

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

ABSTRACT

This research was 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 design using 2 x 3 factorial in completely randomized design. This research was carried out in 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. 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* leaf at 25, 50, and 75 ml concentration were used as seed treatment agent against fungi. The seeds were soaked in prepared 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. *Penicillium sp*, *Fusarium solani*, *Aspergillus niger* and *Aspergillus flavus* were isolated from the untreated maize seed. *Fusarium solani* gave the highest percentage occurrence of 35.48. 50 ml concentration of Turmeric rhizome has antifungal mean value of 3.67 after 20days. The control (untreated) showed antifungal mean value of 6.67 after 20days. 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 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 shown to be good cereal crop due to its low cost of production, wide adaptability and diversified used. Maize suffers from 28 diseases in which are seed borne in nature [2]. Incidence of seed borne pathogen cause germination failure as well as reduction in seedling vigor which ultimately reduces the yields. Control of the seed borne fungal pathogen by chemical fungicide is costly and hazardous for the environment. Therefore, the used of plant materials is important in reducing the

Comment [V1]: Moringa

Comment [V2]: Turmeric

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36 prevalence of the seed borne pathogen associated with the maize seeds and to enhance
37 percentage of maize seeds germination and seedling vigor.

Comment [V10]: pathogens

Comment [V11]: seed

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

Comment [V13]: feed

46 Maize is widely cultivated throughout the world and worldwide production indicated
47 800 million tones in 2007, just slightly more than rice (650 million tones) or wheat (600
48 million tones) in 2007 [5]. Despite its importance, maize has many production constraints
49 which prevent farmers from getting maximum yield. Drought, fire, flood, poor soil fertility,
50 high labour cost, transportation problems and poor agronomic practices are some of the
51 production constraints of maize [6]. Pest and diseases are also form some of the problems that
52 characterize maize production in the world. The diseases of maize seeds include; seed rot
53 (*Fusarium moniliforme*, *Fusarium oxysporum*, *Penicillium species*.) seedling blight
54 (*Aspergillus species*, *Penicilum species*) Biperis leaf spot (*Biperis maydis*), *Curvularia* leaf
55 spot (*Curvularia lunada*) etc. The aim of the study is to determine the effect of different
56 concentration of Turmeric rhizome and *Moringa* leaf extracts in maize seed treatment
57 against fungal pathogens associated with maize seeds.

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58 2 MATERIALS AND METHODS

59 2.1 Study Area

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

64 2.2 Collection of Samples

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

71 2.3 Determination of Fungi Presence on Collected Maize Seed

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

Comment [V18]: leaves

Comment [V19]: Moringa (*Moringa oleifera*)

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84 cultures were obtained. The percentage occurrence of the fungi isolated was determined using
85 the following formula:

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

86 2.4 Identification of Fungi Isolates

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

96 2.5. Preparation of Plant Extract (*Tumeric rhizome and Moringa leaf*)

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

Comment [V25]: Tumeric rhizome and Moringa leaf

101 2.5. Determination of Extract Concentrations

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

104 2.6. Seed Treatment Using *Turmeric rhizome and Moringa* Leaf Extracts

105 Twenty (20) healthy untreated maize seeds were treated using sterilized aqueous
106 extracts of the plant (*Turmeric* and *Moringa*) separately by dipping the maize seeds into
107 different concentrations (25, 50 and 75 ml) of the aqueous extracts for 1 hour. For the

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108 control, twenty (20) healthy untreated seeds dipped in sterilized distilled water for 1 hour
109 served as the control treatment.

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110 2.7. Antifungal Efficacy of Tumeric Rhizome and Moringa Extract

Comment [V30]: Tumeric Rhizome and Moringa

111 After treating the seeds, the seeds were placed in a sterilized Petri dishes containing
112 four (4) layers of blotting papers soaked in sterilized distilled water. Each Petri dish
113 contained 20 seeds and the Petri dishes were replicated three times. The Petri dishes were
114 then kept in incubation chamber at room temperature for 10 days and infected seeds were
115 counted manually after 10, 15, 20 days after sowing [10].

