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Mycotoxic effect of Medicinal Plants Against *Helminthosporium sativum* and *Aspergillus niger*

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Thirty-nine extracts from 10 medicinal plants were tested against *Helminthosporium sativum* and *Aspergillus niger* for their fungitoxicity *in vitro*. Methanol leaves extracts of *Lawsonia inermis, Withania somnifera, Datura metel, Datura stramonium* and stem bark extract of *Bauhinia racemosa* significantly inhibited mycelia growth of both target fungi. Some extracts exhibited greater fungitoxicity than that of synthetic fungicide Dithane M-45.

Key words: *Aspergillus niger*, fungitoxicity, *Helminthosporium sativum, Lawsonia inermis, Withania somnifera.*

Approximately 2.5 to 5 lakhs plant species currently exist on the earth **(**Borris, 1996). But only few of these have been investigated for antifungal activity. However, plants should be an excellent source of potent antifungal, since they are exposed to a wide array of pathogenic fungi present in their surrounding and have had to develop antifungal compounds to survive. In agriculture, fungi are the major disease causing agents in crop plants. Every year lots of crops are damaged due to diseases caused by fungi. Although the use of synthetic fungicides to control plant disease has shown its efficacy for the improvement of agriculture yield, several of these have been found to display side effects. In addition, due to the development of new physiological races of pathogens, many of the synthetic fungicides are gradually becoming ineffective (Wellman, 1977). The presence of naturally occurring substances in plants, with fungicidal properties has been recognized and tested against various phytopathogenic fungi (Begum e*t al.,* 2007; Gomathi and Kannabiran 2000; Thirbhuvanmala and Doraisamy, 2004). In present investigation, an attempt has made to evaluate 10 medicinal plants for their fungitoxic activity against *Helminthosporium sativum* and *Aspergillus niger.*

Materials and Methods

The healthy, infection free, mature parts (i.e. root, stem bark, leaves, flower, seeds and bulbs) of 10 plants were collected from Government Institute of Science campus Aurangabad. Identities of plant species were authenticated by referring standard literature. While *H. sativum* and *A. niger* were isolated from plants and soil respectively.

Preparation of aqueous extracts

The plants parts were washed with fresh water followed by distilled water and finally grounded with sterile distilled water @ 1 g of tissues/ml in mortar with pestle. Macerate was succeeded through cotton wool followed by two layers of muslin cloth and finally through Whatman no.1 filter paper to get the extracts. This formed the standard aqueous extract solution (100 %) from these 10% extracts were prepared and tested for fungitoxic activity.

Preparation of solvent extracts

Collected plant parts were shade dried and ground to a fine powder using grinder mixer. Extracts were prepared in chloroform and methanol at room temperature by simple extraction method (5). Dried powder of various plant parts (10 g) was mixed with 100 ml solvent in 250 ml conical flask. The flasks were plugged tightly with cotton and wrapped with paper. All conical flasks were kept on shaker for 24 h then it was allowed to stand for five hours to settle the plant material. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated at 45^{o}C in vacuum evaporator to make the final volume 1/5th of the original volume. It was stored at 4^{o}C in airtight bottles for further studies.

Fungi-toxic assay

Fungitoxicity of plants were determined by food-poisoned technique. Standard extracts (5 ml) was mixed with 45 ml of sterilized potato dextrose agar (PDA) medium and transferred equally into two Petri plates. Media was allowed to solidify. Then seven days old fungal culture disk of 6 mm diameter was taken and inoculated into the center of Petri plates containing plant extracts in aseptic condition. Only PDA medium, without plant extracts served as control. The fungicide Dithane M-45 (0.2%) was used for comparison. All plates were incubated at 28 ^{0}C and radial growth of colony was measured after seven day of incubation. Each test was performed in triplicate. The percent inhibition of mycelia growth over control was calculated.

Results

Out of 15 aqueous tested extracts, only 10 extract showed some range of antifungal activity against both target fungi. Mycelia growth inhibition ranging from 1.11 percent to 82.22 per cent (Table-1). Highest percent of inhibition of mycelia growth was recorded in *W. somnifera* leaves extract against *H. sativum* followed by *L. inermis* leaves and *D. stramonium* leaves extract against *A. niger* and *H. sativum* respectively. Only four aqueous extracts exhibited significant fungitoxicity (above 70 percent inhibition of mycelia growth) of target fungi.

