Preservation of poultry feed by using different powdered plants

Farhat Ali Khan and Mohammad Zahoor

Department of chemistry, University of Malakand, KP, Pakistan

Abstract: Three selected medicinal plants (Ginger, Onion and Garlic) were evaluated for antifungal activity in poultry feed. The results showed strong antifungal activity of powdered garlic (0.6% w/w) against *A. flavus* and *A. parasiticus* and no growth (Scored 0) of both *A. falvus* and *A.parasiticus* were seen in the tests samples at the end of 6th week. Powdered onion (0.6% w/w) exhibited strong activity against *A. flavus* for four weeks and scored 0, while no growth of A.parasiticus was observed throughout experimental weeks with the same concentration. Powdered ginger was found effective at a concentration of 0.8% w/w against *A. flavus* and 0.7% w/w against *A. parasiticus* with 0 scores during experimental period. The numbers of spores of *A. flavus* were 173×10^4/g at a concentration of 0.2% of powdered ginger whereas 167×10^4/g spores were detected with powdered onion. Powdered garlic of 0.6% was found highly effective against *A. flavus* with spores of 0/g. The spores of *A. parasiticus* were noted 5×10^4/g at a concentration of 0.6% of powdered ginger, while 0/g spore were observed with 0.6% concentration of powdered onion and garlic.

[Farhat Ali Khan and Mohammad Zahoor. **Preservation of poultry feed by using different powdered plants.** *Life Sci J* 2014;11(1s):9-14]. (ISSN:1097-8135). http://www.lifesciencesite.com. 2

Key wards: Toxigenic fungi, Powdered plants, Antifungal activity, spore count

Introduction

Feed add up to approximately 70 percent of the whole cost of poultry production. Its quality has significant effects on growth and performance of poultry birds. Poultry feed consist of various macro and micro components. These components are mostly supplied by different agricultural and industrial by products or wastes. In the last two decades the number of poultry has been raised up to the level that it is not very easy to sustain the growth rate of fifteen percent per anum. In this view the major problem faced by the industry is the availability of quality feed. Furthermore proper storage facilities are insufficient which results the fungal contamination of poultry feed (Bhatti, 1999-2000).

Pakistan is situated in the sub tropical region in the world globe (Williams et al., 2004). Its exact position lies between 24° and 40° North latitudes. As the climatic conditions of the sub tropical regions are warm, moist and damp, therefore it provides best condition for development of fungus. Poultry feed is mainly prepared from agricultural materials therefore it primarily depends on the eminence of these products (Anjum et al., 2000). In the developing countries it is one of the common trends that the high quality cereals and grains kept, stored and exported for human use while the low quality agricultural products are used for manufacturing animal feeds (Jones, 1995). Furthermore, insufficient services for preservation, moist atmospheric conditions and raised temperature especially from month of May till November are favorable for the growth and propagation of fungi particularly *Aspergillus* species, which bring about mycotoxins production under these conditions (Bhatti, 1989).

Mycotoxins are the poisonous substances cause various harmful effects with respect to health and productivity in about all species of domestic animals as well as in poultry birds. Generally, mycotoxicosis in chicks results in reduce intake of feed, low conversion of feed and less productivity. It also increase susceptibility to various diseases and abnormalities depending upon the type of toxins consumed. Mycotoxin contamination in feed is a worldwide problem. Among mycotoxins, aflatoxins are considered one of the most hazardous toxins encounter in animal feeds, throughout the world (Williams, 2004). Aflatoxins are derivative of difurocoumarin, synthesized by various toxigenic species of Aspergillus. Natural contamination of aflatoxin in the poultry feed results in severe economic losses to the poultry industry. Thus Poultry industry is much susceptible to this toxin as compared to other animals. Fungal growth and mcyotoxin production is very common and frequent in poultry feeds as reported by (Farooq, 2008).

