قسم: النسيجات
كلية: العلوم - جامعة أسيوط
رئاس القسم: أحمد حلم
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دراسة الفيروسات الفطرية في مصاورة كرحة الأغنم

محمد مانع، عبد الرحيم عبد المطلب، أحمد عمار، محمد عبد السلام

أمكن جمع وتعريف اثنين وثلاثون نوعًا من الفيروسات التي تتنتمي إلى خمسة عشر جنسًا بالإضافة إلى الفيروسات ذات الخيوط العنقية من عينات عصارة الكرحة المختلفة المستخلصة بعد تغذية الحيوانات على علاجات مختلفة وكانت أكثر الأنواع الفطرية انتشارها هي:

- جيرونيكيم كاند سم، وأسبريلس فلاف، ينسليلام كرزيجنيم
- فوريوزوم مونيليفورم في خليط الكبد وقش القمح (1)
- وصاصة القصب (2) على التوالي، بينما في حالة سيكان الجرسيمة المصري (3)
- كانت جيرونيكيم كاند سم، أسكلا روبس بريفكولا، وينسليلام كرزيجنيم
- أما في حالة تغذية الحيوانات على سيكان الغول الأخضر (4) كانت الأسبريلس بنجر، أسبريلس فيفيجات، فوريوزوم أوكس
- سيروم وموكر راسيموزاس، بينما الفوريوزوم أوكس سيروب وأسبريلس
- نيجر، وموكر راسيموزاس كانت هي السائدة في حالة عروض البصل الخضراء (5).

إذا تم الفحص الأكلينيكي لحيوانات التجربة وذلك لمشاهدة أي تغيير فيسيولوجي لها بعد اتباع النظام السابق للتمييز وقد أظهرت الحيوانات التغيرات التالية:

- المجموعة الأولى (1، 2، 3) من الحيوانات لم تظهر أي تغييرات فيسيولوجية واضحة بينما أظهرت المجموعة الثانية (2) بعض الحمل والفقد النسيجي للشبهة وإيضاً الضعف المتآثر لنقاب الكرحة، أما المجموعة الثالثة (5) فقد أظهرت الحيوانات بعض الحمل والتذرخ مع إسهال وسرعة في التنفس وظهور رائحة تشبه البصل.
STUDIES ON FUNGAL FLORA OF RUMINAL JUICE OF SHEEP GAINED AFTER FED WITH CERTAIN FIELD STUBBLES
(With One Table)

By

M.B. MAZEN, A.A. MOTTELIB*, A.A. AMER* and M.N. ABDEL-SALAM*
(Received at 6/9/1984)

SUMMARY

Thirty two fungal species which belong to 15 genera in addition to fungi with sterile mycelium were collected and identified during this study. Generally the most frequent fungal species encountered from different samples tested were as follows:

Geotrichum candidum; and A. flavus, P. chrysogenum and F. moniliforme, and G. candidum. Scopulariopsis brevicaulis and P. chrysogenum and A. niger, A. fumigatus, F. oxysporum, A. niger and M. racemosus in ruminal juice samples gained after fed with cotton seed cake mixture (I); bagasse (II); Egyptian clover's stems (III); green broad bean's stems (IV), and green onion's stems (V) respectively.

The clinical examination of the experimental animals was also carried out to ensure the sound of physiological activity. Some groups (I, III and IV) ensure soundness of experimental animals but, depression, relative loss of appetite and weak delayed ruminal contractions was observed in group (II) whereas diarrhea, depression and staggering with accelerate breathing of onionic odor in group (IV).

INTRODUCTION

In Egypt, this present investigation to our knowledge is the first suggestion that the colonization of the rumen by microfungi has important implication to the growth and health hazardous of ruminants from microbiological point of view.

On the other hand, HUNGATE et al. (1952), OLTJEN et al. (1962) and BREWER and TAYLOR (1969) stated that any feed stuff eaten by a ruminant were immediately subjected to microbial fermentation in the rumen. It seems that the changes in the feed eventually causes significant changes in microbial activity which in turns may affect in the host. In this respects, CALDER et al. (1962) explained the extreme slow growth rate of sheep in summer and autumn due to the presence of fungi among the ruminal flora.

