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MYCOFLORA ASSOCIATED WITH RUBBER SHEETS AND ITS MANAGEMENT BY COMMON SALT (SODIUM CHLORIDE)

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Abstract

Natural rubber is obtained almost from *Hevea brasiliensis* L. (family Euphorbiaceae). Rubber sheets are infected by fungi within 24 hours of processing and it is a major problem of rubber industry.The present research was undertaken to find out the mycoflora on rubber sheets and to devise a convenient method to protect the rubber sheets from fungal attack. The fungi were isolated from rubber sheets on PDA (Potato Dextrose Agar) medium. The isolated fungi were *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *Cladosporium cladosporioidis* (Fresen) De Vries,, *Corynespora cassiicola* (Berk. & Curt.) Wei., *Colletotrichum* sp., *Fusarium* sp, *Mucor* sp., *Penicillium* sp. and *Trichoderma viride* Pers. Efficacy of common salt (NaCl) was evaluated against the isolated fungal species associated with unprocessed and processed rubber sheets *in vitro*. Radial colony growth of fungi associated with inocula prepared from unprocessed and processed rubber sheets was completely inhibited by salt at the concentrations used (10, 15 and 20 %) up to six months of observation.

Key words: Mycoflora, Rubber sheets, Management, Common salt, Sodium chloride

Introduction

Natural rubber is obtained from the para rubber tree often simply called rubber tree. It is of major economic importance because its sap like extract known as latex is the primary source of natural rubber. It is a tall wood tree indigenous to Brazil. Some common fungal diseases of rubber plants are South American Leaf Blight caused by *Microcyclus ulei,* Powdery mildew caused by *Oidium heveae*, Black stripe caused by *Phytophthora palmivora*, Anthracnose leaf spot caused by *Colletotrichum gloeosporioides* and white root rot caused by *Armilleria mellea* (Wastie 1975 and Lieberei 2007). The world rubber Industry began to develop in the 1800s, with the invention of the masticator and the volcanization process. Demand for rubber grew rapidly with the invention of the solid and later the prematric rubber tire and the demand for rubber insulation by the electric industry. About 48% of the global demand for natural rubber comes from China, India and Malaysia which are three major natural rubber consuming countries within ANRPC (Association of Natural Rubber Producing Countries). Natural rubber and the different types of synthetic rubbers are used usually in many different end products. The most important is the tire sector taking about half of the total elastomex consumption. The other category, general rubber goods, includes shoes, belting, foot ware, surgical goods and

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rubberized cloths. The top five rubber producing countries are Thailand which produces 3,166 m/t, Indonesia 2, 921, 872 m/t, Malaysia 1,072,400 m/t, India 819,000 m/t and Vietnam 659,600 m/t rubber per year (Wikipedia 2013). First attempt to grow rubber in Bangladesh was in 1952 when Forest Department planted some seeds and buds stamp in Chittagong. These seeds were imported from Malaysia and Srilanka (Rahman 1988). In 1959 FAO experts initiated the possibilities of producing rubber plants in large scale and recommended to establish rubber industries in Bangladesh. In 1962 FAO handed over the project to BFIDC. The project for growing rubber as an alternative crop was first introduced in Lungla and Allynuggar Tea estate in 1983. Much research works had been carried out on management of rubber in different parts of the world (Esuruoso 1970, Dayartnes and Munasinghe 1971, Tan *et. al*. 1980 and Chanduayki 2007). In Bangladesh very little work has been done to protect rubber from fungal attack ((Rahman 1988). Present research was under taken to find out the association of fungi with unprocessed and processed rubber sheets and their control.

Materials and Methods

Infected rubber sheets were supplied from Rawjan rubber plantation area of Bangladesh from August to December 2012. Unprocessed and processed rubber sheets were stored at 25-30 ^{0} C (room temperature) and 10 ^{0} C. (refrigerator). The fungi were isolated from rubber sheets on PDA (Potato Dextrose Agar) medium during the period of August 2012 to May 2013. The specimens were cut into small pieces (2mm x 2mm) and surface sterilized by dipping in 10% chlorox for 3-5 minutes followed by rinsing in sterilized water. Surface sterilized rubber pieces were placed on PDA medium (CAB 1968). From each sample 30 inocula were taken and 3 pieces were inoculated on solidified PDA in each Petri dish. Three inocula were placed in each plate and incubated for 5-7 days at 25 ± 1º C. Fungi growing out of the inocula were transferred to separate plates and slants for further studies and storage.

