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Invitro Evaluation of *Boswellia Dalzaelii (Hutch), Prosopis Africana (Itto) and Vachellia Nilotica (L.)* Leaf Extract in Control of *Fusarium Seed Rot* of Maize

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Abstract:- The study aimed to evaluate the efficacy of leaf extract of *Boswellia dalzaelii (hutch), prosopis africana (itto)* and *vachellia nilotica (l),* in control of maize seed rot caused by fusarium *verticillioides*. Phytochemical screening of the plant extract was evaluated for the presence of secondary metabolite, the result revealed the presence of alkaloids, flavonoids, -glycosides, saponins, tannins, Phyto phenol, and terpenes at various degrees of concentration, which are likely the active compound of antimicrobial activities. The effects of leaf extract on the inhibition of spore germination and suppression of mycelial growth were evaluated on the PDA medium amended with extracts. The extracts showed varying degrees of efficacy against the pathogen. The results revealed lower inhibition at a lower concentration of 31.25mg/ml (31.27) and gradually decreased at a maximum concentration of 500mg/ml (11.78). Inhibition and suppression increased with increasing concentration of plant extracts on test organisms (tables 1 and 2. Similarly, the study also revealed that Mycelial growth was significantly ($P \leq 0.05$) reduced with increased concentration of all the extracts. However, extracts of Boswellia dalzielii, Prosopis africana, and Vechelia nilotica show a significant difference ($P \leq 0.05$) at a concentration of 31.25mg/ml, 60.5mg/ml, 125mg/ml, 250mg/ml and 500mg/ml.

Keywords:- *fusarium verticillioides*; maize seed rot; *Boswellia dalzaelii (hutch); Prosopis africana (itto)* and *Vachellia nilotica (l)* leaf extract

I. INTRODUCTION

Maize (*Zea mays* L.), is globally the most important grain crop and dietary staple food after wheat and rice, (Smale, 2001, Chulze 2010). It can alleviate poverty and play a key role in food security and economic well-being, particularly in sub-Saharan Africa (Chaudhary, 1983). the United States Development for Agriculture (USDA), in her 2022 publication reported that the world's total maize production was estimated at 1.2 billion metric tonnes in 2020 on an estimated cultivated area of 197m ha of land globally.

In Africa, Nigeria is the largest producer of maize with nearly 11.6 million tonnes in 2021 (USDA,2022), cultivated on a total land area of about 3.3 million hectares with an estimated yield of about 2.2 metric tonnes per hectare. Yet, its local maize demand continues to surpass supply, creating an annual demand gap of about 4 million metric tonnes. About 60 percent of Nigeria's maize is used for the production of poultry feeds, 25 percent is used up by the food and beverage industry, and the remaining is consumed by households. Ella. (2023) reported that maize corn silk has some health benefits which include; Working as a diuretic agent, treating urinary tract infections, keeping kidney stones at bay, facilitating blood clotting, lowering blood pressure, helping regulate blood sugar levels helping heal inflammatory ailments and conditions, helps fight cholesterol, source of vitamin C and helps fight obesity. Despite the importance of maize, its production is threatened by a microbial attack that often occurs during cultivation, such microbial infections can reduce the yield, and nutritional value of the seeds and negatively affects seed germination and growth (Govender, et al 2010).

Fungal pathogens such as *Fusarium* species, perenosclerospora species, aspergillosis species, penicillium species, and Alternaria are among spoilage pathogens associated with seed rot loss in pre-harvest and post-harvest maize production (Desjarden 2006). However, *Fusarium verticillioides* is the most prevalent pathogen of maize causing root, stalk, and ear rots globally, and it is a major concern to maize producers, consumers, and processing industries due to losses in grain yield and quality, Odebode, et, al, (2005). Another major concern is health complications associated with the consumption of grains contaminated with mycotoxins produced by these species (Desjasden 2006). Mycotoxins may cause fungal keratitis, kidney disorders, and esophageal and liver cancer (Rocha et al.2013). However, several fungal pathogens have been controlled using biological agents such as pruning wounds and other cut surfaces, diseases of leaves and flowers, fruits and vegetables, cereal, and leguminous plants Sobowale (2005). Efforts to control the diseases have not been successfully achieved using antagonistic organisms biopesticides and synthetic fungicides as each has one or more shortcomings.

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The efficacy of some botanicals, antagonistic organisms, and biopesticides on *F. verticillioides* ear rot in maize had been reported by Olowe, et, al, (2015), However, the use of resistant cultivars and metalaxyl fungicide to mitigate the disease, has not been successful due to environmental variability and pathogen resistance (Dalmacio, 2000). Chemical fungicides especially metalxyl and mancozeb exert a negative effect on soil-dwelling microorganisms and biochemical processes in the soil (Wyszkowska and Kucharski 2004; Mahadi F.,2006). The conventional synthetic fungicides as a strategy to manage the pathogen have triggered resistance in the pathogen, and it is not safe for the consumers and the environment (About 80 percent of pesticides used by farmers in Nigeria are highly toxic to humans and the environment (SWOFON 2022), they are not easily degraded and leave chemical residues on agricultural products, Burhanuddin, 2009). Seed treatments with fungicides are expensive and generally beyond the financial reach of resource-poor farmers (Raymundo, 2000). Therefore, the search for botanical fungicides to mitigate/and or manage the disease becomes a necessary evil. Therefore, this study aims to evaluate *in- vitro* antifungal activity of plant extracts obtained from three selected medicinal plants (*Boswelia dazielii*, *Prosopis africana* and *Vachellia nilotica*) leaf extracts in the control of fusarium seed rot of maize.