Various plants have been used traditionally to preserve food in countries like Russia, Japan and India. The extracts and powder of some plants show the ability to inhibit the fungal growth of toxins producing fungi (Meri and Win, 1998). The present study was aimed to analyze the efficacy of powdered ginger (*Zingiber officinale*), onion (*Allium cepa*), and garlic (*Allium satium*) for it's a preservative effects in poultry feed against *A. flavus* and *A.parasiticus*.

Materials and methods

Efficacy of medicinal plants to inhibit fungal growth

Three (3) 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.

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 *Aspergillus flavus* (Accession No 1000) and *Aspergillus parasiticus* (Accession No 1002) 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 number of spores was counted by an improved Neubrour Haemocytometer (Reddy et al., 2009).

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.flavus* (Accession No 1000) and *A.parasiticus* (Accession No 1002) 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. All the petri plates .The incubated plates were then observed for fungal growth weekly (Thanaboripat et al., 1997).

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 (Cuero et al., 1987).

Spore count

The spores of *A. flavus* and *A. parasiticus* were harvested from feed samples with sterile distilled water having 0.1% Tween 80. The spore suspension was filtered and number of spores were calculated using haemocytometer. The filtered spores' suspension 9µl was taken and added to one of the counting four corner chamber. The spore suspension was released slowly to ovoid bubble and overfilling. The spores were then counted in each of the 0.1 mm^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 (Thanaboripat et al., 1997).

Results and Discussion

Effect of different powdered plants on the growth of *Aspergillus flavus* and *Aspergillus parasiticus* 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. (Azzous and Bullerman, 1982; Farag et al., 1989).

The effect of powdered ginger on the growth of *Aspergillus flavus* in poultry feed has been presented in Table 1.The data revealed that as the concentration (w/w) of powdered ginger increased result in decreased of growth of *A. flavus*. The incubated feed without plant powdered and fungus (negative control) was found clear and scored 0 while feed containing *A. flavus* without powdered ginger (positive control) was fully covered by mycelium and scored 5.It was also noted that powdered ginger of 0.6% (w/w) exhibited maximum activity for 2 weeks only whereas 0.7% powdered plant (w/w) was found effective for 4 weeks. Powdered ginger was found highly effective at 0.8% (w/w) and no growth was observed against *A. flavus* throughout the experimental period.

Table-2 revealed that powdered ginger was found effective at concentration of 0.6% (w/w) against *Aspergillus parasiticus* for 4 weeks, while 0.7% (w/w) concentration was found more active and no growth was recorded from 1st to 6th weeks. Moreover the concentration below 0.6% (w/w) was noted less effective and the scores were increased as the concentrations decreased.

Table 1: Effect of powdered Ginger on the growth of *Aspergillus flavus* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered garlic (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.8
	Score observed								
1	0	5	4	3	2	1	0	0	0
2	0	5	4	3	2	1	0	0	0
3	0	5	5	4	2	2	1	0	0
4	0	5	5	5	3	2	2	0	0
5	0	5	5	5	4	3	2	1	0
6	0	5	5	5	4	4	3	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 2: Effect of powdered Ginger on the growth of *Aspergillus parasiticus* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered garlic (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.8
	Score observed								
1	0	5	4	3	2	1	0	0	0
2	0	5	4	3	2	1	0	0	0
3	0	5	5	4	2	1	0	0	0
4	0	5	5	4	3	2	0	0	0
5	0	5	5	4	3	2	1	0	0
6	0	5	5	5	4	3	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

Zingiber officinale (Ginger) is an indigenous plant in the Southeast Asia and is grown in different tropical areas of the globe. The plant has been investigated for its antibacterial, anti-oxidant, antiprotozoa and antifungal activity.The antifungal activity of ginger is due to the presence of thymol, eugenol, 1,8 - cineole, α - and β - pinenes, linalool and α - terpineol as main components (Srivastava et al., 2000).

