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SCREENING AND ISOLATION OF MOLDS FROM *MURGHAS* (SILAGE) BAGS

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Abstract : Cattle farming is a supplementary business for farmers to improve or boost their income along with agriculture. Good health and an appropriate diet for cattle have a direct impact on the economic product obtained from cattle. Availability of forage for the whole year is the biggest challenge in front of farmers and to overcome it making of Murghas (silage) a great option. Ensilage is the conserved and fermented forage. Some microbial contaminants are responsible for the spoilage of ensilage. In this research article, a study has been carried out on the isolation of the fungal contaminants responsible for spoiling the surface of silage. Along with the isolation of fungal contaminants, this research also provides the solution or remedy to overcome or reduce the problem of ensilage spoilage by molds. High salt concentration shows a reduction in the growth of molds.

***IndexTerms* - Silage, contaminants, Molds, Fungi, NaCl, Salinity**

I. INTRODUCTION

Domestic animals such as cattle, sheep, horses, and buffalos have great commercial importance [1]. Cattle farming and milk production from cattle is the supplementary business for farmers to improve or boost their income [2]. The animal feed and appropriate diet of cattle have a direct impact on the health of cattle [3]. The production of milk, flesh, or other products from cattle is also affected by feedings [4]. Forage or fodder can be given to the cattle in fresh form or it can be stored for the future [5]. The traditional method used to store forage was the making of Hay [6]. For lats 50 years making of Murghas (silage) hold popularity and great demand in forage preservation in the regions of intensive cattle farming for milk and flesh [6]. Forage with high moisture content can be preserved by the fermentation process is termed silage [7]. Silage making process comprises more than 50% of the total amount of preserved forage [8]. The main purpose behind silage making is to conserve forage to be used during the seasons of fresh forage unavailability [9]. The crops like maize, sorghum, and wheat can be used for making silage due to their high nutritional value [10]. Instead of the grains, the whole crop is used in silage [11]. The process of making silage involves harvesting the crop, chopping the crop, and storage of chopped crop or forage in a silo or airtight plastic bags by following the addition of required preservatives [12]. In the silage bag, forage has to be filled compactly to reduce the trapped air amount in ensiled forage mass [13]. The rapid achievement of acidic or low pH is the main principle behind the ensilage [14]. The low pH is obtained by lactic acid fermentation and anaerobic condition [15]. The acidification or fermentation process continues for several days from the day of packaging [16]. It also takes several months to complete the acidification of silage. During the fermentation process number of different microorganisms able to grow in an anaerobic condition such as lactic acid bacteria, yeast, clostridia, and enterobacteria compete with each other for available nutrients [6]. The silage microflora is of two types, desirable and undesirable microflora. Lactic acid bacteria fall under the category of desirable microflora and clostridia, enterobacteria are falls under the category of an undesirable one. yeasts, listeria, and molds also fall under undesirable aerobic spoiler of silage [17]. Usually, lactic acid bacteria dominate the process of fermentation and it results in decreased pH [18]. Due to the acidic pH, normal microorganisms which can act as a contaminant or forage spoilers cannot be able to grow in silage [19]. Hence silage can be stored for a longer period until the nutritional value of ensilage reduces due to the breakdown of proteins and amino acids [20]. The presence of enterobacteria, clostridia, yeasts, and acetic acid bacteria in silage can cause the synthesis of some toxic components of silage like biogenic amines production by enterobacteria [21]. The abundant presence of clostridia in silage causes an increase in pH which is susceptible to the growth of other contaminating microorganisms. The presence of yeast causes loss of dry matter and it also reduces the sugar contained for acid production due to excess production of ethanol [22]. Acetic acid bacteria cause spoilage of entire maize crop silage. Molds are usually aerobic microorganisms. Molds show growth on the upper surface of silage [23]. It is also an indication of proper compaction or sealing of silage bags. The successful conservation of silage is primarily depending on the appropriate silage-making technique [24]. The silage quality is also dependent on the composition of the crop including soluble sugar, nitrate, dry matter, lactic acid bacteria, and preservatives added to silage [25].

II. Material and Methods

1. Sampling

Samples from the upper surface of Murghas (silage) bags contaminated with fungus were collected from the village Jawalke of Kopargaon, Maharashtra, India. The sample was collected in clean polythene bags and used for further studies.

Figure 1. Silage bag infected with fungus

2. Physico-chemical test of Murghas (silage) sample

The Murghas (silage) bags were tested for Physico-chemical parameters including pH and Temperature during the sampling [26].

3. Screening of sample

Initially, the Murghas (silage) sample contaminated with the fungus was inoculated on different nutrient media-containing plates [27]. The media used for the screening were Potato Dextrose agar, Congo Red Yeast Extract Mannitol Agar (CRYEMA), and Malt Extract agar [28]. The plates inoculated with the sample were incubated at room temperature for 2 to 3 days and the growth of fungus on the plates was observed.

4. Isolation and Morphological characterization

The pure culture of screened fungus was made using the same growth media. The morphological characterization was done by observing the fungal colonies on respective culture media plates. After morphological analysis, the fungal staining of the isolated fungus was performed using the dye Lactophenol cotton blue, and the results of the microscopic examination were recorded [29].

5. NaCl treatment to reduce the growth of fungal isolate

High salinity can inhibit the growth of microorganisms. To overcome the problem of fungal contamination in silage bags the isolated fungus was grown on high salt-containing media. The growth media was prepared by varying the salt concentration from 5% to 10% and the ability of high salt concentration to inhibit the growth of the isolated fungus was tested. The plates were incubated for 2 to 3 days at room temperature [30] [31].

