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Isolation of Coprophilous Mycoflora from Different Dung Types in Some Local Government Areas of Niger State, Nigeria

Mohammed N.[1], Shinkafi S. A.[1], Enagi M. Y.[2]

[1]Department of Biological Sciences, Federal University Gusau, Gusau, Nigeria
[2]Department of Biological Sciences, Ibrahim Badamasi University, Lapai, Nigeria

Email address:
naibi_muhammad@yahoo.com (Mohammed N.)

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Abstract: This study investigated coprophilous mycoflora on different dung types in some Area of Niger State. Dung samples of four herbivorous animals (Camel, Cow, Donkey and Horse) were collected from three areas (Enagi, Lapai and New Bussa) in Niger State, Nigeria. The dungs were dried in the Department of Biological Sciences Laboratory, Ibrahim Badamasi Babangida University Lapai. A total of six (6) species of macro fungi (*Agaricus campestris, Panaeolus papilionaceus, Agaricus bisphorus, Protostropharia semiglobata, Deconica coprophila, Copelandia cyanescens*) and six (6) species of micro fungi (*Aspergilous. niger, A flavus, A. fumigates, Alternaria sp, Pencillium sp,* and *Rhizopus stolonifer)* were isolated. *A. campestris* has the highest percentage (40%) of macro fungi (from cow dung), while the least was *A. bisphorus* with (0.02%). Alternaria sp has the highest (33.26%) among the micro fungi isolated. From horse dung, *A. fumigates* was isolated with the least percentage of occurrence of 0.10% among the micro fungi. On this note, the animal dung investigated consists of both edible and poisonous fungi. Public enlightenment is therefore recommended on the good and bad effects these fungi. They may also be harnessed for industrial use.

Keywords: Coprophilous, Fungi, Dung, Mycoflora, Microfungi, Macrofungi

1. Introduction

Coprophilous fungus means dung-loving fungi [1]. These are filamentous fungi that exist in a broad range of habitats, fulfilling significant roles in a diversity of ecosystems. Coprophilous fungi release their spores to the surrounding vegetation, which are then eaten up by herbivores. The spores then remain in the animal as the plants are digested, passed through the animal intestines and are finally defecated. The fruiting bodies of the fungi then grow from the animal faeces [2]. The spores themselves survive digestion by being particularly thick-walled, allowing them to germinate in the dung with minimum competition from other organisms [3].

Coprophilous fungi may be useful indicators of habitat diversity [3]. Coprophilous fungi are an artificial group of fungi adapted to life on dung and fecal pellets of herbivorous animals. As the waste product of the digestive process, herbivore faeces are predominantly composed of the most recalcitrant and indigestible parts of the plant: the cell wall polymers cellulose, hemicelluloses, and lignin [4]. Therefore, the potential for the secretomes of coprophilous fungi to contain novel enzymes for efficient plant cell wall degradation is high. The fungal secretomes have been explored to find enzymes and enzyme combinations for various industrial applications, such as paper, textile, and food manufacture [5, 6] and economically and industrially sustainable hydrolysis of plant biomass to fermentable sugars for biofuel production [7, 8, 9]. Dung is a major source of organic matter and a potential home for saprotrophs. From a fungal point of view, herbivore dung is the most interesting dung type for the study of fungi compared to those of carnivore and omnivore that are largely being decomposed by bacteria. Herbivore dung however is known to support a

wide variety of coprophilous fungi [1].

Coprophilous fungi are highly sought after in the textile, detergent, and biofuel industries due to their dispersing effect on solid celluloses and increasing the overall activity in a cellulolytic enzyme array. Nutritionally, they are edible and could serve as an alternative source of protein. Understanding the distribution and growth pattern of these fungi will be of immense benefit to scientists.

2. Aim of the Study

The aim is to study the relationships between coprophilous fungi and fecal substrates of four herbivorous animals (cows, horse, donkey, camel) in some areas in Niger State. The objectives of the study are:

i. To examine the growth of coprophilous mycoflora on four herbivorous animal dungs (cow, horse, donkey and camel) in some areas in Niger State.
ii. To isolate and identify fungal species growing on each dung sample.
iii. To determine the frequencies of occurrence of coprophilous mycoflora growing on each dung sample.

