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Mycoflora Associated with Stored Wild Castor (*Ricinus communis L.*) Seeds in Kogi State of Nigeria

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Stored castor (*Ricinus communis* L.) seeds collected from four different parts of Kogi State, Nigeria were screened for associated mycoflora. Using the direct plating method, samples were plated out on Sabouroud Dextrose Agar (SDA) as growth medium and incubated at room temperature (27 ±1°C) for 7– 12 days. The resulting growth was visually and microscopically screened for fungal species. Results showed that the temperature of the stores ranged from 20-28°C, while the moisture content values of the seeds ranged from 5 – 8%. *Aspergillus* spp were the commonest fungi (66.67%) found in the stored seeds, while species belonging to the *Cephalioplora, Penicillium* and *Syncephalastrum genera* were less common with 11.11% occurrence each. There was significant difference in the percentage occurrence of *Aspergillus flavus* between the various locations. These results are important in developing standard for post harvest practices aimed at discouraging fungal contamination of castor seeds.

Key words: Castor seeds, Mycoflora., *Aspergillums flavus, Cephalioplora, Penicillium, Syncephalastrum,* Fungal contamination.

Castor seeds are obtained from the castor plant (*Ricinus communis L.*) of the family Euphorbiaceae. The plant is important because of the high oil content (40 – 57%) of the seeds (Acheru and Onyeike, 2002; Anjani *et al*, 2004). Basically, the oil is obtained by pressing or solvent extraction methods. Although castor oil is used for frying (Frank, 1974), it has almost unlimited industrial applications and enjoys tremendous world demand in the pharmaceutical, paint, cosmetics. textile,leather, lubricant, chemical,plastic, fibre, automobile and engineering industries (Roetheli *et al*., 1991; Gobin *et al*., 2001; Anjani *et al*., 2004). Also, it has the advantage over other oils because it is a renewable resource, biodegradable and eco-friendly. Many derivatives that have a similar chemical composition as petroleum-based oil can be produced from castor oil. The pomace is used for fertilizer and when detoxified by heating at 140°c for 20 minutes, it can be used as an ingredient for feed formulation (Uzogara *et al.*, 1990).

Today, castor is cultivated in not less than thirty countries on commercial scales and cultivated in more that nineteen out of the 36 sates of Nigeria. A number of workers have carried out work on the

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mycoflora associated with some oil seeds in storage; groundnut (Ward and Diener, 1961; Umechurubu *et al*., 1991), sunflower seeds (Nakai *et al*., 1997); palm kernels (Oso, 1979; Ogundero, 1981), melon seeds (Kuku, 1980; Bankole, 1993) and wild mango (*Irvingia gabonensis*) (Ataga and Ota-Ibe, 2006). Castor seeds, like other oil seeds are vulnerable to attack by fungi in the field, with up to about 80 – 100% damage reported (Ajani *et al.,* 2004). Storage and marketing pre-dispose seeds to microbial infection especially fungi and various species of fungi have been found associated with oil seeds in storage (Ataga and Ota-Ibe, 2006). However, no information appears to be available on the fungi associated with the castor seeds in storage. Hence, the need to investigate the mycoflora associated with castor seeds in storage. The aim of this work is therefore to investigate the mycoflora associated with stored wild castor seeds in Kogi State of Nigeria

MATERIAL AND METHODS

Sample collection

Wild castor (*Ricinus communis L*.) seeds were collected from stores in four locations (Kabba, Ankpa, Ogaminana and Ajaka) in Kogi State, Nigeria.

Two hundred gramme seed lots from each location were collected in triplicates from 100kg sacks of seeds using a grain probe and stored in sterile polyethylene bags. In all, 12 stores in Kogi State were visited and 36 samples were collected and taken to the Department of Microbiology, Ahmadu Bello University, Zaria, laboratory for analysis. The temperature of the stores at the point of sample collection was recorded using 0 – 100^0C thermometer.

Isolation of fungi from the Castor seeds

Ten grammes (approx 24 seeds) from each sample were surface-sterilized by immersion in 1% sodium hypochlorite (NaOCl) solution for 30 seconds and rinsed three times in sterile distilled water (Bankole and Joda, 2004). Then, 6 seeds per dish of the surface-sterilized seeds were directly plated on SDA supplemented with chloramphenicol (100ml/L) in accordance with the procedures of the International Seed Testing Association (1976). The plates were then incubated at room temperature (27±1^0C) for 7 – 12 days. Examination for the presence of fungi on the seeds was carried daily using a dissection microscope and light compound microscope (Magnification X 100).