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116 2.8 Treatment and Experimental Design

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

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119 Treatment combinations = $2 \times 4 = 8$

120 Replications = 3

121 Total plots = $3 \times 8 = 24$.

122 2.9 Data Analysis

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

127 3 RESULTS

128 3.1. Isolation and Identification of Fungi

129 Table 1 below showed the percentage distribution of fungi species. A total number of
130 sixty two (62) fungal species were isolated. The result obtained showed Anagohom with the
131 highest fungi occurrence of 45.16 % compared to Alibah and Anabor which has 29.03 and
132 25.16% respectively (Table 1). The percentage distribution results showed significant
133 difference at $p = 0.05$. The fungi isolated were identified macroscopically and

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134 microscopically as *Penicillium sp*, *Fusarium sp*, *Aspergillus niger* and *Aspergillus flavus*.
 135 *Fusarium solani* (based on the characteristics presented in Table 2) gave the highest
 136 percentage occurrence of 35.48 while the *Penicillium sp* showed the lowest percentage
 137 occurrence of 11.29. The result in Table 4 showed significant difference in fungal percentage
 138 occurrence.

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140 **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

141 a, b, c = means separation indicating level of significance

142 Means within a column followed by the same letters are not significantly difference ($P =$
 143 0.05) using Duncan Multiple Range Test.

144 * = Significant at 95% level of probability.

145 LSD = Least significant difference.

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156 **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 conidiosphores. Appeared leathery, blue green and yellow to orange.
3.	<i>Fusarium solani</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 conidiosphores, conidial head radiate ending to split” into loose columns with time.

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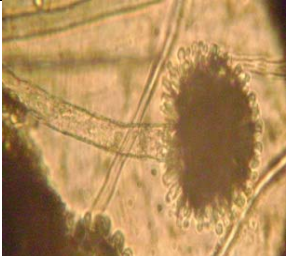
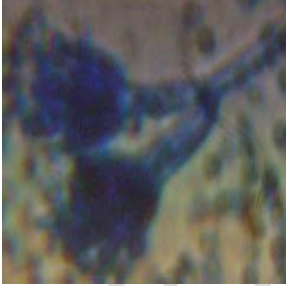
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166 **Table 3: Morphological View of Fungi Isolates**

Appearance on PDA	Photomicrograph	Probable Isolates
		<i>Aspergillus flavus</i>
		<i>Penicillium</i> sp
		<i>Fusarium Solani</i>
		<i>Aspergillus niger</i>

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168
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170 **Table 4: Percentage Occurrence of Fungal Species**

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Fungi Isolates	Number of Occurrence	Percentage Occurrence
<i>Aspergillus niger</i>	19	30.65 ^a
<i>Fusarium species</i>	22	35.48 ^a
<i>Aspergillus flavus</i>	14	22.58 ^b
<i>Penicillium species</i>	7	11.29 ^c
SE±	-	5.86
LSD	-	*
Total	62	100

172 a, b, c = means separation indicating level of significance

173 Means within a column followed by the same letters are not significantly difference ($P =$

174 0.05) using Duncan Multiple Range Test.

175 * = Significant at 95% level of probability.

176 LSD = Least significant difference.

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178 3.2. Antifungal Efficacy of *Moringa* Leaf Extracts

179 The table below showed the mean antifungal effect of *Moringa* extract of 10, 15 and

180 20 days at different concentration of 25, 50 and 75 ml. The control (untreated) maize seeds

181 has the mean values of 4.33 after 10 days, 5.00 after 15 days and 6.7 after 20 day. The 25 ml

182 concentration showed mean values of 2.33 after 10 days and 2.6 after 15 days and 3.00 after

183 20 days. The 75 ml concentration indicated mean values of 1.33 after 10days, 1.7 after 15

184 days and 2.00 after 20 days. The result also showed that *Moringa* leaf extract at higher

185 concentration of 75 ml has the highest effect of 2.00 mean value at 20 days interval. The

186 result obtained showed the effect of *Moringa* extract antifungal activities is directly

187 proportional to the extract concentration and days of exposure.

