

COMPONENT ANALYSIS FOR SEED MYCOFLORA IN FOUR CULTIVARS OF COWPEA (*VIGNA UNGUICULATA* [L] WALP)

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Abstract

Seeds of four cowpea varieties obtained from the National Seed Service, Ilorin, Kwara state were screened for seed borne fungi using agar plate and blotter methods. The localization of the fungi in different parts of the seeds was effected using component plating method. In all, nine fungal species, namely *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Cladosporium* sp., *Fusarium semitectum*, *Fusarium solani*, *Fusarium* sp., *Fusarium oxysporum* and *Penicillium* sp. were found to be associated with the seeds of tested cultivars. However, the fungi were isolated at different rates from the different cultivars as well as the components of the seed. The cotyledon was the main site of infection of cowpea seeds with 57%, 77.8%, 70% and 44.4% infection in IFE BROWN, ITA 397, ITA 90-22-2K and ITA 256 respectively. The embryo was the least affected in all other varieties except in ITA 256, which had the highest embryo infection of 33.3%. *Fusarium solani* was recovered from only the embryo of cv ITA 256. Isolation from the seed coat, cotyledons and embryo axis was respectively at the rate of 42%, 57% and 6% in IFE BROWN; 22%, 77.8% and 0% in ITA 397; 20%, 40% and 10% in ITA 90-22-2K and 22.2%, 44.4% and 33.3% in ITA 256. *Aspergillus flavus* and *Alternaria* sp were common to all cowpea varieties while *Cladosporium* sp was isolated only from ITA 90-22-2K.

Key words: Cowpea cultivars, Component plating and Blotter methods, fungi species

Introduction

Cowpea is a very important grain legume in Africa. In the 1970's, according to the Food and Agriculture Organisation (FAO) about 94% of the total world crop was produced in Africa (FAO, 1973). The situation has not changed much, as the crop continues to serve as cheap source of dietary protein for man and his livestock in the region. Seeds may be used as a concentrate feeds for animals while other parts may be processed as hay or silage. The fodder is always palatable to all type of live stocks (Okafor, 1992).

Notwithstanding the importance of this crop, the incidence of and losses caused by insect pests and diseases are the major constraints to cowpea production in Africa as they have always led to substantial reduction in quantity and quality of yield both on the field and in storage (Booker, 1965; Emechebe and Shoyinka, 1985; Thottapilly and Rossel, 1985; Ofuya and Lagunju, 1998; Adedidire and Ajayi, 2003; Balogun and Aliyu, 2005). Many cowpea diseases are caused by pathogens that are seed-borne. Shetty (1990) observed that among the seed-borne pathogens, fungi cause maximum damage. Many of the seed-borne fungi in cowpea reduce seed germination and they produce symptoms on infected seedlings (Saad *et al*, 1988).

The incidence of seedling diseases continues to rise owing to various biotic and environmental stress factors. Most studies in cowpea yield losses, however, have concentrated on insect pests and field pathogens with little or no attention to seed borne fungi and their eventual pathogenic importance on the field. As a necessary preliminary step in addressing this seeming inadequacy, this study therefore aimed at identifying the fungal flora associated with some cowpea varieties commonly issued to growers and hence widely cultivated in Ilorin, Kwara State Nigeria using two classical seed health testing methods as well as locating sites of infection for each fungus in the different seed components.

Materials and Methods

Source of seeds

The cowpea seeds used for the study (TA 90-22-2K, ITA 256, ITA 397 and IFE BROWN) were obtained from the National Seed Service Ilorin, Kwara State, a subsidiary of the Federal Ministry of Agriculture and Natural Resources. The seeds were sorted to separate apparently healthy ones from those that have been physically damaged.

Isolation and enumeration of fungi from the seeds

Blotter paper and agar plate methods were used to determine the incidence of cowpea seed borne fungi using the procedures of Saad *et al*. (1988). In the blotter method, 30 seeds per cowpea variety were placed on moist blotter paper in 9cm diameter Petri dishes at the rate of 10 seeds per plate. The same was repeated for the Potato Dextrose Agar (PDA) method. The seeds were first surface-sterilized for 10 minutes in 0.5% NaOCl. The PDA used was amended with 1% streptomycin sulphate to suppress bacterial growth. All plates were incubated at $28 \pm 2^\circ\text{C}$ in the dark for 8 days. The population of the fungal isolates was determined by direct counting of the colonies on the plates. The colonies were first designated on the basis of certain morphological characteristics especially colour and forms and were later counted with the use of tallies. Pure cultures were prepared from each of the designated colonies observed on the seeds by transferring hyphae or spores aseptically unto sterile media with alcohol-flamed inoculating needle in an

inoculating chamber. The plates were incubated at $28 \pm 2^{\circ}\text{C}$ in the dark for 8 days. Microscopic structures of fungal isolates were observed under a compound microscope for proper identification at 40x magnification.

Detection of fungi in seed components

The cowpea seeds were separated into component parts (testa, cotyledon and embryo) by soaking in sterile water for 8-12 hours after which the parts were carefully separated using ethanol-flamed dissecting knife. The separated components were later surface-sterilized in 0.5% NaOCl solution for 5 minutes. The components were then placed in 1.4% water agar and PDA in Petri dishes and incubated for 8 days at $28 \pm 2^{\circ}\text{C}$ (Saad *et al.*, 1988). After incubation, the cultivars were examined for fungal growth. Emerging colonies were counted and the data analyzed.

Results and Discussion

Nine fungal species were isolated from the seeds of the four cowpea varieties used in the study. The fungi appeared clearly on the culture plates between 5-7 days of incubation. The incidence of the fungi on blotter was different from that of agar plate. More fungi were isolated with agar plate method than with the blotter method. Only *Aspergillus flavus* and *Alternaria* sp. were recovered on blotter in all cowpea varieties, while agar plates had other species in addition to these (Table 1).

