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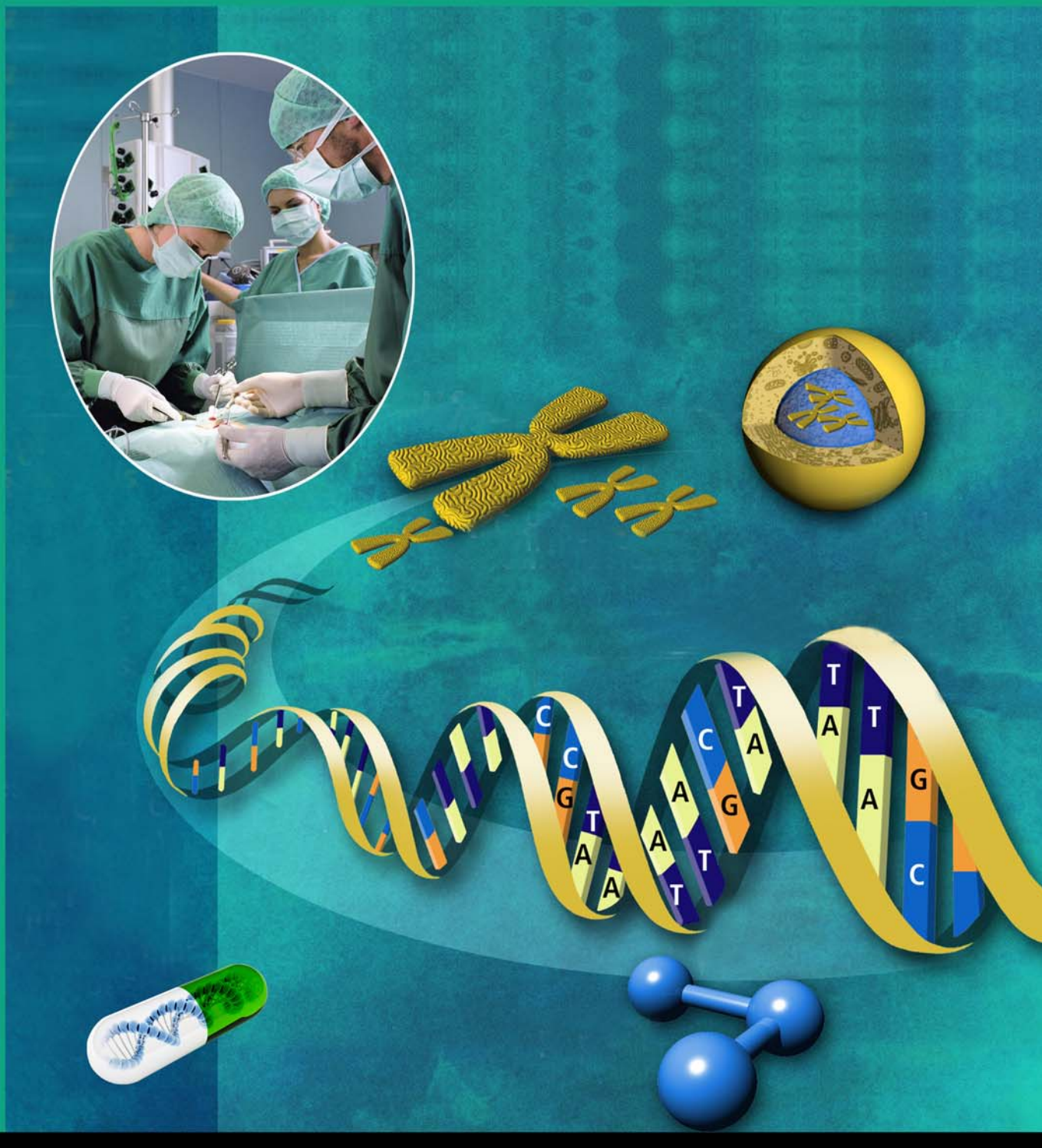

