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Evaluation of Antifungal Activity of Curcumin against *Aspergillus flavus*

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A B S T R A C T

The present study was undertaken to study the antifungal effect of curcumin and other synthetic antifungal agents against *Aspergillus* species isolated from poultry feed. During this study poultry feeds collected from poultry farm of Odisha Veterinary College. Various concentrations of curcumin like 25mg/disc, 50mg/disc, 100mg/disc and 200mg/disc were prepared and were tested by disc diffusion method. In this method antifungal activity of various agents like ketoconazole, voriconazole, itraconazole, clotrimazole, Amphotericin B, miconazole, caspofungin, fluconazole were tested and its zone of inhibition was found to be approximately ≥ 18 mm, ≥ 15mm, ≥ 16mm, ≥ 18mm, ≥ 7 mm, ≥ 10mm, ≥ 18 mm and≥ 10mm respectively. It has been revealed that curcumin treated *Aspergillus* species showed lesser zone of inhibition than synthetic antifungals like ketoconazole, voriconazole, itraconazole, clotrimazole Amphotericin B, caspofungin but in comparison to fluconazole and miconazole curcumin shows larger zone of inhibition. The antifungal sensitivity pattern shows that curcumin (200 mg/disc) has got the highest potential of antifungal action against *A. flavus* and *A. fumigatus* in comparison to synthetic antifungals.

Introduction

Aspergillosis is the major infectious fungal disease of poultry caused by the fungal genus Aspergillus. This disease is most commonly caused by *Aspergillus fumigatus* and less commonly by *A. flavus, A. niger, A. nidulans* and *A. terreus* (Arne *et al.*, 2011; Girma *et al.*, 2016).

Aspergillus is ubiquitous and the common soil saprophyte reported in avian species domestic as well as wild birds (Atlaman, 1997). It also affects human beings and other animals. Avian species are highly susceptible due to the anatomical and physiologic feature of the respiratory system of the bird. The small non-expanding lungs and nine air sacs are the predominant focus of infection (Nardoni *et al.*, 2006). These organisms can sustain on substantial environmental stress condition including the humid environment of the poultry shed, improper ventilation system and damaged egg of hatcheries. These filamentous fungi grow on organic material in warm environment. Fungal proliferation and

sporulation resulted in production of conidiophores from feed, faecal material and soil in suitable conditions. These conidia spread in the air and are possibly inhaled and loaded in the respiratory tract. Aspergillosis usually occurs in young birds as acute aspergillosis, which causes high morbidity and mortality. Older avian species exhibit the sporadic form with lesser mortality (Kunkle *et al.*, 2003). *Aspergillus* species enter the egg shell and infect the embryo which may die or hatch with progressive lesions (Bauk, 1994)

Curcumin is the major curcuminoid found in turmeric and is well known for its multiple pharmacological and biological properties (Gupta *et al.*, 2013; Zhou *et al.*, 2011; Shen and Ji, 2012). It possesses anti-inflammatory, antibacterial, antidiabetic, hepatoprotective, anticancer and anti-fungal properties (Chattopadhyay *et al.*, 2004; Kohli *et al.*, 2005 and Teiten *et al.*, 2010). Dietary inclusion of 222mg/kg curcuminoids had reduced the adverse impact of aflatoxin B1 on serum total protein, albumin and γ – Glutamyl transferase activity (Gowda *et al.*, 2009). The antifungal activity of curcumin has low side effects for that reason it shows synergy with synthetic fungicides. Curcumin affects *Aspergillus* growth and its morphology (Ferreira *et al.*, 2013). Antifungal activity of curcumin is experimented and it has shown its effect against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum* (Jayaprakasha *et al.*, 2001). Antibacterial and antifungal action in poultry is less documented. So in the present study an attempt has been carried out to evaluate action of curcumin against fungus particularly *Aspergillu*s species.

Materials and Methods

Sample collection

In the present study a total of 12 numbers of feed samples were collected from instructional poultry farm of Odisha veterinary College and also from different poultry houses starting from brooder house, broiler house and layer house during the period from December 2018 to May 2019. Four samples from each house were collected and screened for presence of various mycological agents with special reference to *Aspergillus* species. These samples were collected in plastic bottles and were immediately stored a -20°C until analysis.