III. Result and Discussion

1. Physico-chemical characters of Murghas (silage)

After analyzing the silage sample for their Physico-chemical properties, the pH of silage was found to be acidic due to the presence of essential microflora and the anaerobic fermentation process. The presence of lactic acid bacteria in the silage is the main reason for the acidic pH of Murghas (silage). The temperature of the silage bags was found to be increased due to the microbial metabolism and fermentation process in silage bags. The temperature of ensilage was found more than 55℃.

2. Screening of sample

After 2 to 3 days of incubation, the plates of Malt Extract Agar inoculated with silage sample were observed for the morphological characterization. Among the different types of fungi, Molds were observed on culture media plates with white, greenish, and some black colored filamentous colonies.

Figure 2. Screening of Silage samples for the presence of fungal spoilers.

3. **Isolation and Morphological characterization**

after screening and making pure culture of each fungal colony on different growth media, the growth was observed after 2 to 3 days of incubation. Which were further characterized by staining with lactophenol cotton blue for the microscopic examination. Under the microscope, the mycelial structure of all fungal isolates was observed. Some Fungal isolates were showing Septate hyphal structure while some were sowing aseptate hyphal structure under the microscope. The one is showing a long conidiophore structure with globule vesicles under the microscope. The isolated molds which spoil the silage are found to be *Fusarium, Aspergillus, Mucor, and penicillium* based on microscopic and morphological examination.

Figure 3. The isolated fungal colonies on different media-containing plates.

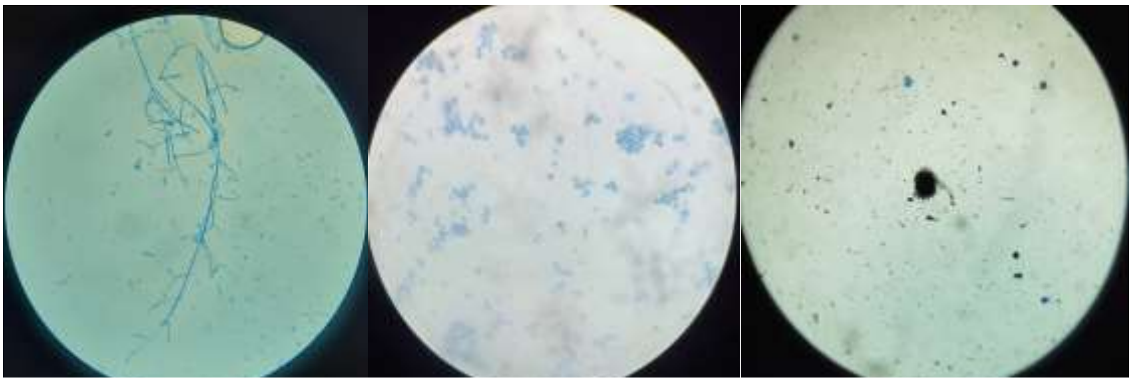

Figure 4. Microscopic examination of fungal isolate using lactophenol cotton blue dye

TABLE 1. Cultural and Morphological Characterization of Fungal Isolates.

Fungal Isolates	Cultural Characters	Morphological Characters
Mucor	Large white-coloured colonies which then turns into black.	Erect Sporangiospores swells at the tip for sporangia formation.
Penicillium spp.	Fast-growing Green coloured colonies.	Branched conidiophores, septate hyaline hyphae
Aspergillus spp.	Olive to lime green colonies.	Septate and hyaline hyphae. Uncoloured and roughened conidiophores.
Fusarium spp.	White creamy to white greyish coloured colonies.	Septate hyphae with erect conidiophore and conidia.

4. **Effect of NaCl treatment on the growth of fungal isolates**
Results were observed after inoculating and incubating the fungal isolates on the culture media containing high salt concentration. By observing culture media plates after 2 to 3 days of incubation, it was found that the growth of the fungi was inhibited. The media containing 5% NaCl shows less inhibition of fungi as compared to the media containing 10% NaCl concentration. It means that the increase in salt concentration or salinity of growth media can be able to inhibit, more growth of fungi. This result can be the remedy to overcome the problem of ensilage spoilage due to fungi. The addition of salt on the upper surface of the silage bag during the forage filling and packaging process can prove effective against spoilage and wastage of ensilage.

IV. Conclusion

Cattle farming is the growing supplementary business for the farmers along with agriculture. To improve the yield of economical products, obtain from cattle, it is important to maintain the good health and diet of the cattle. Availability of forage for the whole year is the biggest challenge in front of cattle farmers. To make forage available for whole years, in many countries silage making practices use to be followed. Ensilage is found to be the best fermented and storage fodder for the cattle. Some microbial contaminants are responsible for the spoilage of the ensilage, which includes yeasts, molds, acetic acid bacteria, enterobacteria, some species of bacilli, etc. In this research, article studies have been carried out on the molds which usually spoil the surface of silage. The isolated molds which spoil the silage are found to be *Fusarium, Aspergillus, Mucor, and penicillium*. To remove or reduce the growth of those fungal contaminants the Sodium Chloride treatment was given to the isolates and it was found that the growth of those isolates can be inhibited by the increased concentration of salt in silage. On the surface of silage, the addition of salt can be a good remedy against those fungal contaminants.

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