Inhibition of toxigenic fungi in Poultry feed using powdered plants

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Abstract: The present study was aimed to evaluate the inhibitory effect of different powdered plant against different toxigenic fungi.The results showed that the powdered coriander was found highly effective against *A.ochraceae* with 0.6% w/w at the end of 6th week and scored 0.Powdered neem also showed inhibitory effect at 0.6% w/w against *penicillim* 1 and 2 while powdered clove showed inhibitory effect against *penicillium* 2 with 0.5%w/w concentration.Total numbers of spores 17×10^4/g of *A.ochracea* was noted by using powdered clove at the end of 3rd week and 31×10^4/g spores of *A.niger* at the end of 4th week.No spores of *A.niger* were detected by using powdred clove at 0.5% w/w whereas 22 $\times10^4$spores/g of *penicillium* 2 were noted at a concentration of 0.4% at the end of 6th week.

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INTRODUCTION

Mycotoxin contamination of agricultural commodities has attracted worldwide attention because of the significant losses associated with its effects on human health, poultry and livestock1,2 . Some mycotoxins are carcinogenic, mutagenic, teratogenic, nephrotoxic and immunosuppressive agents.

Ochratoxin A (OTA) is a mycotoxin that possesses a risk to human health due to its nephrotoxic, immunotoxic, mutagenic, terato- genic and carcinogenic biological properties. This toxin has been classified as a possible human carcinogen (category 2B) by the International Agency for Research on Cancer [3–5]. OTA was originally described as a metabolite of *Aspergillus ochraceus* grown in pure culture [6]. The oldest information indicates that *Penicillium verruca- sum* is the main species associated to OTA production in foods and feeds in temperate climates, while *Aspergillus spp*. predominate in warmer and tropical countries.

Most poultry feeds are prone to fungal growth during different stages of the manufacturing, transportation and storage. For quality control of poultry feed, the identification of contaminating ochratoxin producers is essential. This will provide important information about the production of mycotoxins. Previous studies have determined the mycobiota and mycotoxin formation in final poultry feed obtained from the same factory at which samples are collected [7]. However, little or no information is available on mycological contamination and potential ochratoxin production by strains isolated during poultry feed processing.

OTA-contaminated feed has its major economic impact on monogastric animals and the poultry industries. Turkeys, chickens and ducklings are mostly susceptible to this toxin. Typical signs of poultry ochratoxicosis are reduction in weight gain, poor feed conversion, reduced egg production, poor egg shell quality and nephrotoxicity. OTA fed at various doses (1–5 mg/kg), to poultry birds of various ages, altered their serum biochemistry, including decreases in cholesterol, total protein, albumin, globulin, potassium, and triglyceride levels, and increases in uric acid and creatine levels and in the activities of serum alkaline phosphatase (ALP) and gamma glutamine transpeptidase (GGT) [8–11]. The effects depend on the level of the toxin and time exposure. However, numerous studies showed that even exposure to low levels of OTA 0.5 mg/kg feed) altered performance, including decreased feed consumption and growth rate and poor feed conversion efficiency [12,13].

Keeping in view the importance of ochratoxin in respect of its toxic effect in poultry the present study was designed to to know the effect of different selected powdered plants against *Aspergillus and penecillinium species* in poultry feed to reduce the risk of ochratoxin contamination.

Material and Method:

Efficacy of medicinal plants to inhibit fungal growth

Three samples of different plants, commonly found in Pakistan were selected and identified by Medicinal Botanical Center, Pakistan council of Scientific and Industrial Research (PCSIR), Peshawar Pakistan. The

scientific name, family and part used of the selected plants are presented below.

coriander: Scientific name *coriander sativum*
Family *Apiaceae*
Part used Fruit

Clove: Scientific name *Syzygium aromaticum*
Family *Myrtaceae*
Part used fruit

Neem: Scientific name *Azadirachta indica*
Family *Meliaceae*
Part used Leaves

Preparation of sample

The healthy parts of plant was separated and washed with tap water followed by sterile distilled water. All these plants material were dried in hot air oven at 50°C for 2-3 days. The dried parts of plants were then ground to powder in warring blender and passed through sieve 1mm mesh. The powdered plant samples were transferred into sterile polyethylene bags and kept in refrigerator till farther use.

