

## FUNGI ASSOCIATED WITH *Hibiscus sabdariffa* LINN (YAKWA) SEED FROM PLATEAU STATE

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### ABSTRACT

A study was carried out on fungi associated with *Hibiscus sabdariffa* seeds (Yakwa ) obtained from three L.G.A of Plateau state, namely Jos North, Kanke and Ryom L.G.A. The seeds were collected from different sites which included Markets, farmlands and the Roadside. Standard Blotter Method and Agar method were used for the isolation of Fungi associated with the seeds. The following fungi; *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link Ex fr, *Fusarium oxysporum* Schlecht, *Penicillium chrysogenum* Thom and *Penicillium roqueforti* Thom were isolated from both blotter and agar methods. It was observed that *Fusarium oxysporum* was the dominant fungus in all the study areas with a mean occurrence of (20.3%), followed by *Aspergillus niger* (15.3%), *Penicillium chrysogenum* (14.3%), *Aspergillus flavus* (12.3%) and *Penicillium roqueforti*, which showed the least occurrence with a percentage of (9.6%). The occurrences of fungi isolate according to the L.G.A were observed with *Penicillium chrysogenum* occurring most at the market place in Jos North L.G.A by 31 isolates. In Kanke L.G.A, *Fusarium oxysporum* occurred most at the farmland and at the market place by 25 isolates each. In Ryom L.G.A, *Penicillium roqueforti* occurred most at the market place by 26 isolates.

Keywords: *Hibiscus sabdariffa* and fungi

### INTRODUCTION

Yakwa with English name; Roselle (*Hibiscus sabdariffa* Linn) is an angiosperm of the family Malvaceae. It is an annual herb that grows to 180 cm or more; stems are glabrous, while the lower leaves are ovate with the upper leaves being 3–5 palmately lobed. The flowers are auxiliary or in terminal racemes, the calyx enlarges at maturity and the fruit is fleshy and

white or red depending on the variety (Ojokoh, et. al., 2003). It is a tetraploid species with  $2n = 4x = 72$  (Adekpe et. al., 2000) and widely grown in tropical and subtropical regions of both hemispheres.

In Nigeria, the cultivation and intense utilization of the red and purple genotypes are found mainly in the Guinea and Sudan Savanna ecological zones of the country while the green genotype, hitherto ascribed little utility value is found in the Southwest (Alegbejo, 2000).

Today, roselle is attracting the attention of food and beverage manufacturers and pharmaceutical concerns who feel it may have exploitable possibilities as a natural food product and as a colorant to replace some synthetic dyes (Cook et al., 2000).

Diseases have been reported as a limiting factor to the production of roselle worldwide. The cultivated plants are susceptible to the various pathogens such as *Phytophthora parasitica*, *Phoma sabdariffae*, *Rhizoctonia solani* (Gomez-Leyva et al., 2008) and *F. oxysporum* (Amusa et al., 2005; Agbenin and Ogunlana, 2006).

Ooi and Saleh (1999) reported *F. oxysporum* as the principal causal agent of vascular wilt. For Boulanger et al. (1984), *F. oxysporum* is the causal agent of stem pod of the young plants of *H. sabdariffa*.

Seed borne diseases reduce yield and market value of seeds. Although so much human control efforts and the use of fungicides is being devoted to the preservation of seeds, over 40% of harvested seeds are lost through diseases (Tsai and Ou 2002).

This study was therefore undertaken to isolate fungi associated with *H. sabdariffa Linn* seeds obtained from Plateau state and to determine the pathogenic potentials of the isolated fungi.

## **MATERIALS AND METHODS**

The Yakwa seeds (*H. sabdariffa Linn*) were collected from the markets, farmland and environs of three Local Government Areas; Ryom LGA, Jos North LGA and Kanke LGA in Plateau state.

### **Isolation of Fungi**

The isolation of fungi was carried out using the Standard Blotter methods recommended by International Rules for Seed Testing (ISTA, 1976) and

Agar method (Klement and Voros, 1974)

The apparently healthy seeds of *H. sabdariffa Lin* were put in a beaker and 70% ethanol was poured into the beaker and left for 5min for sterilization purpose. It was then sieved with a sieve and the ethanol drained. Sterile distilled water was poured inside the beaker to rinse the seeds. The sterile seeds were picked with a sterile forceps one by one and plated out in tens on blotter paper moistened with sterile distilled water. The plating was done in replicate of three per area per L.G.A using complete randomized design. It was incubated at room temperature ( $28 \pm 2^{\circ}\text{C}$ ) for 7 days.

### **Inoculation/ Preparation of Pure Culture**

The work area was first surface sterilized using 70% ethanol and cotton wool. An inoculating needle was flamed until red hot then dip in alcohol to cool. (A hot needle will kill the mould that is to be transferred). With the heat-sterilized needle a small portion of the fungi colony were picked and transferred into a sterile plate containing the solidified Potato Dextrose Agar and the needle flamed again until red hot, to kill all adhering spores and hyphae. (Umechuruba and Elenwo 1997). After which, the culture were allowed to grow in a protected place that has as little air movement as possible.

After Incubation period, the developing fungi were identified using Stereobinocular microscope (6-50x) based on their habit characteristic. Single spore pure culture were obtained using Potato dextrose agar incorporated with Streptomycin in dishes. The inoculated Petri dishes were incubated at room temperature for 5-7 days in complete darkness.

