Scholars Journal of Agriculture and Veterinary Sciences **e-ISSN 2348–1854**
Sch J Agric Vet Sci 2017; 4(4):155-159 **p-ISSN 2348–8883**

(An International Publisher for Academic and Scientific Resources) DOI: 10.36347/sjavs.2017.v04i04.005

Evaluation of antifungal activity of some plants against seed-borne fungi

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Abstract: The present study was carried out to screen the antifungal potential of extracts from 9 plant species against six seed-borne fungi. Extraction of shade dried plant materials was carried out by Maceration process using methanol. Antifungal activity of methanol extract of selected plants was determined by Poisoned food technique. All plants exhibited marked antifungal activity and suppressed the mycelial growth of test fungi. Plants namely *Salix tetrasperma* and *Nicotiana plumbaginifolia* displayed marked antifungal activity when compared to other plants. All plants exhibited >50% inhibition of *Aspergillus niger*. Among leaf and flower extracts of *Kigelia africana* and *Clerodendrum philippinum*, leaf extracts exhibited potent antifungal activity. The selected plants can be used for the prevention of seed deterioration and in the management of plant diseases caused by seed-borne fungi.
Keywords: Plants, Maceration, Antifungal activity, Poisoned food technique, Seed-borne fungi

INTRODUCTION

Plants have been used for various by humans since time immemorial. Plants suffer from a number of diseases that are caused by different pathogenic microbes such as bacteria, fungi, viruses and nematodes. Among the pathogenic microbes, fungi are considered to be dominant as they are responsible for causing many diseases in crops leading to huge economic losses. Besides, many fungi are responsible for causing deterioration of grains during storage. The crop diseases can often be seed-borne. Fungi such as *Alternaria*, *Aspergillus*, *Cercospora*, *Bipolaris*, *Curvularia*, *Dreschlera*, *Fusarium*, *Penicillium*, *Pyricularia*, *Pythium*, *Rhizoctonia* and *Rhizopus* are the most common fungi associated with the seeds and many of these are implicated in causing seed abortion, seed rot, seed necrosis, reduction of germination capacity and seedling damage. The use of seed treatment method is the safest and the cheapest approach for controlling seed-borne diseases as well as deterioration of grains. The use of synthetic fungicides is one of the widely used approaches. However, their use is associated with drawbacks such as environmental pollution, adverse effects on humans and emergence of resistant pathogens. Botanicals offer the safest and cheapest alternates and many studies have shown the potential of plants against a wide range of seed-borne fungi [1-14]. The present study was conducted to determine antifungal activity of 9 plant species collected from different regions of Shivamogga district, Karnataka against six seed-borne fungi.

MATERIALS AND METHODS

Collection and identification of plants

The plants were collected from different places of Shivamogga district, Karnataka, India during January-February 2017. The plants were identified by referring flora [15, 16] and with the help of taxonomists. Details on the family, part of the plant used and the place of collection of plants are given in Table 1.

Table 1: Plants selected for this study

Sl. No.	Name of the plant	Family	Part used	Place of collection
1	*Kigelia africana* (Lam.) Benth.	Bignoniaceae	Leaf and flower	Shikaripura
2	*Clerodendrum philippinum* Schauer	Verbenaceae	Leaf and flower	Shikaripura
3	*Salix tetrasperma* Roxb.	Salicaceae	Leaf	Siddarahalli
4	*Azima tetracantha* Lam.	Salvadoraceae	Leaf	Matturu
5	*Kirganelia reticulata* (Poir.) Baill.	Euphorbiaceae	Leaf	Malalakoppa
6	*Ixora brachiata* Roxb.	Rubiaceae	Aerial parts	Shiralakoppa
7	*Rungia repens* (L.) Nees	Acanthaceae	Aerial parts	Matturu
8	*Antigonon leptopus* Hook. & Arn.	Polygonaceae	Leaf	Matturu
9	*Nicotiana plumbaginifolia* Viv.	Solanaceae	Whole plant	Matturu

Extraction of plant materials

The selected parts of the plants were washed well using clean water to remove adhering dirt and dust. The plant materials were dried in shade and powdered in a blender. A known quantity of each of the plant material was extracted by maceration process using methanol in a stoppered container. The plant material was placed in methanol and left for 48 hours with occasional stirrings. The contents were filtered through 4-fold muslin cloth followed by Whatman filter paper No. 1. The filtrates were evaporated at 40^oC to get crude extract. The extract was stored in the refrigerator [9].

Test fungi

Six seed-borne fungi namely *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Curvularia* sp., *Alternaria* sp. and *Fusarium* sp. were tested for their susceptibility to extract of selected plants by Poisoned food technique. The fungi were isolated previously from sorghum and were maintained on Potato dextrose agar (PDA) slants.

