Mycobiology 31(4): 221-225 (2003)

Effects of Plant Extracts on Conidial Germination, Mycelial Growth and Sporulation of Fungi Isolated From Poultry Feed

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(Received August 30, 2003)

Effect of ethanolic extracts of *Lawsonia inermis*, *Azadirachta indica*, *Vinca rosea*, *Tagetes patula*, *Ocimum sanctum*, *Colocasia antiquorum*, *Adhatoda vasica*, *Moringa oleifera*, *Datura metel* and *Curcuma longa* leaf on conidial germination, mycelial growth and sporulation of *Aspergillus flavus*, *A. niger* and *A. fumigatus* were examined. The conidial germination of *A. flavus* and *A. fumigatus* were most inhibited by the extract of *L. inermis*, while that of *A. niger* was inhibited by *A. indica*. Other tested plant extracts have a good effect on conidial germination on the selected fungi. The highest mycelial growth of *A. flavus* (37 mm) was found in *V. rosea*, but in case of *A. niger* and *A. fumigatus* it (38 and 39 mm) was found in *D. metel*. The lowest (4, 9 and 6 mm) respectively mycelial growth of these fungi found in *L. inermis*. The highest sporulation (75×10^4/*ml*) of *A. flavus* was counted in *V. rosea*, but in case of *A. niger* and *A. fumigatus* those (45×10^4 and 55×10^4/*ml*) were in *D. metel* and the lowest (5×10^4, 12×10^4 and 9×10^4/*ml*) respectively sporulation of these fungi counted in *L. inermis* plant extract medium.

KEYWORDS: Conidial germination and sporulation, Fungi poultry feed, Mycelial growth, Plant extracts

Poultry is now a very important and widespread agricultural industry in the tropics (Sastry *et al.*, 1983) and it plays a great role to solve the malnutrition problem (especially of animal protein food) on shortest possible time. Poultry provides protective food in human nutrition in the form of egg, meat and employment opportunities at various levels (Das, 1994). Poultrys feeding is one of the most important branches of poultry farming. Nutritionally balanced diets are provided during phases of productive life in eggers, chicks, grower and layer stages, in broilers starter and finishing stages (Gopalakrishana and Lal, 1985). Mould contamination of poultry feed is particularly high in the tropics due to high relative humidity and temperature that provide excellent condition for fungal growth (Ogundero, 1980). Poultry feed are prepared basically with plants materials such as maize, soybean and sorghum and these materials are known to have fungi as the commonest contaminations (Oyeka and Onochie, 1992). There is absecnce of drying and processing facility at harvest point of the cereals and cakes supports mould and toxin formation (Chauhan and Roy, 1996). Severe mould contamination of the mash (poultry feed) impairs its hygienic quality and makes it unsuitable for fattening purposes (Skrinjar *et al.*, 1995). Toxigenic moulds are often present in feed mixture mycopopulations. These fungi can produce various mycotoxins, which are ingested by the animal with the contaminated feed and can eventually be detected in meat (Skrinjar *et al.*, 1995). Mycotoxins can easily enter in the human bodies by food chain and caused health hazard of human also (Skrinjar *et al.*, 1995). The extracts of plants exhibited marked effect on germination of fungal spores as well (Singh and Singh, 1981; Singh *et al.*, 1983 and Dubey, 1991) and it inhibited the fungal growth (Khair *et al.*, 1995). In the present study, an attempt has been made to observe the effect of different plant extracts on conidial germination, mycelial growth and sporulation of *A. flavus*, *A. niger* and *A. fumigatus* isolated from poultry feed.

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Materials and Methods

Organisms used. *Aspergillus flavus A. niger* and *A. fumigatus* were isolated from poultry feeds, Department of Botany, University of Rajshahi, Bangladesh and cultured on potato dextrose agar (PDA) medium and the conidia were taken for the experiment from 10 days old culture.