Mycological analysis

Isolation of fungus was done as per the methods of Sivakumar *et al.,* (2014). The poultry feeds without additive or preservative were taken for analysis otherwise it might affect fungal growth. Poultry feeds were coarsely ground before analysis.10g of feed was weighed into 250ml of flask and equivalent amount of distilled water was added. Flasks were incubated at 37°C for up to 2 weeks and examined daily for fungal growth (Fig. 1). Distilled water was added to triplicate flasks to maintain the moisture content at day 6 and 14 of the incubation period.

Identification of the mould

After observing the fungal growth in the flask, the fungal conidia, mycelium were carefully removed and inoculated into sabourods dextrose agar and potato dextrose agar. The plates were incubated at 37°C for 48-72 hours. Identification of the fungus was done by observing colony morphology with reference to colour, size of mycelium and nature of colony. Lactophenol cotton blue staining and Gram staining were carried out as per routine procedure.

Antifungal susceptibility test-

Curcumin was obtained from Hi-media, Mumbai and Curcumin disc was prepared as

per the method of Esimone *et al.*, (2006). Various concentrations of curcumin discs were prepared which includes 25mg, 50mg, 100mg and 200mg/disc respectively. Muller Hinton Agar (MHA) obtained from M/S Hi-Media Laboratories Ltd. Mumbai was employed for the *in-vitro* antifungal sensitivity test of fungal isolates as per the commonly used disc diffusion method Bauer *et al.*, (1966) and the zone of inhibition was recorded. The antifungal discussed are ketoconazole (10mcg), voriconazole (1mcg), itraconazole (30mcg), clotrimazole (10mcg), Amphotericin B(20mcg), miconazole (30mcg), Caspofungin (5µg, In house). All the antifungal disks were obtained from HiMedia, Mumbai, India.

Results and Discussion

In the present study *Aspergillus flavus* was the predominant isolates followed by *A. niger, A. fumigatus, A. nidulans* and *A. terreus.* The result is similar to that of the study of Ahmed *et al.*, (2017).A total of 12 randomly selected isolates were identified by studying the colony morphology on Sabouraud dextrose agar and potato dextrose agar plates. Macroscopically *Aspergillus flavus* produces velvety or wooly yellowish green colonies, *A,niger* produced jet black conidia cottony, velvety or powdery colonies were produced by *A. fumigatus*. Velvety buff to yellow colour colonies in case of *A.nidulus* and cinnamon buff colour colonies in case of *A.terreus* were observed (Fig. 2a, 2b). On lactophenol cotton blue staining *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus* were identified (Fig. 3a, 3b, 3c). Club shaped vesicle with uniseriate conidia was identified as A. *fumigatus*, biseriate phialides with long conidiophores in case of *A.flavus*, biseriate elliptical conidia with upward sweeping appearance in case of *A.terreus*, biseriate with profuse conidiation in case of A.niger and biseriate hemispherical vesicles were observed in case of *A.nidulus* under microscope. In gram staining, it was found that the fungal organism and the hyphae were stained purple (Fig. 4). This mycological study is in agreement with the study of Klich (2002) and Mc Clenny (2005).

On analysis of antifungal susceptibility pattern produced by various antifungals and curcumin (at various concentrations), it was found that almost all *Aspergillus* species (n=12/12) were found sensitive to curcumin at the concentration of 200mg/disc. In case of *Aspergillus flavus*, synthetic antifungals like itraconazole, AmphotericinB ketoconazole, voriconazole, clotrimazole, caspofungin, miconazole and fluconazole with minimum inhibitory zone of 22, 8, 21, 16, 20, 2111 and 10mm respectively whereas curcumin shows zone of inhibition of ≥ 10mm diameter at 200mg/disc and ‹ 10 mm in case of 100mg/disc and 50mg/disc (Table 1 and 2).

