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MYCOLOGICAL SURVEY OF BAJRA KARBI *(PENISETUM TYPHOIDES* [BURMF.] S&H) COMMONLY USED FODDER IN RELATION TO POSSIBLE HEALTH HAZARDS

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ABSTRACT

Fungi constituting the mycoflora are universally distributed in nature. They are the plants without leaves, chlorophyll they can`t perform photosynthetic activity. As such they are not able to make their own food, which they have to obtain from living or dead tissues and simultaneously produce a wide variety of secondary metabolites called mycotoxins. Considerable amount of work on moulds and their ill effects on food and feed crops have also been undertaken in India. About a dozen mycotoxins are known to be elaborated by different moulds contaminating various foods and feeds. The toxic effects of fungal toxins on man and animal arise from the ingestion of food and feed stuff contaminated by them.

KEYWORDS: Mycoflora, Photosynthetic activity, Secondary metabolites, Mycotoxins, Moulds, Contaminated

INTRODUCTION

Moulds, invading a large number of substrates of plants and animal origin, are major cause for the spoilage of agriculture crops and commodities. According to Johnson (1948) 1 to 2 percent of the worlds grain produce is lost due to microbial spoilage. They are important, not only from the point of view of deteriorating the food quality and quantity but also from the health hazards point of view. (Christensen & Kaufmann 1965).

Association of moulds with foods and feeds is a constantly occurring natural phenomenon world over Species of Aspergillus, Penicillium, Fusarium, Alternaria etc. affect our foods and feeds both during storage and in the field. Christensen (1957) has systematically divided fungi into two district groups- field fungi and storage fungi. The main genera of field fungi are *Alternaria, Fusarium, Helminthosporium and Cladosporium,* the storage fungi includes species of *Penicillium, Aspergillus and Sporendonema*.

In congenial environment moulds grow on variety of substracts. During their growth they not only deprive the substrates of their valuable nutrients, but also contaminates them with toxins produced as a result of their metabolic activity. The word mycotoxins, which means fungal

poison in used to designate Secondary fungal metabolites causing pathological and physiological abnormalities in man and warm blooded animals (Forgacs and Carll, 1962).

Considerable amount of work on moulds and their ill effects on food and feed crops have also been undertaken in India. It gained momentum with the sudden outbreak of aflatoxicosis amongst Bhil tribals of western India in 1974, which stimulated our mycologist to study the mycotoxin problem. (Krishanamachari et al., 1975). While their surveys with groundnut have been reported by Rao et. A. (1965 & 1979) from Andhra and by Sreenivasamurthy et. A. (1965) from Mysore, their cakes have been investigated by Dwarkanath et. al. (1969) and Yadgiri and Tulpule (1974). Fungal succession on groundnut fodder was investigated by Surekha and Reddy (2003). Seed borne mycoflora was detected in maize by Ishrat Naiz and Shahnaz Dawar (2009). Similary survey of mycoflora and aflatoxin production has been studied by Singh and Shukla (2008).

Further, some mycologists have also drown their attention to livestock feeds and their contamination with aflatoxins and other mycotoxins. Bilgrami (1985) Kumar and Singh (1988), Verma et. al. (1989) and Sharma et. al. (1990) have concentrated their attention to livestock feeds, in general. The variety of mycoflora has been responsible for a number of disease malformations and their outbreaks have adversely affected man and his animals from time to time. Periodic disease called Ergotism (Barger, 1931) is caused by Claviceps purpurea infesting barley and rye. It has also been named as 'St Anthony's Fire' or Holl fire which resulted in thousands of deaths in Europe (Forgacs 192).

Animal mycotoxicoses associated with mouldy feed have also occurred sporadically throughout Japan since 1953. Hori and Yamamoto (1953), investigated an out break of poisoning among dairy cows involving over 100 deaths and demonstrated with calves that the causative agent was patulin produced by P. urticae in the feed.

