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A biorational approach for management of key plant pathogens

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ABSTRACT

The antifungal potential of solvent extracts of four plants, viz. *Polyalthia longifolia* (Sonn.) Thw. (PL)*, Paederia foetida* (PF)*, Limonia acidissima* L. (LA) and *Balanites aegyptiaca* (L.) Del. (BA)*,* were screened during the year 2010 and 2011 against important plant pathogenic fungi. The methanol, petroleum ether, chloroform and water extracts, of the plants were bio-assayed against test fungi, *Rhizoctonia bataticola*, *R. solani*, *Sclerotinia sclerotiorum, S. rolfsii*, *Fusarium oxysporum*, *Alternaria alternata* and *Pythium aphanidermatum*. Out of 16 plant extracts, extracts of *P. longifolia* recorded significant antifungal activity (up to 84% inhibition) against almost all test fungi. The methanol extract of *P. longifolia* gave most promising results for inhibiting the test fungi, resulting in inhibition concentration (IC_{50}) in the range of 200-600 ppm. Hence, a single methanol extract of the plant *P. longifolia* could act as a promising biofungicide for inhibiting the growth of different fungi responsible for crop losses. Thus these plants extracts have potential to act as effective fungicide.

Key words: Disease management, Fungitoxicity, Plant extracts, Plant pathogens, Screening

The farmers of Gujarat, India are practising certain indigenous technologies for the management of pests under plant protection. The National Innovation Foundation (NIF), Ahmedabad, Gujarat, an autonomous institution of Department of Science and Technology, Government of India, provides institutional support to grassroot innovators and traditional knowledge holders from the unorganized sector of the society. NIF encourages farmers/innovators by inviting their innovations, and has provision to award the best innovation. The indigenous technologies are then scientifically validated under lab conditions for further validation. The paper presents screening of extracts of four plants, which were practiced by farmers since years for crop pest management.

The global agricultural production sustains annual loss of about 20 to 30% on an average due to plant diseases on various crops in different countries. Occasionally, the losses rise even to 100% in the most favorable circumstances or when no control measures are taken in case of some important diseases. Plant diseases are one of the major bottlenecks in agricultural production particularly in irrigated crops, in monoculture cultivations and in certain widely grown rainfed crops as well (India agronet 2012).

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Synthetic chemicals may be used in plant protection programs to limit crop damage by pests and pathogens. But because of growing concerns about health and environmental safety, the use of toxic, carcinogenic, and/or environmentally damaging chemicals are being discouraged. These chemicals leave toxic residues in consumable agricultural commodities. The survey of monitoring of farm-gate samples in different parts of the country recorded pesticide residues above Maximum Residue Limit (MRL) (Kole *et al.* 2002, Madan *et al.* 1996, Mandal and Singh 1996).

The solvent extracts of four plants, viz. *Polyalthia longifolia* (Sonn.) Thw. (Annonaceae family) (English name: False Ashoka, Buddha tree, Indian mast tree, Indian fir tree)*, Paederia foetida* L. (Rubiaceae family) (English name: Skunk vine tree), *Limonia acidissima* L. (Rutaceae family) (English name: Wood apple or curd fruit) and *Balanites aegyptiaca* (L.) Del. (Zygophyllaceae family) (English name: soap berry tree or bush or Thron tree) from different families were screened for their antifungal potential against important plant pathogenic fungi. The solvent extracts, namely methanol, petroleum ether, chloroform and water, of all four plants were tested against test fungi, *Rhizoctonia bataticola*, *R. solani*, *Sclerotinia sclerotiorum, S. rolfsii*, *Fusarium oxysporum*, *Alternaria alternata* and *Pythium aphanidermatum* (Lalitha *et al.* 2011, Upadhyaya 2013, Naidu 2014).

Keeping in gravity of problems of environmental contamination by use of synthetic pesticides and yield losses due to above mentioned plant pathogens, the authors decided

to screen *in vitro* a large number of plants extracts for antifungal activity against these important plant pathogenic fungi.

MATERIALS AND METHODS

Fresh disease free plant parts (fruits and leaves) were collected from different regions of country. The plant parts used for extraction were, fruits of *Balanites aegyptiaca*, aerial parts (leaves with stems; without roots) of *Paederia foetida*; leaves of *Limonia acididssima*; and seeds of *Polyanthia longifolia.* The preparation of extracts followed three different methods.