For microscopic observations fungal structures like mycelia, spore bearing structures and spores were scrapped off from the surface of rubber sheets with a scalpel and was mounted in lacto phenol over a clean slide. In case of hyaline structures, a little amount of aniline blue (cotton blue) was added to the mounted fluid.

The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Barnett and Hunter 1972, Ellis 1971, 1976, Ellis and Ellis 1997 and Sutton 1980). Prevalence (%) of fungi in different specimens was also recorded.

The experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka. All the specimens were preserved in the Herbarium, Mycology and Plant Pathology section, Department of Botany, University of Dhaka, Bangladesh.

Efficacy of common salt (NaCl) was evaluated against fungal species associated with unprocessed and processed rubber sheets *in vitro*. Rubber inocula were placed for 24 hours in test tubes with 5, 10, 15 and 20% NaCl solution then plugged with sterilized cotton. PDA medium with 5, 10, 15 and 20% NaCl was prepared and poured into sterilized Petri plates and was allowed to solidify. Each Petri plates was inoculated centrally with a 2×2 mm rubber inocula. In control set, Petri plates containing PDA medium with the requisite amount of distilled water instead of salt was also inoculated with rubber inocula in the same way as described above. Three replications were maintained for both treatment and control sets. The inoculated Petri plates were incubated at 25 ± 1ºC. The radial growth of the colonies was measured after 5 days of incubation.

The percentage growth inhibition of each test pathogen was calculated by using the following formula:

$$I = \frac{C - T}{C} \times 100$$

Where, I = percent growth inhibition, C = growth in control and T = growth in treatment

Results and Discussion

A total of 10 species of fungi belonging to 8 genera of Deuteromycetes was isolated from Rubber sheets. The isolated fungi were *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *Cladosporium cladosporioidis* (Fresen) De Vries,, *Corynespora cassiicola* (Berk. & Curt.) Wei., *Colletotrichum* sp., *Fusarium* sp, *Mucor* sp., *Penicillium* sp. and *Trichoderma viride* Pers. In order of their prevalence they were *Mucor* sp., *Aspergillus niger, C. cladosporioidis, Colletotrichum* sp., *Fusarium* sp., *A. flavus, A. fumigatus, T. viride*, *Penicillium* sp. and *C. cassiicola.* Their prevalence was 66.00, 50.00, 25.00, 20.00, 10.00 and 2.00% respectively.

A total of 8 species of fungi was found to be associated with unprocessed rubber stored in room temperature. Prevalence of *A. niger* was the highest (40.00%) followed by *C. cladosporioidis,, Fusarium* sp. (25.00%), *Mucor* sp., *T. viride* (20.00%) respectively. Prevalence of other fungi ranged from 2.00 to 10.00%. Only 3 species of fungi, namely *Mucor* sp., *A. niger* and *A. fumigatus* were associated with unprocessed rubber placed in refrigerator, their prevalence was 66.00%, 34.00% and 10.00% respectively.

Seven species of fungi isolated from processed rubber sheets were stored in room temperature. The isolated fungi were A. *niger, A. flavus*, *A. fumigatus*, *C. cladosporioidis, Fusarium* sp. *Mucor* sp. and *T. viride.* Prevalence of *A. niger* was the highest (50.00%) followed by *A. flavus*, *A. fumigatus*, *C. cladosporioidis,* and *Fusarium* sp (20.00%), *Mucor* sp. and *T. viride* (10.00%) respectively. Three species of fungi isolated from processed rubber were stored in refrigerator. The isolated fungi were *Mucor* sp., *A. niger* and *Colletotrichum* sp. their prevalance was 50.00%, and 25.00% respectively. *C. cassicola* was associated with rubber sheets but did not grow on PDA medium. Frequency percentage