Fungal strains

The fungal strains of A. *ochraceus*, A. *niger* and *P. verrucosum* and *P. carbonarius* were procured from Food Microbiology section, PCSIR laboratories complex, Peshawar, Pakistan.

Preparation of spore suspension

Potato dextrose agar (PDA) slants (Oxide, USA) were used for maintaining the cultures of each fungus at 25°C for seven days. The spores of fungi were removed from the sporulating colonies, poured into sterile distilled water containing Tween 80 (0.1% v/v) and transferred into sterile distilled water bottle. The numbers of spore were counted by an improved Neubrour Haemocytometer.

Efficacy of powdered plants on the growth of Fungi

Known amounts of powdered plant (0.2-0.8%) were mixed with autoclaved poultry feed (30g) in sterile petri plates. A dilute spore suspension of 0.1ml ($25x10^7$ CFU/ml) of A. *ochraceus*, A. *niger* and *P. verrucosum* and *P. carbonarius* were added to the center of petri plates containing sterile poultry feed and incubated at room temperature for 6 weeks. The poultry feed without powdered plant was used as control. The incubated plates were then observed for fungal growth weekly.

Observation of fungal growth

Inhibition of fungal growth in poultry feed were assessed visually by scale.

0 = No growth
1 = Very little growth
2 = 25% of feed covered
3 = 50% of feed covered
4 = 75% of feed covered
5 = 100% of feed covered

Spore count

The spores of A. *ochraceus*, A. *niger* and *P. verrucosum* and *P. carbonarius* were harvested from feed samples with sterile distilled water having 0.1% Tween 80. The spore suspension was filtered and numbers of spores were calculated using haemocytometer. The filtered spore suspension 9µl was taken and added to one of the counting four corner chamber. The spore suspension was released slowly to ovoid bubble and over filling. The spores were then counted in each of the $0.1mm^3$ square.

Calculation:

Numbers of spores were calculated by using the formula:

Spores/ml = (n) $x10^4$

Where

n = the average cell count per square of the four corner squares counted.

Effect of different powdered plants on the growth A. *ochraceus*, A. *niger, P. verrucosum* and *P. carbonarius* in poultry feed.

Powdered medicinal plants converted into powder were commonly used by old civilizations to preserve foods. The Egyptians got benefit of the preservative properties of these plants and were utilized in man food materials. In the same way, Greek and Roman literatures have many references of the use of plants for medicinal purposes. Several authors cited the antifungal activity of many medicinal herbs 14-19.

Table 1: Effect of powdered coriander on the growth of *Aspergillus ocharaeceus* in poultry feed

Incubation period(weeks)	-ve control	+ve Control	Percent Concentration of powdered coriander (w/w)						
	Score observed		0.2	0.3	0.4	0.5	0.6	0.7	0.9
1	0	5	3	3	2	0	0	0	0
2	0	5	3	3	2	0	0	0	0
3	0	5	4	3	2	0	0	0	0
4	0	5	4	4	2	1	0	0	0
5	0	5	5	4	3	1	0	0	0
6	0	5	5	5	3	1	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered bymycelium, Score 5= 100% of the feed covered by mycelium

As can be seen from table1 the feed containing no powdered plant and fungus was found clear and no growth were obsereved throughout experimental periods and scored 0.The petri plates containing feed plus fungus were fully covered by *A. ocharaeceus* mycelium and scored 5.The coriander powder at a concentration of 0.4% w/w were found less effective as compared to that of 0.5%.The concentration of coriander powder at 0.5% were noted effective upto 3 weeks and no growth were observed however fungal growth were found in the 4,th , 5th and 6th weeks.The powdererd coriander at a concentration of 0.6% w/w were highly effective against A. ocharaceas and inhibited the growth throughout experimental weeks.