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Research Paper

ISOLATION AND CHARACTERIZATION OF FUNGI ASSOCIATED WITH THE SPOILAGE OF CORN (*ZEA MAYS*)

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Fungi spoiling organisms are silently invading, acidifying, fermenting, discolouring, and disintegrating microbes that render corn (*Zea mays*) unpalatable and unsafe for human consumption. Fungi spoilage is caused by two factors, (biotic) living which includes insects, birds, rodents and microorganisms and (non-biotic) non-living which includes temperature, humidity and time. Two samples of spoilt corn, red (treated) and white were taken from the store and field respectively for investigation in order to ascertain the fungi that were associated with the spoiling of the corn. Different methods were used in the identification and five genera were isolated. The frequency of occurrence for the fungi isolates were *Mucor* spp 6%, *Aspergillus* spp. 9%, *Rhizopus* spp. 15%, *Penicillium* Spp. 33% and *Fusarium* Spp. 36%. Fusarium spp. had the highest frequency and was the predominant fungus.

Keywords: *Zea mays*, Fungi, Spoilage organisms, Biotic and abiotic factors

INTRODUCTION

The world is concerned with food safety that has enhanced interest in fungal and subsequent food spoilage. Contamination with mould causes deterioration of product which affects human and animal health. In relation to this, interest is continuously focused on corn because it is one of the most important dietary staple foods (Pitt and Hocking, 2002).

Corn (*Zea mays*) is the most important raw material used in food production. The contamination of the raw materials by mould and mycotoxin are very frequent. This contamination could lead to nutrient losses and detrimental effect on animals and production. Drought, humidity, temperature, insect, infestation and rough handling have been suggested as factors which contribute to the presence of fungi and subsequently toxins in agricultural products (Jay, 1998).

Fungal spoilage of corn reduces the nutritional value and palatability of the feed, thereby

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increasing its allergic potential and may result in mycotoxic contamination (Scudamore and Livesay, 1998).

Another issue is that some fungi species present in corn have been linked to mystic infection of cattle, particularly *Aspergillus fumigatus* (Jay *et al*., 1998).

Corn grows in ears, each of which is covered in rows of kernel that are protected by the silk-like threads called corn silk. Corn is scientifically known as *Zea mays*. Though corn is usually associated with yellow color, it actually comes in host of different varieties featuring an array of different colors. There are red, pink, purple and blue corns. (Pitt and Hocking *et al*., 2009).

Fuzzes, powders and slimes of white, black, green, orange, red and brown colors are signs of silently invading, acidifying, fermenting, discoloring and disintegrating microbes that render commodities unpalatable and unsafe (Pitt and Hocking, 2002). This research work focused on corn and its spoilage organisms. It set out to document current knowledge on the interaction of corn and fungi in the context of spoiling.

Corn (*Zea mays*) is a grain grown by farmers in various parts of the world especially in Africa. Its spoilage could limit its availability to consumers thereby causing economic waste to the farmers. The aim of this research was to microbiologically evaluate and identify the fungi that are responsible for corn spoilage.

MATERIALS AND METHODS

Autoclave, Refrigerator, Incubator, Colony counting chamber, Hot air Oven, Wire loop Bunsen Burner, Test tube rack, pH meter, staining Rack, Cotton Wool, Spatula, Beakers, Electronic Weighing Balance, Washing bottles, Swab sticks, Slides, Cover slip, Glass rod, Conical flask, Petridishes, Test tubes, Pipettes and Aluminium Foil.

Reagents

Methyl red indicator, Phenol red, Distilled water, Peptone, Ethanol, Lactophenol cotton blue and Urea.

Collection of Sample

Samples of spoilt corn were selected from the field and stores at Abakpa, Enugu State of Nigeria.

Sterilization of Glass Wares

Properly washed petri-dishes, test tubes, conical flasks, beakers, pipettes, knives, spatulas, etc., were sterilized in hot air oven at 180^{o}C for two hours (2 h) and stored at 4^{0}C.