Among chloroform extract, 83.34 percent extract revealed some degree of fungitoxicity. Only two extract showed significant fungitoxicity (i.e. above 70 percent) against phytopathogens. Again *W. somnifera* leaves extract showed greatest percent inhibition of mycelia growth in *H. sativum* followed by *L. inermis* leaves against same fungi (Table-2).

Most of the methanol extracts exhibited significant fungitoxicity against both target fungi. Of which *L.inermis* leaves showed highest percent inhibition of mycelia growth of *H. sativum* and *A. niger* (88.88 % and 87.8 %) respectively followed by stem bark extract of *Bauhinia racemosa* restricted growth of *A. niger* upto 85.36 percent. Whereas, *C. equisetifolia* leaves extract revealed lowest percent inhibition in *H. sativum* (2.22%) among methanol extracts (Table-3).

Table 1. Inhibitory effect of some aqueous plant extracts (10 % v/v) on mycelia growth of *Aspergillus niger* and *Helminthosporium sativum*.

Plant	Part(s) used	*Aspergillus niger*		*Helminthosporium sativum*	
		Mycelia growth (mm)	%inhibition over control	Mycelia growth (mm)	%inhibition over control
Withania somnifera	Lv	38	53.65 ± 1.37	16	82.22 ± 0.94
	Rt	35	57.31 ± 0.40	90	00.00 ± 0.00
Lycopersicon esculentum	Lv	40	51.21 ± 0.91	89	01.11 ± 1.16
Datura metel	Lv	28	65.85 ± 1.47	82	08.88 ± 1.82
Datura stramonium	Lv	60	26.82 ± 1.20	23	74.44 ± 1.38
	Sd	40	51.21 ± 1.29	90	00.00 ± 1.10
Thevetiaperuviana	Lv	40	51.21 ± 1.29	90	00.00 ± 0.00
	Sd	59	28.04 ± 1.10	80	11.11 ± 0.91
Catharanthusroseus	Lv	65	20.73 ± 1.11	27	70.00 ± 1.29
	Fl	37	54.87 ± 1.77	90	00.00 ± 0.00
Lawsoniainermis	Lv	20	75.60 + 0.75	23	74.44 + 1.79
Bauhinia racemosa	Lv	79	03.65 + 0.91	36	60.00 + 0.19
	Sb	58	29.26 + 0.85	57	36.66 + 1.08
Casuarinaequisetifolia	Lv	63	23.17 + 1.11	90	00.00 + 0.00
Allium Cepa	Bb	68	17.07 + 1.31	60	33.33 + 2.16
Dithane M-45	-	53	35.36 + 0.19	53	35.36 + 0.70
Control	-	82	00.00	90	00.00 + 0.00

Where :- Values expressed in mean ± S.E.M. of triplicate, Lv = Leaves, Rt = Root, Sd = Seed, Fl = Flower, Sb =Stem bark and Bb = Bulb

Table 2. Inhibitory effect of some chloroform plant extracts (10 % v/v) on mycelia growth of *Aspergillus niger* and *Helminthosporium sativum*.

Plant	Part(s) used	*Aspergillus niger*		*Helminthosporium sativum*	
		Mycelia growth (mm)	%inhibition over control	Mycelia growth (mm)	%inhibition over control
Withania somnifera	Lv	28	65.85 ± 1.11	22	75.55 ± 0.85
Lycopersicon esculentum	Lv	60	26.82 ± 0.85	82	08.88 ± 0.67
Datura metel	Lv	47	42.68 ± 1.25	48	46.66 ± 1.11
Datura stramonium	Lv	48	41.46 ± 1.55	35	61.11 ± 0.94
	Sd	62	24.39 ± 1.71	52	42.22 ± 0.90
Thevetia peruviana	Lv	51	37.80 ± 1.08	54	40.00 ± 1.20
	Sd	69	15.85 ± 1.04	90	00.00 ± 0.00
Catharanthus roseus	Lv	69	15.85 ± 1.58	52	42.22 ± 1.37
Lawsonia inermis	Lv	53	35.36 + 0.63	27	70.00 + 1.49
Bauhinia racemosa	Lv	54	34.14 + 0.48	50	44.44 + 1.37
	Sb	68	17.07 + 0.32	50	44.44 + 2.11
Casuarina equisetifolia	Lv	72	12.19+ 1.77	90	00.00 + 0.00
Dithane M-45	-	53	35.36 + 0.19	53	35.36 + 0.70
Control	-	82	00.00	90	00.00 + 0.00

Where :- Values expressed in mean ± S.E.M. of triplicate, Lv = Leaves, Sd = Seed, Sb =Stem bark and Bb = Bulb

Table 3. Inhibitory effect of some methanol plant extracts (10 % v/v) on mycelia growth of *Aspergillus niger* and *Helminthosporium sativum*.