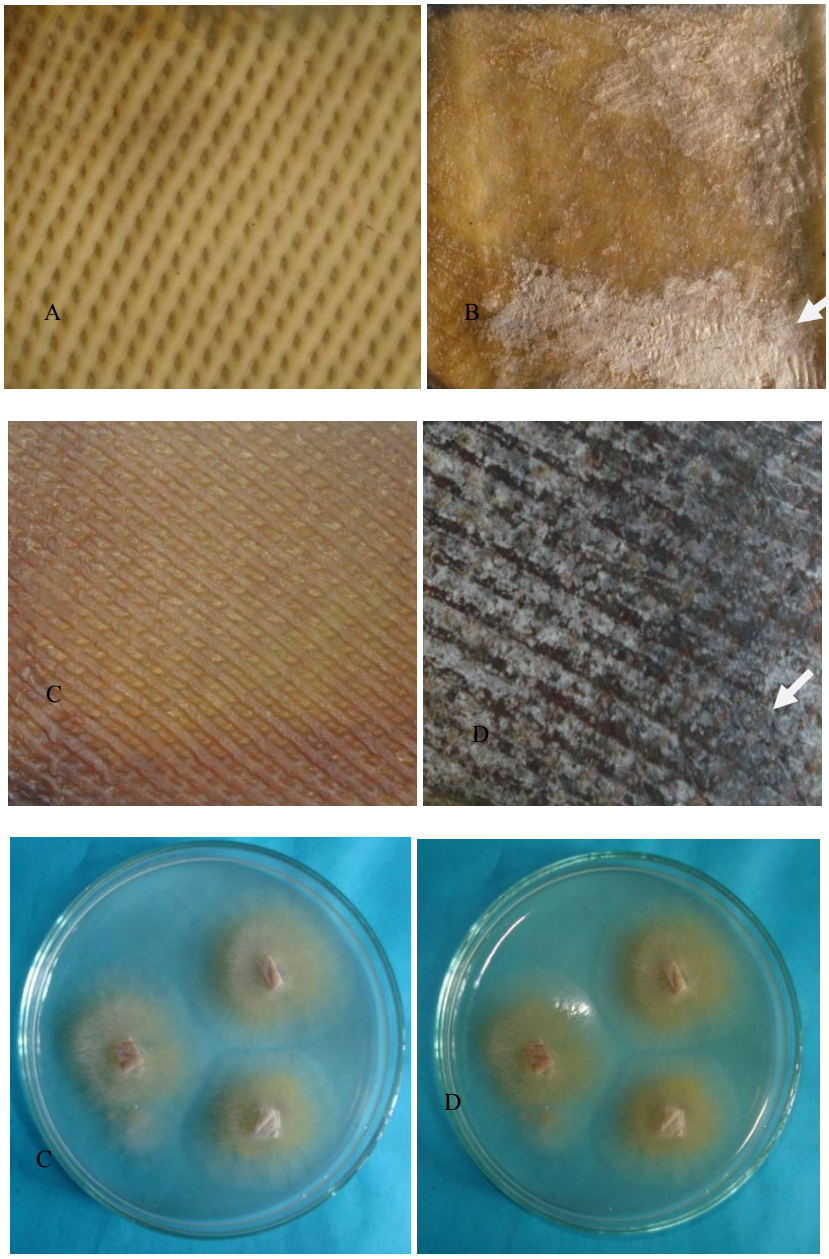

Plate 1. A. unprocessed rubber, B. unprocessed infected rubber, C. processed rubber D. processed infected rubber, E. *Mucor* sp. on unprocessed rubber and F. *Mucor* sp. on processed rubber.

