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MYCOFLORA OF COW DUNG USED AS ORGANIC MANURE

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ABSTRACT
Cow dung is one of the most important sources of organic manure which is often used by local farmers due to its low cost and easy availability. It is therefore necessary to isolate and identify the mycoflora present in it so as to know whether such mycoflora are pathogenic or not. The cow dung sample used in this study was collected from some fields within the University of Ilorin Main Campus and taken to the Biology laboratory for mycoflora analysis using standard methods. Five fungal species were isolated and their pure cultures were identified as Aspergillus niger, A. flavus, A. fumigatus, Saccharomyces cerevisiae and Mucor sp. A. niger, A. flavus and A. fumigatus are pathogenic while S. cerevisiae and Mucor sp. are saprophytic. It is therefore recommended that cow dungs be properly treated so as to eliminate these pathogens from them if they are to be used as organic manure.
KEY WORDS: *cow dung, isolates, mycoflora, organic manure, pathogens.*

INTRODUCTION

Manure is anything that can be added to the soil to increase its fertility and enhance plant growth **(**Boller & Hani, 2004). Manures contribute to the fertility of the soil due to addition of organic matter and inorganic matters such as nitrogen (Haynes, 2003). It also provides carbon and other constituents that affect soil humus content, biological activity and soil physical structure (Rono *et al.,* 2012). Manures may be organic or inorganic. Organic manures are derived from decaying material of plant or animal origin while inorganic manures, also known as fertilizers, are derived from chemical processes that are most often man-made (Boller & Hani, 2004). Cow dung is one of the most important sources of bio-fertilizer (Kesavan & Swaminathan, 2008).

The use of cow dung as organic manure increases soil organic matter content leading to improved water infiltration and water holding capacity as well as an increase in the cation exchange capacity of such soil (Kesavan & Swaminathan, 2008). Also, cow dung is a very effective manure for reducing bacterial and fungal pathogenic diseases as it suppresses mycelial growth of plant pathogenic fungi such as *Fusarium solani, F. oxysporum* and *Sclerotinia sclerotiorum* (Basak & Lee, 2002; Misca *et al.,* 2014). Despite the advantages of cow dung as an organic manure, some of its disadvantages include unequal distribution of essential nutrients in it, tedious nature of its collection and processing as well as the presence of some plant pathogens in some of them (Maynard, 1991).

Richardson (2001) isolated 137 ascomycetes on the dung samples of sheep, horse, cow, roe deer, rabbit and hare; these included *Phomatospora coprophila., Ascophanus microsporus, Podospora cumula, Coprobia granulate, Ascobolus immerses*, *A. albidus, Podospora appendiculata, Thelebolus stercoreus*, *T. nanus, Saccobolus versicolor* and

Sporormia intermedia while Onuma *et al*. (2006) studied hyphomycetes fungi from dung samples of wildlife and domestic animals including deer, elephant, guar, rabbit, camel, goat, horse, buffalo, cow, mouse and toad collected from eighteen provinces in Thailand. Four-hundred and six isolates (406) belonging to 24 genera and 28 species of hyphomycetes were identified including *Arthrobotrys oligospora, Cephaliophora irregularis*, *Nodulisporium gregarium* and *Oidiodendron griseum*. Potential plant pathogenic fungal isolates reported by them included *Alternaria alternata, Curvularia lunata, Exserohilum rostratum, Fusarium oxysporum, F.solani, Nodulisporium gregarium* and *Thielaviopsis* sp. (Onuma *et al*., 2006).

Obire *et al.* (2008) isolated *Alternaria* sp., *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Geotrichum* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Sporotrichum* sp., *Thamnidum* sp., *Candida* sp., *Rhodotorula* sp. and *Torulopsis* sp. in their investigation of saprophytic and crude oil degrading fungi from cow dung and poultry droppings. Lakshmi *et al.* (2015) carried out an investigation on coprophilous fungi from different dung samples of domesticated hybrid cow around Lawspet area of Puducherry, India and reported 44 isolates from the dung samples. *Aspergilli* were the most common with seven species viz., *Aspergillus awamori, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. sydowii* and *A. terreus*. Other isolates were identified as *Absidia* sp*., Curvularia intermedia, Drechslera* sp*., Rhizopus stolonifer* and *Saccharomyces cerevisiae* (Lakshmi *et al.,* 2015).

Local farmers use cow dung as organic manure, which goes a long way in improving yield. This cow dung serves as substrates for many microorganisms some of which are pathogenic to some plants. This study is purely qualitative, not quantitative. It was aimed at isolating and identifying fungal isolates from cow dung so as to determine whether they are pathogenic or not based on available literatures and textbooks.

MATERIALS AND METHODS

Collection of sample. Fresh cow dung samples from cattle grazing on the University of Ilorin farmland were collected into sterile black polythene bags and transported to the Biology laboratory of University of Ilorin for mycoflora analysis.

Isolation of fungi from the cow dung. Fungi were isolated from the cow dung sample using serial dilution techniques as described by Fawole & Oso (2007). Serial dilution was done up to 10^{-4}. A measure (0.1ml) of aliquot from 10^{-2}, $10^{-3}$and 10^{-4} dilutions were inoculated on the surface of sterile streptomycin – amended potato dextrose agar (PDA) plates using spread plate method. The plates were then incubated at room temperature for 72 hours.