According to Sushil and Mamta (2013) , Ginger oil *(Zingiber officinale* Rosc.*)* possesed potent antifungal activity against various fungi. The minimum fungicidal activity with concentrations of 1.0ml/ml was found to be active against *Alternaria alternata, Botrytis cinerea, Cladosporium cladosporioides, Colletotrichum capsici, C. falcatum, Fusarium cerealis, F. culmorum, Gloeosporium fructigenum, Penicillium digitatum* and 1.2ml/ml against *Aspergillus flavus, A. fumigatus, A. niger*, *A. parasiticus.* Krishna reddy et al., (2011), investigated the effect of some local plant against *Aspergillus flavus* and aflatoxin contamination. The aqueous extract of *Zingiber officinalis inhibited* aflatoxin synthesis to a significant level, while other solvent extracts of *Zingiber officinalis* suppressed both the growth and aflatoxin production by *A. flavus.* The antimicrobial activity of ginger has been attributed to gingerol and shagelol derived from the ethanolic extracts of ginger.

The results of Table-3 showed that the powdered onion was less effective at concentration of 0.2% to 0.5% (w/w) against *A. flavus.* A trend was noted that growth was slowly decreased with the increase in concentration of powdered onion. The results indicated that no growth was present in 1st, 2nd, 3rd and 4th weeks at a concentration of 0.6 % (w/w) against *A. flavus*, while total inhibition was noted at 0.7% (w/w) concentration during all experimental weeks.

The results (Table-4) revealed that powdered onion was highly effective at a concentration of 0.6% (w/w) against *A.parasiticus* and no growth was observed in all experimental weeks. It was also noted that powdered onion was less effective at concentration below 0.6% and the scores were found increased with the decreased of powdered onion concentration in poultry feed.

Table 3: Effect of powdered Onion on the growth of *Aspergillus flavus* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered garlic (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.8
	Score observed								
1	0	5	3	2	2	1	0	0	0
2	0	5	3	3	2	1	0	0	0
3	0	5	4	4	3	1	0	0	0
4	0	5	5	4	3	2	0	0	0
5	0	5	5	5	4	2	1	0	0
6	0	5	5	5	4	2	2	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 4: Effect of powdered Onion on the growth of Aspergillus parasiticus in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered garlic (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.8
	Score observed								
1	0	5	3	2	1	0	0	0	0
2	0	5	3	2	2	0	0	0	0
3	0	5	4	3	2	0	0	0	0
4	0	5	4	4	3	1	0	0	0
5	0	5	5	4	3	1	0	0	0
6	0	5	5	5	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

Allium genus contains more than 500 members which are differed in maturing, color and taste and similar with biochemical, phytochemical and neutraceutical content. *Alliums* were considered to possess strong antimicrobial activities (Benkeblia, 2004; Haciseferogullari et al., 2005). Antifungal activity of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) essential oils against various fungi has also been studied. The low concentration of essential oil of green and yellow onions were found less effective against *A. niger,* whereas red onion and garlic essential oils showed strong antifungal activity against *A. niger* (Benkeblia, 2004).

Antifungal studies about onions are very limited however some *authors have studied the antifungal activity of* thiosulfonates compounds in onions against *A. niger*, *P. italicum*, *Tryptophyton gypseum* and *Microsporon audouini*. According to researchers ether extracts of onion show strong antifungal activity against *A. flavus* and *A. parasiticus* (Pruthi, 1980).

Table 5: Effect of powdered Grlic on the growth of Aspergillus flavus in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered garlic (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.8
	Score observed								
1	0	5	3	2	1	0	0	0	0
2	0	5	3	2	2	0	0	0	0
3	0	5	4	2	3	0	0	0	0
4	0	5	4	3	3	1	0	0	0
5	0	5	5	3	3	2	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

A similar trend was observed in Table-5 as in Table-4. The results showed that as the concentration of powdered garlic increased, results in decrease of score values. The powdered garlic was scored 0 at concentration of 0.5% (w/w) in first three weeks whereas the growth of *A.flavus* appeared in the 4th week and covered 25% of the feed in 5th and 6th weeks. The powdered onion at concentration of 0.6% (w/w)

was found more effective and scored 0 throughout experimental weeks.