3. Materials and Methods

3.1. Selected Sample Areas

Three areas were chosen for this research work. These are: Enagi, (Edati Local Government headquarters), Lapai, (Lapai Local Government headquarters) and New Bussa, (Borgu Local Government headquarters) all in Niger State. These areas are known for the settlement of Nomadic Fulanis who rear and use these animals for crop cultivation and the Buzus (a clan within Hausas) who are seasonal migrants that are into produce transportation using camels (beasts of burden).

3.2. Collection of Dungs

Dungs of four domestic animals namely: *Bos primigenius* (cow), *Camelus dromedaries* (camel), *Equus ferus callabus* (Horse) and *Equus asinus* (Donkey) were collected for this work. Most of these dungs were collected between February and April 2015 in Lapai, Enagi and New Bussa all in Niger State.

3.3. Methods

Each dung sample was placed on a sterile polythene bag in the field, labeled and taken to the Department of Biological Sciences laboratory, Ibrahim Badamasi Babangida University Lapai, Niger state. The dung collections were shade dried in the laboratory and stored at room temperature (28°C) in paper envelops.

3.4. Isolation of Mycoflora

The collected dung samples were ground properly into loose materials. Then, five grams (5gms dry weight) of each dung powder was placed on sterile filter paper moistened with sterile distilled water in 9cm Petri dishes and incubated at room temperature (28+2°C). After three days the samples were examined for the fruiting bodies with a dissecting microscope. Longer incubation periods of up to 60 days were necessary for certain species of fungi to develop their fruiting bodies. From time to time, a small amount of sterile water was added to the dishes to keep the dung continually moist [10, 11]

3.5. Observation of Micro Fungi

Slides were prepared from the moist dungs and observed using the lower x10 and higher x40 magnification of light microscope.

3.6. Percentage Frequency

The presence of coprophilous fungi was recorded based on the appearance of their fruiting bodies. The percentage frequency for each fungus was calculated as follows [10]

$$\frac{\text{No of Samples on which fruiting body was observed}}{\text{Total number of dung samples of that animal examined}}$$

4. Results

Figures 1 and 2 indicated that, a total of six macrofungi and six microfungi were isolated from four different animal dungs (Cow, Horse, Camel and Donkey) in Enagi area. The result from Cow dung exhibited a high significant difference ($P<0.05$) among all the isolated fungi. The percentage frequency of occurrence of *A. campestris*, was the highest (40%) followed by *P. papilionaceus* (30%) while *C. cyancescens* was not isolated from cow dung. In horse dung (Figure 1), the percentage occurrences of *A. campestris* (25.06%). *P. papilionaceus* (25.00%) and *D. Coprophila* (26.00%) were not significantly different ($P<0.05$). From camel dung (Figure 1), *D. Coprophila* had the highest percentage of occurrence (33.30%) and was significantly different ($P<0.05$) from the remaining macrofungi isolated. *P. semiglobata* was not isolated from this dung. Donkey dung sampled (Figure 1) from Enagi area had the highest percentage of occurrence of *A. campestris* (30.30%) and the lowest percentage of occurrence of *A. bisporus* (19.66%). No growth of *D. coprophila* from such sample.

The result from Cow dung showed that among the microfungi, *Alternaria sp* had the highest percentage (25.26%) followed by *R. stolonifer* with percentage frequency of (25.00%), *Penicillium sp* (17.10%), *A. niger* (17.03%), *A. fumigates* (8.10%) and *A. Flavus* (8.03%). In horse, among the microfungi isolated *Alternaria sp* with (33.26%) had the highest percentage occurrence and significantly different ($P<0.05$), followed by *R. stolonifer* (22.30%), *A. fumigates* (22:16%), *A. niger* (11.26%), *Penicillium sp* (11.03) and the least was *A. flavus* (0.25%). In camel dung, among the microfungi *Penicillium sp* had the highest percentage of occurrence (30.03%) which was significantly different ($P<0.05$) from other isolated fungi. *A. fumigates* (19.96%), *R. stolonifer* (20.13%), *A. flavus* (10.16%), *Alternaria sp* (20.06%) and *A. niger* (0.13%) were

not significantly different (P<0.05).