About a dozen mycotoxins are known to be elaborated by different moulds contaminating various foods and feeds. Out of these, aflatoxins, ochratoxins, zearalenone, patulin, citrinin, sterigmatocystin and alternarial toxins happen to be more important because of their adverse effects on man and his animals (Krogh, 1983). Among these, aflatoxins are reported to have received maximum attention because of their hepatocarcino genicity (Lancaster et al., 1961). Zearalenone, which ranks second to this series is commonly called F-2 toxins. These are followed by ochratoxin and patulin as far as their importance is concerned. Species of *Aspergills* and *Penicillium* investigated for ability to produce ochratoxin in poultry feed (Rosa, et.al.,2006).

CONTROL MEASURES

Considering the ill effects of fungi and their consequent toxins on man, his plants and animals, efforts to control or keep them within limits, have been emphasized. Looking to the abundance and omnipresence of the fungal infection, it is practically impossible to eliminate them to keep our foods and feesds absolutely free of fungal contamination. Moreover they being important in

the overall cycle of life in nature, they may have to co-exist and are to be tolerated. The only thing which is needed is to keep them limited and not let their infection cross the permissible limits.

Control methods are, therefore, directed either to reduce the concentration of toxins to safe limits or to produce non-toxic degradation products without reducing the nutritional value of the treated commodities (Doyle et al., 1982). Methods of control have been classified into two main categories.

1. Prevention of mould contamination and growth.
2. Detoxification of toxic products.

Prevention of mould contamination and growth is to be archieved by one or more of the following methods.

(i) Improved farm management.
(ii) Use of antifungal agent.
(iii) Genetic engineering approaches.
(iv) Rapid screening techniques.
(v) Control of environment conditions like moisture, temperature and gaseous atmosphere etc.

Detoxification by physical methods involved extraction, with solvent, heat inactivation, irradiation and adsorption (Doyle et al., 1982 and Masimango et al., 1978).

In chemical detoxification, a wide range of chemicals have been used for the degradation of toxins. These include acids, bases, oxidizing agent aldehydes, several gases and bisulfites (Moss Smith, 1985).

Similarly, degradation of mycotoxins by biological methods involving micro- organisms, including bacteria, actinomycetes, yeasts, moulds and algal have also been reported by Karunaratne et al. (1990) and Mohana and Raveesha (2007).

Reddy and Nusrath (1986). While working on the Sorghum, stage susceptible to fungal infection & muycotoxin production, reported that premature and late mature stages had the maximum contamination as they had longer exposure to wet periods. This may help us to decide about the effective use of fungicides to combat their infections.

Prajapati and Neelakantan (1992), on the basis of their studies on wheat straw treated with urea and whey reported on the lignocellulolytic fungi. According to them, maximum lignocellulolytic activity was observed in case of urea and whey treated straw.

MATERIALS AND METHODS

The materials & methods employed in this investigation remained as under :

It involved crop residues available in abundance in our country and form a major component of live stock feed called fodders/roughages.

1. Bajra (*Penisetum typhoides [Burmf.]*) S & H Karbi (Stumps)

Approximately 50 gm samples of the above fodders were collected from farmers field while still growing and also from stores when harvested and stored. These came from Bharatpur and its sub-urbs. Samples were taken to the laboratory for study and analysis. A total of 90 samples collected had the following distribution.

S.No.	Name of Fodder	Samples from		Total
		Field	Store	
1	Bajra Karbi (BK)	15	30	45

In each case, the samples were collected in sterilized polythene bags alongwith relevant details and brought to the laboratory for detailed processing, examination and analysis. All samples, as a rule, were screened within a week to 10 days but never later than a fortnight of their collection.

ISOLATION OF MYCOFLORA ASSOCIATED WITH LIVESTOCK FODDERS

Isolation of mycoflora associated with fodder samples of Bajra Karbi was done following the method outlined by Graves and Hesseltine (1966). According, I gm fodder from each samples was aseptically weighed and transferred to 250 ml Erlenmeyer flask containing 100 ml sterilized distilled water and wrist-action shaken for 30 minutes. Subsequently, serial dilutions were prepared from the original flask to obtain final dilution of 1/1000. From this final dilution, 1ml was then transferred into the sterilized petridishes, to which 15ml of sterilized either Czapek`s Agar Medium or Potato Dextrose Agar (PDA) medium containing antibiotic was poured and shaken gently to disperse the medium and the spore suspension uniformly. Ten replicates and one control (only Czapek`s medium) was then prepared for each sample. After the medium had solidified, the dishes were kept in sterilized polythene bags and incubated at 28+1^{o}C for 6 to 7 days.