Fungal isolates were obtained directly from fungal colonies on the seeds by subculturing of the fungal spores or mycelium on Sabouroud Dextrose Agar (SDA) supplemented with chloramphenicol (100mg/L). Pure cultures of the fungal isolates were transferred on to fresh SDA slants and stored at room temperature.

Percentage frequency of each fungus was calculated as total number of seeds on which a particular fungus appeared per plate divide by the total number of seeds per plate.

Identification of Fungal Isolates

Identification of the isolated fungi was carried out at the Department of Crop protection, Ahmadu Bello University, Zaria with reference to mycological texts (Barnett and Hunter, 1972) and confirmed by the Global Plant Clinic of the Common Wealth Agricultural Bureau International (CABI), London.

Moisture content

The moisture content (Mc) value of the samples was determined using the high constant temperature oven method according to AOAC (1990). Twenty grammes of the samples were taken and slowly ground in a porcelain mortar to avoid heat build up and minimize moisture loss. Ten grammes portions were then weighed into crucibles and dehydrated in pre-heated oven at 130°C for 30 minutes. The average weight loss was then recorded and expressed as a percentage of the initial weight.

Data analysis

Data were subjected to analysis of variance using the SUPERANOVA (Abacus concepts Inc. CA, USA) computer and significant difference between means was determined by the least significant difference technique at 95% confidence level (Peterson, 1985).

RESULTS

Percentage of fungal genera on castor seeds

The percentage occurrence of genera of fungi isolated from the fungal-colonized castor seeds are presented in Fig. 1. The genus, *Aspergillus* had the highest percentage occurrence

Table 1. Average monthly environmental temperatures and relative humidity of the four sampled locations in Kogi State, Nigeria

Zone	Location	Date of collection	Mean (°C) Temperature	Mean relative humidity (%)
A	Kabba	May 12-16, 2005	28	68
B	Ankpa	May 10-14, 2005	27	70
C	Ogaminana	June 24-28, 2005	28	66
D	Ajaka	June 12-16, 2005	27	72

(66.67%) while *Penicillium*, *Cephaliophora* and *Syncephalastrum* had the same percentage occurrence (11.11%).

Percentage occurrence of fungal species on castor seed samples

In all the experimental zones, *Aspergillus flavus* was found to have the highest percentage frequency (35.00% in zone A, 20.69% in zone B, 24.32% in zone C and 25.88% in zone D). The details are presented in Fig. 2.

Distribution of fungal isolates in the locations

The distribution of fungal isolates in the four locations is shown in Fig. 3.The result showed

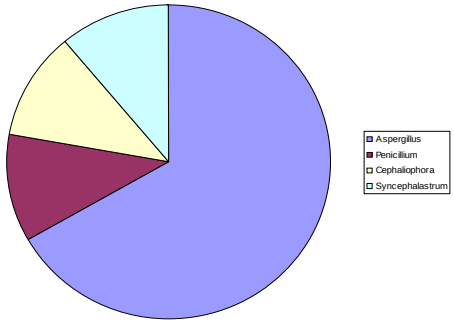

Fig. 1. Percentage fungal genera on castor seeds in Kogi state

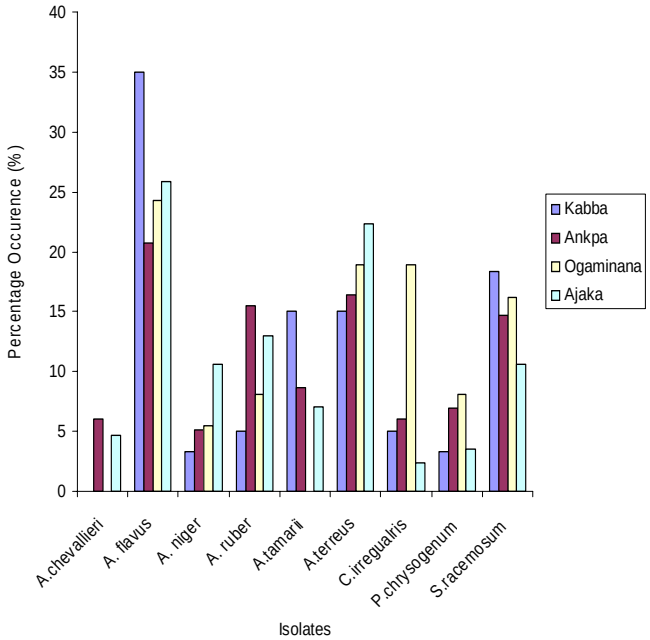

Fig. 2. Percentage of ocourrence of fungal species on naturally-infected castor seeds in Kogi state