Recent studies revealed that most of fungal species frequently found in the air or growing on substrates use as food or feed were almost pathogenic and capable for producing toxic metabolites (mycotoxins) (AUSTWICK and VENN, 1961; AUSTWICK and EL-PHICK, 1964; RAPER and FENNELL, 1965; WOGAN, 1965; JUNGERMAN and SCHWARTZMAN, 1972; WILSON, 1973; HESSELTINE, 1974; EMMONS et al., 1977; YLIMAKI et al., 1979; EL-KADY and MOUBASHER, 1981; EL-KADY and EL-MARAGHY 1982 a & b and several others).

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METHODS

To accomplish this study, different diets were offered to 5 groups of 48 balady ewes (3-5 years old). Each diet was given for 30 days, with 15 days interval between each diet. Of these, three diets: bagasse (II), green broad bean's stems (IV) and green onion's stems (V) represent field stubbles, the other two diets used were commercial concentraat ration and wheat straw mixture (I) in autumn and Egyptian clover's stems (III) in summer. This study was extended from September 1979 to May 1980.

The ruminal juice samples were taken 5 hours post feeding, three times at 10 days interval. The stomach tube apparatus essentially as described by ABDEL-SALAM (1981) was employed. About 300 - 500 ml of ruminal juice was collected for each sample. Immediately, the whole ruminal sample was placed in water bath at 30°C during the period of examination. About 50 ml was drawn out under complete clean precautions into a sterile container and kept in the refrigerator for fungal examination. Also the clinical examination of the animals before and during each experiment was observed.

For estimation of ruminal juice fungi, the dilution plate technique as described by JOHANSON et al. (1959) was employed.

One ml of the ruminal juice sample was transferred to 20 ml sterilized test tube containing 9 ml sterile distilled water and then the tube was shaken for 5 minutes. One lm of this suspension (1/10) was drawn (while in motion) into a sterile 1 ml pipette and transferred into 9 ml sterile water blank container to give a dilution of 1/100 which was found to be appropriate in most cases giving about 25 colonies per plate.

2. One ml of desired dilution was then transferred immediately into sterile Petri-dishes (5 replicates were used for each sample). About 15 ml of Czapek's agar medium were poured into each Petri-dish. Also rose-bengal at the rate of 1: 30.000/L and streptomycin (30 ug/ml) were added to each plates just before solidification of agar as bacteriostatic agents (MARTIN, 1960).

3. The plates were incubated at 28°C for 7 days and the resulting colonies were counted and identified. Single colonies may be transferred to slant tubes or plates of Czapek's agar medium for further study.

RESULTS and DISCUSSION

The results presented in Table (1) revealed that the highest fungal population was encountered in ruminal juice gained after fed with green onion's stems (78200 colonies calculated per/mL ruminal juice) followed by bagasse (Wheat straw) and cotton seed cake mixture (63640 and 53540 colonies respectively). The least fungal population was estimated in case of Egyptian clover's stems (32120 colonies) and green broad bean's stems (32800 colonies).

Thirty two species which belong to 15 genera in addition to fungi with sterile mycelium were isolated and identified during present study. The broadest spectra of fungi (15 genera and 32 species) was recorded in case of Egyptian clover's stem's (least fungal count). The least spectrum (6 genera and 13 species) was estimated in case of green onion's stems (highest fungal population). The other samples appeared relatively high fungal spectra (about 13 genera and 26 species) as shown in Table (1).

Aspergillus was the most abundant and frequent genus recovered in all ruminal juice samples tested. Its highest count was almost behind the high fungal population of the ruminal juice.
influenced after fed with green broad-bean's stem (yielding 52.2% of total fungi); green onion's stem (37.9%) and bagasse (37.6%). The least count of Aspergillus was determined in case of Egyptian clover's stems (17.6% of total fungi).

