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TREATMENT OF FUNGI ASSOCIATED WITH MAIZE SEEDS USING EXTRACT OF TURMERIC RHIZOME (*CURCUMA LONGA*) AND MORINGA LEAF (*MORINGA OLEIFERA*)

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ABSTRACT

This research focused on the use of different concentration of aqueous extract of Moringa leaf and Turmeric rhizome as seed treatment agents against fungi associated with maize seeds. The study was designed using 2 x 3 factorial in completely randomized design. This research was carried out in the Biology Laboratory of Federal College of Forestry, Jos from April to June 2018. Untreated maize seeds were collected from farmers in three different areas of Mazah village in Jos North LG. Aqueous extract of Turmeric rhizome and Moringa leaf at 25, 50, and 75 ml concentration were used as seed treatment agent against fungi. Maize seeds were soaked in prepared extracts for 1 hour and then cultured on petri dishes having moist filter paper, control samples were soaked in sterile water. Infested seeds were counted manually after twenty days. Results were analyzed using one-way analysis of variance at 95% confidence level. Penicillium sp, Fusarium solani, Aspergillus niger and Aspergillus flavus were isolated from the untreated maize seeds. Fusarium solani gave the highest percentage occurrence of 35.48. Fifty milliliters (50ml) concentration of Turmeric rhizome had an antifungal mean value of 3.67 after 20days. The control (untreated) showed the antifungal mean value of 6.67 after 20 days. The results obtained revealed significance between the treatment and the control. The results indicate Turmeric rhizome and Moringa leaf extracts could be used in seed dressing against maize infection caused by fungi.

Keywords: *Treatment, Fungi Associated, Maize Seeds, Extract, Turmeric Rhizome (Curcuma Longa), Moringa Leaf (Moringa Oleifera).*

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in the world and rank third next to wheat and rice [1]. Maize suffers from several fungal diseases, which are seed borne in nature [2]. The incidence of seed-borne pathogen cause germination failure as well as a reduction in seedling vigor which ultimately reduces the yield. Control of the seed borne fungal pathogen by chemical fungicide is costly and hazardous for the environment. Therefore, the use of plant material is important in reducing the prevalence of the seed borne pathogens associated with the maize seed and to enhance percentage of maize seed germination and seedling vigor. Maize is an important cereal in many developed and developing countries of the world. It is widely used for animal feed and industrial raw materials such as starches, acids and alcohols in the developed countries, whereas it serves as food in developing countries. Recently there has been interest in using maize for the production of ethanol in the United States and China, as a substitute for petroleum-based fuels [3]. In Nigeria, maize is the most popular food crop in the domestic market. It is a good substitute for other food crops like sorghum, millet, plantain, yam and cocoyam when they are in short supply. It is the main feedstuff for poultry and other livestock [4]. In 2012, the total world production of maize was 875,226,630 tons, with the United States, China, and Brazil harvesting 31%, 24%, and 8% of the total production of maize, respectively [5]. Despite its importance, maize has many production constraints which prevent farmers from getting maximum yield. Drought, fire, flood, poor soil fertility, high labour cost, transportation problems and poor agronomic practices are some of the production constraints of maize [6]. Pest and diseases also formed some of the problems that characterize maize production in the world. The diseases of maize seeds include; seed rot (*Fusarium moniliforme, Fusarium oxysporum, Penicillium* species), seedling blight (*Aspergillus*

species, *Penicillium* species) Biperis leaf spot (*Biperis maydis*), Curvularia leaf spot (*Curvularia lunada*) etc. The study aims to determine the effect of different concentration of Turmeric rhizome and Moringa leaf extracts in maize seed treatment against fungal pathogens associated with maize seeds.

MATERIALS AND METHODS

Study Area

The experiment was carried out in the Biology Laboratory of Federal College of Forestry Jos, North Local Government Area of Plateau State. Jos is located between latitude 7-11° North and longitude 7 -8° East. Temperature ranges between 10-32°C and main annual rainfall is about 1340 mm, with an average elevation of 1200 mm above sea level.

