

Original Research Article

THE EFFICACY OF TURMERIC RHIZOME (*Curcuma longa*) and MORINGA LEAVES (*Moringa oleifera*) AQUEOUS EXTRACT IN SEED TREATMENT AGAINST FUNGI ASSOCIATED WITH MAIZE SEEDS

ABSTRACT

AIM: This research was focused on the use of different concentration of aqueous extract of Moringa leaves and Turmeric rhizome as seed treatment agents against fungi associated with maize seeds

Study Design- Completely randomized design

Place and Duration of Study: This research was carried out in Biology laboratory of Federal college of Forestry, Jos from April to June, 2018.

Methodology: Untreated maize seeds were collected from farmers in three areas of Mazah village in Jos North LG. Maize seed were tested for the presence of fungi by culturing on Petri dish having moist filter paper. Aqueous extract of Turmeric rhizome and Moringa leaves at 25, 50, and 75ml concentration were used as seed treatment agent against fungi. The seeds were soaked in the extracts for 1 hour and then cultured on petri dish having moist filter paper, control samples were soaked in sterile water. Infested seeds were counted manually after twenty days. Results were analysed using one way analysis of variance at 95% confidence level

Result: *Penicillium sp*, *Fusarium sp*, *Aspergillus niger* and *Aspergillus flavus* were isolated from the untreated maize seed. *Fusarium* species gave the highest percentage occurrence of 35.48. 50ml concentration of Turmeric rhizome has antifungal mean value of 3.67 after 20days. The control (untreated) maize seeds has antifungal mean value of 6.67 after 20days. The results obtained revealed significance between the treatment and the control.

Conclusion: The results indicate Turmeric rhizome and Moringa leaves extracts could be used in seed dressing against maize infection cause by fungi.

Keywords: Turmeric; Moringa; Maize; Fusarium; Jos

1 INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crop in the world and rank third next to wheat and rice [1]. From the recent past, it has a good potential as a cereal crop

33 due to its low cost of production, wide adaptability and diversified used. Maize suffers from
 34 28 diseases in which are seed borne in nature [2]. Among the diseases; seed rot (*Fusarium*
 35 *moniliforme*, *Fusarium oxysporum*, *Penicillium species*.) seedling blight (*Aspergillus species*,
 36 *Penicilium species*) *Biperis* leaf spot (*Biperis maydis*), *Curvularia* leaf spot (*Curvularia*
 37 *lunada*) etc. Incidence of seed borne pathogen cause germination failure as well as reduction
 38 in seedling vigor which ultimately reduces the yields. Control of the seed borne fungal
 39 pathogen by chemical fungicide is costly and hazardous for the environment. Therefore, the
 40 used of plant materials is to reduce the prevalence of the seed borne pathogen associated with
 41 the maize seeds and to enhance percentage of maize seeds germination and seedling vigor.

42 Maize is an important cereal in many developed and developing countries of the
 43 world. It is widely used for animal feed and industrial raw materials such as starches, acids
 44 and alcohols in the developed countries where as the developing countries use it in general
 45 for food. Recently there has been interest in using maize for the production of ethanol in the
 46 United States and China, as substituted for petroleum based fuels [3]. In Nigeria maize is the
 47 most popular food crops in the domestic market. It fills in other food crops like sorghum,
 48 millet, plantain, yam and cocoyam when they are in short supply. It is the main feeds stuff
 49 for poultry and other livestock [4].

50 Maize is widely cultivated throughout the world, and a greater weight of it is
 51 produced each year than any other grain. Worldwide production was around 800 million
 52 tones in 2007, just slightly more than rice (650 million tones) or wheat (600 million tones) in
 53 2007, over 150 hectares of maize were planted worldwide, with yield of 4970. 9
 54 kilogram/hectare [5]. Despite is importance, maize has many production constraints which
 55 prevent farmers from getting maximum yield. Drought, fire, flood, poor soil fertility, high
 56 labour cost, transportation problems and poor agronomic practices are some of the production
 57 constraints of maize [6]. Pest and diseases are also some of the problems that characterize

maize production in the world. The aim of the study is to determine the effect of different concentration of Turmeric rhizome and Moringa leaf (*Curcoma longa* and *Moringa oleifera*) extracts in maize seed treatment against fungal pathogens associated with maize seeds.

2 MATERIALS AND METHODS

2.1 Study Area

The experiment was carried out in Biological Laboratory of Federal College of Forestry Jos, North Local Government Area of Plateau State. Jos is located between latitude 7-11⁰ North and longitude 7 -8⁰E. Temperature ranges between 10-32⁰C and main annual rainfall is about 1340mm, with an average elevation of 1200mm above sea level.