Table.1 Prevalence and isolation of different *Aspergillus* sp. from poultry feed samples

Name of the fungal isolates	No. of isolation	Isolation (%)
Aspergillus flavus	12/12	100
Aspergillus niger	9/12	75
Aspergillus fumigatus	8/12	67
Aspergillus nidulans	6/12	50
Aspergillus terreus	5/12	42

Table.2 Comparative study of synthetic antifungal agents with Curcumin disc (at various concentration)

Name of fungal isolates	Zone of inhibition in mm											
	Curcumin(CUR)				ITR	AMB	KT	VCZ	MIC	FLC	CC	CAS
	25mg	50mg	100mg	200mg								
A.flavus	10±0.05	11±0.00	12±0.04	14±0.1	22 ±0.5	8±0.56	21 ±0.7	16±0.05	11±0.02	10±0.05	20±0.02	21±0.00
A.niger	10±0..01	10±0.04	11±0.00	12±0.00	19 ±1.0	8 ±0.6	19 ±0.8	20±0.05	12±0.5	11±0.04	18±0.00	18±0.05
A.fumigatus	9±0.00	11±0.02	12±0.02	13±0.04	18 ±0.7	9 ±1.0	20 ±0.65	15±0.00	10±0.45	12±1.00	21±0.02	19±0.01
A.nidulans	10±0.05	10±0.00	11±0.03	11±0.12	19 ±0.5	8±1.2	18 ±0.75	20±1.0	11±0.00	10±0.00	19±0.02	18±0.75
A.terreus	11±0.04	12±0.01	11±0.05	13±0.57	17 ±1.2	7 ±0.8	20 ±1.0	19±1.2	11±0.05	13±0.00	18±0.00	20±0.02