After incubation, the plates were studied and the colonies of different fungi finally studied. For the identification of different fungi, they were subcultured and maintained on Czapek`s/ PDA medium for further details. Whenever possible figures and photographs have also been used to support the text.

IDENTIFICATION, PURIFICATION AND MAINTENANCE OF FUNGAL CULTURES

The different fungal isolate were purified and studied thoroughly for their morphological features using standard techniques and maintained on Czapek`s Agar and Potato Dextorse Agar (PDA)

medium. The identification of fungal isolates was made following the illustrations and descriptions given by Gilman (1957) ; Barnett (1960). Smith (1969) and Subrahmaniam (1971) and also with the help of the isolates maintained in the R.B.S. College laboratory.

Composition of culture media used in the study.

Czapek`s Agar Medium

NaNo3	-	3.00g
K2 HPo4	-	1.00g
KCl	-	0.50g
MgSo4	-	0.50g
FeSo4	-	0.01g
Sucrose	-	30.00g
Agar Agar	-	20.00g

All dissolved in 1 litre distilled water & sterilized.

POTATO DEXTROSE AGAR (PDA) MEDIUM

Potato	-	200g
Dextrose	-	20g
Agar Agar	-	20g

All dissolved in 1 litre distilled water & sterilized.

OBESERVATIONS AND DISCUSSIONS

The observations made during the course of this investigation have been presented in this chapter. In order to present the observations logically tables and photographs has also been appropriately use in this chapter.

The spoilage of feeds and fodders by moulds is a common process. Mould contamination of agricultural commodities is an on going problem in the field, right from seed sowing to harvesting and their handling during storage and distribution (Saucer, 1978). This calls for a proper mycological surveillance of feeds and feed ingredients destined for the use by man and his domestic animals as the mould infestation is generally associated with mycotoxin contamination.

Considerable awareness developed about the presence of mycotoxins in foods and feeds and their relationship with man and livestock consuming them. A few surveys made on mycobial

infestation of oil seeds, oil cakes, cereal grains, pulses and mixed feeds and their contamination with mycotoxins, have been reviewed by Hesseltine (1974) ; Bhat et. al. (1978) ; Rao et.al. (1979) ; Bilgrami et. al. (1981) and Mirocha et. at. (1981) emphasing their importance in relation to health hazards.

The present investigation is, therefore, an attempt to identify the association of various fungi with Jowar Karbies (Serghum shumps). This study was under taken to assess their mycobial contamination during growth while green in the fields and dry when stored in the heaps godowns.

It is seen from Table1 that green (field) and dry samples of Bajra stumps (Karbi) revealed a total of 45 fungal species associated with them. It includes 12 species of *Aspergillus* 5 species of *Fusarium.* 4 species each of *Alternaria* and *Penicillium,* 2 species each of *Acremonium Chaetomium, Cladosporium, Curvularia, Mucor* and one species each of *Bipolaris, Cephalosporium, Cladotrichum, Helminthosporium, Humicola, Monilia, Paecilomyces, Phoma, Trichothecium* and *Verticillium.*

15 green samples of Bajra Stumps infected with total of 30 fungal species which includes 10 species of *Aspergillus,* 4 species of *Alternaria,* 2 species of each *Acremonium, Curvularia, Fusarium, Penicillium,* and one species each of *Bipolaris, Chaetomium, Cladosporium, Helminthosporium, Mucor, Paecilomyces. Trichothecium,* and *Verticillium.*

30 Dry samples of Bajra stumps infested with 42 fungal species. like Jowar, Bajra samples are also mostly contaminated with *Aspergillus.* It includes 10 species of *Aspergillus,* 5 species each of *Acremonium, Chaetomium, Cladosprorium, Curvularia, Mucor* and one species each of *Bipolaris, Cephalosporium, Cladotrichum, Helmintho sporium, Humicolta, Paecilomyces, Phoma, Trichothecium,* and *Verticillium.*

After having considered the overall picture of fungi infesting the common live-stock fodders and their distribution pattern in general, it would be desirable to highlight the status of the toxin producing ones. It is further observed from Table-l that mycotoxini producing Aspergilli, were the most numerously (in 13 species) present and abundantly found associated with both the fodder studied irrespective of their stage and weather conditions.