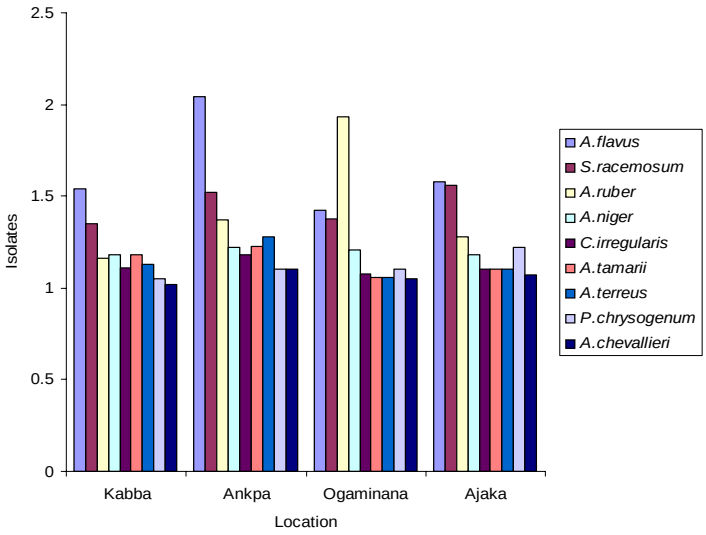

Fig. 3. Effect of location on the distribution of fungal species on castor seeds in Kogi state

that *A. flavus* had the highest occurrence in three locations (Kabba, Ankpa and Ajaka). This was followed by *Syncephalastrum raceomosum*, except in Ogaminana, were *A rubber* had the highest occurrence.

Table 1 showed the average environmental temperatures and relative humidity of the four sample locations in Kogi State. The temperature of 28±1°C was obtained in both Kabba and Ogaminana and 27±1°C was obtained in Ankpa and Ajaka. The highest relative humidity of 72% was obtained in Ajaka and 66% in Ogaminana..

DISCUSSION

The study showed that a total of four fungal genera (*Aspergillus*, *Cephaliophora*, *Penicillium* and *Syncephalastrum*) and nine fungal species belonging to the four genera were isolated and identified as the most predominant mycoflora of castor seeds in storage. The fungal species included: *Aspergillus chevallieri, Aspergillus niger, Aspergillus ruber, Aspergillus flavus, Aspergillus tamarii, Aspergillus terreus, Cephaliophora irregularis, Penicillium charysogenum* and *Syncephalastrum racemosum*. Although some of the fungal species had been previously isolated from some Nigerian oil seeds (Kuku, 1980; Bankole, 1993; Oso, 1979; Ataga and Ota-Ibe, 2006), this appears to be the first report of *Cephaliophora irregularis* on any Nigerian oil seed.

The high occurrence of fungal species on the stored castor seeds agrees with previous reports of high occurrences of fungal species on stored seeds; Bankole (1993), Oso (1979), Ataga and Ota-Ibe, (2006) on melon seeds, palm fruits and wild mango respectively. The predominant fungal genus found in the seeds was *Aspergillus sp* with 66.67% occurrence, contrary to the finding of Nakai *et al* (1997) who reported the genus *Fusarium* (67.70%) as predominant on stored pistachio nuts in Turkey.

Although the study showed no significant difference ($P > 0.05$) in the occurrence of *Aspergillus niger* between castor seeds from all the zones. The higher percentage frequency of *Aspergillus flavus* obtained is in contrast with Dilek *et al* (1994) who had reported *Aspergillus niger* as the most predominant in stored pistachio nuts. This variation could be due to preference for specific crop by the invading fungal species or that members of *Aspergillus* were more competitive than species of other genera in the locations (Talley *et al.*, 2002). Also, the moisture content of the seeds which ranged from 5 – 8% in the locations could be attributed to this variation. Since, *Aspergillus* species had reported to be more competitive under

reduced conditions of moisture content (Talley *et al*., 2002).

The effect of location on the distribution of fungal species showed that *Aspergillus flavus* had the highest occurrence (P<0.05) in Akpa in relation to other locations. This could be attributed to the possibility that the fungus could have had a higher inoculum leading to its higher occurrence in the location. Also higher relative humidity observed at this location could also have had more adverse effects on other species than on *A. flavus*. Also *Aspergillus ruber* in Ankpa, Ogaminana and Ajaka were statistically similar and also significantly (P de 0.05) higher than Kabba, while seeds from Ajaka were found to have the highest occurrence of *Syncephalastrum raceomosum*. This study therefore showed significant variation in the distribution of the fungal isolates with locations. This is supported by Talley *et al.* (2002), who reported that fungal abundance and richness are clearly affected by weather conditions in the locations or habitats.