II. MATERIALS AND METHODS

➢ *Source of Plant Materials and Preparation of Leaf Extract*

Fresh leaves of *Boswellia dalzaelii (hutch), Prosopis africana (into) and Vachellia nilotica (l.)* were collected from the natural surroundings of Biu local Government Area, Borno State, Nigeria in September 2024 and identified by a taxonomist in the Department of Plant Science and Biotechnology University of Jos, Nigeria

The collected leaves were sterilized in (10% NaOCL for 2 min) rinsed in five times with sterile distilled water and air dried at 28ºC for 7 days at room temperature. The air-dried leaves were pounded with the aid of a pestle and motor to obtain 500g of powder of each plant species. Water extract was obtained by adding each powder (100g) to 500 ml of sterile distilled water in 1000ml conical flasks. Each suspension was hand shaken for two minutes and allowed to stand for 10- 12 hours before being filtered using muslin cloth. The extract was evaporated to dryness in a water bath. Ten grams of the extract were reconstituted in 50 ml of sterile distilled water and used as the test solution. Each plant extract was placed separately in a sterilized, filtered sealed container and stored in the refrigerator at a temperature of 5°C for further use.

About 10ml part of each plant extract was dissolved separately in 0.5ml of Tween 20 droplets added as a diffusion material to obtain different concentrations of 31.25mg/ml,62.5mg/ml,125mg/ml,250mg/ml and500mg/ml of plant extracts.

➢ *Isolation and identification of pathogen.*

A rotten maize seed showing a typical symptom of the disease was collected from local farmers at Biu market, Borno state, Nigeria. The infected maize seeds were surface-sterilized in 10% sodium hypochlorite (NaOCL) solution for 2 minutes to remove surface contaminations and rinsed twice in sterile distilled water. Five infected seeds were plated out in Potato Dextrose Agar (PDA). The plates were incubated at 28ºC for 7 days. Five subcultures were made on PDA to obtain a pure culture of the fungus. Morphological identification was done to ascertain its identity with the aid of an electron microscope and identification guide (Barnett and Hunter, 1999). *Fusarium verticilliodes(sacc)* were observed under the microscope to possess white and pale colonies salmon colored with low and often ropy mycelium and a powdery texture due to the production of chained microconidia. However, on the PDA, is variable, pale salmon, greyish violet.

➢ *Phytochemical Analysis*

Phytochemical analysis was carried out on the three plant extracts to reveal the presence of secondary metabolites in them using standard procedures of Evans, (1996). Evan and Trease (2002). table1

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Table 1: Qualitative Phytochemical Screening of Crude Plant Extracts.Plants Extract

Metabolite	*B.dalzealii*	*P.africana*	*V.nilotica*
Alkaloids	+	+++	++
Saponins	++	-	+
Tannins	+++	+	+++
Flavonoids	+++	+	+++
Carbohydrate	++	-	++
Polyphenol	+++	+	+++
Steroids	+++	+	+
Anthraquinones	-	-	-
Cardiac glycosides	++	+++	++
Terpenes	+++	+++	-

Key.
- not detected
+ slightly present
++ moderately present
+++ highly present

➢ *Effect of Plant Extract on Spore Germination*

To determine the effect of extracts against spore germination. Plates with each extract were inoculated with one drop (0.1 ml) of spores. Spore count was done by cutting 1cm of agar from treated and untreated plates, and transferring the spores into a 50ml beaker containing 10 ml distilled water with the aid of a 1-inch brush. The suspension was stirred vigorously with a glass rod, each concentration was counted with a hemocytometer slide and a light microscope. Thus, percentage spore germination was obtained as;

$$\%\text{spore} = \frac{sc - st}{sc} \times \frac{100}{1}$$

where; sc spore number of control plates
st spore number of treated plates

III. EFFECTS OF EXTRACT ON SPORE GERMINATION

Table 1. Effect of Plant Extracts on Spore's Germination of *fusarium verticilliodes* Isolated on Zea Mays Infected Seed.