For aqueous extractions, the leaf samples (100 g) of plants were thoroughly washed, blot dried and powdered using blender. Ten g dry powder was soaked in 200 ml distilled water and allowed to boil on water bath at 100ºC till the total volume of water was reduced to half. The solution was filtered using stainless steel sieve followed by Whatman filter paper No 1. The excess water was evaporated under reduced pressure at 50^0C using rotary evaporator. The sample was sterilized at 120^0C for 30 min., which served as the mother extract. Dry extracts were stored in air tight glass vials. The Method 1 was followed to prepare water extracts of all plants. (Darout *et al.* 2000, Turi *et al.* 1999).

For Method 2, the mature leaves of all the test plants were thoroughly washed and shade dried and then powdered with the help of a blender. The powder (10 g) was extracted with 100ml of organic solvents of varied polarity methanol, chloroform and petroleum ether in 250 ml screw capped flask. The flasks were put on rotary shaker for 3-4 hours, at 120 rpm. After shaking the materials in solvents, the flasks were left overnight for cold maceration. The extracts were filtered through Whatman filter paper No. 1, impregnated with same solvent. During filtrations, small amount (2 g) of anhydrous sodium sulphate was put on filter paper to avoid any water contents in filtrate. The organic solvents were concentrated to near dryness under reduced pressure at temperature below 40°C. Dry extracts were stored in airtight brown bottle until further use. All the extracts were subjected to antifungal activity against the test fungi. The methanol and petroleum ether extracts were prepared following Method No. 2 (Mongelli *et al.* 1997, Mingarro *et al.* 2003).

Thoroughly washed mature leaves of all the test plants were shade dried and then powdered with the help of a blender. The plant powder (10 g) was extracted with 200 ml organic solvent, in a flask of 500 ml capacity, using soxhlet apparatus. Three repeat reflux were carried out for each plant sample. After extraction, the organic solvent was concentrated to near dryness under reduced pressure maintaining temperature below 40ºC. Chloroform extracts were prepared using Method No. 3.

The mother culture of test fungi, *Rhizoctonia bataticola*, *R. solani*, *Sclerotinia sclerotium, S. rolfsii*, *Fusarium oxysporum*, *Alternaria* spp. and *Pythium aphanidermatum,* were procured from plant pathology division of IARI and NCIPM, New Delhi, followed by their sub-culturing on Potato Dextrose Agar (PDA) medium in laboratory. Fungal-bioassay studies were carried out in laboratory by poison food technique. In this technique PDA media was poisoned with different concentrations of the plant extracts (0.1, 0.05, 0.5, 0.01, 0.005% and control).About 15 ml of this medium was poured into each petriplate and allowed to solidify. Five mm disc of 7-day-old culture of the test fungi were placed at the center of the petriplates and incubated at 25±2 ºC for seven days. After incubation the colony diameter was measured in millimeter. For each treatment four replicates were maintained. PDA medium without the extract served as control. The inhibition of fungal growth in treated medium, compared to control gave the evaluation of fungitoxicity of

Table 1 Inhibition of test fungi against various plant extracts

Plant extracts	Solvent name	% inhibition of test fungi						
		R. bataticola	*R. solani*	*P. aphanidermatum*	*S. rolfsii*	*S. sclerotiorum*	*A. alternata*	*F. oxysporum*
LA	Water	No	No	No	No	No	No	No
	Methanol	41.3	13.8	46.3	38.8	20.0	45.0	22.5
	Pet. ether	No	No	No	No	No	No	No
	Chloroform	No	No	No	No	No	No	No
BA	Water	17.5	37.5	17.5	23.8	18.8	17.5	22.5
	Methanol	53.8	85.0	73.8	33.8	23.8	18.8	35.0
	Pet. ether	16.3	43.8	23.8	21.3	No	40.0	32.5
	Chloroform	32.5	83.8	56.3	100.0	No	No	No
PF	Water	17.5	22.5	18.8	21.3	21.3	17.5	20.0
	Methanol	18.8	17.5	18.8	20.0	18.8	20.0	15.0
	Pet. ether	No	No	No	No	No	No	No
	Chloroform	No	55.0	31.3	26.3	No	No	No
PL	Water	41.3	55.0	66.3	53.8	43.8	46.3	37.5
	Methanol	81.3	68.8	83.8	36.3	82.5	36.3	37.5
	Pet. ether	75.0	62.5	65.0	55.0	No	57.5	45.0
	Chloroform	82.5	68.8	61.3	62.5	68.8	No	No

LA- *Limonia acidissima;* BA- *Balanites aegyptica*; PF- *Paederia foetida*; PL- *Polyalthia longifolia*

tested extracts (Table 1).The fungitoxicity of extracts in terms of percentage inhibition of mycelial growth was calculated by using the formula:

$$\% \text{ inhibition} = dc - dt/dc \times 100$$

where dc, Average increase in mycelial growth in control, dt, average increase in mycelial growth in treatment (Arora and Gopal 2006).

