairchild *et al*., 2013) [10]. Furthermore, the inappropriate use of fungicides was increased in more frequent dosage units (Genet *et al*., 2006) [12]. It was resulted in the occurrence of fungal resistance (Brent and Hollomon, 1998; Mcgrath, 2001; Haouala, 2008) [7, 20, 13] and also in hazardous effects in human and animal health and on the environment resulting in ecological imbalances (Pramila and Dubey, 2004) [25]. Many agriculturally important pesticides have been banned by World Health Organization (WHO) due to their wide range of toxicity against nontarget organisms including humans, which are known to cause pollution problem (Barnard *et al*, 1997) [6].

Recently aqueous leaf extracts as natural products were widely used to control pests (Lale *et al*., 1999; Islam *et al*.; 2004) [17, 15]. The natural fungicides are easily accessible and relatively cost effective, in the perspective of sustainable methods of plant. Various reports on plants extracts have shown inhibitory effects against phytopathogenic fungi *in vitro* (Ashwani Tapwal *et al*., 2011; Abdulrahman A. Aba Alkhail, 2005; Mangang H.C. and Nchhetry G.K., 2012; Inampudi Sailaja, 2014; Umesh P.Mogle, 2013; Raji R. and Raveendran K., 2013**)** [5, 17, 1, 18, 14, 28, 26]. In view of this, the present work was done to determine the *in vitro* antifungal activity of the aqueous leaf extracts of *Tamarindus indica* L. collected from Shendra M.I.D.C. area Aurangabad (MS).Their antifungal properties were tested against the five pathogenic fungi- *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum sp.* and *Gibberella avenaceum* at 10, 20, ...100% concentrations.

2. Materials and Methods
2.1 Isolation of Pathogens
The five fungal pathogens viz. *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum sp.* and *Gibberella avenaceum* of some ornamental plants were isolated on PDA. The pure cultures were maintained on PDA media and allowed to grow at 28°±1°C for 7 days and preserved on slants in laboratory in a refrigerator at 4°C.

2.2 Collection of Plant Material
Fresh and healthy leaves of *Tamarindus indica* L. were collected from Shendra MIDC area, Aurangabad (M.S.). The leaves were washed with running tap water and finally rinsed with distilled water. After that leaves were blotted with filter paper and used in the preparation of leaf extract.

2.3 Preparation of Aqueous Leaf Extract
Aqueous leaf extract was obtained by grinding 100 gm of leaves in 100 ml of distilled water. It was strained through double layered muslin cloth. The leaf extract were allowed to settle for a while and supernatant were passed through Whatman's filter paper No.1. The filtrate was used for the test.

2.4 Antifungal Activity
Antifungal activity studied by Poisoned Food Technique. Potato Dextrose Agar (PDA) medium with 10, 20, ... 100% concentration of the aqueous leaf extracts of *Tamarindus indica* L. were prepared and poured into sterile petrilpates and allowed to cool and solidify. Then 5 mm mycelial discs of seven days old cultures of *Fusarium incarnatum,*

Alternaria citri, *Colletotrichum musae*, *Colletotrichum sp.* and *Gibberella avenaceum* were placed at the centre of the petriplates and incubated at 28°±1°C for seven days. The PDA medium without the aqueous leaf extract but with the same concentration of sterile distilled water treated as a control. The colony diameter was measured in mm. These antifungal activities were done in triplicates. The percentage inhibition of mycelia growth was calculated by the following formula (Dissanayake, M.L.M.C., 2014).

Percentage Inhibition = [(dc – dt)/ dc] × 100 Where, dc= Average colony diameter in control. dt = Average colony diameter in treatment.