Table 2: Effect of powdered coriander on the growth of *Aspergillus niger* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered coriander (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score observed								
1	0	5	3	2	1	0	0	0	0
2	0	5	3	3	2	0	0	0	0
3	0	5	3	3	2	0	0	0	0
4	0	5	4	3	2	0	0	0	0
5	0	5	4	3	3	1	0	0	0
6	0	5	4	4	3	1	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Table 2 data reveals that the powdered coriander was more effective at a concentration of 0.5% up to 4 weeks and no growth of *A. niger* was observed, while after 4th week A.niger growth was noted in the 5th and 6th weeks. The powdered coriander showed maximum activity at 0.6% w/w against *A.niger* and no growth was observed throughout experimental weeks.

Table 3: Effect of powdered coriander on the growth of *penicilium*in *verrucosum* poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered coriander (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score observed								
1	0	5	2	1	0	0	0	0	0
2	0	5	2	1	0	0	0	0	0
3	0	5	2	1	0	0	0	0	0
4	0	5	3	1	0	0	0	0	0
5	0	5	4	2	1	0	0	0	0
6	0	5	4	2	1	0	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4 = 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Powdered coriander at 0.3% was found highly effective against *P. verrucosum* and a very little growth was found from 1st to 4th weeks while 25% area was covered by the mycelium in the 5th and 6th weeks. .No growth was observed at concentration of 0.4% from 1st to 4th weeks and scored 0 whereas a very little growth was determined at the end of 5th and 6th weeks. The growth of *penicilium*in *verrucosum* was fully inhibited throughout experimental time and scored 0.

Table 4: Effect of powdered Coriander on the growth of *Penicillium carbonarius* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered coriander (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score observed								
1	0	5	4	2	1	0	0	0	0
2	0	5	4	2	1	0	0	0	0
3	0	5	4	3	1	0	0	0	0
4	0	5	5	4	1	0	0	0	0
5	0	5	5	4	2	1	0	0	0
6	0	5	5	4	3	2	1	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

The effects of powdered coriander highly effective1st, 2nd, 3rd and 4th weeks and scored 0 at a concentration of 0.5% with no growth at the end of ,however very little growth was noted in the 5th week and about 25% area was covered by mycelium at the end of 6th week.0.6% powdered coriander was effective up to 5th weeks and very little growth was observed at the end of 6th week.

Table 5: Effect of powdered neem on the growth of *Aspergillus 0chareceus* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered neem (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score observed								
1	0	5	5	3	2	1	0	0	0
2	0	5	5	3	2	2	0	0	0
3	0	5	5	4	2	2	2	0	0
4	0	5	5	4	2	2	2	0	0
5	0	5	5	4	3	2	2	0	0
6	0	5	5	5	4	3	2	1	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Table 5 data shows that neem powdered plant was effective in the concentration of 0.8% and scored 0 while 0.7% powdered plant was effective up to 5th weeks with 0 score. Very little growth was found at the end of 6th week at 0.7% concentration (w/w).

Table 6: Effect of powdered neem on the growth of *Aspergillus niger* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered neem (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	5	4	3	2	1	0	0
2	0	5	5	4	3	2	1	0	0
3	0	5	5	4	3	2	1	0	0
4	0	5	5	5	3	2	1	0	0
5	0	5	5	5	4	2	1	0	0
6	0	5	5	5	4	3	1	1	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Effect of powdered neem on the growth of *Aspergillus niger* has been presented in table 6.Result reveals that powdered neem inhibited total growth at concentration of 0.8% (w/w) throughout the experimental period of six weeks however 0.7% (w/w) concentration was found effective up to 5th week. A trend was noted that as the concentration of powdered garlic decreases the number of score increases.