Interestingly, fungal species found infesting fodders in this study, fungi like Aspergillus clavatus, A flavus, A. ochraceous, A. parasiticus, A.sulphureus, A. terreus, Fusarium equisetii, F. moniliforme, F. nivale, F. oxysporum, F. semittictum, F. solani, Penicillium cyclopium and P. expansum have been found associated with mycotoxiin producing.

The experimental findings of present investigation indicates that mycotoxin producing Aspergilli were the most numerously present and abundantly found associated with Bajra Karbies studied irrespective of their stage and weather conditions.

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TABLE 1: Mycoflora Associated with green and dry samples of Bajra Stumps (Karbi)

S. No.	Mycoflora	Green B.K. (15)	Stored B.K. (30)
1	2	3	4
1	*Acremonium glaucum*	+(8)	+(8)
2	*A.roseum*	+(4)	+(8)
3	*Alternaria alternata*	+(6)	+(18)
4	*A. humicola*	+(4)	+(9)
5	*A. tenuissima*	+(5)	+(20)
6	*Aspergillus acuteatus*	+(2)	+(7)
7	*A. flavus*	+(11)	+(21)
8	*A. fumigatus*	+(3)	+(17)
9	*A. japonicas*	-	+(18)
10	*A. nidulans*	+(3)	+(20)
11	*A niger*	+(6)	+(19)
12	*A. ochraceous*	+(3)	+(9)
13	*A. parasiticus*	-	+(8)
14	*A. sulphureus*	+(4)	-
15	*A. Sydowii*	+(11)	+(20)
16	*A. tamariti*	+(4)	-
17	*A. terreus*	+(3)	+(19)
18	*A. varsicolor*	+(7)	+(12)
19	*Bipolaris Sp.*	-	+(4)
20	*Cephalosporium acremonium*	-	+(7)
21	*Chaetomium globosum*	-	+(10)
22	*Chaetomium sp.*	+(4)	+(4)
23	*Cladosporium cladosporioides*	-	+(7)
24	*C. herbarum*	+(8)	+(20)
25	*Cladotrichum sp.*	-	+(11)
26	*Curvularia geniculata*	+(5)	+(13)
27	*C. lunata*	+(12)	+(21)
28	*Fusarium equisetu*	-	+(5)
29	*F. moniliforme*	-	+(4)
30	*F. oxysporum*	+(4)	+(18)
31	*F. semitectum*	+(6)	+(20)
32	*F. Solani*	-	+(8)
33	*Helminthosperium sp.*	+(2)	+(14)
34	*Humicola sp.*	-	+(4)
35	*Monilia sp.*	+(7)	-
36	*Mucor circinelloides*	-	+(4)

37	*Mucor hiemalis*	+(2)	+(11)
38	*Paecilomyces fusisporus*	+(8)	+(4)
39	*Penicillium chrysogenum*	-	+(13)
40	*P. citrinum*	+(5)	+(10)
41	*P. expansum*	-	+(12)
42	*P. raistrickii*	+(7)	+(11)
43	*Phoma sp.*	-	+(8)
44	*Trihothecium roseum*	+(4)	+(4)
45	*Verticillium terrestre*	+(3)	+(20)
	Number of fungal species occurred	30	42

(Figures in parentheses denote number of samples)

CONCLUSION

The fact that moulds produce biologically active substances called mycotoxins, is known since times immemorial, but their association with health hazards of man and animals, is relatively of recent origin. About a dozen mycotoxins are known to be elaborated by different moulds, contaminating various foods and feeds. Out of these aflatoxins, Ochratoxins, Zearalenone, Patulin, Citrinin, Sterigmatocystin and the Alternarial toxins happen to be more important because of their adverse effects on man and animals (Krogh, 1983).