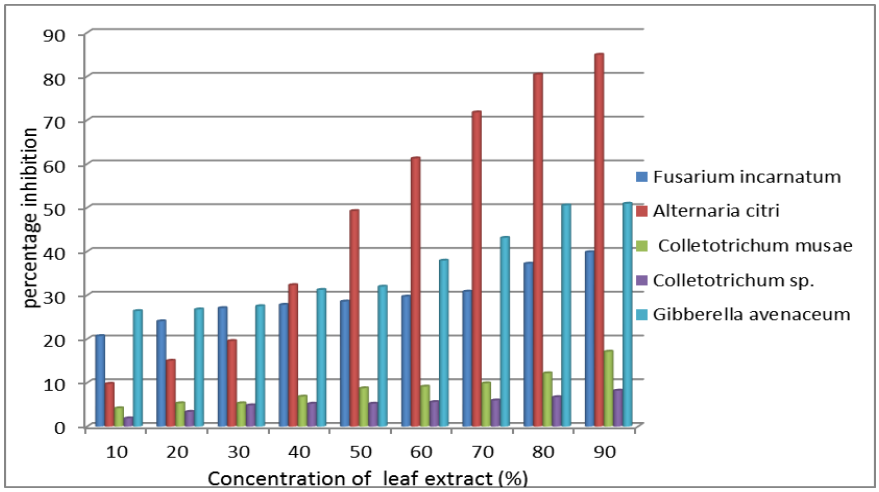
3. Results and Discussions

The antifungal activity was determined by measuring the percentage inhibition in radial growth. The percentage inhibition was corroborated at different concentrations of aqueous leaf extract against five pathogenic fungi i.e *Fusarium incarnatum*, *Alternaria citri*, *Colletotrichum musae*, *Colletotrichum sp. and Gibberella avenaceum.* At 100% concentration, aqueous leaf extract of *Tamarindus indica* L. shows excellent inhibitory activity against *Alternaria citri* (87.59 %), *Gibberella avenaceum* (51.67%), *Fusarium incarnatum* (42.11%) (fig.2). At 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% concentration of leaf extract, the percentage inhibition was 20.68, 24.06, 27.07, 27.82, 28.57, 29.70, 30.83, 37.22, 39.85, and 42.11 % respectively against *Fusarium incarnatum*, the percentage inhibition was 9.77, 15.04 , 19.55, 32.33, 49.25, 61.28 , 71.80 , 80.45, 84.96, and 87.59 % respectively against *Alternaria citri*, the percentage inhibition was 4.18 , 5.32 , 5.32, 6.84, 8.75, 9.13, 9.89, 12.17, 17.11, and 17.87 % respectively against *Colletotrichum musae*, the percentage inhibition was 1.87, 3.36, , 4.85, 5.22, 5.22, 5.60, 5.97, 6.72, 8.21, and 8.96 % respectively against *Colletotrichum sp.* and the percentage inhibition was 26.39, 26.77, 27.51, 31.23, 31.97, 37.92, 43.12, 50.56, 50.93 and 51.67 % respectively against *Gibberella avenaceum* (Table1, fig.1).

Table 1: Effect of *Tamarindus indica* L. aqueous leaf extract on pathogenic fungi.

Conc. of Leaf Extract (%)	Percentage inhibition of the mycelial growth of pathogenic fungi.				*Gibberella avenaceum*
	Fusarium incarnatum	*Alternaria citri*	*Colletotrichum musae*	*Colletotrichum sp.*	
10	20.68	9.77	4.18	1.87	26.39
20	24.06	15.04	5.32	3.36	26.77
30	27.07	19.55	5.32	4.85	27.51
40	27.82	32.33	6.84	5.22	31.23
50	28.57	49.25	8.75	5.22	31.97
60	29.70	61.28	9.13	5.60	37.92
70	30.83	71.80	9.89	5.97	43.12
80	37.22	80.45	12.17	6.72	50.56
90	39.85	84.96	17.11	8.21	50.93
100	42.11	87.59	17.87	8.96	51.67

Fig 1: Effect of *Tamarindus indica* L. leaf extract on pathogenic fungi.