Table 7: Effect of powdered neem on the growth of *penicilium verrucosum* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered neem (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	3	2	0	0	0	0	0
2	0	5	3	2	0	0	0	0	0
3	0	5	3	2	1	0	0	0	0
4	0	5	4	2	1	0	0	0	0
5	0	5	4	3	2	0	0	0	0
6	0	5	5	4	3	1	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

The effect of powdered neem showed maximum activity against *p. verrucosum* at a concentration of 0.5% up to 5th weeks while a very little growth was recorded at the end of 6th week. At 0.6% powdered neem plant was examined highly effective throughout experimental periods and scored 0.

Table No.8: Effect of powdered neem on the growth of *Penicillium carbonarius* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered neem (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	2	1	0	0	0	0	0
2	0	5	2	1	0	0	0	0	0
3	0	5	2	1	1	0	0	0	0
4	0	5	2	2	1	0	0	0	0
5	0	5	3	2	2	0	0	0	0
6	0	5	4	3	2	1	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Table 8 data reveals that very little growth was recorded during 3rd and 4th weeks and scored 1 whereas 25% area was covered by *P. carbonarius* at the end of 5th and 6th weeks. Powdered neem at 0.5% (w/w) concentration was effective up to 5th weeks whereas 0.6% powdered neem (w/w) showed maximum activity throughout the weeks and scored 0.

Table No.9: Effect of powdered clove on the growth of *Aspergillus ochareus* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered clove (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	5	4	2	1	0	0	0
2	0	5	5	4	2	1	0	0	0
3	0	5	5	5	2	1	0	0	0
4	0	5	5	5	3	2	1	0	0
5	0	5	5	5	3	2	2	0	0
6	0	5	5	5	3	2	2	1	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

The effect of powdered clove against *A.ochareus* has been presented in table 9. The results revealed that no growth was observed at concentration of .6% up to 3 weeks and scored 0.The 0 scored was noted with 0.7%

powdered neem (w/w) at the end of 5th weeks however a very little growth was found during 6th week and scored 1.

Table No.10: Effect of powdered clove on the growth of *Aspergillus niger* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered clove (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	5	3	1	0	0	0	0
2	0	5	5	3	1	0	0	0	0
3	0	5	5	3	1	0	0	0	0
4	0	5	4	2	0	0	0	0	0
5	0	5	5	4	3	1	0	0	0
6	0	5	5	4	4	2	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

No fungal growth was recorded with 0.5% powdered neem at the end of 4th weeks whereas very little growth was noted at the end of 5th week and scored 1.Upto 25% area was covered during 6th week with the same concentration and noted scored 2.The maximum activity was observed with 0.6% powdered neem through experimental periods and scored 0 (table 10).

Table No.11: Effect of powdered clove on the growth of *penicilium*in *verrucosum* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered clove (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	2	1	0	0	0	0	0
2	0	5	2	1	0	0	0	0	0
3	0	5	3	1	0	0	0	0	0
4	0	5	4	2	0	0	0	0	0
5	0	5	4	3	1	0	0	0	0
6	0	5	5	4	2	1	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Powdered clove was found highly effective at concentration of 0.4% up to 4 weeks and scored 0 whereas score 1 was noted in the 5th and 2 in the 6th weeks. The concentration of powdered neem at 0.6% was examined highly effective against *penicilium*in *verrucosum* and scored 0 in all experimental weeks (table 11).