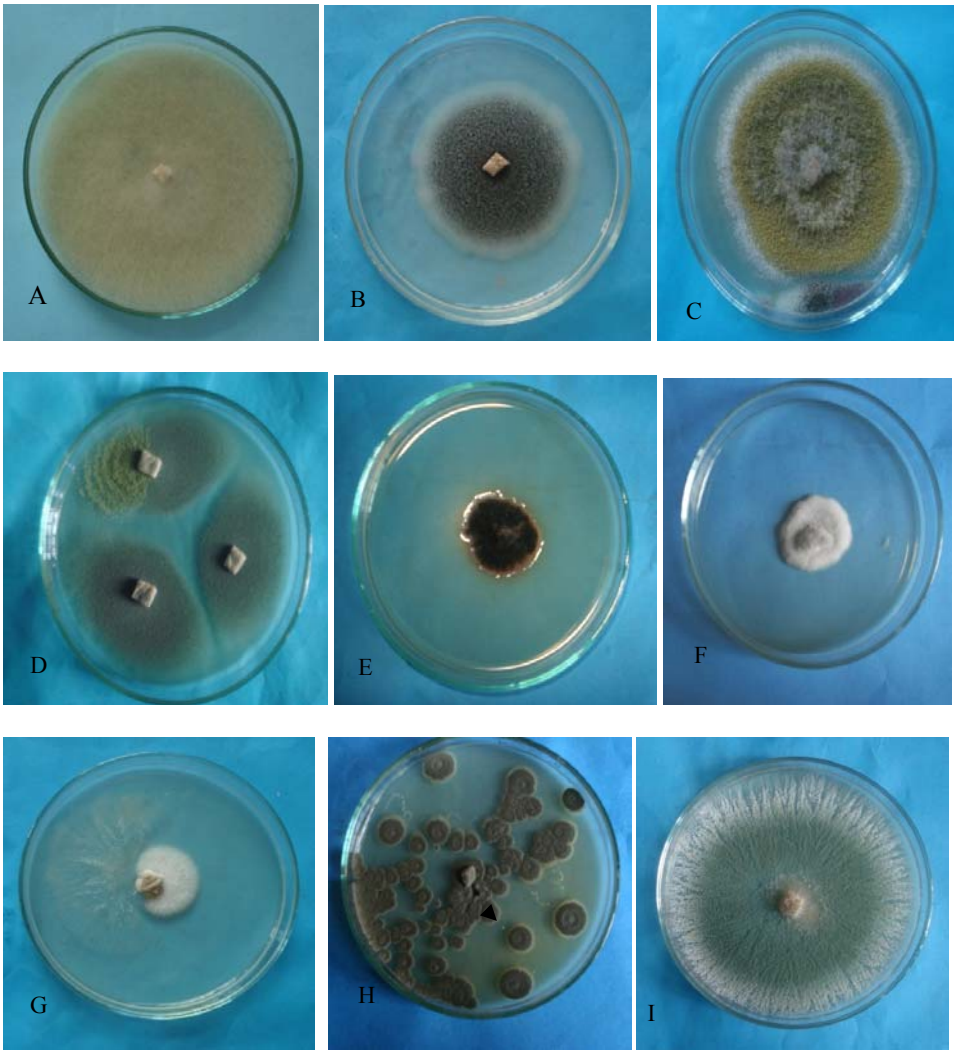

Plate 2. Fungal colony isolated from rubber sheets: A. *Mucor* sp., B. *A. niger,* C. *A. flavus,* D. *A. fumigatus*, E. *C. cladosporioides,* F. *Colletotrichum* sp. G. *Fusarium s*p., H. *Penicillium* sp. and I. *T. viride*.

of association of fungi with rubber sheets are presented in Table 1. The predominating fungus with rubber sheets was *Mucor* sp. Growth of *Mucor* sp. on rubber sheets and PDA plates are presented in Plate 1. Fungal colony isolated from rubber sheets are presented in plate 2.

Mucor sp. and *T. viride* were very fast growing fungi, followed by *Aspergillus* spp. whereas growth of *Fusarium* sp. and P*enicillium* sp. was moderate. *Colletotrichum* sp. and C.

cladosporioidis were found to be slow growing fungi. *Mucor* sp. was the predominating fungi associated with rubber fungi and due to its fast growth it suppressed the growth of other fungi on rubber sheets.

Table 1. Frequency percentage of association of fungi associated with rubber sheets.

Isolates	% of fungi			
	unprocessed rubber		processed rubber	
	25-33^0 C	10^0 C	25-33^0 C	10^0 C
Aspergillus flavus	10	-	20	-
A.fumigatus	-	10	20	-
A.niger	40	34	50	25
C. cladosporioidis	25	-	20	-
Colletotrichum sp.	-	-	-	25
Corynespora cassicola	2.0	-	-	-
Fusarium sp.	25	-	20	-
Mucor sp.	20	66	10	50
Penicillium sp.	10	-	-	-
Trichoderma viride	20	-	10	-

'-'= No isolate

Present paper is the first report of fungal contamination of rubber sheets and its management from Bangladesh. Fungal contamination of rubber had been recorded as early as 1919 by De Vries who observed that the fungi which normally occur on rubber in the tropics are various species of *Penicillium* sp. and *Aspergillus* sp. Esuruoso (1970) reported association of *Aspegillus flavus*, *A. fumigatus* and *A. aculeatus* with rubber sheets from Nigeria.