Preparation Of Culture Media (Sample)

The media for culturing were aseptically prepared when needed according to the manufacturer's instruction and autoclaved at 121^{0}C for 15 min.

Preparation of Sample

Five fold serial dilutions of the sample were prepared.

Plating Technique

The diluted sample was used to inoculate the prepared media using pour plate method. The agar plates were allowed to solidify and placed in an inverted position for 7 days at 25^{0}C, thereafter, their colonies were observed.

Identification and Characterization of Isolates

Cultural Characteristics

The growth pattern, pigmentation and size of colonies were recorded at the incubation period to aid identification of the organisms.

Colony Morphology

A drop of lactophenol (LP) was placed on a clean microscopic slide. A small portion of the isolate was placed in the drop of lactophenol (LP) and suspended. A clean cover glass was placed over the suspension and observed microscopically.

Spore Staining

The staining procedure for identification of spore was carried out by placing heat-fixed slide (containing the smear of the isolate) over a steaming water bath and placing of blotting papers over the area of the smear without sticking out past the edges of the slide. The blotting paper was then saturated with 5.6% solution of malachite green and steamed for 5 min. Following this, the slide was cooled to room temperature and then rinsed thoroughly with tap water. Safari was then applied for one minute and rinsed briefly but thoroughly before blotting dry. After which the slide was examined microscopically.

Motility Test

Fungal motility was determined by transferring a small drop of live isolates to the centre of a slip of a depression slide using petroleum jelly or 2-3 drops of peptone water with growth of the organism replaced on a clean slide with wire loop. Then cover slip was placed over the slide, the slide was left for sometime and then examined microscopically with the high power objective. Motile organisms were seen swimming around.

Biochemical Test

Carbohydrate Assimilation Test: Filtered and sterilized carbohydrates were added to the medium at concentration of 1% while the pH was adjusted to 5.4 by addition of NaOH or HCl. 2 ml of the media were dispensed into 10 ml test tube. The tubes were also inoculated with isolates and carbohydrates. All tubes were incubated at 20^{o}C for 14 days. A change in the color of the medium of orange and yellow were taken as positive result. A change to pink or purple was considered negative result.

Amino-acid Assimilation Test: Medium preparation and indication were as described for the carbohydrate assimilation test. 10 mm test tubes containing 2 ml of the media were inoculated with the isolate and control tubes for each fungus and amino acid. Also tubes were incubated at 20^{o}C for 14 days. A change to pink or purple was considered positive result while a change in color of the medium to orange was taken as negative result.

Hydrolysis Test: The basal medium was similar to that of amino acid assimilation test with addition of 0.05 mg milk and 1.2 mg agar. After autoclaving at 110^{o}C for 30 min, the medium was poured into petri dish. Isolates were inoculated at the centre of the plate and incubated at 20^{o}C for 14 days. The appearance of a clear zone around the fungal colony was taken as a positive result.

Lipase Activity Test: The medium of 0.5% peptin, 0.3% yeast extract and 1.0% agar were autoclaved at 121^{o}C for 10 min. It was filtered and dispensed into sterilized test tubes. Isolates were inoculated into the surface of the medium and incubated at 20^{o}C for 7 days. The occurrence of clearance in the medium column was taken as a positive result.

Fungal Identification

The isolates were identified using cultural characteristics and morphology with reference to De Hoog *et al.* (2000) and Jay (1992).

RESULTS

The microorganisms isolated from two (2) corn samples together with their frequency of

occurrences are shown in Tables 1, 2 and 3 and Figure 1. The isolated organisms are *Mucor* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp. *and Penicillium* spp.

Identification

Identification of the isolate was based on cultural characteristics, colony morphology (cell size, shape, pigmentation and arrangements), motility and general biochemical characteristics and the results were presented in Table 1.