2.2 Collection of Samples

Fresh disease free leaves of Moringa (*Moringa oleifera*) and rhizomes of Tumeric (*Curcoma longa*) 100g each was collected from Farin - gada market of Jos North Local Government area, Plateau State. 500 seeds of Healthy local maize (Waxy corn (F1) colourful) was collected from farmers at three different areas (Alibah, Anagohom and Anabor) in Mazah village of Jos North Local Government Area at different locations. Waxy corn (F1) colourful was collected due to its availability and farmers choice in study area [7].

2.3. Preparation of Plant Extract (Tumeric rhizome and Moringa leaves)

The collected plant materials (Moringa leaves and Tumeric rhizome) samples (100g each) were washed very carefully with distilled water. The plant parts were grounded using mortar and pestle differently. Then the grounded plant materials was dipped into 100ml of distilled water for 48 hours (2 days) for complete extraction of the active ingredient from the extracted sample each. After that, the water and grounded materials were filtered differently using a very fine and clean sieve for each plant extract, the sieve was washed with distilled water for further usage [8].

2.4. Determination of Fungi Presence on Collected Maize Seed

Three pieces of 9cm filter paper (whatman No 1) were soaked in distilled water and place at the bottom of the petri dish. Untreated healthy maize seeds collected from farmers at three different areas in Mazah village was used for fungi testing. This was done using Blotter method, maize seeds collected from different locations was randomly selected and 20 seeds were placed in each petri dish containing moist filter paper base on the number of the areas and the seeds were incubated at room temperature for 10 days. Potato Dextrose Agar (PDA) was prepared according to manufacturer's instruction. 80g of Gentamycin was added to the prepared 500ml of agar (PDA) media in order to inhibit bacteria growth. Fungi developed from incubated maize seeds were inoculated in the solidified potato Dextrose agar using inoculating needle flamed over a burner flame, the solidified inoculated plates was kept at room temperature ($28 \pm 2^{\circ}\text{C}$) until visible growth appeared on the plates. The fungal colonies that grew from the incubated plates were further sub-culture into fresh medium until pure cultures were obtained.

2.5. Identification of Fungi Isolates

Physical and Microscopic examination was used for examining the colony characteristic. A sterile needle was used to pick a little portion of the different species of fungi in the colony and placed on a sterile glass slide separately, the slide was stained with lactophenol cotton blue and was viewed under the microscope, using x10 and x40 magnification. Identification was achieved using an atlas.

2.6. Seed Treatment Using Turmeric rhizome and Moringa Leaves Extracts

The required amount of seeds (20) was treated in the aqueous botanical (Turmeric and Moringa) extract separately for 1 hour. The concentration of the solution was 100% (v/v) for all the two individual plant species, the treatment was done separately under different concentration such as 25ml, 50ml and 75ml respectively. Healthy untreated seeds were dipped in distilled water for 1 hour as control treatment.

2.7. Antifungal Efficacy of Tumeric and Moringa Extract

After treating the seeds, the seeds were placed in a sterilized Petri dishes, four (4) layers of blotting papers were soaked into sterilized distilled water and placed into sterile Petri dishes. Each dish contained 20 seeds and the petri dishes were replicated three times. The petri dishes were then kept in incubation chamber at room temperature for 10 days and data were collected (recorded) after 10, 15, 20 days after sowing (DAS). Infected seeds were counted manually and percentage infestation determined [8].



Fig: Experimental plates

2.8 Treatment and Experimental Design

The experimental layout used was complete random design (CRD) whereby the three (3) treatments were replicated three times (25ml, 50ml, 75ml,) respectively.

C1	C2	C3
T1R1	T1R2	T1R3
T2R1	T2R2	T2R3
T3R1	T3R2	T3R3

Keys

122 C = Control
 123 T1 = Treatment 1 (25ml)
 124 R = Replicate
 125 T2 = Treatment 2 (50ml)
 126 T3 = Treatment 3 (75ml)
 127

128 2.9 Data Analysis

129 Data analysis was carried out using turkey pair-wise comparison one way analysis of
 130 variance (ANOVA) to compared means. Means separation was determine using Duncan
 131 multiple range test. Significance difference was determine at 95% confidence.

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133 3 RESULTS

134 3.1. Isolation and Identification of Fungi

135 Table 1 below showed the percentage distribution of fungi species. A total number of
 136 sixty two (62) fungal species were isolated. The result obtained showed Anagohom with the
 137 highest fungi occurrence of 45.16 % compared to Alibah and Anabor which has 29.03 and
 138 25.16% respectively. The fungi isolated were identified microscopically as *Penicillium sp*,
 139 *Fusarium sp*, *Aspergillus niger* and *Aspergillus flavus*. *Fusarium* species gave the highest
 140 percentage occurrence of 35.48 while the *Penicillium sp* showed the lowest percentage
 141 occurrence of 11.29.