Rubber plant suffers from various fungal diseases. Mukerji and Bhasin (1986) reported seed rot and stem rot (*Botryodiplodia theobromae* Pat.), leaf spot (*Corynespora cassicola* (Berk. & Curt.) Wei, *Drechslera heveae* (Patch.) Eill., *Gloeosporium albo-rubrum* Petch., *Hypoxylon rubiginosum* Pers. ex Fr., *Pestalotiopsis guepini* (Desm.) Stay), Powdery mildew (*Oidium hevea* Steinman), *Colletotrichum ficus* Koord., *Colletotrichum heveae* Petch.), root rot (*Fomes lamaoensis* (Murril) Sacc. & Trea), wood and root rot (*Polysticta occidentalis* Klotzsch, *P. persoonii* Fr.), keleroga disease on fruit (*Phytophthora arecae* (Colem.) Pethybridge and stem rot (*Pythium vexans* de Bary, *Trametes corrugeta* (Pers.) Bres) diseases of rubber plant from India.

Mucorrecemous, *Mucor* sp., *A. niger*, *Aspergillus* sp. and *Rhizopus* sp. were isolated from Natural rubber waste serum (NRWS) and washing effluents obtained from Green park Rubber Industries Ltd. Umuta, Delta State, Nigeria (Atagna *et al.* 1999).

Tan *et. al.* (1980) reported 2% Captafol or 3% Sodium pentachlorophenate incorporated in Shellkote gave fairly good control of fungal attack of rubber at storage for a period of 4 weeks. Chanduaykit (2007) isolated *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Cladosporium* sp., *Mucor* sp., *Trichoderma* sp., *Rhizopus* sp., *Geotrichum* sp.,

Tritirachium sp., *Daldania eschscholzii* and *Shizophyllum commune* from rubber sheets. The isolated fungi were selected to test against various antifungal agents such as calcium propionate, calcium hydroixids, potassium sorbate, potassium benzoate, sodium metabisulphite, sodium acetate sodium nitrate, ammonium bicarbonate, acetic acid and smoked acid from different kinds of woods (bamboo, Koa, kaole and eucalyptus) by a hyphal extension inhibition assay. (Most agents were takes at the concentrations of 01-10% (w/v or v/v) except smoked acids at 10-100% (v/v). The effective agents were 10% sodium metabisulphite, potassium sorbate, potassium benzoate and 100% smoked acid