A birds eye view of what has been introduced above, clearly indicates that apparently the insignificant looking moulds play an exceedingly important role in respect of us, our crops and animals in nature. While fungi that grow on foods, shoes, books, clothes and even bodies of man and his animals, are no doubt that they provide antibiotics are of value as life saving medicines. As foas are considered more important than friends from the safety and security point of view, the ones that produce mycotoxins have been more thoroughly investigated throughout the word.

India, with its rich livestock population but por feed resource, can not afford to neglect any of its feeds and/or fodders. Since our huge livestock population primarily subsists on crop residues and agricultural wastes, concerted efforts are warrented to protect any wastage of their quantity and spoilage of their quality.

It was, therefore, decided to study mould infestation of fodder providing important crop residue of Bajra (Pennisetum Sp.) which make bulk of the livestock fodders in Bharatpur and its suburbs.

REFERENCES

1. Barger, G. (1931). Ergot and Ergotism, Gurney and Jackson, London,
2. Barnett, U.L. (1960). Illustrated genera of imperfect fungi. IInd ed. Minneapolis Burgess Publ.Co.
3. Bhat, R.V.V ; Nagaraunjan and Tulpule, P.G. (1978). Health hazards of Mycotoxin in India. Indian council of Medical Research, New Delhi. P. 58.

4. Bilgrami, K.S.; Prasad, T.; Misra, R.S. and Sinha, K.K. (1981). Changes in protein and amino acids contents of maize seeds infested with aflatoxin producing strain of *Aspergillus parasiticus*. Seed Research, 9 : 52-56.
5. Bilgrami (1985). Mycotoxins in dry-fruits and spices. Narendra Publishing House, Delhi.
6. Christensen, E.M. (1957) Deterioration of stored grains by fungi.
7. Doyle, M.P. ; Applebaum, R.S. ; Brakett, R.E. and Marth, E.H. (1982). Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. J.Food Prot, 45 : 964.
8. Dwarkanath, C.T. ; Sreenivasamurthy. V. and Parpia, H.A.B.(1969). Aflatoxin in Indian peanut oil, J.Food Sci. Technol., 6: 107-109.
9. Forgacs, J. (1962) Mycotoxicoses : The neglected diseases. Food stuffs, 34 (18) : 124-134.
10. Forgacs, J. and Carll., W.T. (1962) Mycotoxicoses Adv. Vet. Sc., 7 : 273-382.
11. Gilman, J.C. (1957). A manual of soil fungi. The lowa State Univ. Press. Ames, lowa, P.450.
12. Graves. R.R. and Hesseltine. C.W. (1966). Fungi in flour and refrigerated dough products. Mycopathol, et Mycol. Appl., 29:277-290.
13. Hesseltine, C.W. (1974). Natural occurrence of mycotoxins in cereals. Mycopathol. Mycol. Appl., 53: 141- 153.
14. Hori, M. and Yamamoto, Y. (1953) J.Pharm. Soc. Japan (Japanees), 73: 1097. C.f. Uraguchi, K. and Yamazaki, M.(1978). Toxicology: biochemistry and pathology of mycotoxins. Ed. John Wiley & Sons. New York, p.288.
15. Ishrat Niaz and Shahnaz Dawar (2009). Detection of seed Borne mycoflora in maize (Zea maysl). Pak.J.Bot. 41 (l) : pp. 443-451.
16. Karunaratne, A. ; Wezenberg, E. and Bullerman, L.B. (1990). Inhibition of mould growth and aflatoxin production by Lactobacillus sp.,J.Food Prot., 53 : 230.
17. Krishnamachari, K.A.V.R. ; Bhat, R.V. ; Nagarajan, V. and Tilak T.B.G. (1975). Investigation into an outbreak of hepatitis in parts of western India. Indian J. Med. Res., 63 : 1036-1048.
18. Krogh, P. (1983). In, Postharvest physiology and crop preservation (ed. Mortis Lieberman). Plenium Press, London, pp. 315-329.
19. Kumar, A. and Singh, S. (1988). Effect of penicillin on aflatoxini elaboration by *Aspergillus flavus* in liquid medium. Nat. Acad. Sci. Letters, 11 (10) : 309-310.
20. Lancaster, M.C., Jenkins, F.P., Philip, J,Mc. L.; Sargeant, K., Sheridan, A.; O' Kelly.J. and Carnaghan, R.B.A. (1961). Toxicity associated with some samples of peanuts, Nature, 192:1095-1097.
21. Masimango, N. ; Remacle, J. and Ramaut, J.L. (1978). The role of adsorption in the elimination of aflatoxin B, from contaminated media. Eur. J. Appl. Microbial. Biotech., 6:101.
22. Mirocha, C.J.; Pathre, S.V. and Robinson, T.S. (1981). Comparative metabolism of zearalenone and transmission into bovine milk. Food Cosmet. Toxicol., 19: 25-30.
23. Moss, M.O. and Smith, J.E. (1985). Mycotoxins: Formation. Analysis and significance, John Wiley and Sons, Chichester, P.7.