Plant	Part(s) used	*Aspergillus niger*		*Helminthosporium sativum*	
		Mycelia growth (mm)	%inhibition over control	Mycelia growth (mm)	%inhibition over control
Withania somnifera	Lv	35	57.31± 0.13	12	86.66 ± 0.32
Lycopersicon esculentum	Lv	69	15.85 ± 0.91	34	62.22 ± 2.16
Datura metel	Lv	22	73.17 ± 1.08	32	64.44 ± 0.32
Datura stramonium	Lv	28	65.85 ± 0.85	26	71.11 ± 1.19
	Sd	42	48.78 ± 0.37	50	44.44 ± 1.37
Thevetia peruviana	Lv	27	67.07 ± 0.85	46	48.88 ± 1.29
	Sd	50	39.02 ± 1.49	50	44.44 ± 0.67
Catharanthus roseus	Lv	27	67.07 ± 0.64	41	54.44 ± 2.16
Lawsonia inermis	Lv	10	87.80 + 0.57	10	88.88 + 0.85
Bauhinia racemosa	Lv	35	57.31 + 1.08	72	20.00 + 1.04
	Sb	12	85.36 + 0.2	23	74.44 + 0.64
Casuarina equisetifolia	Lv	50	39.02 + 2.16	88	02.22 + 1.64
Dithane M-45	-	53	35.36 + 0.19	53	35.36 + 0.70
Control	-	82	00.00	90	00.00 + 0.00

Where :- Values expressed in mean ± S.E.M. of triplicate, Lv = Leaves, Sd = Seed, Sb =Stem bark and Bb = Bulb.

Comparatively, most of the methanol extracts showed significant antifungal activity on targeted fungi than aqueous and chloroform extracts.Out of 39 extracts, 33 (84.6%) extracts inhibited mycelia growth of *H. sativum* at varying percentage. Of which, methanol extracts of *W. somnifera, L. inermis, B. racemosa* inhibited more than 85 percent mycelia growth of either one or both the target fungi. The greatest percent inhibition of mycelia growth was observed in mathanol extract of *L. inermis* (88.8%) against *H. sativum*. Out of 39 tested extracts, 23 % extracts showed more than 75 % inhibition of mycelia growth of fungi, 25.6% plant extracts revealed 60-76 % inhibition of mycelia growth and 30.7% plant extracts inhibited 45-65% mycelia growth. While some plant extracts revealed poor fungitoxic activity.

A. niger was found to be more susceptible to plant extracts than *H. sativum.* Some plants extracts showed greater fungitoxicity than that of the synthetic fungicide Dithane M-45. Overall *L. inermis, W. somnifera, B. racemosa, D. metel* and *D. stramonium* were found to be potent fungitoxic plants.

Discussion

Pandey *et al.,* (2002) reported fungitoxic effect of *L. inermis, D. stramonium, T. peruviana* and *A. cepa* against *H. sativum.* Present result was slightly differing as *T. peruviana* did not inhibited mycelia growth of *H. sativum*, which was not according to his finding. Fungitoxicity of these plants were reported against various fungi (Benkeblia, 2004; Gomathi and Kannabiran, 2000). The difference in fungitoxic activity due to the difference in yield constituents and concentration of active principle compared to those previously reported in the literature collected in other geographic areas. The other factors could be attributed to such as climate, time of collection, age of the plants, methods of extraction, membrane permeability of microbes, etc. (Chimanga *et al.,* 2002).

Successful prediction of botanical compounds from plants material is largely depends on the type of solvent used in extraction procedure. Traditionally, most of the people used

water primarily as a solvent but in present investigation, methanol is certainly superior. This may be due to the better solubility of antifungal compounds in organic solvent. This observation rationalized in terms of polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity and their ability to dissolve or diffuse in different media used in the assay. The growth media also seems to play an important role in determination of fungitoxic activity.

A. niger and *A. flavus* reported to cause severe deterioration in paddy, maize grains and other food commodities during storage (Tiwari, 1995). The plant extracts can be used to save the paddy, maize grains and other commodities from infection of *A. niger.* Thus, the present finding indicates that the plant extracts offer a practicable and safer eco-friendly, alternative to the use of costly fungicides also could possibly be exploited for effective management of plant diseases caused by *A. niger* and *H. sativum.* Further studies are needed to isolate and identify pure active compounds from the potent plants.

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