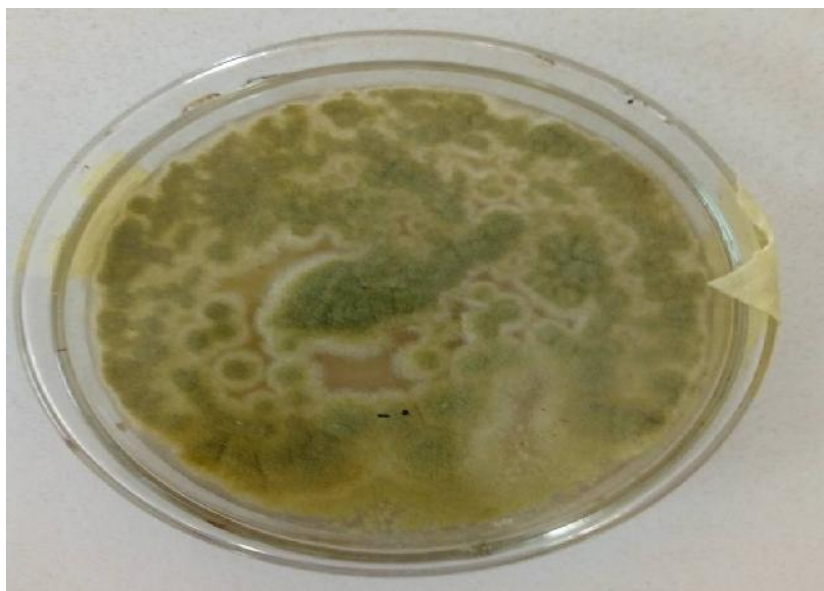
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143 **Table 1: Percentage Distribution of Fungi Species in Alibah, Anabor, Anagohom**

Area	No of fungi Occurrence	Percentage Distribution
Alibah	18	29.03
Anabor	16	25.81
Anagohom	28	45.16

Total	62	100
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Fig 2: *Aspergillus flavus*



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Fig 3: *Penicillium*

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Fig 4: *Fusarium sporotrichioides*

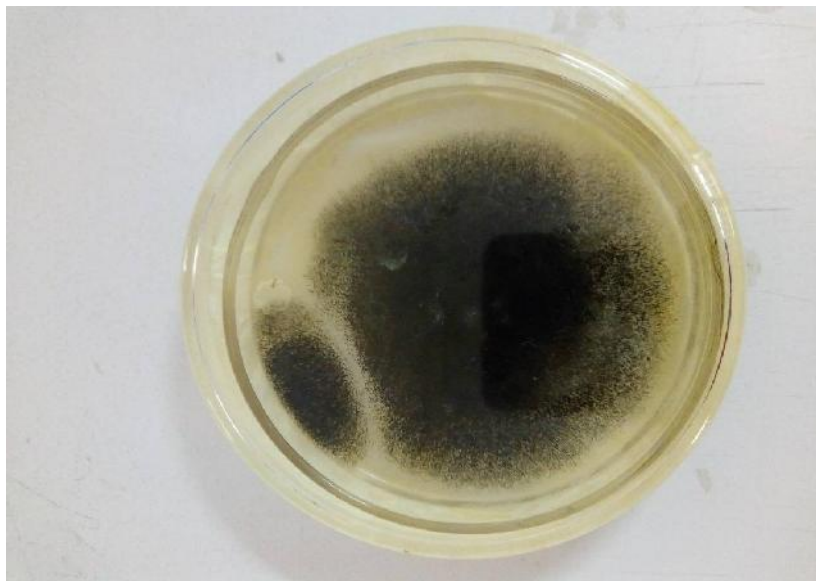


Fig 5: *Aspergillus niger*

158 **Table 2: Fungi Features/ Characteristics**

S/NO	FUNGI	FEATURES/CHARACTERISTICS
1.	<i>Aspergillus flavus</i>	It consist of a dense of yellow – green conidiophores and conidials with a head typically radiated, and splitting into several loose columns. Conidiophores becomes dark yellow-green, hyaline and roughened.
2.	<i>Penicillium species</i>	Consist of a dense felt of conidiospores. Appeared leathery, blue green and yellow to orange.
3.	<i>Fusarium species</i>	Cottony, whitish, letter becoming yellow, pink- red. Reverse in yellow to brownish shades.
4.	<i>Aspergillus niger</i>	It consist of a compact white basalfelt with a dense layer at dark brown to black conidiospores, conidial head radiate ending to split” into loose columns with time.