Effect of powdered garlic on the growth of *A.Parasiticus* has been presented in Table-6.Results revealed that powdered garlic inhibited total growth at concentration of 0.6% (w/w) throughout six weeks however 0.5% (w/w) concentration was found effective up to 5th week. A trend was noted that as the concentration of powdered garlic decreased the number of score increased.

Table 6: Effect of powdered Garlic on the growth of *Aspergillus parasiticus* in poultry feed

Incubation period(weeks)	-ve control	+ve control	Percent Concentration of powdered garlic (w/w)						
			0.2	0.3	0.4	0.5	0.6	0.7	0.8
	Score of different concentration								
1	0	5	3	2	1	0	0	0	0
2	0	5	3	2	1	0	0	0	0
3	0	5	3	2	1	0	0	0	0
4	0	5	4	3	1	0	0	0	0
5	0	5	5	3	2	0	0	0	0
6	0	5	5	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

Extracts of garlic (*Allium sativum* L.) and onions (*Allium cepa* L.) contain volatile antimicrobial substances which inhibit *Aspergillus flavus, A. parasiticus, Candida albicans, Cryptococcus, Penicillium, Rhodotorula, Saccharomyces, Torulopsis* and *Trichosporon* species. The main compounds obtained from this extract is allicin (Davidson., 1997). Ahmad et al., (2012) presented the antifungal activity of aqueous extract prepared from Egyptian garlic (*Allium sativum* L.) against *Aspergillus flavus*. The recorded minimum inhibitory concentration (MIC) for growth inhibition was 3.60 mg/ml.

Table 7: Number of spores of *A. flavus* 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	219±3.31
Ginger	173±3.21	127±1.47	89±2.31	56±1.53	12±0.58	4±0.76	0	
Onion	167±1.98	105±1.62	73±1.18	38±0.52	7±0.65	0	0	
Garlic	153±2.11	97±0.99	59±0.93	13±0.46	0	0	0	

Number of spores *of A. flavus* has been presented in table 7.Results showed that the numbers of spores were 28×10^4/g at a concentration of 0.5% of powdered ginger whereas 38×10^4/g spores were detected with powdered onion. Powdered garlic was found more effective than powdered ginger and onion with spores count 13×10^4/g. It had been noted that powdered garlic at 0.6% were found highly effective against *A. flavus* with spores of 0/g. The number of spores 4×10^4/g of *A. flavus* were observed with a concentration of 0.7% of powdered ginger, while powdered onion with the same concentration showed 0/g spores of *A. flavus*.

Table 8: Number of spores of *A.parasiticus* 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	171±2.41
Ginger	141±1.86	102±1.33	69±0.93	28±0.92	5±0.14	0	0	
Onion	129±2.15	83±0.89	38±0.43	11±0.14	0	0	0	
Garlic	120±0.78	61±0.81	25±0.56	6±0.13	0	0	0	

.Table-8 data revealed that 28×10^4 spores/g of *A.parasiticus* were observed with 0.5% of powdered ginger followed by 11×10^4 and 6×10^4 spores/g with powdered onion and powdered garlic respectively. The results showed that powdered onion and garlic were highly effective with concentration of 0.6% resulting score 0 however powdered ginger gave 5×10^4 number of spores/g of *A.parasiticus*.

Conclusion

A number of chemicals have been used as antifungal agent to preserve food and feed. Due to health and economic considerations natural products may provide an alternative method to protect food and feed from fungal contamination. Ginger, onion and especially garlic powder can be used as antifungal agents to preserve poultry feed.