Fig.1 Isolation of fungus from feed

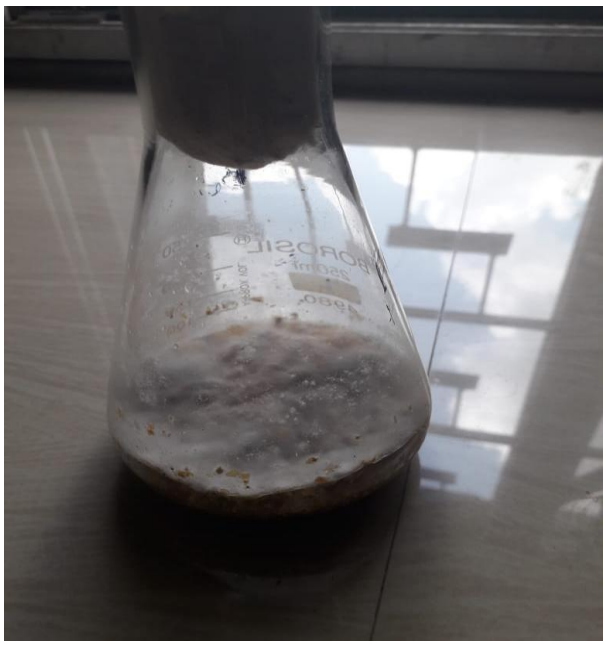


Fig.2(a) showing mixed culture of fungus

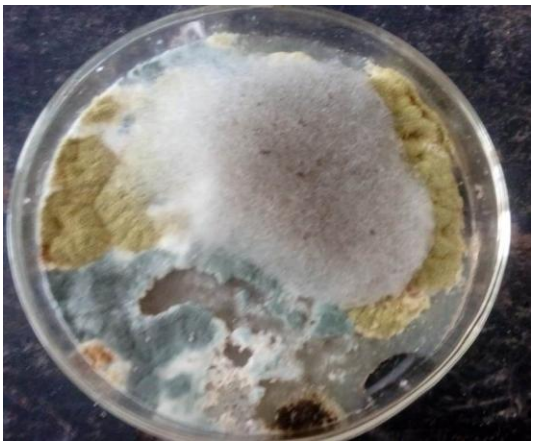


Fig.2(b) showing pure culture

Fig.3 showing lactophenol cotton blue staining of fungal culture

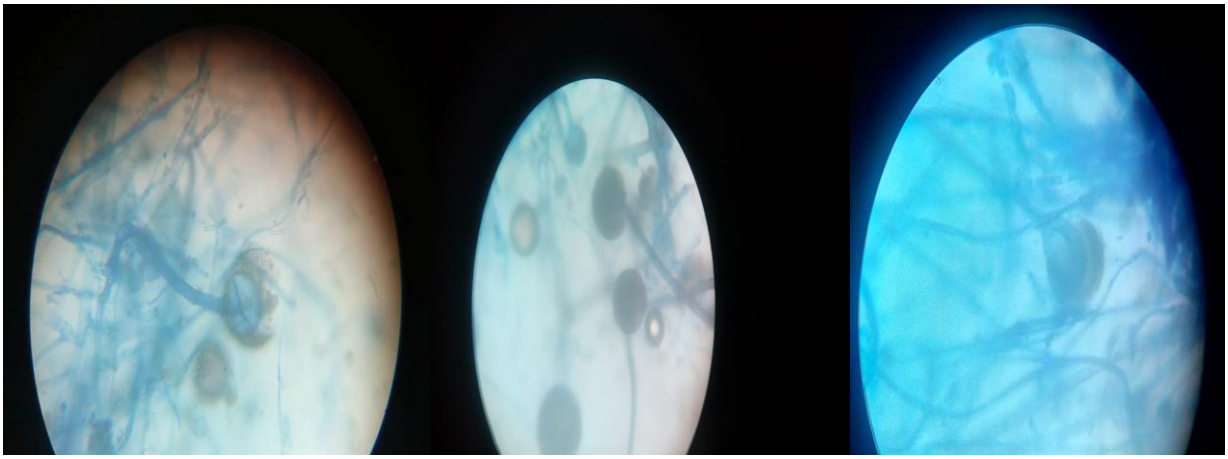
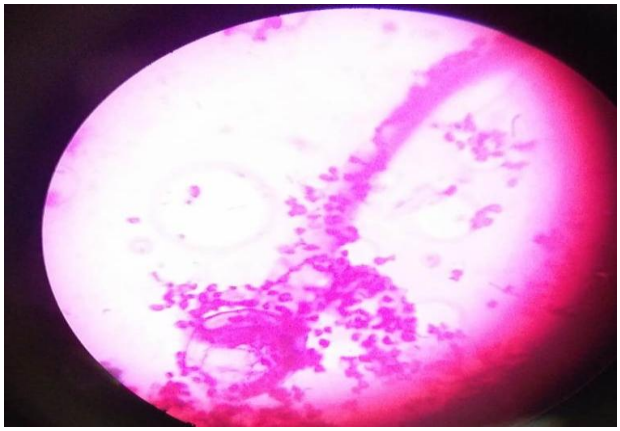


Fig.4 Gram staining of *Aspergillus* species



In case of *Aspergillus niger* synthetic antifungals like itraconazole, AmphotericinB, ketoconazole, voriconazole, clotrimazole, caspofungin miconazole, fluconazole showed minimum inhibitory zone of 19,8,19,20,12,18, 12 and 11mm respectively where as curcumin shows zone of inhibition of approximately ≥ 10mm diameter. In case of *Aspergillus fumigatus* synthetic antifungals like itraconazole, AmphotericinB, ketoconazole, voriconazole, clotrimazole, caspofungin miconazole, fluconazole showed minimum inhibitory zone of 18, 9, 20, 15, 21, 19, 10 and 12 mm respectively whereas curcumin shows zone of inhibition of ≥9mm diameter. In case of *Aspergillus nidulans* synthetic antifungal like itraconazole, AmphotericinB, ketoconazole, voriconazole, clotrimazole, caspofungin miconazole, fluconazole showed minimum inhibitory zone of 19, 8, 18, 20, 19, 18, 11 and 10 mm respectively whereas curcumin shows zone of inhibition of ≥10mm diameter. In case of *Aspergillus terreus* synthetic antifungals like itraconazole, AmphotericinB, ketoconazole, voriconazole, clotrimazole, caspofungin miconazole, fluconazole showed minimum inhibitory zone of 17, 7, 20, 19, 18, 20, 11 and 13 mm respectively whereas curcumin shows zone of inhibition of ≥11mm diameter. The present study revealed that Amphotericin B is resistant to almost all *Aspergillus* species

isolated from poultry feed is in agreement with study of Ramesh *et al.*, (2013).In the present study, curcumin shows better zone of inhibition than that of miconazole, fluconazole and amphotericinB. More investigation is required in order to use curcumin as an potential antifungal agent for poultry.

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