Antifungal activity of selected plants

The antifungal potential of plants, in terms of inhibition of radial growth of test fungi, was assessed by Poisoned food technique. In brief, the test fungi were inoculated aseptically at the centre of control (without extract) and poisoned PDA (1mg extract/ml of medium) plates by point inoculation method. The plates were incubated for 96 hours at room temperature. After incubation, the diameter of fungal colonies in control as well as poisoned plates was measured in mutual perpendicular directions. Antifungal effect of extracts, in terms of inhibition of mycelial growth of test fungi, was determined using the formula:

Inhibition of mycelial growth (%) = (C – T / C) x 100, where C and T denotes the colony diameter of test fungi in control and poisoned plates respectively [9].

RESULTS AND DISCUSSION

Interest in higher plants with antifungal activity has intensified due to some drawbacks that are associated with the use of synthetic chemicals. A number of studies have highlighted the potential of several plant species to exhibit antifungal activity against a range of phytopathogenic fungi including seed-borne fungi [1, 3, 8, 9, 11, 17-21]. In the present study, we evaluated the antifungal activity of 9 plant species against 6 seed-borne fungi by Poisoned food technique. Poisoned food technique is one of the most widely used in vitro antifungal assays being used by several researchers to screen the antifungal effect of plants. A considerable reduction in the mycelial growth of test fungi occurs if the test sample contains antifungal principles [1, 4, 8, 9, 22, 23]. The result of antifungal potential of extracts from selected plants is shown in Table 2 and Figure 1. Among *Aspergillus* species, *A. Niger* was shown to be highly susceptible to all extracts. All extracts were shown to cause >50% inhibition *A. Niger*. The inhibition of *A. Niger* by selected plants was in the range 52.63 to 68.42%. Highest inhibitory activity was shown by extract of *S. tetrasperma*, *N. plumbaginifolia* and *A. tetracantha* (68.42%) while least inhibitory effect was displayed by flower extract of *C. philippinum* (52.63%). The inhibition of *A. flavus* by extracts ranged from 27.90 to 53.48%. Extract of *S. tetrasperma* and *N. plumbaginifolia* exhibited marked inhibition of *A. flavus* (53.48%) while leaf extract of K. reticulata showed least inhibitory activity against *A. flavus* (34.88%). The inhibition of *A. fumigatus* was in the range 33.33 to 58.97%. Extract of *S. tetrasperma* inhibited *A. fumigatus* to high extent (58.97%) while least inhibition of *A. fumigatus* was caused by *R. repens* and *K. africana* flower (33.33%).

The range of inhibition of *Curvularia* sp. was 46.6 to 71.1%. Out of 11 extracts, 9 extracts exhibited >50% inhibition of *Curvularia* sp. Three plants namely *S. tetrasperma, A. tetracantha* and *A. leptopus* displayed higher inhibitory activity against *Curvularia* sp. (71.1%) while least inhibition of *Curvularia* sp. was displayed by flower extracts of *K. africana* and *C. philippinum* (46.6%). The extent of inhibition of *Alternaria* sp. by extracts ranged from 30.7 to 75.0%. High inhibitory activity against *Alternaria* sp. was shown by extract of *N. plumbaginifolia* (75%) while flower extract of *K. africana* displayed least inhibition of *Alternaria* sp. (30.7%). The inhibition of *Fusarium* sp. by extracts was in the range 30.2 to 60.4%. Flower extracts of *K. africana* and *C. philippinum* displayed least inhibition of *Fusarium* sp. (30.2%) while extract of *N. plumbaginifolia* displayed high inhibitory activity against *Fusarium* sp. (60.4%). Among leaf and flower extracts of *K. africana*, leaf extract exhibited marked antifungal activity when compared to flower extract. In case of *C. philippinum* also, leaf extract displayed high antifungal potential when compared to flower extract. Studies have shown the potential of several plants to inhibit mycoflora isolated from seeds of plans such as Sorghum [1, 24, 25], Maize [1, 24, 26], *Solanum gilo* [27], Rice [1, 24, 28], tomato [29], *Solanum melongena* [30], green gram [31], soybean [32, 33], barley [34] and ground nut [35].