188 **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

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Comment [V44]: *Moringa*

Comment [V45]: *Moringa*

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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	*	*	*

a, b, c = means separation indicating level of significance

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

* = Significant at 95% level of probability.

LSD = Least significant difference.

3.3 Antifungal Efficacy of Turmeric Rhizome Extract

The table below showed the means effect of Turmeric rhizome extracts at 10, 15, and 20 days of the experiment at different concentration of 25, 50 and 75 ml. 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 25 ml concentration showed the mean values of 2.00 after 10 days, 2.33 after 15 days and 3.00 after 20 days. The 50 ml concentration has the mean value of 2.33 after 10 days, 3.00 after 15 days and 3.67 after 20 days. The 75 ml concentration showed mean value of 3.67 after 10 days, 4.33 after 15 days, and 5.67 after 20 days. The result shows that Turmeric rhizome at smaller concentration is more effective in control of fungal pathogen associated with maize seeds as it showed least mean value of 2.00, 2.33 and 3.00 of infected seeds after 10, 15 and 20 days which is followed by 50 ml concentration. The control has the highest mean value of infected maize seeds, which shows that Turmeric rhizome is effective in controlling fungal pathogen associated with maize seeds.

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

4. 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 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 [11]. Plant extracts of many plant parts has to possess antifungal activities *in vitro* [12]

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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 cultivation period of maize. In the present work antifungal activity of two plants extracts, *Moringa* leaf and Turmeric rhizome

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236 was assessed at the concentration of 25, 50 and 75 ml against fungal growth by blotter
237 technique. Similar investigation on antifungal activity of plant extracts against seed-borne
238 fungi has been reported by many researchers [15-17]. Between these, Turmeric rhizome
239 extract was found more effective in inhibition of fungal growth than *Moringa* leaf at lower
240 concentration. Antifungal activity of the extract at different concentrations are found to be
241 effective [18,19] this could be due to the activities of essential oils, a mixture of terpenoids,
242 aromatic phenols and many other compounds such as 1, 8-Cineole which has proved to have
243 fungicidal properties [20].

244 The result obtained using Turmeric extract at 25 ml concentration showed means
245 value of 3.00 infestation which is the least compared to 50 ml and 75 ml concentration.
246 Turmeric at small concentration after 20 days of the experiment is more effective than the
247 other two concentrations (50 ml and 75 ml). This result agrees with the finding of Abiamere
248 *et al.* [21] which evaluated the efficacy of *Moringa*, Ginger and *Chromolena odorata* plant as
249 treatment agent against seed borne fungi associated with Cowpea. The 75 ml concentration
250 after 20 days of seed incubation has high means value infestation of 5.67, this shows that
251 turmeric at higher concentration is less effective in control of fungal pathogen associated
252 with maize seed, this result is in line with the findings of Lee *et al.* [22] which says
253 fungicidal activities of Turmeric has been found effective in controlling certain
254 agricultural and animal pest or pathogens due to the presence of a variety and bioactive
255 constituents that interfere with fungi behavior and growth. This present study has therefore
256 shown that plant extract of *Moringa* and Turmeric can be used as fungicidal seed treatment
257 for the control of seed borne fungi of maize seeds and for increasing seed germination.

258 5. CONCLUSION

Comment [V51]: *Moringa*

Comment [V52]: How these plant extracts are increasing seed germination? There is no data related to this finding in whole text.

The result obtained in this experiment showed *Penicillium sp*, *Fusarium solani*, *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 (concentration 25 and 50 ml) and *Moringa* (concentration 75 ml) extract can be used in seed dressing against fungal pathogen associated with maize seed.

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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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