Organism	Concentrations (mg/ml) 31.25 mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500 m/ml
B.dalzealii	31.27± 0.03^{d}	27.49± 0.10^{d}	17.29± 0.10^{d}	13.46± 0.07^{d}	11.78± 0.07^{d}
P.africana	62.26± 0.18^{c}	60.27± 0.04^{c}	53.35± 0.55^{c}	45.61± 0.09^{c}	37.83± 0.08^{c}
V.nilotica	81.44± 0.07^{b}	72.64± 0.29^{b}	57.48± 0.38^{b}	50.39± 0.10^{b}	43.45± 0.10^{b}
Metalaxyl	15.53± 0.06^{e}	11.25± 0.03^{e}	8.47± 0.07^{e}	7.78± 0.07^{e}	6.73± 0.05^{e}
Water	95.02± 0.33^{a}	95.02± 0.33^{a}	95.02± 0.33^{a}	95.02± 0.33^{a}	95.02± 0.33^{a}
L.S.D	0.62				
P-value	<0.0001 ****				

At P≤0.05 there was a significant difference in the effect of plant extracts on spore germination of fusarium verticilliodesi on *Zea mays* infected seed. Values are presented as mean±standard error of means. The ranking was done across the organisms and values with the same superscript are not significant.

Table 2 Effect of Plant Extracts on Inhibition of Mycelial Growth of Fusarium Verticillioides of Zea Mays Infected Seed.

Organism	31.25 mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500 m/ml
B.dalzealii	41.51± 0.46^{d}	33.37± 0.08^{d}	25.27± 0.33^{d}	21.33± 0.06^{d}	17.41± 0.13^{d}
P.africana	67.46± 0.24^{c}	62.73± 0.71^{c}	57.48± 0.10^{c}	47.74± 0.05^{c}	39.31± 0.17^{c}
V.nilotica	87.53± 0.06^{b}	76.12± 0.16^{b}	67.39± 0.26^{b}	58.32± 0.19^{b}	53.39± 0.04^{b}
Metalaxyl	19.59± 0.11^{e}	12.26± 0.04^{e}	15.47± 0.05^{e}	13.77± 0.04^{e}	9.59± 0.19^{e}
Water	96.25± 0.48^{a}	96.25± 0.48^{a}	96.25± 0.48^{a}	96.25± 0.48^{a}	96.25± 0.48^{a}
L.S.D	0.84				
P-value	<0.0001 ****				

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At P≤0.05 there was a significant difference in the effect of plant extracts on spore germination of fusarium verticilliodesi on *Zea mays* infected seed. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same super script are not significant.

IV. RESULTS AND DISCUSSION

The results of the phytochemical analysis showed that the three plant extracts had flavonoids, saponins polyphenols which have strong antifungal properties among others, as well as essential oils and their constituents (Table 1). The inhibitory effects of these plant extracts on the pathogen may be due to the presence of the above phytochemicals. This observation conformed with a report of Haralampidis et al., (2001) who reported that secondary metabolites have been implicated as chemical defense against attack by soil fungi.

The effect of plant extract on spore germination was examined under different concentrations (13.25mg/ml, 60.5mg/ml, 125mg/ml, 250mg/ml, and 500mg/ml), of plant extracts of *Boswellia dalzielii*, Prosopis *africana*, *and Vachelia nilotica*. The extracts were tested for their efficacy against *fusarium Verticillioides*. The results revealed higher germination of spores at a lower concentration of 31.25mg/ml (31.27) and gradually decreased to a minimum at a higher concentration of 500mg/ml (11.78), of Boswellia dalzielii extract. Similarly, it was the same in Prosopis Africana and vechelia nilotica extract. Inhibition of spore germination increased with increasing concentration of plant extracts on test organisms (Table 1). However, there was a significant difference among all the extract at(P≤0.05) at different concentrations. This result confirmed the finding of Fredrick, et, al. (2024) The extract becomes more effective at 500mg/ml of inoculation. There were significant differences between *Boswellia dalzielii, Prosopis africana*, and *Vachelia nilotica* at(P≤0.05), Table 1. The result also revealed that Boswellia dalzielii was more effective in suppressing the pathogen's spore germination. The higher effect of the extract of B.dalzielii on F. verticilliodes is consistent with the study of Ajaiyeoba(2002), who reported a similar higher effect of ethanol extract of P. biglobosa on S. aureus.

The effect of plant extracts on suppressing mycelial growth of the pathogen revealed that extract promote significantly at (P ≤ 0.05) suppression of fusarium verticillioides in all the tested concentrations. The results show that there was increased suppression with increased concentration among all the extracts, at31.25 mg/ml (41.51) was the lowest suppression of all the concentrates. The highest maximum suppression at 500mg/ml was (17.41 , 39.31 53.39 for all the extract Table 2.The control did not show any level of suppression, but metalaxyl effectively controlled the pathogen. The study suggested that farmers can effectively manage the disease with the plant extract, as there are environmentally friendly.

V. CONCLUSION

The study reveals the potential of natural plant products in managing maize seed rot caused by *fusarium Verticillioides*. The study also revealed that a plant extract that could inhibit the growth of mycelia and spore germination of the pathogen is *Boswelia dalzielii* followed by *vachelia nilotica* This can be recommended to smallholder farmers since it is available, cheap, and environmentally friendly and they /are highly degradable as compared to the synthetic fungicide which leaves their residues in the crops.

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