24. Prajapati, J.B. and Neelakanta (1992). Solid state fermentation of Urea and whey treated wheat straw with Lignocellulolytic fungi. India J. Microbiol., 32 (1): 85-90.
25. Rao, K.S., Madhavan, T.V. and Tulpule, P.G. (1965). Incidence of toxigenic strains of A.*flavus* affecting groundnut crop in certain coastal district of India. Indian J. Med. Res., 53 : 1196-1202.
26. Rao, P.V.; Rao, C.R. and Murty, K.N. (1970). Outbreak of fungal diseases in areas of Andhra Pradesh. Sorghum Newsletter, 22 :114.
27. Reddy, B.N. and Nusrath, M. (1986). Stage of Sorghum in the field susceptible to fungal invasion and mycotoxin formation. Indian Phytopath., 39 : 106-109.
28. Riazonov (1947). Lecture on the Republic conference on Alimentary toxic aleukia, Moscow.
29. Rosa C.A.R., Ribeiro, J.M.M., Fraga, M.J., Gatti, M., Cavaglierri, L.R. Magnoli, C.E., Dalcero, A.M. and Lopes, C.W.G. (2006).
Mycoflora of Poultry feeds and Ochratoxin producing ability of isolated *Aspergillus* and *Penicillium* species, In press, Veterinary Microbiology.
30. Sauer, D.B. (1978). Contamination by mycotoxins : When it occurs and how to prevent it. In, Mycotoxic fungi, mycotoxins, Mycotoxicoses, Vol. 3, Wyllie, T.D. and More house, L.G. (eds.), pp. 147-158.
31. Sharma D.K., Gupta, M.N. and Garg, P.K. (1990), Occurrence of mycotoxins in some common foodstuffs of Agra region (Abstract).
In, proc. 2nd Asia Pacific Conference of Animal, Plant and Microbial Toxins, P. 107.
32. Smith, G. (1969). An introduction to industrial mycology. Edward Arnold Ltd., London, P. 390.
33. Singh, P.K. and Shukla, A.N. (2008). Survey of mycoflora counts, aflatoxin production and induced biochemical changes in walnut kenels. J. of stored product research, Volume 44, Issue 2, pages 169-172.
34. Sreenivasamurthy, V. ; Jayaraman, A. and Parpis, H.A.B. (1965). Aflatoxin in Indian peanuts, analysis and extraction. In, 'Mycotoxins in feed stuffs', Wogan, G.N. (ed.) MIT Press, Cambridge, pp. 251-260
35. Subrahmaniam, C.V. (1971). 'Hyphomycetes', ICAR. New Delhi, p.930.
36. Surekha, M. and Reddy. S.M. (2003) Succession of Mycoflora on groundnut fodder, Crop Protection, 22: p. 837-851.
37. Verma, R.J., Raval, P.J. and Dube, H.C. (1989). Incidence of aflatoxin producing fungi in oilseeds, oil cakes and animal feeds in Bhavnagar. In, International Symp. On Agricultural and biological aspects of aflatoxin related health hazards, Delhi, India. P-21.
38. Yadgiri, B. and Tulpule, P.G. (1974). Aflatoxin in buffalo milk. Indian J. Dairy Sci., 27: 293-297.