Collection of Samples

Fresh disease-free leaves of *Moringa oleifera* and rhizomes of Tumeric (*Curcuma longa*) 100 g each were purchased from Farin - gada market of Jos North Local Government area, Plateau State. Five hundred seeds of healthy local maize (Waxy corn (F1) colorful) previously stored were collected from farmers at three different areas (Alibah, Anagohom and Anabor) in Mazah village of Jos North Local Government Area at different locations. Maize variety Waxy corn (F1) colorful was used for the research due to its availability and farmer's choice in the study area [7].

Determination of Fungi Presence on Collected Maize Seed

Three pieces of 9 cm filter paper (Whatman No 1) were soaked in distilled water and placed at the bottom of the Petri dish. Untreated healthy maize seeds collected from farmers at three different areas in Mazah village were used for fungi testing. This was done using Blotter method, maize seeds collected from different locations were randomly selected and 20 seeds were placed in each Petri dish containing moist filter paper based on the number of the areas. The seeds were incubated at room temperature for 10 days. Potato Dextrose Agar (PDA) was prepared according to the

manufacturer's instruction. Eighty gram of Gentamycin was added to the prepared 500 ml of agar PDA media to inhibit bacteria growth. Fungi developed from incubated maize seeds were inoculated on the solidified PDA using inoculating needle flamed over a burner flame, the solidified inoculated plates were kept at room temperature (28± 2°C) until visible growth appeared on the plates. The fungal colonies that grew from the incubated plates were further sub-cultured onto fresh medium until pure cultures were obtained. The percentage occurrence of the fungi isolated was determined using the following formula:

$$\text{Percentage occurrence} = \frac{\text{Number of isolates}}{\text{Number of total isolates}} \times 100 \quad (1)$$

Identification of Fungi Isolates

Macroscopic and Microscopic examination were used to determine the morphological characteristic of the fungi isolates. For macroscopic identification, colony characteristics such as appearance and change in color were observed on the Petri plates. For microscopic examination, the sterile inoculating needle was used to pick a little portion of five-day-old isolate and placed on a sterile glass slide, the slide was stained with lacto phenol cotton blue, mixed and covered with a slip. The slide was then viewed under the microscope, using x10 and x40 magnification. Shapes of the conidia and conidiophores were taken note of. These features were matched with standards described by Barnett and Hunter [8] and Booth [9] and identified by the help of an expert.

Preparation of Plant Extract (Turmeric Rhizome and Moringa Leaf)

Collected fresh plants were detached and washed first in tap water than in distilled water and air dried at room temperature. 100 g of fresh sample was chopped and then crushed in a surface sterilized mortar and pestle by adding 100 ml distilled water (1:1 w/v). The extract was filtered through two layers of Muslim cloth and was used as a stock solution [10].

Determination of Extract Concentrations

The plant extracts were used at 25, 50 and 75 % concentrations, for which 25, 50 and 75 ml of stock solution was mixed with 75, 50 and 25 ml of distilled water respectively.

Seed Treatment Using Turmeric rhizome and Moringa Leaf Extracts

Twenty healthy untreated maize seeds were treated using sterilized aqueous extracts of the plant (Turmeric and Moringa) separately by dipping the maize seeds into the different concentrations (25, 50 and 75 ml) of the aqueous extracts for 1 hour. For the control, twenty healthy untreated seeds dipped in distilled water for 1 hour served as the control treatment.

Antifungal Efficacy of Turmeric Rhizome and Moringa Extract

After treating the seeds, the seeds were placed in sterile Petri dishes containing four layers of blotting paper soaked in distilled water. Each Petri dish contained 20 seeds in replicates. The Petri dishes were then kept in an incubation chamber at room temperature for 10 days and infected seeds counted manually after 10, 15, 20 days after sowing [10].

Treatment and Experimental Design

The experimental layout used was 2 x 3 factorial in complete random design (CRD) whereby the three treatments were replicated three times (0, 25, 50 and 75 ml).

Treatment combinations = $2 \times 4 = 8$ Replications = 3 Total plots = $3 \times 8 = 24$.

Data Analysis

Data analysis was carried out using turkey pairwise comparison one-way analysis of variance (ANOVA) and T-test to compare means. Means separation was determined using Duncan multiple range test. Significance difference was determined at 95% confidence.