References

1. Ahmed, A; Ismaiel; Gamal, H; Rabie; Saied; E.M; Kenawey; Marwa A.(2012): Efficacy of aqueous garlic extracts on growth, aflatoxin b1 production, and cyto morphological aberrations of aspergillus flavus, causing human ophthalmic infection: topical treatment of a. Flavus keratitis. *Brazilian Journal of Microbiology*, 29:1355-1364
2. Anjum, A.D and Naseem,M.(2000): Scenario of mycotoxins in poultry feed ingredients and finished feed in Pakistan. Biomin Gmbh, Industriestrasse, Herzogenburg, Austria, Newsletter, *Biomin Natural*, 14: 6-8.
3. Benkeblia, N.(2004):Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum).*Lebensm.-Wiss.u-Technol*, 37: 263-268.
4. Bhatti, B.M. (1989): Incidence of poultry diseases and their importance in poultry production in Pakistan. *Pakistan Vet J*, 9: 194-197.
5. Bhatti, B.M: (1999-2000): Poultry Development Centre Rawalpindi, Pakistan.
6. Cuero, R.G; Smith, J.E; Lacey, J. (1987): Stimulation by H.burtonii and B.amyloliquefacian of aflatoxin production by A.flavu in indicated maize and rice grain.Applied environmental microbiology. 21 (4), 354-364.
7. Davidson, P.M. (1997): Chemical preservatives and natural antimicrobial compounds. In: Doyle, M.P., Beuchat, L.R. & Montville, T.J. (Eds.). *Food Microbiology-Fundamentals and Frontiers*. Washington D.C., ASM Press: 520-556.
8. Farag, R. S.; Daw, Z. Y; Abo-Raya, S. H. (1989): Influence of some spice essencial oils on Aspergillus parasiticus growth and production of aflatoxinas in a synthetic medium. *Journal of Food Science*, 54: 54-74.
9. Farooq, M. (2008): Prevalent disease and mortality in egg type of layers.www.priory.com/vet/egg.htm.6.2.08. 10.30 a.
10. Haciseferogulları, H; Ozcan, M; Demir, F; Calısır, S.(2005): Some nutritional and technological properties of garlic (Allium sativum L.). *J.Food Eng.* 68: 463- 469
11. Jones, B.D. (1995): The role of aflatoxins in human and animal health. In: *Proceedings of National Seminar on Hazards of Mycotoxins in Human and Animal Health*, April 5-6 Karachi, Pakistan.
12. Krishna, R.V; Srinivas, M; Rajende,r A; Reddy; Srujana, G; Surekha, M and Reddy S. M.(2011): Plant extracts in the management of aflatoxin production by aspergillus flavus. *International Journal of Pharma and Bio Sciences.* 2: 2.
13. Pruthi, J.S.(1980): Natural antimicrobials from plants.Spices and Condiments Chemistry,Microbiology ,Technology.Academic Press, San Fransisco,pp 35-305.
14. Reddy, K.R.N; Reddy, C.S; Muralidharan, (2009): Potential of botanical and biocontrol agents on growth and aflatoxin production by Aspergillus flavus infecting rice grain. *Journal of food control* 35 : 173-178.
15. Srivastava, A; Shukla, Y.N; Kumar, S. (2000): Recent development in plant derived antimicrobial constituents - A Review. *J Med Arom Plant Sci*. 22:349 - 405
16. Sushil, K. S; Mamta, P. S . (2013): Pesticidal activity of Ginger oil against post harvest spoilage in Malus pumilo L. *Journal of Natural Products*. Vol. 667-72
17. Thanaboripat,D; Nontabenjawan,K; Leein,K; Teerap, D; Sukcharoen,O; Ruangrattanametee, V. (1997): Inhibition of aflatoxin production and growth of A.flavus by citronella oil. *Journal of forestry research*. 8: 39-42.
18. Williams, J.H; Phillips, T.D; Jolly, P.E; Stile, J.K; Jolly, C.M. and Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a revivew of toxicology, exposure, potential health consequences and interaction. *American Journal of Clinical Nutrition*. 80: 1106–1122.

12/24/2013