From Aspergillus 12 species were collected and identified of which A. flavus and A. niger were the most common (12 cases each) in the ruminal juice samples obtained after fed with bagasse and green onion's stems which contributing 35.1 and 35.5% of total fungi respectively. In case of ruminal juice influenced after fed on green broad bean's stems, A. niger (31.5%) and A. fumigatus (17.2%) were only the most prevalent species of genus Aspergillus. A. sydowi (8.4%), A. niger (8.4%) and A. versicolor (5.2%) and A. niger (7.5%), A. sydowi (5.5%) and A. terreus (1.3%) were collected in moderate frequencies of occurrence (6 - 8 cases out of 12) from ruminal juice samples gained after fed with cotton seed cake mixture and Egyptian clover's stems respectively. The other remaining species of Aspergillus recorded in this investigation were isolated but in less frequencies of occurrence than the preceding ones (Table 1).

The importance of Aspergillus spp. lies in that many of them are pathogenic species growing on substrates used as food and feed for man and animals and may produce mycotoxins which were appeared during digestion and act on one or more body systems. The diseases produced by this type of activity was known as aspergillotoxicois (AUSTWICK and EL-PHICK, 1966; RAPER and FENNELL, 1965; TRENK et al., 1971; JUNGERMAN and SCHWARTZMAN, 1972; WILSON, 1973, EMMONS et al. 1977 and several others). BREWER and TAYLOR (1969) and LUNDT (1974) come in agreement with our results in which A. fumigatus is found in ruminal juice of female sheep. Its endotoxins may explain the haemorrhagic lesions or abortion produced in animals especially in cattle and sheep (JUNGERMAN and SCHWARTZMAN, 1972 and EMMONS et al. 1977). Furthermore Aspergillus which are most frequently found on mouldy hay, straw and cereal grains are of the most commonly pathogenic e.g. A. nidulans, A. flavus, A. terreus A. sydowi and several other (RAPER and FENNELL, 1965; TRENK et al., 1971 and HESSELTINE, 1974). On the other hand, pathological effect of A. ochraceus including acute nephrosis, hepatic degeneration and enteritis when animals fed with contaminated cereal grains with "Ochratoxins" had been reported by TRENK et al. (1971). A. niger was one of those isolated by WILSON and WILSON (1961) as present in mouldy feeding stuffs associated with owalate poisoning. Also, RAPER and FENNELL (1965) reported that the American and French workers stated that the isolates of A. clavatus obtained from mouldy feed pellets when grown on maize and wheat and fed to calves and dairy cows produced depression with hbornmage and congestion of the liver, inopetence and many animals were died after short time of the appearence of these symptoms.

The aflatoxins can be regarded as highly toxic, potent hepatocarcinogens and are mutagenic (WOGAN, 1965, ENOMOTO and SATIO, 1972; EL-ZAWAHRY et al. 1977 and several others). However aflatoxins are produced mainly by strains of A. flavus in cereals or other food and feed stuffs (AUSTWICK and EL-PHICK, 1964; RAPER and FENNELL, 1965; ENOMOTO and SATIO, 1972; HESSELTINE, 1974; MOUBASHER, et al. 1977 a & b and several others).

The other Aspergillus spp. recovered in the present study were mostly recorded by several workers as human and animal pathogenic, but doubt still exists of the ability of them to cause disease for the digestive system of ruminates.

Penicillium Chrysogenum was also isolated in high frequency of occurrence (12 cases) from the ruminal juice samples gained after fed with bagasse and Egyptian clover's stems (representing 30.0 and 14.9% of total fungi respectively). In other ruminal juice samples, it was isolated either in moderate (6 - 8 cases) or low (3 - 5 cases out of 12) frequencies of occurrence but its count was sharply reduced with regard to the other ones (Table 1).
AUSTWICK and EL-PHICK (1964), HESSELTINE (1974) and EMMONS et al. (1977) stated that many animals were died after eating feed naturally contaminated with Penicillium spp., which were responsible for toxicosis (Paxilline) outbreak. Congestion and haemorrhage especially in liver was prominent features of the disease.

