



SEASONAL EVALUATION OF MYCODIVERSITY ASSOCIATED WITH MILKING ANIMALS FROM INDOOR CATTLE SHED

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Abstract: There is limited information on fungal diversity compared with bacterial biodiversity associated with milking animals from indoor dairy shed. This work aimed to identify and evaluate the diversity of mycoflora associated with milking animals that may act as one of the sources of contamination of dairy products. The study of mycodiversity associated with milking animals was performed from 5 different indoor cattle shed in Thane district of Maharashtra, India. The mycoflora was investigated by skin swabbing method using Sabouraud's glucose agar medium supplemented with 500mg/l chloramphenicol and ampicillin. A total of 100 isolates were obtained from the skin swabs of cattle. Mycodiversity was contributed by eleven different types of fungi *Aspergillus* spp. (26.5%) was the most predominant fungi followed by *Penicillium* spp. (21.7%), *Alternaria* spp. (14.5%) and *Cladosporium* spp. (10.8%) on skin of the cattle. Seasonal variation was noted in the prevalence of fungi associated with the skin of milking animals *Aspergillus* spp. was more prevalent during monsoon followed by summer and winter season. Some recorded fungi were earlier reported as allergenic, toxic, and pathogenic for occupational workers as well as cattle population and may adversely affect human health through allergy, infection, and toxicity and indicates the need for improved hygienic standards.

Index Terms - Mycoflora, cattle shed, *Aspergillus* spp, *Penicillium* spp.

I. INTRODUCTION

Today, India is one of the prominent milk producing country in the world. The assessment of microbial load at various stages of manufacture or processing is useful tool for quality assessment and improvement which will result in longer shelf life which is a desirable market requirement. The presence of microbial community at high concentration in indoor environment confers more serious risks in indoor environments than those related to outdoor exposures. In recent years, many farmers are following to keep their cows indoors throughout the year. Indoor housing of milking animals provides benefits as protection from predators, parasites, and exposure to extreme weather conditions. Assessment of microbial contaminants from indoor environment is always a challenging and worthwhile subject of great concern (Matkovic *et al.* 2006). Detection and quantification of culture based microbial analysis is useful in order to assess the exposure of variety of microbial contaminants in the occupational environment. Intensity of microbial contaminants and exposure assessment have been reported from various environment (Adhikari *et al.* 2004).

In India much attention has not been paid towards the hazards posed from exposure to filamentous fungi in indoor cattle shed environment and also the flora from the skin surface of cattle in cattle sheds. Detailed information of fungal types in the cattle shed environment would be helpful in effective diagnosis and treatment of allergic ailments, thus reducing infections in indoor environment of cattle sheds. The fungal spores being light in weight can easily disseminate in environment leading to contamination of cattle sheds. Many infectious fungal strains have been reported from indoor cattle shed environment (Adhikari *et al.*, 2004; Pavan, 2015; Prashant, S.P., & Ganesh, K.R. 2019). There is inadequate information available about mycoflora association with the skin surface of milking animals and impact of seasonal changes on mycodiversity. Hence, the present study would help to identify the diversity of mycoflora associated with indoor dairy cattle which will lead to early diagnosis and treatment of ailments caused by fungi.

II. MATERIALS AND METHODS

Site Selection

Five different local indoor cattle sheds were selected for the study from Thane district, Maharashtra, India. The cattle sheds with the capacity of 50 milking animals from Ulhasnagar, Ambernath, Badlapur, Kalyan and Dombivli locality were consider for the study.

Characterization of indoor cattle shed environment

Each selected indoor cattle shed was studied for their details w.r.t infrastructure and dairy workers. During the study period microclimate parameters, temperature (°C), and relative humidity (Rh%) of indoor air were measured in order to assess the influence of meteorological variations on the concentration of different types of fungi.

Sample Collection from skin of milking animals

Samples were collected during a period of 11 months from May 2019-March 2020. Sampling was performed two times a month at an interval of 15 days. The samples were collected by skin swabbing method using sterile cotton swabs moistened with physiological saline. The collected samples were immediately carried to the laboratory for further analysis.

Seasonal assessment of mycoflora associated with the skin of milking animals

Sterile Sabouraud's glucose agar medium supplemented with 500mg/l chloramphenicol and ampicillin was used for isolation of fungi. The skin swabbing samples were inoculated on media and the plates were incubated at room temperature for 48 hours. The fungal isolated were studied microscopically. The fungi were stained in lactophenol cotton blue and identified on the basis of morphological features. (Williams, 2001; Stevens, 1974; Goyer, 2001).

III. RESULTS AND DISCUSSION

Quality management on dairy farms becomes more and more important regarding the different areas of animal health, animal welfare and food safety. Monitoring animals, farm conditions and farm records can be useful in risk identification and management. The hazard analysis critical control point system is extended as an on farm strategy to control the product as well as the production process on the areas of animal health, animal welfare and food safety.

In the present study, the assessment period was divided into three seasonal cycles, monsoon (June to September), winter (October to January) and summer (February to May). All the selected indoor dairy cattle shed were studied for sanitary conditions. Out 5, one cattle shed located Ulhasnagar had a poor drainage system and wet floor. Fodder was stored in adjoining area next to the cattle shed. Ventilation was found to be adequate. Table I indicates the average temperature and relative humidity (%) during monsoon, winter, and summer season.