Frequency of Visible Colonies

The total plate count of the visible colonies reveals that *Fusarium* genera were higher in number among the five fungi genera observed. It could also be concluded from the result that the rate of involvement of *Fusarium* in the spoilage of corn in the field and in the store was high.

Y=33, therefore to calculate

$$(\%\ \text{Frequency}) = \frac{x}{y} = \frac{100}{1}$$

DISCUSSION

Fungi isolates were identified by their cultural and morphological characteristics as presented in Table 1. These isolates include *Mucor* spp, *Fusarium* spp, *Rhizopus* spp, *Aspergillus* spp and *penicillium* spp. In all analyzed samples, the most prevalent genera was *Fusarium*, its dominance could have been that the maize was incorrectly dried. Insufficient drying and precarious condition

Table 1: Cultural, Morphological Characteristics And Identification

Isolate	Cultural Characteristics	Morphological Characteristics
Rhizopus spp	Large fluffy white milky colonies which later turns black as culture ages.	Non-septate hyphal with up right *sporagioshere* connected by stolon and rhizoids, dark pear-shaped *sporaregium* on hemispherical columella.
Mucor spp	Cream white/large fluffy white colonies almost covering the whole surface	*Sporangium* comes out directly from the hyphal without stolon or rhizoids collumella.
Penicillium spp	Large fluffy white colonies almost covering the whole surface.	Non – septate branched hyphal enlarge at the apex to form *cornidophorex* they produce brownish black *ceridia* in chains.
Fusarium spp	Rapidly growing wooly to cottonly lemon and yellow	Multicellular distinctive sickle shaped macro *coniclia.*
Aspergillus spp	Very common colours of colony (black and white)	*Conidia* borne in 360 arrangements covering the upper 2/3 of the *conidiophores*.

Table 2: Spore Formation, Motility and Biochemical Identification

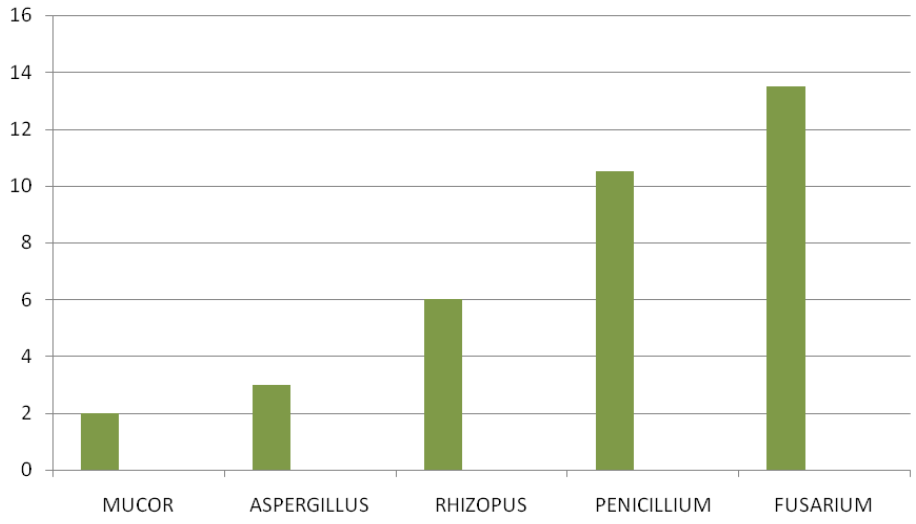
S. No.	Isolate	Carbohydrate Assimilation	Spore Formation	Amino Acid Assimilation	Motility	Hydrolysis	Lipase Activity
1.	*Mucor* spp.	+	+	-	-	-	-
2.	*Rhizopus* spp.	+	+	+	-	-	
3.	*Fusarium*	+	-	+	-	-	+
4.	*Aspergillus* spp.	+	-	+	-	-	+
5.	*Penicillium* spp.	+	-	+	-	-	+

Key: + = Positive; - = Negative

Table 3: Frequency of Visible Colonies

Isolate	(X)Frequency/Number of Occurrence From Both Samples	% Frequency
Mucor spp.	2	6.06
Aspergillus spp.	3	9.09
Rhizopus spp.	5	15.15
Penicillium spp.	11	33.33
Fusarium spp.	12	36.36

Figure 1: Frequency of Isolates



of storage could promote *Fusarium* growth as *Fusarium* genera need water for growth (Harrigan *et al*., 1988).