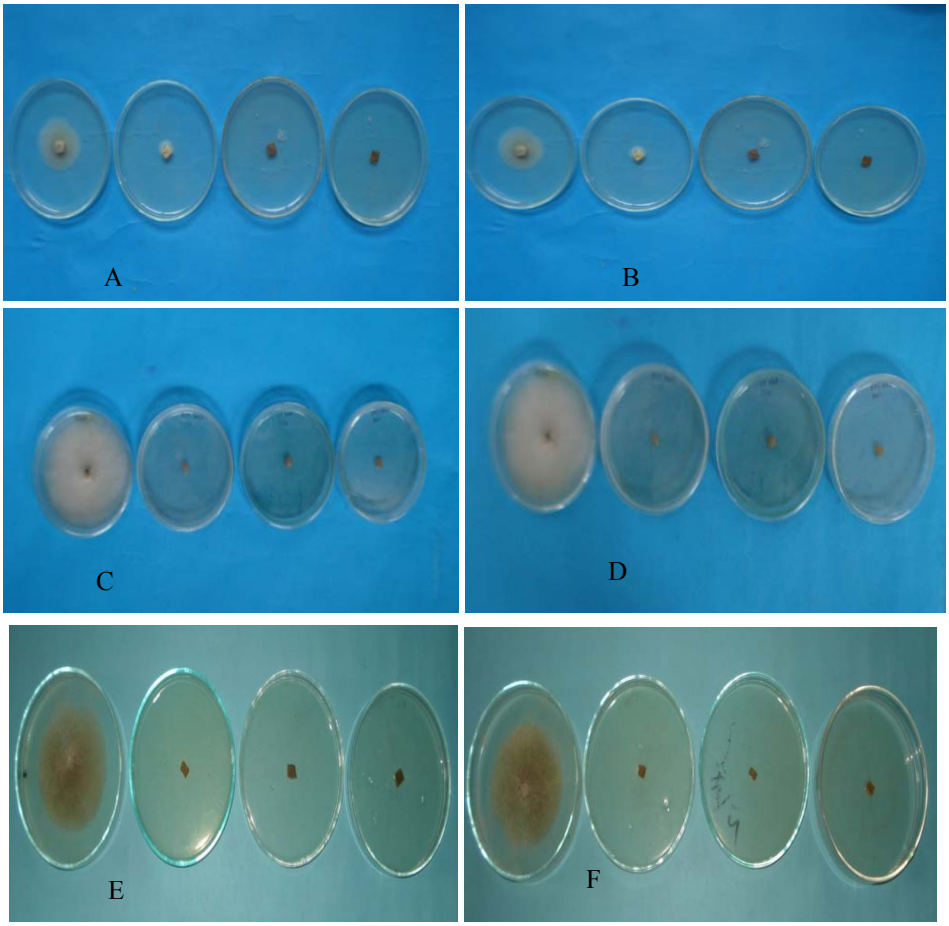

Plate 3. A-B. Effects of NaCl on radial colony growth of *Mucor* sp. associated with unprocessed and processed rubber at 5, 15 and 20% conc. after 5 days of incubation. C-D. Effects of NaCl on radial colony growth of *Mucor* sp. associated with unprocessed and processed rubber at 10, 15 and 20% conc. after 4 weaks of incubation. E-F. Effects of NaCl on radial colony growth of *Mucor* sp. associated with unprocessed and processed rubber at 10, 15 and 20% conc. after six months of incubation.

from bamboo. The minimal inhibitory concentrations (MIC) of these agents against 27 test fungal isolates were determined. *Aspergillus* sp. SR9 was the most tolerant to all antifungal agent tested for 72 h at room temperature with MIC values of 10% (w/v) potassium sorbate 5% (w/v) sodium metabisulphite, 5% (w/v), potassium benzoate 0.313% (v/v) acetic acid and 6.25% (v/v) smoked acid from bamboo. In addition, sodium metabisulphite at a concentration of 2 MIC could prevent fungal growth on para rubber sheets for more than 7 days.

Antifungal activity of sodium chloride was reported by Esam (2009) on *Saprolegnia diclina* and *Aphanomyced* sp. Presently sodium chloride at the concentrations of 5, 10, 15 and 20% was used to control the fungi associated with unprocessed and processed rubber sheets. Sodium chloride at 5% concentration inhibited 50% radial growth of *Mucor* sp., *A. flavus*, *A. fumigatus* and *A. niger* on rubber sheets. Sodium chloride at 10, 15 and 20% concentrations completely inhibited colonial growth of fungi on processed and unprocessed rubbers stored at room temperature (25-28 ^{0}C) and refrigerator for a period of six months (Plate 3).

Rubber is an elastomer that is, a polymer that has the ability to regain its original shape after being deformed. Rubber is also tough and resistant to weathering and chemical attack. Elastomer can be naturally occurring polymers, such as they can be synthetically produced substances e,g, a butyl rubber, Thical rubber or neoprene. For a substance to be a useful elastomer it must possess a high molecular weight and a flexible polymer chain. Fungal contamination not only down- grade the rubber by discoloration of it, it can lead to lose weight which may amount to more than 30%. Weight loss is caused by the decomposition of carbohydrate in the rubber. The fungus attack unprocessed and processed rubber sheets and spread on the surface of the sheet as well as enter within it. Fungi growing on rubber sheets not only reduced quality of rubber they also affects on health of farmers and producers.

Management of fungi with rubber sheets by Sodium chloride is an easier, cheaper and convenient way without any side effects for farmers, workers and producers. In this study it is suggested to use sodium chloride to protect rubber sheets from fungal contamination.

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