These results were confirmed by many authors and found that plant extract shows antifungal activity against plant pathogenic fungi. (Ashwani Tapwal *et al.*, 2011; Abdulrahman A. Aba Alkhail, 2005; Mangang, H.C.,and chhetry G.K.N. 2012; Inampudi Sailaja, 2014; Umesh P.Mogle 2013 ; Raji R. and Raveendran K. 2013 ; Doughari J.H. 2006; Shehu K. *et al.*, 2016; Garcia L. 2011; Aram Abuzied, Mawadda Adam, *et al.*, 2014; Abubakar M.G. *et al.*, 2010 ; Maria Diana Cerqueira Sales *et al.*, 2016 ; Paola Díaz Dellavalle ,2011 ; Julio César Escalona-Arranz , 2010 ; Nehad M. Gumgumjee 2012) [5, 17, 18, 14, 28, 26, 9, 27, 11, 4, 2, 19, 22, 16, 21]. Finally, from the result concluded that, leaf extract of *Tamarindus indica* L. is best alternative to the harmful chemical fungicides and can be effectively used as eco-friendly fungicides against *Alternaria citri, Gibberella avenaceum* and *Fusarium incarnatum,* (fig.2) which can help to controlled pollution caused by chemical fungicides.

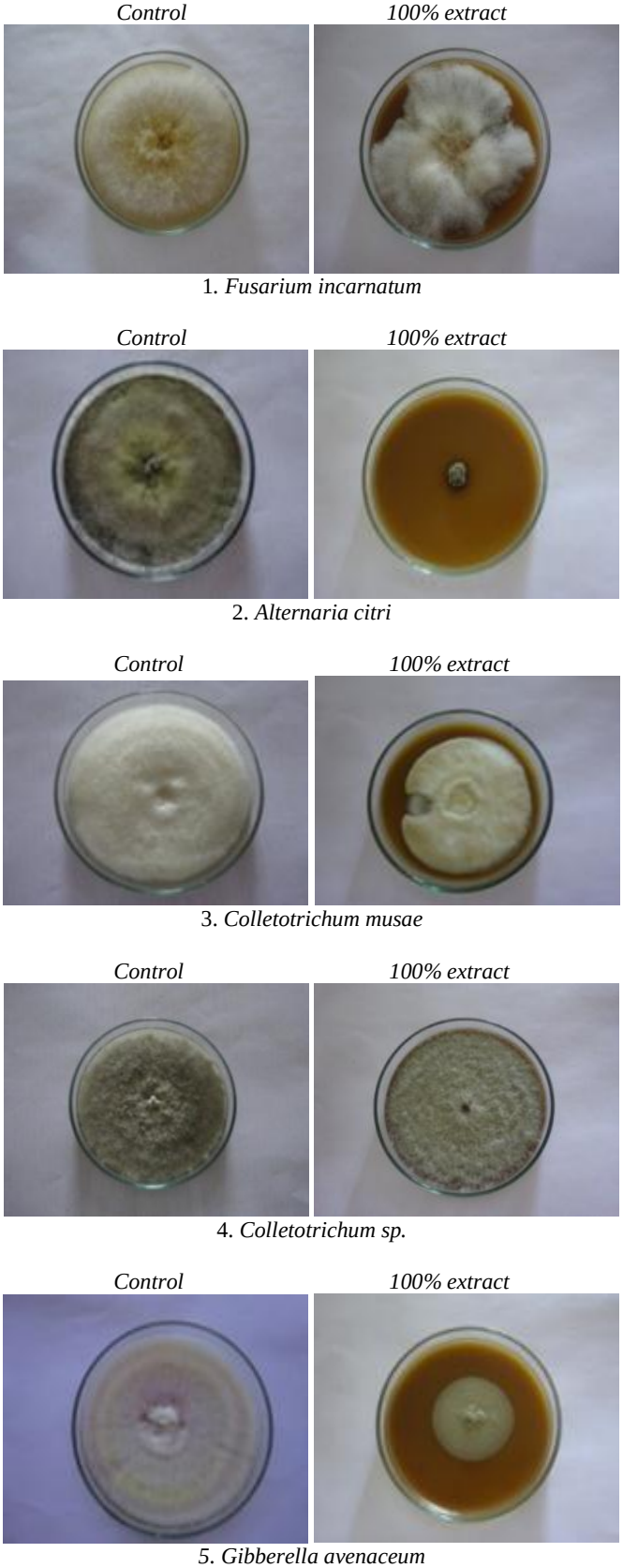


Fig 2: Control and treatment set of *Tamarindus indica* L. extract on pathogenic fungi.

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