Table 1: Antifungal activity of methanol extract of selected plants

Treatment	**Colony diameter in cm (% Inhibition)**					
	A. niger	***A. flavus***	***A. fumigatus***	***Curvularia* sp.**	***Alternaria* sp.**	***Fusarium* sp.**
Control	5.7	4.3	3.9	4.5	5.2	4.3
K.africana leaf	2.3 (59.64)	2.2 (48.83)	2.4 (38.46)	1.6 (64.4)	2.2 (57.6)	2.4 (44.1)
K.africana flower	2.6 (54.38)	2.7 (37.20)	2.6 (33.33)	2.4 (46.6)	3.6 (30.7)	3.0 (30.2)
C.philippinum leaf	2.6 (54.38)	2.1 (51.16)	2.1 (46.15)	2.0 (55.5)	2.8 (46.1)	2.8 (34.8)
C.philippinum flower	2.7 (52.63)	2.6 (39.53)	2.3 (41.02)	2.4 (46.6)	3.4 (34.6)	3.0 (30.2)
S.tetrasperma	1.8 (68.42)	2.0 (53.48)	1.6 (58.97)	1.3 (71.1)	2.0 (61.5)	2.3 (46.5)
K.reticulata	2.2 (61.40)	2.8 (34.88)	1.9 (51.28)	1.5 (66.6)	2.2 (57.6)	2.1 (51.2)
A.leptopus	2.1 (63.15)	2.6 (39.53)	2.2 (43.58)	1.3 (71.1)	1.9 (63.4)	2.5 (41.9)
A.tetracantha	1.8 (68.42)	2.3 (46.51)	2.1 (46.15)	1.3 (71.1)	2.7 (48.0)	2.3 (46.5)
I.brachiata	2.0 (64.91)	2.5 (41.86)	2.4 (38.46)	1.4 (68.8)	2.5 (51.9)	2.2 (48.8)
R.repens	2.3 (59.64)	2.6 (39.53)	2.6 (33.33)	1.5 (66.6)	2.9 (44.2)	2.1 (51.2)
N. plumbaginifolia	1.8 (68.42)	2.0 (53.48)	2.1 (46.15)	1.4 (68.8)	1.3 (75.0)	1.7 (60.4)

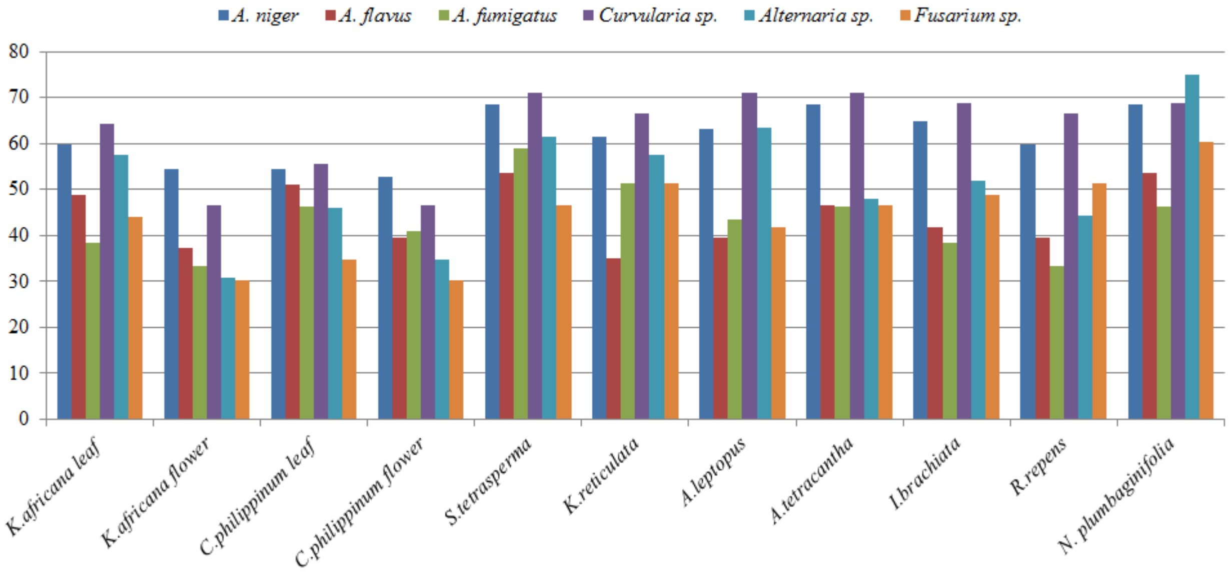

Fig 1: Extent of inhibition (%) of test fungi by selected plants

CONCLUSION

Exploitation of plants and plant based formulations in crop protection and prevention of biodeterioration of grains caused by fungi appear to be promising. In the present study, methanol extract from the selected plant species exhibited marked antibacterial activity against the seed-borne fungi. Treatment of seeds with these plants can be effective in reducing fungal infections and promoting seedling emergence and better growth. In suitable form, the plants can be exploited as antifungal agents against seed-borne fungi.

ACKNOWLEDGEMENTS

Authors thank Head of the Department of Microbiology and Principal of S.R.N.M.N College of Applied Sciences for providing facilities to conduct work. Authors thank N.E.S for moral support. Authors also thank Dr. Vinayaka K.S and Prof. D. Rudrappa for assisting in collection and identification of plant materials.

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