Fusarium represented by three species of which F. oxysporum was the most frequent species in ruminal juice samples influenced after fed with green onion's and green brood bean's stems (cam first and second before and behind A. niger with regard to its count which accounting 54.5 and 22.4% of total fungi respectively). F. moniliforme only came third (21.6 %) behind A. flavus and P. chrysogenum in ruminal juice obtained from animals fed with bagasse (sugar cane). Its count in the other ruminal juice samples were either sharply reduced or completely absent (Table, 1).

On the other hand, Fusarium species is widely distributed in stored grains and feed stuffs and has shown to be involved in a number of serious outbreaks of mycotoxices among farm animals. Two types of mycotoxins are usually produced by the different species of Fusarium and these are an estrogenic metabolite called F-2 or Zearalenone and the other is the trichothecenes mycotoxins. The F-2 toxin was found in samples of hay that had been fed to dairy cattle. This hay caused an increase in the number of artificial insemination.

Most of the trichothecane are local irritant causes extensive hemorrhaging necrotic foci of the liver and intestines (KOSURI et al., 1970; JUNGERMAN and SCHWARTZMAN, 1972; HESSELTINE, 1974; EMMONS et al., 1977; YLIMAKI et al., 1979 and EL-KADY and EL-MARAGHY, 1982 a).

Geotrichum candidum (yeast-like fungus) came first with regard to its count in ruminal juice samples gained after fed with cotton seed cake (71.4% of total count) and Egyptian clover's stems (35.1%) before A. sydowi (8.4%) and S. brevicaulis (17.9%) respectively. Its count was sharply declined or completely absent from the other ruminal juice samples tested. However, EMMONS et al. (1977) reported that Geotrichum had a low degree of verulence for animals.

Scopulariopsis was represented by two species, S. brevicaulis and S. candida. The former was the second most frequent species behind G. candidum and before P. chrysogenum in the ruminal samples influenced after fed with Egyptian clover's stems (17.9% of total fungi). Its count was sharply declined or completely disappeared from the plates of other ruminal juice samples.

ELLIS (1971) and EMMONS et al. (1977) stated that S. brevicaulis was considered as one of the most common and wide spread fungus isolated from air, animals and food stuffs.

Mucorales (Phycomycetes) which represented in our study by 3 genera and 6 species and the most frequent mucors were estimated in the ruminal juice samples gained after fed with green broad bean's (6.5%) and Egyptian clover's stem (6.3%) and these counts were mainly to the high population of Mucor racemosus (42.6% of total mucors) and Rhizopus stolonifer (34.2%) and Mucor racemosus (75.4%) and M. hiemalis (36.5%) respectively. These previous species in addition to M. pusillus, M. circinelloides and Syncephalastrum racemosum were also encountered in the other ruminal juice samples but in low frequent than the preceding ones or completely missed from the plates tested (Table 1).

Ulcerative lesions similar to those seen in gasteric and intestinal phycomycosis due to Rhizopus spp. and Mucor (M. circinelloides, M. pusillus and M. racemosus), had been observed by AUSTWICK and VENN (1961); MORGUE et al. (1965); CLARK (1968); JUNGERMAN.
and SCHWARTZMAN (1972) and EMMONS et al. (1977).

*Stachybotrys chartarum* was mostly recovered from all ruminal juice samples but in relatively low fungal population ranged between 0.2 - 1.5% of total fungi (Table 1).