CONCLUSION

A great variation occurred in the distribution of fungi with location of castor seed sampled. High occurrence of *Aspergillus* species was observed with *Aspergillus flavus* as the predominant mycoflora of stored castor seeds.

Recommendations

There is the need for mass enlightenment on the storage practices and conditions that will minimize growth of fungi on stored seeds especially in states where castor seeds are produced. It is also important to properly monitor and discourage the use of fungal-infected seeds in human and animal nutrition to avoid toxins usually associated with the growth of these fungi.

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REFERENCES

1. Acheru, G.N. and Onyeike, E.N. Chemical composition of selected Nigeria oilseeds and physicochemical properties of the oil extracts. *Food Chemistry*I, 2002; **77**: 431-437
2. Anjani, K., Raoof, M.A., Ashoka, P., Reddy, V. and Rao, C.H., Sources of resistance to major castor (*Ricinus communis* L.) diseases. *Plant Genetic Resources Newsletter, No*. 2004; **137**: 46-48.
3. Ataga, A.E. and Ota-Ibe, G., Seed-borne fungi of the wild mango (ogbono) [*Irvingia gabonensis* (Aubry-Leconte ex. Rorke) Bail] and their effects on food composition. *Nigerian Journal of Botany,* 2006; **19**: 54-60.
4. Bankole, S.A., Moisture content, mould invasion and seed geminability of stored melon seeds. *Mycopathologia*, 1993; **122**: 123-126.
5. Bankole, S.A. and Joda, A.O., Effect of lemon grass (*Cynbopogun citratus* Stapf.) Powder and essential oil on mould deterioration and aflatoxin contamination melon seeds (C*olosynthis citrulus* L.) *African Journal of Biotechnology,* 2004; **3**: 52-59.
6. Denizel, T., Jarvis, B. and Rolfe, E.J., A field survey of pistachio (*Pistachio vera*) nut production and storage in Turkey with particular reference to aflatoxin contamination. *Journal of Science and Food*, 1976; **27**: 1021-1026.
7. Dilek, H., Neda, A. and Mahmut, A., Mycoflora and aflatoxin contamination in shelled pistachio nuts. *Journal of Science Food Agriculture*, 1994; **66**: 273-278.
8. Frank, C., Baker castor oil Co., Bayonne, New Jersey 07002. *Journal of the American Oil Chemists Society*, 1974; **51**: 65-71.
9. ISTA (International Seed Testing Association). International Rules for Seed Testing. Seed Science and Rules and Annexes Technology, 1976; **4**: 1-177
10. Kuku, F.O., Fungal deterioration of Nigerian melon seeds (*Citrulus vulgaris* Schrad). Report of Nigerian Stored Product Research Institute. *Technical Report*, 1980; 7: 63-70.
11. Nakai, S. and Balatincz, J., Comparative examination of biologically active compounds of fatty oils of medicinal and alternative herbs separated by cold-moulding. *Journal of Agriculture and Food Chemistry,* 1997; **43**: 2850-2853.
12. Peterson, R. G., Design and Analysis of Experiments. Marcel Dekker Inc. New York and Bassel, 1985; 429pp.
13. Roetheli, J.C., Glaser, L.K. and Brigham, R.D.,

Castor: Assessing the feasibility of U.S. Production. Workshop Summary, Plain view, Tx, Sept. 1991; 18-19, 1990.

14. Talley, S.M., Coley, D.P. and Kursar, T.A., The effect of weather on fugal abundance and richness among 25 communities in the intermountain West. *Biomed Central Ecology*, 2002; **2**: 1-11.
15. Umechuruba, C. I. Out, K. A. and Ataga, A. E., The Role of Seed-borne *Aspagillus Flavus, A. Niger* and *Cacrophomina Phascolina* on Deterioration of Groundunt Seed: *International Biodeterioration and Biodegredatio*, 1992; **26**(1): 57-63.
16. Uzogora, S.G., Agu, L.N. and Uzogora, E.O., A review of traditional fermented foods condiments and beverages in Nigeria: their benefits and possible problems. *Ecology of Food and Nutrition*, 1990; **24**: 267-288.
17. Ward, H. S. and Diener, U. L., Biochemical changes in shelled peanuts caused by storage fungi. 1. Effect of *Aspergillus tamarii*, four species of *Aspergillus glaucus* group and *Penicillium citrinum*. *Phyptopathology,* 1961; **51**: 244-250.