Table No.12: Effect of powdered clove on the growth of *Penicillium carbonarius* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered clove (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.9
	Score of different concentration								
1	0	5	2	0	0	0	0	0	0
2	0	5	3	1	0	0	0	0	0
3	0	5	4	2	0	0	0	0	0
4	0	5	4	2	0	0	0	0	0
5	0	5	4	3	1	0	0	0	0
6	0	5	4	3	1	0	0	0	0

Score 0=No growth, Score 1= Very little growth, Score 2=25% of the feed covered by mycelium, Score 3= 50% of the feed covered by mycelium, score 4= 75% of the feed covered by mycelium, Score 5= 100% of the feed covered by mycelium

Table 12 data shows that although the growth of *Penicillium carbonarius* was 50% at the end of 5th and 6th weeks however abrupt decreased in mycelium was noted with 0.4% concentration and scored o at the end of 6th week. The results also indicated that 0.5% inhibited the growth throughout experimental weeks and scored 0.

Table 13: Number of spores of *A. ochraeceus* in poultry feed (spores/g) at the end of 6th week

Plant	Number of spores/gram							Control
	Percent concentration of powdered plants (w/w)							
	0.2	0.3	0.4	0.5	0.6	0.7	0.8	214±2.37
Coriander	198±3.31	168±2.1	53±2.9	13±1.6	0	0	0	
Neem	151±2.6	92±2.2	41±6.3	10±4.2	0	0	0	
clove	101±3.1	62±2.9	17±2.8	0	0	0	0	

The number of spores of *A.ochraeceus* has been depected in table 13.The results revealed that the numbers of spores were 53×10^4/g at a concentration of 0.4% of powdered coriander while 41×10^4/g with the same concentration was detected with powdered neem.Numbers of spores 17×10^4 was detected with 0.4% of powdered clove at the end of 6th week.The best result was shown by powdered clove with 0.5% at the end of 6th week and scored 0 followed by 10×10^4/g by powdered neem and 13×10^4 number of spores by powdered coriander.

Table 14: Number of spores of *A. niger* in poultry feed (spores/g) at the end of 6th week

Plant	Number of spores/gram							Control
	Percent concentration of powdered plants (w/w)							
	0.2	0.3	0.4	0.5	0.6	0.7	0.8	217±3.37
Coriander	179±3.2	102±3.7	78±1.02	31±2.6	17±2.2	0	0	
Neem	198±4.1	126±1.7	105±3.2	63±2.1	21±2.9	10±1.06	0	
Clove	181±3.3	120±6.1	81±3.9	31±2.93	0	0	0	

The highest number of spores 63×10^4/g were found at 0.5% powdered neem whereas equal number of spores 31×10^4 were found at a same concentration at the end of 6th week (table 13).Less spores count 17×10^4/g were found at a concentration of 0.6% of powdered coriander followed by 21×10^4/g while no spores were detected at a concentration of 0.6% with powdered clove and scored 0.

Table 15: Number of spores of *peniciliumin verrucosum* in poultry feed (spores/g) at the end of 6th week

Plant	Number of spores/gram							Control
	Percent concentration of powdered plants (w/w)							
	0.2	0.3	0.4	0.5	0.6	0.7	0.8	201±3.41
Coriander	179±3.4	97±3.06	53±4.1	19±2.2	0	0	0	
Neem	193±2.77	112±1.63	68±3.9	21±1.01	0	0	0	
Clove	181±1.16	103±3.61	61±2.3	17±1.88	0	0	0	

The number of spores of *P. verrucosum* 1 /g has been shown in table 11.The result showed that 19×10^4 spores/g of penicillium 1 were found with 0.5% of powdered Coriander whereas the powdered Neem was found more effective 17×10^4 spores/g of penicillium 1 at a concentration of 0.5% than powdered Clove at the end of 6th week.0.6% concentration of all the tested powdered samples were found highly effective and scored 0 at the end of 6th week.