Leaf extraction. The ethanolic extraction of *Lawsonia inermis*, *Azadirachta indica*, *Vinca rosea*, *Tagetes patula*, *Ocimum sanctum*, *Colocasia antiquorum*, *Adhatoda vasica*, *Moringa oleifera*, *Datura metel* and *C. longa* leaf were done following the method described by Mahadevan and Sridhar (1982). Five g tissues were cut into pieces and immediately plunged in sterilized distilled water in a beaker and allowed to boil for 5~10 min. using five to ten *ml* of sterilized distilled water for each g of tis-

sue. The extractions were done on top of a steam bath. The extracts were cooled in a pan of cold water. The tissues were crushed thoroughly in a morter with a pastle and then passed through two layers of cheesecloth. The ground tissues were re-extracted for 3 minutes in sterilized distilled water and 2~3 *ml* of sterilized distilled water were used for every g of tissues of the said plant materials. The extracts were cooled and passed through cheesecloth and filtered through Whatman's no. 1 filter paper. The volume (10 *ml*) of the extracts were evaporated on a steam bath to dryness and 1.25 *ml* of sterilized distilled water was added for 5 g of tissues and the extracts were used for experiment.

Preparation of conidial suspension and conidial germination. Conidia of different fungi cultured on PDA plates were taken and conidial suspensions (10^5/*ml*) were made separately with different plant extracts. These suspensions (1.25 *ml*) were taken in small sterilized Petri dishes (65 mm) and were kept at 28±2°C for 5~30 minutes. A drop of treated conidial suspension (from different plant extracts) was taken on separate slides continue 5 min. interval and were kept at 28±2°C in a moisture chamber for 24 hrs of incubation. Then a drop of lactophenol cotton blue was placed on the conidial suspension on the slides. The slides were examined under high power microscope (×400) for recording the percentage of conidial germination.

Media with plant extracts. Two hundred gram of each leaves was washed thoroughly fresh water, cut into bits and steamed in 500 *ml* distilled water for 30 min, strained through a cheese cloth and the decoction was a added to the melted agar (which was steamed in 500 *ml* of distilled water) and the volume was made upto 1,000 *ml*. All media were adjusted to pH 6.5 by using 0.1 N HCl and 0.1 N NaOH and autoclaved at 15 lb/in.^{2} pressure for 20 min. An equal quantity of each medium was poured in Petri plates (90 mm). After solidification, triplicate plates of each leaf extract agar medium were inoculated by using 5 mm inoculum disc cut from the margins of actively growing colonies of the fungus on PDA medium and kept them at 28±2°C for incubation. The measurement of radial growth of mycelium was taken in mm at intervals of 24 hrs. The diameter of the colony was measured in two directions at right angles to each other, whereas in case of irregular colonies the measurement was taken along the longest and the shortest directions and the average was taken as the growth of the colony (Brown, 1923).

Sporulation test. The level of sporulation of the fungi were measured 10 days after incubation at different concentrations of media. Five mm mycelial mat was cut randomly at four different places and was macerated in 1 *ml* of sterile distilled water and 0.1 *ml* of this suspension was placed on a clean glass slide and the numbers of spores were counted with the help of haemocytometer under low power of microscope. Statistical analysis of data given as percentage was carried out from angular transformed values and performed using Microsoft Excel Software. LSD were determined, whenever, the calculated F values were significant at 5% level (Snedecor and Cochran, 1980).