In donkey, among the microfungi, *R. stolonifer* had the highest percentage of occurrence (33.6%) and there was high significant different (P<0.05) while *penicillium sp* (0.13%) had the least (Figure 2). In Lapai area, a total of six macrofungi (Figure 3) and six microfungi (Figure 4) were isolated from four different animal dungs (Cow, Horse, Camel and Donkey). The result from Cow dung showed no significant difference, (P<0.05), between the percentage of occurrence of macrofungi *P. papilionaceus* (25.10%) and *D. coprophila* (25.00%). In horse dung (Figure 3) *A. campestris* had percentage frequency of occurrence of (21.46%), *A. biporus* had (21.20%) *C. cyanescens* (21.40%) had the highest percentage of occurrence and was not significantly different (P<0.05). *P. semiglobata* (6.96%) had the least percentage of occurrence and was significantly different from the rest of the isolates.

From Camel dung (Figure 3) the occurrence of *A. bisporus* (28.50%), *D. coprophila* (28.53%) were not significantly different (P<0.05). From Donkey dung (Figure 3) percentage of occurrence of *P. papilionaceus* (20.20%), *A. bisporus* (19.96%), *D. coprophila* (30.05%), *C. cyanescens* (20.03%) were not significantly different (P<0.05).

From cow dung, among the microfungi isolated *A. niger* had the highest percentage of occurrence (30.03%) which was significantly different (P<0.05) from other isolates. The percentage of occurrence of *Penicillium sp* (20.16%), *R. stolonifer* (20.00%) *Alternaria sp* (20.26%) were not significantly different (P<0.05) (Figure 4). Among the microfungi isolated from horse dung, *R. stolonifer* had the highest percentage of occurrence (25.10%) which was significantly different (P<0.05) from the rest isolates. While *A. flavus* (0.16%) was the least (Figure 4). The microfungi isolated from camel shows that *A. fumigates had* (30.10%) frequency of occurrence, *Alternaria sp* (33.33%) had the highest percentage of occurrence and was not significantly different (P<0.05). *A. flavus* (0.16%) had the least percentage of occurrence and was significantly different from the other isolates (Figure 4). From donkey dung, *Alternaria sp* is significantly different (P<0.05), *R. stolonifer* (0.13%) was the least and was significantly different from other isolates (Figure 4). Figure 5 indicated that a total of six macrofungi and six microfungi were isolated from four different animal dung (Cow, Horse, Camel and Donkey) at New Bussa Area. The results of isolates from Cow dung exhibited high significant difference (P<0.05), the percentage of occurrence of macrofungi *A. campestris* (38.63%), the percentage of occurrence of macrofungi *A. campestris* (38.63%) was the highest, followed by *D. coprophila* (22.66%) and was significantly different (P<0.05) from other isolates. *A. bisporus* (0.16%) was the least (Figure 5).