RESULTS

Isolation and Identification of Fungi

A total number of sixty-two fungal species were isolated. The result obtained showed Anagohom with the highest fungi occurrence of 45.16% compared to Alibah and Anabor which had 29.03 and 25.16% respectively

(Table 1). The percentage distribution results showed a significant difference at p = 0.05. The fungi isolated were identified macroscopically and microscopically as *Penicillium* sp, *Fusarium* sp, *Aspergillus niger* and *Aspergillus flavus*. *Fusarium solani* (based on the characteristics presented in Table 2) gave the highest percentage occurrence of 35.48 while the *Penicillum* sp showed the lowest percentage occurrence of 11.29. The result in Table 4 showed a significant difference in fungal percentage occurrence.

Antifungal Efficacy of Moringa Leaf Extracts

Table 5 below showed the mean antifungal effect of Moringa after 10, 15 and 20 days at a different concentration of 25, 50 and 75 ml. The control (untreated) maize seeds have the mean values of 4.33 after 10 days, 5.00 after 15 days and 6.7 after 20 days. The 25 ml concentration showed mean values of 2.33 after 10 days and 2.6 after 15 days and 3.00 after 20 days. The 75 ml concentration indicated mean values of 1.33 after 10 days, 1.7 after 15 days and 2.00 after 20 days. The result also showed that Moringa leaf extract at a higher concentration of 75 ml has the highest effect of 2.00 mean value at 20 days' interval. The result obtained showed that the effect of Moringa extract antifungal activities is directly proportional to the extract concentration and days of exposure.

Table 1: Percentage Distribution of Fungi Species in Alibah, Anabor, Anagohom

Area	No of fungi occurrence	Percentage distribution
Alibah	18	29.03[a]
Anabor	16	25.81[a]
Anagohom	28	45.16[b]
SE±	-	5.64
LSD	-	*
Total	**62**	**100**

a, b, c = means separation indicating level of significance; Means within a column followed by the same letters are not significantly different (P = 0.05) using Duncan Multiple Range Test. * = Significant at 95% level of probability. LSD = Least significant difference.

Table 2: Fungi Features/ Characteristics

S/NO	FUNGI	FEATURES/CHARACTERISTICS
1.	*Aspergillus flavus*	It consists of a dense of yellow-green conidiophores and conidials with a head typically radiated, and splitting into several loose columns. Conidiophores become dark yellow-green, hyaline and roughened.
2.	*Penicillium species*	Consist of a dense felt of conidiosphores. Appeared leathery, blue green and yellow to orange.
3.	*Fusarium solani*	Cottony, whitish, letter becoming yellow, pink-red. Reverse in yellow to brownish shades.
4.	*Aspergiluus niger*	It consists of a compact white basal felt with a dense layer at dark brown to black conidiosphorses, conidial head radiate ending to split" into loose columns with time.

Table 3. Morphological View of Fungi Isolates

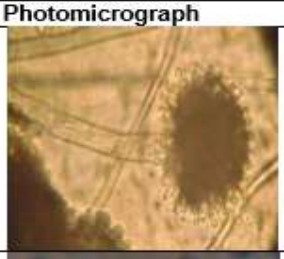
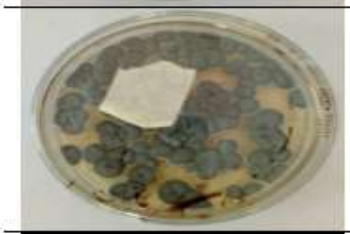
Appearance on PDA	Photomicrograph	Probable Isolates
		*Aspergillus flavus*
		*Penicillum* sp
		*Fusarium Solani*
		*Aspergillus niger*

Table 4. Percentage occurrence of fungal species

Fungi isolates	Number of occurrences	Percentage occurrence
Aspergillus niger	19	30.65[a]
Fusarium species	22	35.48[a]
Aspergillus flavus	14	22.58[b]
Penicillium species	7	11.29[c]
SE±	-	5.86
LSD	-	*
Total	62	100

Table 5: The Antifungal Mean Effect of Moringa Leaf Extract

Concentration (ml)	Number of Days		
	10	15	20
Control	4.33[c]	5.00[b]	6.67[b]
25	2.33[b]	2.67[a]	3.00[a]
50	2.00[ab]	2.33[a]	2.67[a]
75	1.33[a]	1.67[a]	2.00[a]
SE±	0.41	0.58	0.53
LSD	*	*	*