RESULTS AND DISCUSSION

Out of total 16 plant extracts (basically four solvent extracts of four plants each), extracts of only *Polyalthia longifoila* exhibited maximum potency for *in-vitro* fungal inhibition of all test fungi (Table 1). The water extracts of all plants except *P.longifolia* could not inhibit any test fungi. The *Polyalthia longifolia* (methanol) extract was observed to give promising and significant results for fungal inhibition of all test fungi except *S. rolfsii*, *Alternaria* and *Fusarium* (36-37%).The water extract could inhibit all test fungi in the range 41-66% except non-promising results for *Fusarium* (37%).The petroleum ether extract showed no bioactivity against *S. sclerotiorum* but 45-75% for rest of the fungi. Similarly its chloroform extract could not inhibit the growth of *Alternaria* and *Fusarium* but inhibited growth for rest of the fungi significantly.

None of the solvent extracts of *Limonia acidissima,* could inhibit growth of any test fungi, except methanol, which inhibited the growth of *Pythium aphanidermatum*, *Rhizoctonia bataticola* and *A. alternata* up to 40-46%. *Balanites aegyptiaca* (methanol) was observed for fungitoxicity against the growth of *R. solani* and *P. aphanidermatum* and to some extent *R. bataticola;* while its chloroform extracts inhibited growth of *R. solani* and *S. rolfsi*, significantly. Besides petroleum ether extract of *P. foetida* which did not show any bioactivity against test fungi, other solvent extracts, except chloroform, did not give any significant bioactivity. The chloroform extract inhibited growth of *R. solani* for more than 50%.

A software EC_{50} calculator was used to calculate IC_{50} (Inhibition concentration) of all extracts to get an idea about their efficacy as antifungal fungicides (Table 2).

Although, all four crude *P. longifolia* extracts could inhibit test fungi up to 84% with significant antifungal activity, but its methanol extract was observed with maximum potency to inhibit *P. aphanidermatum*, *R. bataticola* and *S. sclerotiorum* with inhibition concentration (IC_{50}) in the range of 200-600 ppm, compared with water, chloroform and petroleum ether extracts; which was 800 and more than 2500 ppm, respectively, for tested fungi. For rest of the test fungi the IC_{50} values were observed in the range of 1500-5000 ppm. The values seem to be slightly towards higher side, but the test material being a crude plant extract is environmentally safer too. Once the crude samples are fractioned using chromatography techniques, the bioactive fractions would be more potent with less concentration. Methanolic extracts of *Polyalthia longifolia* have yielded 20 known and two new organic compounds, some of which

Table 2 Antifungal activity (IC_{50} values) of plant extracts against various fungi**

Test fungi	IC_{50} values (%)		
	Estimate (ppm)*	95% conf intervals	
Pythiuma phanidermatum			
Polyalthia longifolia (water)	0.08 (800)	0.0676	0.1023
Balanites aegyptiaca (water)	6.38	1.7413	23.3955
Paederia foetida (water)	4.55	2.3549	8.8047
Polyalthia longifolia (methanol)	0.06 (600)	0.0480	0.0800
Limonia acidissima (methanol)	0.34 (3400)	0.2174	0.5436
Paederia foetida (methanol)	4.35	2.2856	8.2848
Rhizoctonia bataticola			
Polyalthia longifolia (water)	0.37	0.2730	0.5129
Balanites aegyptiaca (water)	4.38	2.0319	9.4626
Paederia foetida (water)	4.82	2.2304	10.4163
Polyalthia longifolia (methanol)	0.03 (300)	0.0275	0.0443
Limonia acidissima (methanol)	0.52	0.4028	0.6600
Paederia foetida (methanol)	3.92	2.1390	7.1748
Rhizoctonia solani			
Polyalthia longifolia (water)	0.16 (1600)	0.1294	0.1886
Balanites aegyptiaca (water)	0.55	0.3435	0.8752
Paederia foetida (water)	2.09	1.2126	3.6153
Polyalthia longifolia (methanol)	0.14 (1400)	0.1240	0.1650
Limonia acidissima (methanol)	7.88	3.1345	19.8264
Paederia foetida(methanol)	3.57	2.1540	5.9110
Sclerotinia sclerotiorum			
Polyalthia longifolia (water)	0.27 (2700)	0.1752	0.4127
Balanites aegyptiaca (water)	5.02	1.8237	13.8085
Paederia foetida (water)	3.11	1.5729	6.1563
Polyalthia longifolia (methanol)	0.02 (200)	0.0140	0.0280
Limonia acidissima (methanol)	4.06	1.7588	9.3696
Paederia foetida (methanol)	4.42	2.1854	8.9353
Sclerotinia rolfsi			
Polyalthia longifolia (water)	0.15 (1500)	0.1126	0.2018
Balanites aegyptiaca (water)	2.17	1.1788	4.0055
Paederia foetida (water)	2.88	1.5662	5.2881
Polyalthia longifolia (methanol)	0.41 (4100)	0.3000	0.5800
Limonia acidissima (methanol)	0.63	0.4293	0.9177
Paederia foetida (methanol)	3.22	1.8678	5.5448
Fusarium oxysporum			
Polyalthia longifolia (water)	0.54 (5400)	0.3252	0.8835
Balanites aegyptiaca (water)	3.46	1.5126	7.9086
Paederia foetida (water)	4.39	1.9785	9.7379
Polyalthia longifolia (methanol)	0.53 (5300)	0.3110	0.9220
Limonia acidissima (methanol)	2.99	1.6979	5.2579
Paederia foetida (methanol)	5.16	2.9214	9.1244
Alternaria alternata			
Polyalthia longifolia (water)	0.24 (2400)	0.1740	0.3416
Balanites aegyptiaca (water)	4.39	2.1032	9.1821
Paederia foetida (water)	4.34	2.2649	8.3020
Polyalthia longifolia (methanol)	0.23 (2300)	0.1380	0.3910
Limonia acidissima (methanol)	3.24	1.2772	8.2410
Paederia foetida (methanol)	3.60	2.0689	6.2557