In horse dung (Figure 5) macrofungi, *A. campestris* (30.06%) and *P. papilionaceus* (29.93%) had the highest percentage of occurrence and showed no significant difference (P<0.05), followed by *P. semiglobata* (20.00%) and was significantly different (P<0.05) from other fungi. From Camel dung (Figure 5), *A. bisporus* had the highest percentage of occurrence (25.10%) and was significantly different (P<0.05) from the remaining macrofungi isolates. The occurrence of *A. campestris* (16.60%) and *D. coprophila* (16.66%) were not significantly different (P<0.05). From Donkey dung, the occurrence of *A. campestris* (27.33%) and *D. coprophila* (27.13%) were not significantly different (P<0.05). The percentage of occurrence of *P. semiglobata* (18.16%) and *C. cyanescens* (18.33%) were not significantly different (P<0.05), while *A. bisporus* (0.16%) was the least from among macrofungi isolated from the dung types in New Bussa area (Figure 5). On microfungi isolated from cow dung, the occurrence of *A. niger* (22.96%) and *A. flavus* (23.20%) were not significantly different from other isolates (Figure 6). From horse dung, among the microfungi isolated, *A. niger* had the highest percentage of occurrence (30.10%) and was significantly different (P<0.05) among the microfungi isolated. The microfungi isolated from the camel dung indicated that *A. fumigates* had the highest percentage of occurrence (33.00%) and was significantly different from the remaining microfungi isolated *A. niger* had 22.16% of occurrence, *Pencillium sp* (21.93%) *Alternaria sp* (0.16%) was the least (Figure 6). From the donkey dung, among the microfungi isolated, *Alternaria sp* had the highest percentage of occurrence. The percentage of occurrence of *A. niger* (20.03%), *R. stolonifer* (20.06%) and *A. flavus* (20.03%) were not significantly different (P<0.05), while *A. fumigates* (0.23%) was significantly different (P<0.05) from other microfungi isolates. (Figure 6).

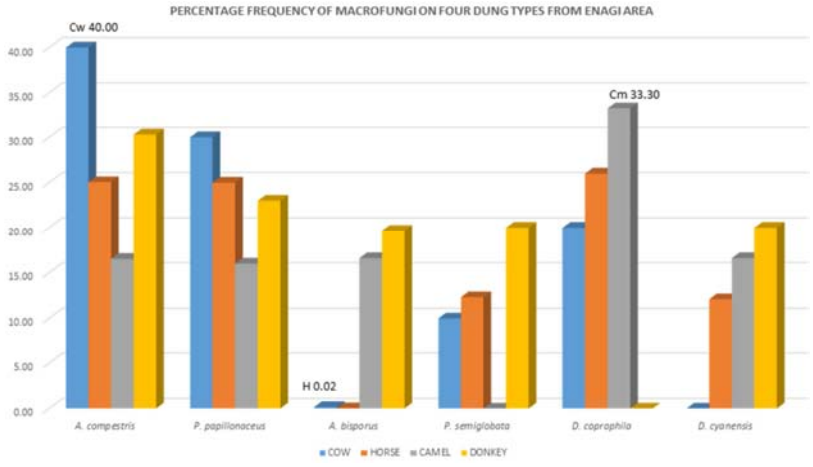

Figure 1. *Percentage frequency of coprophilous macrofungi on four dung types from Enagi Area.*

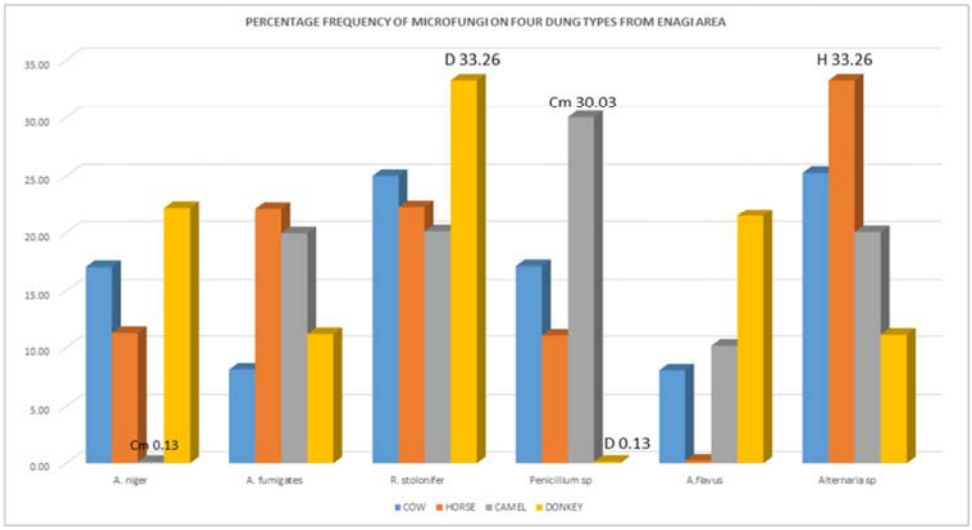

Figure 2. *Percentage frequency of microfungi on four different dung types from Enagi Area.*