Table I. Seasonal average values of temperature and relative humidity of cattle sheds.

Parameter	Monsoon	Winter	Summer
Temperature (°C)	26 ± 1.8	22 ± 1.5	32 ± 1.3
Relative humidity (%)	84.4 ± 4.5	57.6 ± 1.8	52.1 ± 2.9

The skin swabbing was done just before the milking time. The collected samples were immediately plated on Sterile Sabouraud's glucose agar medium supplemented with 500mg/l chloramphenicol and ampicillin. A total of 100 different isolates were obtained from the skin swabs of cattles. Mycodiversity was contributed by eleven different types of fungi isolated from the skin surface of the milking animals viz., *Alternaria spp.*, *Aspergillus spp.*, *Chaetomium spp.*, *Cladosporium spp.*, *Curvularia spp.*, *Epicoccum spp.*, *Mucor spp.*, *Penicillium spp.*, *Rhizopus spp.*, *Trichoderma spp.*, *Phoma spp.* The analysis of mycofloral abundance revealed that *Aspergillus spp.* (26.5%) was the most predominant fungi on the skin surface of cattles followed by *Penicillium spp.* (21.7%), *Alternaria spp.* (14.5%) and *Cladosporium spp.* (10.8%) (Fig 1.). Similar findings have been reported from the indoor cattle shed environment (Sharma, 2010; Bernardo. *et al.*, 2005; Wang, 2007).

The seasonal prevalence of fungi isolated from the skin swabs of dairy cattles is depicted in Fig 1. Significant variation in the prevalence of fungi associated with skin of milking animals were observed during all the three seasons. Highest percentage of fungal isolates were obtained during the monsoon season probably due to high humidity and moderate temperature. Similar results have been reported by Simas *et al.*, (2007), Adhikari *et al.*, (2004) and Chakrabarti *et al.*, (2012).

Fig 3. and Fig 4. indicates seasonal distribution of dominant fungal species associated with milking animals. *Aspergillus spp.* and *Penicillium spp.* were found to be the dominant fungal organisms. *Aspergillus spp.* was more prevalent during monsoon followed by summer and winter season. Comparable results were observed by Priyamvada *et al.*, (2017). High concentration of *Penicillium spp.* was reported in monsoon, followed by winter and summer season. Karmarkar *et al.*, (2020) and Verma (2011) has reported that allergy reactions were elicited by *Aspergillus spp.*, *Alternaria spp.*, *Cladosporium spp.* and *Penicillium spp.* Presence of these pathogenic fungi on skin of milking animals indicates the potential health hazards to the cattles as well as the animal handlers

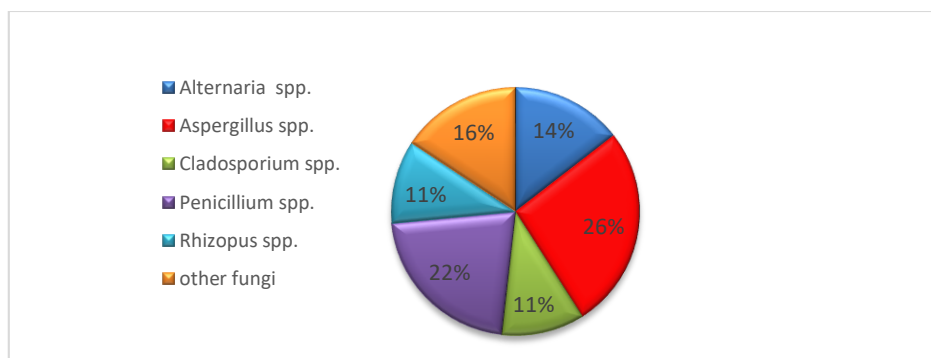
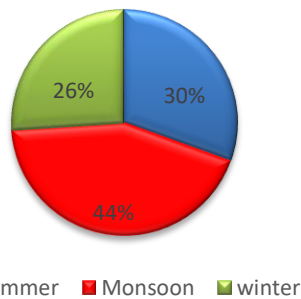
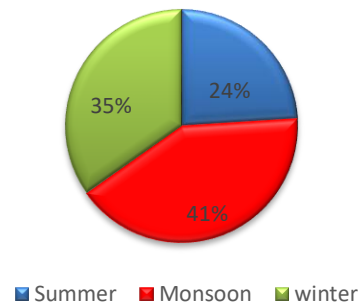


Fig. 1. Predominant fungal flora associated with the skin surface of milking cattles.

Fig. 2. Seasonal variation in the *Aspergillus* spp.Fig. 3. Seasonal variation in the *Penicillium* spp.

IV. CONCLUSION

The present study revealed a significant seasonal variation in the diversity of cattle skin mycoflora. Temperature and humidity is correlated with the presence of large number of fungal load during monsoon. Presence of considerable load of allergenic fungi on cattle integument may lead to increased risk of allergies in animals and animal handlers. Our study confirms that despite the seasonal variation potential allergenic fungi are present throughout the year, this may eventually contribute to higher occurrence of occupational ailments and environmental pollution with health risk.

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