Results and Discussion

Ten plant extracts were tested as inhibitor against conidial germination of *A. flavus, A. niger* and *A. fumigatus.* All of the extracts were showed more or less inhibitory effect against conidial germination of the tested fungi after immersing 5~30 min (Table 1). The conidial germination (95%) of *A. flavus* was inhibited (or reduced) by the extract of *L. inermis*, while that of *A. niger* was most inhibited by *A. indica.* For the *A. fumigatus,* the lowest (19% inhibition) and the highest (99% inhibition) conidial germination were found in *M. oleifera* and *L. inermis* respectively. Rests of the plants extracts were showed intermediary effect. Inhibitory effect was observed on germ tube formation of *A. flavus, A. niger* and *A. fumigatus*, when the fungi were immerged in *L. inermis* and *A. indica* extracts. The highest inhibitory effect on germ tube formation of *A. flavus* was measured in *L. inermis* extracts. Correlation (r) values were –0.999 to –0.975, –0.999 to 0.981 and –0.999 to 0.979 for *A. flavus, A. niger* and *A. fumigatus* respectively, indicating that there was highly negative significant relationship between immersion (in every plant extracts) period (5~30 min) and conidial germination. Calculated 'F' value of conidial germination of selected fungi in different types of plant extracts and immersion period is greater than table value. It indicated that there was significant different role of plant extracts and immersion period on conidial germination (Table 1). Alam *et al.* (2002) tested the effect of ten plant extract as fungicides on conidial germination of *C. gloeosporioides* and found *T. erecta* (leaf) and *A. indica* (bark) extracts were most effective in inhibition of conidial germination after inmmersing 5~30 min in 5:1.5 (w/v) concentration. Alam *et al.* (2002) used extracts of different parts of *V. rosea* and *A. indica* against spore/conidial germination of *B. sorokiniana, F. oxysporum* f. sp. *vasinfectum, R. artocarpi* and *B. theobromae* and found *V. rosea* root extract inhibited 100% spore germination of *B. sorokiniana* and *R. artocarpi* when they were immersed from 5~30 minutes at 5:1.25 (w/v) concentration. They also reported *A. indica* (leaf, root and seed) extracts showed good (100%) inhibition results on *B. sorokiniana* and *R. artocarpi.* Alam *et al.* (1999) reported the antifungal effects of leaf and root extracts of *Vinca rosea* and

Table 1. Effect of different plant extracts (leaf) on the inhibition of conidial germination of *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* after immersing conidia for 5 to 30 minutes

Name of fungi	Name of plant	% of conidial germination inhibition after immersed in plant extracts (minutes)[a]						Length of germ tube in (mm)
		5[b]	10	15	20	25	30	
Aspergillus flavus	***Vinca rosea***	7	10	15	22	29	35	8.03-218.28
	Tagetes patula	22	23	31	38	41	48	19.26-208.05
	Azadirachta indica	10	13	18	22	27	33	16.05-215.07
	Ocimum sanctum	11	13	16	21	25	28	11.24-215.07
	Lawsonia inermis	65	69	73	80	88	95	6.42-93.16
	Colocasia antiquorum	6	10	14	22	27	32	32.1-250.38
	Adhatoda vasica	4	8	11	16	21	25	12.84-215.07
	Moringa oleifera	2	8	13	18	23	28	9.63-218.28
	Datura metel	7	13	18	23	31	37	16.05-138.03
	Curcuma longa	12	16	21	27	32	38	9.63-243.96
Aspergillus niger	*Vinca rosea*	9	12	15	19	22	26	16.05-224.7
	Tagetes patula	7	11	14	17	20	23	19.26-231.12
	Azadirachta indica	66	70	74	79	83	87	6.42-138.03
	Ocimum sanctum	39	45	52	58	63	69	9.63-208.05
	Lawsonia inermis	28	31	35	39	44	48	8.03-218.28
	Colocasia antiquorum	19	25	30	36	41	47	11.24-227.90
	Adhatoda vasica	13	17	22	27	33	38	12.84-243.96
	Moringa oleifera	18	24	29	34	40	47	9.63-215.07
	Datura metel	6	6	13	17	21	26	16.05-304.95
	Curcuma longa	7	13	17	23	28	33	12.84-250.38
Aspergillus fumigatus	*Vinca rosea*	5	9	12	17	23	29	13.05-254.6
	Tagetes patula	7	11	15	20	25	32	17.23-220.02
	Azadirachta indica	6	11	13	17	22	26	7.42-137.08
	Ocimum sanctum	12	18	24	29	34	38	11.65-198.02
	Lawsonia inermis	56	65	76	84	88	99	9.23-223.25
	Colocasia antiquorum	3	5	8	13	16	20	21.23-242.76
	Adhatoda vasica	6	11	15	18	21	24	13.33-232.64
	Moringa oleifera	5	8	11	13	16	19	12.53-221.61
	Datura metel	11	14	17	20	24	28	26.30-294.34
	Curcuma longa	2	5	9	12	16	21	24.83-264.31