*The values in parenthesis is ppm; **Results at 0.25%.

show cytotoxic properties (Chen *et al.* 2000).

Antifungal activity was reported in leaf extracts of *Polyalthia longifolia* (Sonn.) Thw. (Nair and Chanda 2006) and the antibacterial and antifungal activity was observed in methanol, acetone and 1,4-dioxan fractions of leaves of *Polyalthia longifolia* **(**Chanda and Nair 2010).

The antifungal activity of ethanolic extract of 40 higher plants representing 23 families were tested against some phytopathogenic fungi. Out of these, leaf extracts of *Paederia foetida* was also found to be antifungal in nature. The ethanolic extracts of several higher plants could be used as alternative source of antifungal agents for protection of plants or crops against fungal infection (Begum *et al.* 2007).

The main constituents of the essential oil isolated from the leaves of *Limonia acidissima* were found to be methyl chavicol (27.2%), thymol (24.4%), t-anethol (10.9%), *p*-cymen-7-ol (7.3%) and isohumulene (4.8%) that also exhibited microbial activity (Ahmad *et al*. 1989). Antifeedant and toxic effects of leaf extracts of *Syzygium cumini* L., *Ocimum basilicum* L, and *Limonia acidissima* were evaluated in the laboratory and the results were reported to have microbial activity (Peta and Usha 2008). *In vitro* antibacterial activity of the ethanol extract of leaves of *Paederia foetida* (Rubiaceae) were also reported (Borhan *et al.* 2007).

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Verma and Dubey 1999). The plants extracts have been found to contain broad spectra of phytochemicals (secondary metabolites) such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, phlobatannins, polyphenols and steroids. Secondary metabolites constitute plants' weaponry against pests and pathogens invasion. These groups of phytochemicals possess wide ranging chemical functional groups; by which they establish contact with and bind to sites on target pathogens to ineffectuate them. This will aid in either synergizing or synthesizing them so as to combat the enormous and obvious challenges of fungicide resistance threatening agricultural production and food security (Enyiukwu *et al.* 2014a, 2014b). The methanol crude extract of aromatic ginger, wild basil and neem exhibited strong fungistatic and fungicidal activities against *F. oxysporum*, whereas aromatic ginger and wild basil could be used as an effective antifungal agent (Disanayake 2014).

Out of 16 plant extracts, the methanol extract of *P. longifolia* gave most promising results for inhibiting the test fungi, inhibition concentration (IC_{50}) values in the range of 200-600 ppm. Hence, a single methanol extract of the plant *P. longifolia* could serve as a promising biofungicide for inhibiting the growth of different fungi responsible for crop losses. The *P. longifolia* extracts, especially of methanol solvent, could be worked for fractional analysis using column chromatography followed by bio-assay of its solvent fractions against test fungi. This could lead to a promising bioactive fraction which can be formulated for field validation studies based on *in-vitro* results; so as to replace synthetic fungicides and their hazardous impact on environment.

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