Table 16: Number of spores of *Penicillium carbonarius* in poultry feed (spores/g) at the end of 6th week

Plant	Number of spores/gram							Control
	Percent concentration of powdered plants (w/w)							
	0.2	0.3	0.4	0.5	0.6	0.7	0.8	210±2.41
Coriander	190±1.39	111±3.2	71±1.39	42±3.3	15±1.090	0	0	
Neem	171±2.21	93±3.02	53±2.98	20±1.17	0	0	0	
Clove	151±1.11	71±2.21	22±3.13	0	0	0	0	

Table 12 data showed that powdered neem was not effective against *P. carbonarius* at a concentration of 0.5% and 0.6% while powdered CLOVE was found highly effective at a concentration of 0.5% and scored 0 at the end of 6th week however powdered Coriander inhibit the *P.carbonarius* spores production at a

concentration of 0.6% throughout the experimental weeks and scored 0.

Discussion;

Powdered neem and clove were found highly effective at 0.5 and 0.6% w/w against the tested fungi, where as many researchers have also reported the activity of different plants against different pathogenic fungi.

According to S.Centeno (2010) the extracts of *Rosmarinus officinalis* and *Thymus vulgaris* possesses antifungal properties against *Aspergillus flavus* and *A.Ochraceus* and could be used as an alternative for the decontamination of food by fungi.20

Marian B et al (2013)studied the antifungal activity of the alcoholic extracts obtained from three native plant species: burdock (Arctium *lappa*), thyme (*Thymus vulgaris*) and rough cocklebur (*Xanthium strumarium*). The demonstration of the antifungal activity was realized using *Aspergillus ochraceus* and *Acremonium chrysogenum* isolates. The antifungal activity of the selected plant extracts was evaluated by radial growth measurement on potato dextrose agar amended with plant extracts of different concentrations. Fungi were completely inhibited in the variant using plant extracts of the following concentration: 0.3 g plant material / ml to 70o ethanol. When using concentration of 0.075 g plant material / ml to 70o ethanol, the development of fungi was significantly inhibited.21

According to Shubhi Avasthi et al (2013), Eight commonly used spices *Syzygium aromaticum, Cinnamonum zeylanicum, Zingiber officinale, Murraya koenigii, Piper nigrum, Trachyspermum ammi, Allium sativum* and *Allium cepa* were tested for in vitro antifungal activity on Aspergillus niger, a causative agent of different destructive disease. Out of eight plant materials used, five showed significant antifungal activity against the test pathogen by poisoned food technique. *Syzygium aromaticum* and *Allium sativum* showed 100% inhibition of mycelial growth at 20% concentration. Results of the present investigation indicates that spices possess antifungal activity and can be exploited as natural fungitoxicant to control the growth of storage or spoilage fungi, A. niger and thus reduce the dependence on the synthetic fungicides.22

Five different plants species such *as Abrus precatorius* L. (Papilionaceae), *Aegle marmelos* (L.) *Correa ex Roxb*. (Rutaceae), *Aporosa lindleyana Baill* (Euphorbiaceae), Areca catechu L. (Arecaceae), *Brassica juncea* (L.) *Czern*. (Brassicaceae) were used in traditional Indian medicine were examined against *Aspergillus niger* by Raji R et al (2013) . The extracts of 5 plants exhibited varying degrees of inhibition activity against the fungi. Among the 5 plants studied 4 plants showed maximum antifungal activity and the remaining one show minimum antifungal activity were taken for the antifungal study.23

Sibel Özçakmak (2012)reported the antifungal effects of essential oil isolated from Heracleum platytaenium Boiss on the growth of ochratoxigenic *Penicillium verrucosum* (D-99756) isolated from Kashar cheese were in- vestigated. Minimal inhibitory concentration (MIC) and Minimal lethal concentration (MLC) tests were performed by using broth dilution method. The MIC and MFC values were determined as 31 and 125 µL/mL, respectively.24

Conclusion

From the present it was concluded that the powdered coriander at 0.6%, 0.5% and 0.5% w/w were found highly active against *A.ochraceae, A.niger* and of *penicilium*in *verrucosum* respectively whereas powdered clove showed highest activity at 0.5% against *Penicillium* 11 at the end of 6th week and scored 0..

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