This observation was apparently due to the fact that PDA supplied nutrients for the growth of fastidious organisms. It can also be observed in the table that only eight out of the nine fungal isolates described are represented, while the ninth isolate (*Fusarium solani*) was recovered from the embryo of one of the cowpea varieties used during component plating experiment. Cowpea variety ITA 90-22-2K had the highest number of isolates while ITA 397 had the lowest number of isolates. *Aspergillus flavus* and *Alternaria* sp. were common to all cowpea varieties used. *Cladosporium* sp. was recovered only from ITA 90-22-2K.

The component plating study on PDA indicated that the cotyledon is the main site of infection of cowpea seeds with 57%, 77.8%, 70% and 44.4% infection in IFE BROWN, ITA 397, ITA 90-22-2K and ITA 256 respectively. The embryo is the least affected in all other varieties except in ITA 256 which had the highest embryo infection (33.3%) of the cowpea varieties used (Table 2). Zannmeyer and Thomas (1957) reported a similar result for *Colletotrichum* sp. (Sacc and Magn) Bri and Cav. in bean. Prasana (1985) had a contrary result with cowpea when he observed 92% fungal infection of the seed coat in one sample compared with 27% and 11% of embryo. Agarwal *et al.* (1972) attributed the difference in the degree of infection of the seed components to NaOCl pre-treatment. *Fusarium solani* was recovered only from the embryo of ITA 256.

On plain agar, fungal pathogens were detected only in the cotyledons of ITA 397, ITA 90-22-2K and in the testa of ITA 256. The fungi detected tallied with those isolated from whole cowpea seeds plated on butter (Table 3).

The infection of the cotyledon and embryo probably leads to deterioration of food reserves in cowpea seeds. Vijaya and Karam (1981) reported deterioration in the protein content of cowpea seeds infected by *A. flavus*. Protein and amino acids are also decreased in peanut seeds infected by *A. flavus* (Cherry et al., 1975). On the whole, infection of the embryo and the deterioration of food reserves in the cotyledon may have negative consequence on the percentage germinability of the seeds. The pathogenic evaluation of the fungi herein isolated would be carried out subsequently.

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Table 1: Mean population of fungal isolates from whole cowpea seeds on blotter and agar plates

Variety	Fungal isolates	Mean population on PDA	Mean population on blotter
IFE BROWN	<i>Aspergillus flavus</i>	0.50 ± 0.02	0.25 ± 0.00
	<i>Cladosporium</i> sp.	0.38 ± 0.22	-
	<i>Alternaria</i> sp.	0.13 ± 0.01	0.75 ± 0.00
	<i>Aspergillus niger</i>	0.13 ± 0.01	-
ITA 397	<i>Aspergillus flavus</i>	0.69 ± 0.09	1.00 ± 0.00
	<i>Alternaria</i> sp.	0.23 ± 0.00	2.00 ± 0.00
	<i>Fusarium semitectum</i>	0.08 ± 0.00	-
ITA 90-22-2K	<i>Aspergillus flavus</i>	0.33 ± 0.00	0.20 ± 0.00
	<i>Alternaria</i> sp.	0.13 ± 0.00	0.80 ± 0.00
	<i>Fusarium</i> sp.	0.07 ± 0.01	-
	<i>Aspergillus niger</i>	0.20 ± 0.00	-
	<i>Penicillium</i> sp.	0.07 ± 0.01	-
	<i>Fusarium oxysporum</i>	2.20 ± 0.00	-
ITA 256	<i>Aspergillus flavus</i>	0.19 ± 0.00	0.40 ± 0.00
	<i>Alternaria</i> sp.	0.69 ± 0.00	0.60 ± 0.00
	<i>Aspergillus niger</i>	0.06 ± 0.01	-
	<i>Fusarium oxysporum</i>	0.60 ± 0.01	-

Values are means from 10 seeds $\pm$ standard deviation

Table 2: Percentage infection of cowpea seed component by fungal isolates on PDA

Variety	Seed component	Isolates	Population on PDA	Total	%
IFE BROWN	Testa	<i>Alternaria</i> sp.	3	3	42
	Cotyledon	<i>Aspergillus flavus</i>	1		
		<i>Fusarium semitectum</i>	3	4	57
ITA 397	Testa	<i>Cladosporium</i> sp.	2	2	22.2
	Cotyledon	<i>Aspergillus flavus</i>	1		
		<i>Alternaria</i> sp.	6	7	77.8
ITA 90-22-2K	Testa	<i>Aspergillus flavus</i>	2	2	20
	Cotyledon	<i>Aspergillus flavus</i>	2		
		<i>Penicillium</i> sp.	5	7	70
	Embryo	<i>Alternaria</i> sp.	1	1	10
ITA 256	Testa	<i>Aspergillus flavus</i>	1		
	Cotyledon	<i>Alternaria</i> sp.	1	2	22.2
		<i>Aspergillus flavus</i>	1		
		<i>Fusarium oxysporum</i>	3	4	44.4
		<i>Alternaria</i> sp.	1		
	Embryo	<i>Fusarium solani</i>	2	3	33.3

Table 3: Percentage infection of cowpea seed component by fungal isolates on plain agar

Variety	Seed component	Isolates	Population on Plain Agar	Total	% infection
ITA 397	Cotyledon	<i>Alternaria</i> sp.	6	6	100
ITA 90-22-2K	Cotyledon	<i>Aspergillus flavus</i>	6	6	100
ITA 256	Testa	<i>Alternaria</i> sp.	6	6	100