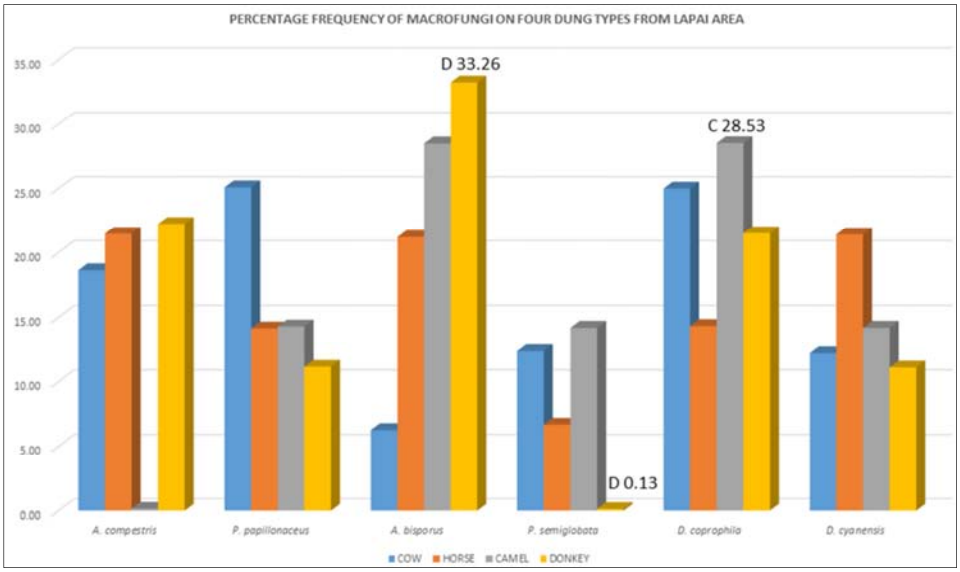

Figure 3. *Percentage frequency of macrofungi on four different dung types from Lapai Area.*

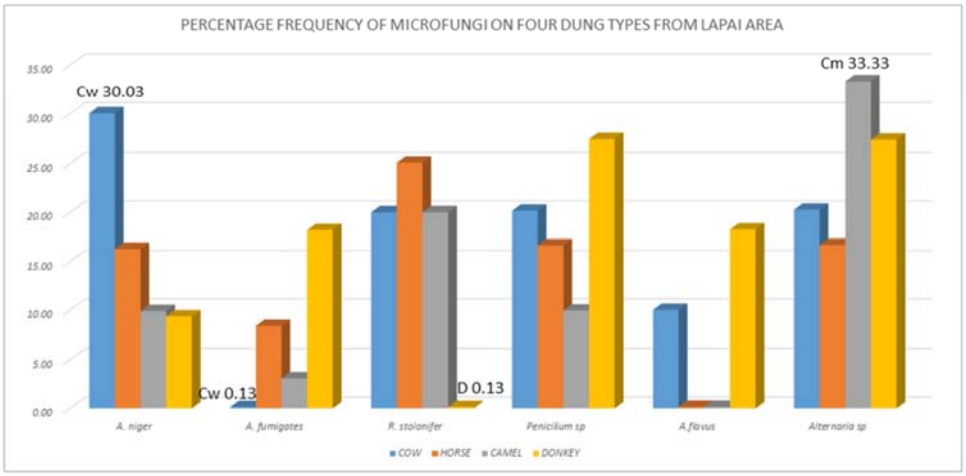

Figure 4. *Percentage frequency of microfungi on four different dung types from Lapai Area.*