Many workers reported that straw, hay, cotton and sugar cane roods (used either as feed or bedding) contaminated with toxin producing strains of *S. chartarum*. *Stachybotryotoxico- ses* is a mycotoxicoses produced by *S. chartarum*, it usually effects horses and humans but the lesions can be produced in sheep, calves and swine (STANKOUSHEV et al., 1965; PVLOV et al., 1967; GOLDBLATT, 1969; SARKISOV et al., 1971; NIKOV et al., 1974; JONG and DAVIS, 1976; HINTIKKA, 1977 and EL-KADY and MOUBASHER, 1981). Symptoms include salivation, swelling and cracking of the lips, prolonged prothrombin time, leukopenia, diarrhea and death (FORGAES et al., 1958; and WOGAN, 1965). HINTIKKA (1977) reported that wild animals were usually able to avoid the ingestion of feed stuffs contaminated with *S. chartarum* therefore there was no records of *Stachybotryotoxico- ses* among them.

*Chaetomium globosum*, *Botryotrichum piluliferum*, *Paecilomyces terricola*, *Cephalosporium acremonium*, *Myrothecium verrucaria* and *Gliocladium roseum* in addition to fungi with sterile mycelium were also collected and identified from different ruminal juice samples but mostly less frequently than the preceding ones (Table 1).

Generally most of these species were considered as pathogens or capable for elaborating mycotoxins (WILSON, 1973 and JONG and DAVIS, 1976).

The clinical examination of experimental animals was also carried out to ensure the sound of physiological activities. The results reveal that ensure soundness of animals in groups I, 11 and IV, but depression, relative loss of appetite and weak, delayed ruminal contractions was observed in sheep of group II. Diarrhea, depression and staggering with accelerate breathing of onionic odour in sheep of group V.

CALDER et al. (1962) and BREWER and TAYLOR (1969) attributed the extreme slow growth rate of sheep in summer and autumn due to the presence of fungi among the ruminal flora.

However the results must therefore be regarded as preliminarily data and further work in progress.
REFERENCES