[a]mean of three replications.
[b]Period of incubation (days).

leaf, root and seed extracts of *A. indica* against chilli fruit rot pathogen *Alternaria tenuis*. Singh *et al.* (1993) reported the antifungal activities of leaf extracts against *B. theobromae*, *F. oxysporum*, *Helminthosporium spiciferum*, *Curvularia lunata*, *A. flavus* and *Trichothecium roseum*. They used some medicinal plants such as, *C. procera*, *V. negundo*, *L. camara*, *A. indica*, *F. religiosa*, *O. sanctum*, *T. orientalis*, *A. mexicana*, *A. aspera*, *D. fastuosa* and *R. communis* and observed good control against these pathogens. Of the 11 leaf extracts, those of *A. indica* and *O. sanctum* were most effective in controlling the fungi. Singh *et al.* (1990) reported, ajoene, a compound derived from garlic, inhibited spore germination of some fungi such as *Alternaria solani*, *A. tenuissima*, *A. triticina*, *A.* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium lini*, *F. oxysporum*, *F. semitectum* and *F. udum*, which cause serious disease in some important crop plants in India. The compound was very effective in inhibiting spore germination at concentration of 25 mg/*ml* in some fungi and, in most cases there was 100% inhibition of germination at 100 mg/*ml*. The present study indicates that *L. inermis* and *A. indica* posses (or contain) some antifungal compounds.

Effect of plant extracts on mycelial growth and sporulation of tested fungi were examined and found that the highest (37 mm) mycelial growth of *A. flavus* was found in *V. rosea* extracts on comparison with PDA (control) 41 mm (Table 2). But in case of *A. niger* and *A. fumigatus*, the highest mycelial growth (38 and 39 mm) was measured in *D. metel* on comparison with PDA were 62 and 53 mm. The mycelial growth of *A. flavus*, *A. niger* and *A. fumigatus* were most inhibited by *L. inermis* extracts. The mycelial growth in other tested plant extracts media were intermediary. The highest (75×10^4/*ml*) spores of *A. flavus* was counted in *V. rosea* extracts, but in case of *A. niger* and *A. fumigatus* it (45×10^4 and 55×10^4/*ml*)

Table 2. Effect of different plant extracts media on radial mycelial growth and sporulation of *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* 10 days after incubation at 28±2°C

Name of fungi	Name of the plant extracts (leaf).	Radial growth of mycelium (mm) on different media after incubation period (days)[a]										Sporulation/*ml*
		1[b]	2	3	4	5	6	7	8	9	10	
Aspergillus flavus	*Vinca rosea*	0	2	5	8	12	17	23	28	33	37	75×10^4
	Tagetes patula	0	1	3	7	11	15	19	23	25	28	41×10^4
	Azadirachta indica	0	2	5	8	12	16	21	26	30	34	53×10^4
	Ocimum sanctum	0	1	4	7	10	13	17	21	25	28	40×10^4
	Lawsonia inermis	0	0	0	1	1.5	2	2.5	3	3.5	4	5×10^4
	Colocasia antiquorum	0	1	3	6	10	14	18	22	26	30	43×10^4
	Adhatoda vasica	0	1	4	7	10	14	19	24	29	33	47×10^4
	Moringa oleifera	0	0	2	5	8	12	16	21	25	29	49×10^4
	Datura metel	0	1	3	5	8	11	15	20	26	31	65×10^4
	Curcuma longa	0	0	1	2	4	7	10	14	18	21	29×10^4
	PDA (Control)	0	2	5	9	14	20	26	32	37	41	83×10^4
Aspergillus niger	*Vinca rosea*	0	1	3	6	10	14	19	24	28	32	35×10^4
	Tagetes patula	0	1	3	7	11	16	21	26	30	34	38×10^4
	Azadirachta indica	0	0	1	3	7	10	14	18	21	25	19×10^4
	Ocimum sanctum	0	1	3	7	12	18	24	29	33	37	43×10^4
	Lawsonia inermis	0	0	1	2	3	5	7	8	8.5	9	12×10^4
	Colocasia antiquorum	0	0	1	4	8	12	17	21	24	27	29×10^4
	Adhatoda vasica	0	1	2	5	9	13	17	22	27	31	33×10^4
	Moringa oleifera	0	1	4	7	11	16	21	26	30	34	36×10^4
	Datura metel	0	1	3	8	11	17	23	29	34	38	47×10^4
	Curcuma longa	0	0	1	3	6	10	14	18	22	25	20×10^4
	PDA (Control)	0	2	7	14	22	32	40	48	55	62	52×10^4
Aspergillus fumigatus	*Vinca rosea*	0	1	2	4	8	13	18	23	27	31	23×10^4
	Tagetes patula	0	1	3	5	8	11	15	19	23	26	20×10^4
	Azadirachta indica	0	1	4	8	12	16	21	26	31	35	40×10^4
	Ocimum sanctum	0	1	3	6	10	14	18	22	26	29	39×10^4
	Lawsonia inermis	0	0	1	2	2.5	3	3.5	4	5	6	9×10^4
	Colocasia antiquorum	0	1	3	5	9	14	20	26	32	37	45×10^4
	Adhatoda vasica	0	1	3	7	11	15	19	25	30	35	51×10^4
	Moringa oleifera	0	1	3	6	10	14	18	23	28	32	47×10^4
	Datura metel	0	1	3	7	12	17	22	28	34	39	55×10^4
	Curcuma longa	0	1	2	5	8	12	17	22	26	30	27×10^4
	PDA (Control)	0	1	4	8	13	19	28	36	45	53	72×10^4