It is important to note that *Fusarium* spp. includes diverse producing toxigenic lineage in maize and derived products (Larone, 1998). It was shown that the predominating fungi genera in the analyzed samples were *Fusarium spp* (36.4%), followed by *Penicillium* spp. (33.4%), *Rhizopus* spp. (15.2%), *Aspergillus* spp. (9.09%) and *Mucor* spp. (6.06%) according to Table 3 above.

This research work has established that corn can spoil under any weather condition. However, the rate and degree of spoilage has been shown to be higher under moist or high humidity conditions. Moreover, all the fungal organisms identified, characterized, and isolated in this study are capable of causing death to man and animals resulting from mycotoxins which they are capable of producing. Also, the results of this study show that the conditions to which corn is exposed in the field and store, as well as the storage method used to preserve it have effects on the type, rate, and extent of infection of the corn by fungi.

CONCLUSION

In conclusion, maize can be infected by fungi irrespective of storage condition.

REFERENCES

1. Alexsander M (1999), "The Mycoflora of Corn Silage", *Journal of Veterinary Medicine,* Vol. 23, No. 1, p. 57.
2. A D (2009), *Fungi and Food Spoilage,* 3rd Edition, Spring or Dorchrecht Heidepberg London, p. 90.
3. Barath H, Knabe O and Lepom P (1990), "Occurrence of Fusarium Species and their Pycotoxins in Maize Silage: Studies on the Fusarium Infestation of maize Silage Plants" *Arch Animal Nutrition,* Vol. 40, No. 1, pp. 397-298.
4. Bilgrami K S and Choudhery A K (1998), *Mycotoxins and food Safety*, Marcel Dekker Inc., New York, p. 399.
5. De Hoog G S, Guarre J and Gene J F (2000), *Atlas of Clinical Fungal,* 2nd Edition, The Netherland Publishers, pp. 450-453.
6. Harrigan W F and MeCance M C (1988), *Laboratory Methods in Food and Diary Microbiology*, Academic press Inc. London, p. 495.
7. Hunt J, Boddy J, Randergon P F and Rogers H J (2004), "An Evaluation of DNA Approaches for the study of Fungal Diversity in Grassland Soils", *Journal of Microorganism and Ecology,* Vol. 160, No. 1, pp. 385-388.
8. Jay J M (1998), *Food Spoilage in Modern Food Microbiology,* 4th Edition, Chapman and Hall Inc. New York, p. 195.
9. Larone D H (1998), *Medically Important Fungi: A Guide to Identification,* 3rd Edition, ASM Press, Washington DC, pp. 205-209.
10. Marin S, Sanchis V and Magan N (1999), "Water activity Temperature and pH effects on growth of Fusarium", *Journal of Moniliforme and Proliferation*, Vol. 41, No. 3, p. 1063.
11. Marsh S F and Payne G A (1998), "The colonization of sent corn by Aspergillus Flanus", *Journal of Phytopaology,* Vol. 74, No. 1, p. 557.
12. Morrison E, Runderberget T, KosiaK B, Aastveitm A H and Bernhoft A (2001), "Cytotoxicity of Trichothences strains Isolated from Nerwegian Cereals", *Journal of Mycopathology*, Vol. 153, No. 3, p. 49.
13. Pitt J I and Hocking A D (2002), *Fungi and Food Spoilage,* 2nd Edition, London Brackie Academic and Professional Chapman and Hall Cooperation London.

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