### Pathogenicity test:

One hundred grams of healthy *Hibiscus sabdariffa* seeds were weighed out into 250ml conical flasks, plugged with non –absorbent cotton wool and covered with foil and then autoclaved at 15PSI at 121°C for 15 minute to eliminate any internal and external seed borne micro- organism. After autoclaving the flasks were allowed to cool and 100ml of sterile distilled water was added to each flask and shaken gently to wet all the seeds and to create a humid and conducive environment for the micro organisms to be inoculated to have an even distribution. Each flask containing seeds was inoculated with a disc of 7 day old mycelium spores of each fungus obtained from the pure culture of isolated fungi from infected seeds. This was done with a 1.5cm diameter sterile cork borer. The flask was left for 14days after which the seeds were plated out. Pathogenicity test was carried out on the healthy seeds to ensure that the organisms were actually associated with the seeds.

The identification of the isolated fungi was carried out with the aid of Manual on the description of fungi (Burnett and Hunter 1972). Frequency of occurrence of fungi was determined based on the Score method recommended by Ataga and Akueshi, 1986). The data generated were analyzed by one way analysis of variance.

### RESULTS

From this experiment, the following fungi pathogens were isolated. These organisms were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Penicillium roqueforti* from the three L. G. A. of Plateau State. The results are represented in Table 1.

Species compositions of fungi isolate according to the L.G.A were observed with *Penicillium chrysogenum* occurring most at the market place in Jos North L.G.A by 31 isolates. In Kanke L.G.A, *Fusarium oxysporum* occurred most at the farmland and at the market by 25 isolates each. In Ryom L.G.A, *Penicillium roqueforti* occurred most at the market place by 26 isolates.

**Frequency of Occurrence of Fungi :** Table 2 shows the frequency of occurrence of the fungi isolated. It was observed that *Fusarium oxysporum* was the dominant fungus in all the study areas with a mean occurrence of (20.3%), followed by *Aspergillus niger* (15.3%) occurrence, *Penicillium chrysogenum* (14.3%), *Aspergillus flavus* (12.3%) and *Penicillium roqueforti*, which showed the least occurrence with a percentage of (9.6%).

**Table 1:** Fungi isolated from *Hibiscus sabdariffa* Seed from three different locations each from three L.G.A of Plateau State

Fungi	Jos North L.G.A			Kanke L.G.A			Ryom L.G.A			Mean
	Farm Land	Market Place	Road Side	Farm Land	Market Place	Road Side	Farm Land	Market Place	Road Side	
<i>Fusarium oxysporum</i>	*2.00	1.60	1.65	1.75	1.75	1.60	1.50	1.70	1.95	1.72a
<i>Penicillium chrysogenum</i>	1.80	1.80	1.80	1.20	1.20	1.25	1.00	1.55	1.50	1.46c
<i>Aspergillus niger</i>	1.75	1.85	1.65	1.45	1.45	1.50	1.65	1.45	1.80	1.61b
<i>Penicillium roqueforti</i>	1.45	1.15	1.00	1.50	1.50	1.00	1.00	1.20	1.20	1.20d
<i>Aspergillus Flavus</i>	1.65	1.35	1.00	1.00	1.00	1.50	1.30	1.00	1.00	1.20d

\*Each value is the mean of two tests, each consisting of three replicates. Mean followed by the same letter within columns are not significantly different (P=0.05)

Scores based on a Scale in which 1= absence of fungus and 2 = presence of fungus, therefore any mean score above 1 indicates the presence of fungi.

**Table 2:** Frequency of Occurrences of Fungi according to L.G.A in %

Fungi Organism	Location and Percentage Frequency			
	Jos North	Kanke	Ryom	Mean
<i>Aspergillus niger</i>	10.5	14.5	21.0	*15.3
<i>Aspergillus flavus</i>	11.0	10.5	15.5	12.3
<i>Fusarium oxysporum</i>	31.5	37.0	23.5	20.3
<i>Penicillium chrysogenum</i>	15.5	12.5	15.0	14.3
<i>Penicillium roqueforti</i>	7.5	8.5	13.0	9.6

\*Mean value fungi for the three locations

## DISCUSSIONS

The above organisms were pathogenic and they cause a great damage to both the live of individuals and animals. Also these same organisms caused seed borne diseases and diseases in the growth of Yakwa plant. These diseases range from rots, fungal spots, necrosis, chlorosis, wilting of plants and plant death (Ooi and Saleh, 1999). These pathogenic fungi aid in the reduction of yield. This is one of the challenges hindering the growth of yakwa plant due to attack of seeds by seed borne pathogens.

This research agrees with the findings of the above author that *F. Oxysporum* is one of the major pathogen of *H. sabdariffa* seed.

In conclusion, this study emphasizes the role yakwa seeds play as a good medium for the growth of pathogenic fungi. These fungi are of economic importance to the farmer in the sense that when seeds are not healthy, we do not get healthy plant. Also there is the decrease in yield of the plant.

The Yakwa plant is a very important crop because of its so many uses. The need to get healthy seeds is of paramount importance. It is recommended that there should be adequate seed testing and scrutiny before planting so as to get healthy plants.

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