Table 6. The Antifungal Mean Effect of Turmeric Rhizome Extracts

Concentration (ml)	Number of Days		
	10	15	20
Control	4.33[b]	5.00[c]	6.67[b]
25	2.00[a]	2.33[a]	3.00[a]
50	2.33[a]	3.00[ab]	3.67[a]
75	3.67[ab]	4.33[bc]	5.67[b]
SE±	0.71	0.67	0.71
LSD	*	*	*

a, b, c = means separation indicating level of significance; Means within a column followed by the same letters are not significantly different (P = 0.05) using Duncan Multiple Range Test. * = Significant at 95% level of probability. LSD = Least significant difference.

DISCUSSION

Seed is the most important unit of crop production and its health plays important role in agriculture, which determines the plant population and final yield. One of the major constraints that deteriorate the seed quality is the seed-borne fungi present inside or on the surface of seeds [7]. Leaf

extracts of many higher plants have been reported to possess antifungal activity under laboratory trails [10]. Farmers are faced with challenges of fungal infestation which has led to great economic losses. Botanical seed dressing agents is gradually gaining attention due to the problems associated with the use of synthetic pesticides, synthetic pesticides are toxic, not friendly to the environment and also not affordable by most farmers especially the local farmers that own small farms due to its expensive nature [11]. Plant extracts of many plant parts have to possess antifungal activities in vitro [12]. The finding of this research revealed that fungal pathogen associated with stored maize seeds include; *Penicillium* sp, *Fusarium solani*, *Aspergillus niger* and *Aspergillus flavus*, which is in line with the findings of Nirmal *et al.* [13] which says Maize seeds diseases are produced by many species of fungi such as *Aspergillus, Fusarium, Penicillium*, *Claviceps* and *Altermaria* genera that are associated with maize seeds. The result of percentage occurrence of fungi isolates showed that *Fusarium* species has the highest percentage occurrence of 35.48 %, the result agrees with the work conducted by Forsberg *et al.* [14] which state that *Fusarium* occurs throughout the cultivation period of maize. In the present work antifungal activity of two plants extracts, Moringa leaf and Turmeric rhizome were assessed at the concentration of 25, 50 and 75 ml against fungal growth by blotter technique. Similar investigation on the antifungal activity of plant extracts against seed-borne fungi has been reported by many researchers [15-17]. Turmeric rhizome extract was found more effective in inhibition of fungal growth than Moringa leaf at a lower concentration. Antifungal activity of the extract at different concentrations are found to be effective [18,19] this could be due to the activities of essential oils, a mixture of terpenoids, aromatic phenols and many other compounds such as 1, 8-Cineole which has proved to have fungicidal properties [20].

The result obtained using Turmeric extract at 25 ml concentration showed means value of 3.00 infestation which is the least compared to 50 ml and 75 ml concentration. Turmeric at small concentration after 20 days of the experiment was more effective than the other two concentrations (50 ml

and 75 ml). This result agrees with the finding of Abiamere *et al*. [21] which evaluated the efficacy of Moringa, Ginger and *Chromolena odorata* plant as treatments agent against seed borne fungi associated with Cowpea. The 75 ml concentration after 20 days of seed incubation had high mean value infestation of 5.67, showing that turmeric at higher concentration is less effective in control of fungal pathogen associated with maize seed. According to Lee *et al*. [22] fungicidal activities of Turmeric has been found effective in controlling certain agricultural and animal pest or pathogens due to the presence bioactive constituents that interfere with fungi behavior and growth. This present study has therefore shown that plant extract of Moringa and Turmeric can be used as a fungicidal seed treatment for the control of seed borne fungi of maize seeds.

CONCLUSION

Based on data from the present study, Moringa leaf and Turmeric rhizome extracts at different concentrations showed a significant difference at 95% confidence level. The result showed that Turmeric (concentration 25 and 50 ml) and Moringa (concentration 75 ml) gave a mean antifungal effect of 2.00, 2.33 and 1.33 respectively. This showed the extracts can be used in seed dressing against fungal pathogens associated with maize seeds.

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