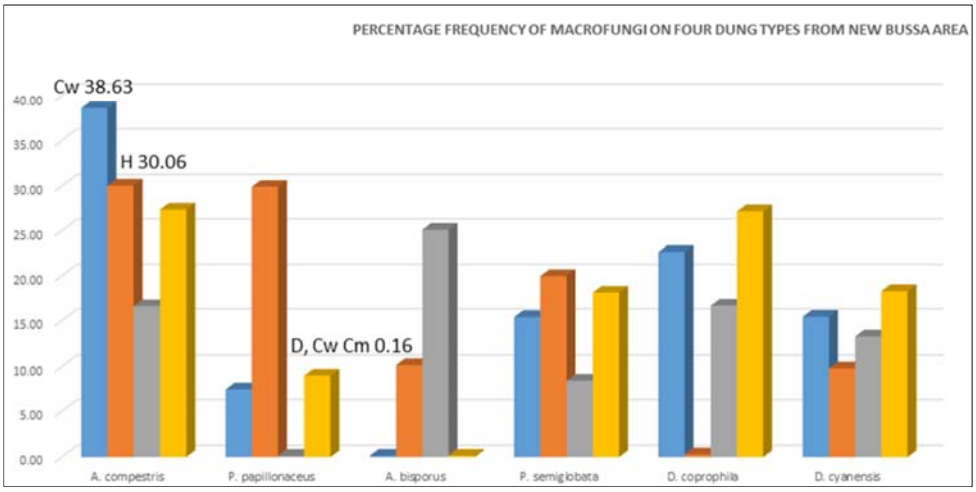

Figure 5. *Percentage frequency of macrofungi on four different dung types from New Bussa Area.*

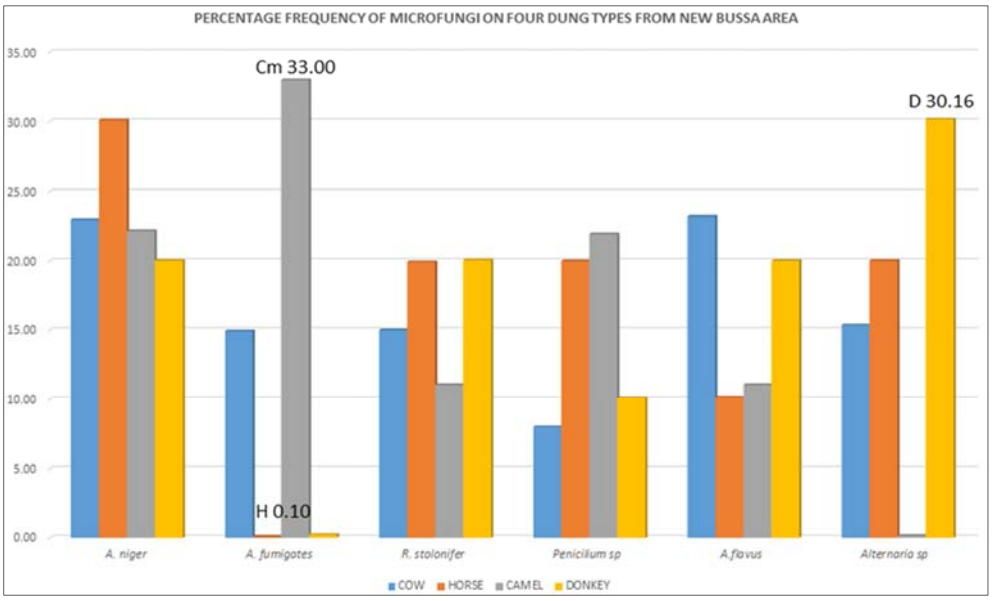

Figure 6. *Percentage frequency of microfungi on four different dung types from New Bussa Area.*

Key
Cw = Cow, Cm = Camel, H = Horse, D = Donkey

5. Discussion

In this research work, a total of six macrofungi (Agaricus campestris, Panaelous papilionaceus, Agaricus bisporus, prototropharia semiglobata, Deconica coprophila and Copelandia cyanescens) and six macrofungi, (Aspergillus niger, Aspergillus fumigates, Rhizopus stolonifer, Penicllium sp, Aspergillus flavus and Alternaria sp. were isolated from four herbivorous animal (Cow, Horse, Camel and Donkey) from dungs collected from three areas (Enagi, Lapai and New Bussa) in Niger State.