Table (17): Percentage count (calculated to total count and number of cases of isolation out of 12 samples) of fungal genera and species recovered from ruminal juice samples after fed with certain diet on Czapek's agar medium at 28°C.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%C</td>
<td>NCI</td>
<td>%C</td>
<td>NCI</td>
<td>%C</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>21.7</td>
<td>12H</td>
<td>37.7</td>
<td>12H</td>
<td>17.6</td>
</tr>
<tr>
<td>A. niger Van Tieghem</td>
<td>6.8</td>
<td>6M</td>
<td>1.9</td>
<td>6M</td>
<td>7.5</td>
</tr>
<tr>
<td>A. flavus Link ex Fr.</td>
<td>0.3</td>
<td>2R</td>
<td>-</td>
<td>-</td>
<td>0.6</td>
</tr>
<tr>
<td>A. fumigatus Fresenius</td>
<td>0.3</td>
<td>2R</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>A. pyrowi (Bain, &amp; Sart,) Thom &amp; Church</td>
<td>8.4</td>
<td>6M</td>
<td>0.1</td>
<td>1R</td>
<td>5.5</td>
</tr>
<tr>
<td>A. verricolor (Vuill,) Tirab.</td>
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<td>6M</td>
<td>0.1</td>
<td>1R</td>
<td>0.3</td>
</tr>
<tr>
<td>A. terreus Thom</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>1R</td>
<td>1.3</td>
</tr>
<tr>
<td>A. candidus Link ex Pr.</td>
<td>0.3</td>
<td>2R</td>
<td>0.1</td>
<td>1R</td>
<td>0.5</td>
</tr>
<tr>
<td>A. nidulans (Eid.) Winter</td>
<td>0.1</td>
<td>1R</td>
<td>0.1</td>
<td>1R</td>
<td>0.4</td>
</tr>
<tr>
<td>A. ochraceus Wilhelm</td>
<td>0.1</td>
<td>2R</td>
<td>0.1</td>
<td>1R</td>
<td>0.5</td>
</tr>
<tr>
<td>A. clavatus Desm.</td>
<td>0.1</td>
<td>1R</td>
<td>0.1</td>
<td>1R</td>
<td>0.6</td>
</tr>
<tr>
<td>A. natac (Bain,) Thom &amp; Church</td>
<td>0.1</td>
<td>1R</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>A. manetoladi Thos &amp; Church</td>
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<td>1R</td>
<td>-</td>
<td>-</td>
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<td>Penicillium chrysogenum Thom</td>
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<td>6H</td>
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<td>P. moniliforme Scheldon</td>
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<td>2R</td>
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<td>12H</td>
<td>0.8</td>
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<tr>
<td>P. solani (Kart.) Sacc.</td>
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<td>1R</td>
<td>-</td>
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<tr>
<td>Geotrichum candidum Link</td>
<td>71.4</td>
<td>12H</td>
<td>0.7</td>
<td>5L</td>
<td>35.1</td>
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<tr>
<td>Scopulariopsis brevicaulius (Sacc,) Bain</td>
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<td>6M</td>
<td>4.4</td>
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<td>17.9</td>
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<td>S. candida (Guez.,Vuill)</td>
<td>-</td>
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<tr>
<td>Mucor</td>
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<td>Mucor hiemalis Wehmer</td>
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<td>W. cirrincelluloides Van Tieghem</td>
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<td>1R</td>
<td>-</td>
<td>-</td>
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<td>W. racemosa Prezen.</td>
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<td>1R</td>
<td>2.7</td>
<td>6M</td>
<td>1.8</td>
</tr>
<tr>
<td>W. pusillus Lindt</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>2R</td>
<td>1.4</td>
</tr>
<tr>
<td>Rhizopus alcalifon (Ef. ef. ex. Fr.) Lindt</td>
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<td>2R</td>
<td>0.1</td>
<td>1R</td>
<td>0.5</td>
</tr>
<tr>
<td>Syncephalastrum racemosum (Cohn.) Schröel</td>
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<td>1R</td>
<td>0.3</td>
<td>2R</td>
<td>0.3</td>
</tr>
<tr>
<td>Stachybotrya chartarum (Ef. ef. ex. Links)</td>
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<td>2R</td>
<td>0.3</td>
<td>2R</td>
<td>0.9</td>
</tr>
<tr>
<td>Hughes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Chaetonium globosum Kunze ex. Fr.</td>
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<td>6M</td>
<td>0.4</td>
<td>3L</td>
<td>1.0</td>
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<tr>
<td>Botryotrichum pilluliferum Sacc.,March.</td>
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<td>2R</td>
<td>0.4</td>
<td>3L</td>
<td>0.9</td>
</tr>
<tr>
<td>Bolomyces terecola (Mill., Cidd., Post)</td>
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<td>3L</td>
<td>0.4</td>
<td>3L</td>
<td>0.8</td>
</tr>
<tr>
<td>Acremonium strictum W. Gams</td>
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<td>2R</td>
<td>0.3</td>
<td>3L</td>
<td>0.9</td>
</tr>
<tr>
<td>Kytheroseum verrucarum (Alb. &amp; Schw.) Dit ex. Fr.</td>
<td>0.1</td>
<td>2R</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>Gliocladium roseum Bain Sterile m</td>
<td>-</td>
<td>0.1</td>
<td>1R</td>
<td>0.1</td>
<td>1R</td>
</tr>
<tr>
<td>Sterile mycelium</td>
<td>0.1</td>
<td>2R</td>
<td>0.1</td>
<td>2R</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Total fungi/ml ruminal juice: 53540 63640 32120 32800 76800

Fungal spectrum: lenders 14 & 24 15 & 32 13 & 26 6 & 13

| Group | I = Cotton seed cake and wheat straw mixture | II = Sugar cane (sugar-cane) | III = Egyptian clover's stem's | IV = Green broad bean's stems | V = Green onion's stems | NCI = number of cases of isolation | %C = percentage count (per total count) | OR = occurrence remarks | H = high occurrence, more than 5 cases out of 12 (75-100%) | M = moderate occurrence, between 6-8 cases (50-74%) | L = low occurrence, between 3-5 cases (25-49%) | R = rare occurrence, less than 3 cases (less than 25%) |