[a]mean of three replications.
[b]Period of incubation (days).

were observed in *Datura metel.* The lowest (5×10^4, 12×10^4 and 9×10^4/*ml*) spores of *A. flavus*, *A. niger* and *A. fumigatus* were counted in *L. inermis* plant extracted medium, other cases it was intermediary. Correlation (r_2) values are 0.966 to 0.998, 0.978 to 0.992 and 0.878 to 0.992 for *A. flavus*, *A. niger* and *A. fumigatus* respectively, indicating that there was highly significant relationship between incubation period and radial mycelial growth of these fungi. Calculated 'F' values of radial mycelial growth of selected fungi in different types of plant extracts media and incubation period are greater than table value. It indicated that there was significant different role of plant extracts media and incubation period on redial mycelial growth of these fungi (Table 2). Biswas *et al.* (1995) reported that the effects of 10% alcoholic water extracts of fresh plant parts from twenty (20) different species were studied on the development of powdery mildew *P. corylea* (Pers.) Karst., leaf spot (*Pseudocercospora mori* Hara Deignton) and leaf rust (*C. fici* (Cast.) Arth.) diseases in mulbery during 1992~93 and 1993~94. The pooled data of these two years studies revealed that extracts of *A. vasica* was the most effective in decreasing the severity of all the diseases followed by extracts of *A. indica*, *L. coromandelica* and *O. corniculata.* Which significantly minimized two diseases, namely, powdery mildew and leaf rust, while those of *C. argentia* and *E. odoratum* reduced leaf spot and leaf rust diseases. Extracts from several other plant species exhibited ability to reduce either leaf rust or powdery mildew disease. Chauhan and Joshi (1990) reported the efficacy and persistence of 14

plant extracts and found carbendazim (0.05%) as mango fruit dip treatments were compared in controlling mango fruit anthracnose caused by *Colletotrichum gloeosporioides* was the most effective control treatment. Eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for >2 weeks when fruit were inoculated and were significantly better than the other plant extracts tested. Castor oil (5%), eucalyptus oil (1%), garlic bulb, mango, turmeric and lantana leaves also significantly controlled the disease. Mohawed *et al.* (1996) studied on the antifungal activity of garlic extract against fungi, isolated from cow and poultry feedstuffs of Egyptian origin and they reported garlic extract (0.5%) inhibited the growth of all tested fungi. In the present experiment it also indicates that antifungal reagent are present or rich in *L. inermis*, which inhibit the radial mycelial growth and sporulation of the tested fungi.

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