According to Abdullah and Rattan [10], also isolated from his study were other forty species among which are Saccobolus crenulatus, A. immerses, Chaetomium circinatum, C. globosum, Podospora communis, P. decipiens, Mucor sp, Aspergillus candidus and others from sheep, donkey and camel. In Enagi area, the result shows that cow dung had the highest percentage of occurrence of macrofungi, A. campestris (40%) while among the microfungi, R. stolonifer and A. Flavus were found to have highest percentage of occurrence on horse and donkey dungs with (33.26%) each. This could probably be related to the presence of forests in the area where these animals grazed. This was earlier reported by Lundqvist [12] that there is a positive association between forest-dwelling animals and certain coprophilous fungi.

From Lapai area, the results showed that donkey had the highest percentage of occurrence of macrofungi *D. coprophila* (30.05%). While among the microfungi, *Alternaria sp*, had the highest percentage of occurrence (33.33%) from camel dung. This may be due to different

grasses collected to feed the animals and proper sanitation of the environment in which these animals are kept, Heino Lepp and Fagg [2] reported that coprophilous fungi release their spores to the surrounding vegetation, when then, eaten by herbivores, The spores then remain in the animal as the plant are digested, passed through the animal intestines and are finally defecated. The results from New Bussa showed that cow dung had the highest percentage of occurrence of macrofungi *A. campestris* (38.63%). While among the Macrofungi *A. fumigates* had the highest percentage of occurrence (33.00%). This may be as a result of forest areas in which these animals are found. This is related to the report of Lundqvist [12] who pointed out that there is a positive association between forest dwelling animals and certain coprophilous fungi.

In this research work, a group of fungi *Agaricus campestris* and *Panaelous papilionaceus*, were found growing on all the dungs examined from Enagi area. Also, *Panaelous papilionaceus, Agaricus bisporus, Deconica coprohila* and *Copelandia cyanescens* were found growing on all dungs examined from Lapai area, *Agaricus campestris, Protostropharia semiglobata* and *Copelandia cyanescens* were found growing on all the dungs examine from New Bussa. This is similar to the findings of Richardson [3], that "Coprophilous fungi are an artificial group of fungi adapted to life on dung and fecal pellets of herbivorous animals. These fungi developed certain special features which help them to reappear on dung and adapted to a cyclic relationship between dung-herbage animal gut-dung in the course of evolution. It was observed that *Agaricus spp* was found growing on all the dungs examined from the three areas. This was in line with the report of [13] that *Agaricus sp* are known to occur in almost all environments; the fungi growing on the dung are those that have passed through the animal and saprotrophs that land after deposition of these faeces. Some fungi have specific dungs on which they commonly grow such as *Agaricus bisporus* which grows on cow dung, while like *Panaelous papilionaceus* was commonly found on Cow and Horse dungs. Other fungi have a worldwide distribution such as *Agaricus campestris* [13]. These fungi were also isolated from these dungs in this study.

6. Conclusion

From this study, it was established that animal dungs are good substrates for the production of fungi. A total of twelve species of coprophilous fungi were collected out of which six species were macrofungi and six species were microfungi. The difference in number and type of fungi isolated is probably reflections of physicochemical nature or type of plant species consumed by these animals. Considering the importance of these fungi, some are edible; some of them are poisonous, while the microfungi are toxins producers. As saprotrophs they play a significant role in decomposition of organic matters, hence increasing soil fertility. They are as well used to produce drugs in pharmaceuticals, also employed in textiles industries for their secretomes producing protease capable of degrading hemicelluloses, lignin and pectin.

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