Purification and identification of fungal isolates. Each fungal colony obtained was sub-cultured on fresh sterile streptomycin - amended PDA plates several times until a pure culture was obtained. The pure culture of the isolates obtained were then transferred onto sterile PDA plates and stored in the refrigerator at 4 to 8^{o}C prior to their identification. The fungal isolates from cow dung samples in this study were labelled F1, F2, F3, F4 and F5 respectively. The labelled fungal isolates were identified morphologically based on their macroscopic and microscopic features. For the macroscopic features, the naked eyes and hand lens were used whereas, glass slides were prepared using the procedures of Fawole & Oso (2007). They were then viewed under a light microscope at X40 objective for the microscopic features. The macroscopic and microscopic features of each of the fungal isolates were recorded and compared with those listed by Funder (1968) and Ellis & Ellis (1985).

RESULTS AND DISCUSSIONS

Macroscopic features of each of the fungal isolates in this study are listed in Table 1 while the microscopic features observed are presented in Table 2. Based on the macroscopic and microscopic features listed in Tables 1 and 2, F1 was identified as *Aspergillus niger*, F2 as *A. flavus,* F3 as *A. fumigatus*, F4 as *Saccharomyces cerevisiae* and F5 as *Mucor* sp. The results obtained in this study agreed with those of previous studies by Onuma *et al*. (2006), Obire *et al*. (2008) and Lakshmi *et al.* (2015). *A. niger* is known to cause various plant diseases such as black rot of onions, crown rot of peanuts, tuber rot of yam, mango rotting, rot of tomatoes, kernel rot of grapes, fruit rot of grapes and fruit rot of banana (Ajay *et al*., 2011). *Aspergillus flavus* is a saprotrophic and pathogenic fungus with a cosmopolitan distribution, colonizing cereal grains, legumes, and tree nuts. They cause pre- or post- harvest infections during storage and/or transit (Machida & Gomi, 2010; Ramírez-Camejo *et al*., 2010). In addition to causing infections, many strains of *A. flavus* produce significant quantities of toxic compounds known as mycotoxins, which, when consumed, are toxic to mammals (Agrios, 2005). *A. flavus* is also an opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals (Amaike & Nancy, 2011; Ianovici, 2016). *A. fumigatus* also causes allergic diseases, respiratory illnesses, and bloodstream infections in humans. *A. niger, A. flavus* and *A. fumigaus* are pathogenic (Table 3). *Saccharomyces cerevisiae* is known to have strong potential as plant growth promoter, it is considered to be a new promising plant growth promoting yeast for different crops (Moustafa & Mohamed, 2008) while *Mucor* sp. is a saprophytic and crude oil degrading fungus. Both *Saccharomyces cerevisiae* and *Mucor* sp are not pathogenic.

TABLE 1: Macroscopic features of fungal isolates from cow dung sample in this study

Fungal isolates	Surface texture	pigmentation	Reverse side colour
F1	Cottony	Blackish	Creamy
F2	Powdery	Greenish yellow	Creamy
F3	Powdery	Blue – green with white margin	Creamy
F4	Smooth	Creamy	Creamy
F5	Cottony	White	Pale white

TABLE 2: Microscopic features of fungal isolates from cow dung in this study

Fungal isolates	Microscopic Features
F1	The conidiophores have branched, septate hyphae; conidia are glubose, very rough or irregular in surface and very blackish brown in colour; the stipes colour is slightly brown; the surface is smooth walled; the vesicle is large, round and biseriate.
F2	Conidia are glubose ellipsoid, smooth finely roughened; conidiophores are hyaline and coarsely roughened; the surface is quietly spherical; hyphae are septate; the stipes colour is pale brown; vesicle serration is biseriate.
F3	Conidia are glubose and small in columns; Smooth walled; the stipes colour is grayish near the apex. The conidia surface is smooth or spinose. Vesicle serration is uniserate pyriform.
F4	Chains of cells (blastonidia) which exhibited pseudohyphal morphology; the cells are large, dispersed, bright and smooth; they are globose to ellipsoidal and yeast – like.
F5	White cottony colonies which become grayish brown due to the brownish sporangiophores; sporangiophores are tall and irregular in shape; hyphae are coenocytic and branched.

CONCLUSIONS

Aspergillus niger, *A. flavus, A. fumigatus, Saccharomyces cerevisiae* and *Mucor* sp. were isolated from samples of cow dung used as organic manure in this study. *Saccharomyces cerevisiae* and *Mucor* sp are non pathogenic while the remaining three isolates are pathogenic.

This does not negate the importance of cow dung as an organic manure. Cow dung should be well treated in order to reduce its microbial load before using it as an organic manure.

TABLE 3: Status of the fungal isolates in this study

Fungal isolates	Pathogenic	Non pathogenic
F1	*Aspergillus niger*	
F2	*Aspergillus. flavus*	
F3	*Aspergillus. fumigatus*	
F4		*Saccharomyces cerevisiae*
F5		*Mucor* sp.

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