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160

161 **Table 3: Percentage Occurrence of Fungal Species**

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Fungi Isolates	Number of Occurrence	Percentage Occurrence
<i>Aspergillus niger</i>	19	30.65
<i>Fusarium species</i>	22	35.48
<i>Aspergillus flavus</i>	14	22.58
<i>Penicillium species</i>	7	11.29
Total	62	100

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164 **3.2. Antifungal Efficacy of Moringa Leaves Extracts**

165 The table below showed the mean antifungal effect of Moringa extract at 10, 15 and

166 20 days of the experiment at different concentration of 25ml, 50ml and 75m. The control

(untreated) maize seeds has the mean values of 4.33 after 10 days, 5.00 after 15 days and 6.67 after 20 day. The 25ml concentration showed mean values of 2.333 after 10 days and 2.57 after 15 days and 3.00 after 20 days. The 75ml concentration has the mean values of 1.33 after 10days, 1.67 after 15 days and 2.000 after 20 days. The result also showed that Moringa extract at higher concentration of 75m has the highest effect of 2.00 mean value at 20 days interval. The result obtained showed the effect of Moringa extract is directly proportional to the extract concentration and days of exposure.

Table 4: The Antifungal Mean Effect of Moringa Leaves Extracts

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

Means within a column followed by the same letters are not significantly difference (P = 0.05%) using Duncan Multiple Range Test.

* = Significant at 0.05% level of probability

LS = level of significant

3.3 Antifungal Efficacy of Turmeric Rhizome Extracts

The table below showed the means effect of *Curcuma longa* extracts at 10, 15, and 20 days, of the experiment at different concentration of 25ml, 50ml and 75ml. The control (untreated) maize seeds has the mean value of 4.33 after 10 day, 5.00 after 15 days, 6.67 after 20days. The 25ml concentration has the mean values of 2.00 after 10days, 2.333 after 15 days and 3.00 after 20days. The 50ml concentration has the mean value of 2.33 after 10days, 3.00 after 15 days and 3.67 after 20days. The 75ml concentration showed mean value of 3.67 after 10days, 4.33 after 15 days, and 5.67 after 20 days. The result shows that *Curcuma longa*

at smaller concentration is more effective in control of fungal pathogen associated with maize seeds since it has the least value of 2.00, 2.33 and 3.00 of infected seeds after 10, 15 and 20 days which is followed by 50m concentration and the lastly the 75ml concentration. The control has the highest mean value of infected maize seeds, which shows that *Curcuma longa* is effective in controlling fungal pathogen associated with maize seeds.

Table 5: The Antifungal Mean Effect of Turmeric Rhizome Extracts

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

Means within a column followed by the same letters are not significantly difference (P = 0.05%) using Duncan Multiple Range Test.

* = Significant at 0.05% level of probability

LS = level of significant

4. DISCUSSION

Farmers are faced with challenges of fungal infestation which has led to great economic lose. 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 owns small farms due to it expensive nature [9]. Plant extracts of many plant parts has to possess antifungal activities in vitro [10]

The fungal pathogen associated with maize seeds base on this research are has been found to be *Penicillium sp*, *Fusarium sp*, *Aspergillus niger* and *Aspergillus flavus*, which is

in line with the findings of [11] which says Maize diseases are produced by many species of fungi such as *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Altermaria* genera of fungi 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 [12] which state that *Fusarium* occurs throughout cultivation period of maize.

The infestation failure of the maize seed was influenced by the increase in the level plant extract concentration used. From the result obtained, *Moringa*, at 25ml concentration recorded the highest mean value of infestation of the seeds. 75ml concentration of *Moringa* extract recorded the smallest mean value of infestation of the seeds. This shows that *Moringa* extract at high concentration is more effective in control of fungal pathogen associated with maize seeds, this shows that the plant extracts has the ability to inhibit mycelia growth. The result obtained using Turmeric extract at 25ml concentration showed means value of 3.00 infestation which is the least compared to 50ml and 75ml concentration. Turmeric at small concentration after 20 days of the experiment is more effective than the other two concentrations (50ml and 75ml). The 75ml concentration after 20 days of seed incubation has high means value infestation of 5.67, this shows that turmeric at higher concentration is less effective in control of fungal pathogen associated with maize seed, this result is in line with the findings of [13] which says fungicidal activities of Turmeric has been found effective in controlling certain agricultural and animal pest or pathogens due to the presence of a variety and bioactive constituents that interfere with fungi behavior and growth.

5. CONCLUSION

The result obtained in this experiment showed *Penicillium sp*, *Fusarium sp*, *Aspergillus niger* and *aspergillus flavus* associated with untreated maize seed. The two (2)

different botanical (plant materials) at different concentration showed a significant different between the two botanicals and also has a significant different between the different concentrations of the two (2) botanicals. It can be concluded that different fungal pathogen such as *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* species and *Penicillium* species are among the pathogen that are responsible for failure in germination of maize seeds. The result obtained from the research indicate that turmeric and Moringa extract can be used in seed dressing against fungal pathogen associated with maize seed.

6 RECOMMENDATION

Further research should be carried out on these same botanicals by combining the two together to determine their synergic effect.

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