



World News of Natural Sciences

An International Scientific Journal

WNOFNS 58 (2025) 15-27

EISSN 2543-5426

***In vitro* Evaluation of Plant Extracts for Post Harvest Management of *Fusarium oxysporum* f. sp. *cepae* causing Fusarium Basal Rot of Onion (*Allium cepa* L.)**

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ABSTRACT

Fusarium basal rot disease of onion caused by *Fusarium oxysporum* f. sp. *cepae* is an economically vital disease-causing threat to onion cultivation and storage. This study evaluated the effect of three plant extracts, Eucalyptus (*Eucalyptus camaldulensis*), Ginger (*Zingiber officinale*), and Moringa (*Moringa oleifera*), on the management of the Fusarium basal rot pathogen (*Fusarium oxysporum* f. sp. *cepae*). The study was conducted at the pathology laboratory of the Department of Crop Protection, Bayero University, Kano. The treatments comprised of three plant extracts (Ginger, Moringa, and Eucalyptus) and five levels of concentration (0, 25%, 50%, 75%, and 100%) and synthetic chemical fungicide (Ridomil Gold), which were factorially combined and replicated three times in a completely randomized design (CRD), were used to evaluate for their inhibitory effects on *F. oxysporum* growth *in vitro*. The result from the mycelial growth revealed that Moringa and Ginger at 100% concentration significantly ($P < 0.05$) recorded the lowest mycelial growth rates compared to control. Furthermore, Ginger and Moringa recorded a higher percent of growth inhibition. Lower concentrations of 25 and 50% exhibited the least percent growth inhibition compared to control. It was concluded that the plant extracts used at different concentrations showed promising prospects for control of *F. oxysporum* growth *in vitro*. Moringa and ginger extracts at 75% and 100% concentration significantly ($P < 0.05$) inhibited the growth of the fungus and could therefore be recommended for further studies in the screen house and field to evaluate their efficacies against Fusarium basal rot under field conditions in combating the post-harvest losses of onions

Keywords: Plant extracts, Concentration, Mycelial growth, Percent growth inhibition, Onions, Post harvest

1. INTRODUCTION

Onion (*Allium cepa* L.) belongs to the genus *Allium* of the family Alliaceae, which was believed to have originated in South Western Asia, being the center of domestication and variability, from where it was spread first across the world and has been cultivated for over 4700 years as annuals for bulb production's purpose (Brewster, 2002). The crop is used either as a mature bulb or green vegetable when harvested early. It is second to tomatoes among other vegetables in use as cooking condiments (Dantata, 2014). Nigeria is ranked 17th among producing countries of the world but 4th highest producer in Africa (Inuwa, 2001). Onion is an important vegetable crop extensively grown in many parts of the world for fresh market use and for processing (Bansal and Gupta, 2000). Onion becomes infected from many diseases from pre-harvest to post-harvest period; about 35-40% of onion is lost due to damage caused by different diseases. A number of microorganisms are responsible for bulb rotting of onions, but among them, fungi are the main causal agent responsible for pre- and post-harvest spoilage and losses in the onion (Mrema and Rolle, 2002).

Onion bulb losses caused by postharvest rot in onions are greater than is often realized and avoidable between farm gate and consumers. The major fungal storage diseases of onions are *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus* spp., and *Alternaria porri*.

Fusarium basal rot can occur in the field or during storage, and their losses during storage were greater than those observed in the field (Cramer, 2000). Also, various species of *Aspergillus* pathogens are reported to cause blue mold on onion bulbs during storage. The blue molds are frequently isolated from stored diseased bulbs of local cultivars of onion (Hussain *et al.*, 1997). The fungus causing black mold is the main member of *Aspergillus* and is predominantly a plant pathogen responsible for post-harvest deterioration of stored food materials (Marziyeh *et al.*, 2010).

It affects the availability of onions for domestic and international trade. The infection of fungi causes spoilage and ultimately decreases the qualitative attributes and quantity of food (Randle *et al.*, 1997; Marziyeh *et al.*, 2010). *Fusarium* basal rot pathogen contamination begins at the germination stage and remains up until the storage period (Hayden *et al.*, 1994). The pathogen transmission is by infected soil or seed, and the infected bulbs show neck discoloration along with black-colored mycelia and the hiding spores in the outer dry scales (Pelligrini *et al.*, 1999). Onion bulb losses as a result of *Fusarium* basal rot may be high because of the favorable weather conditions for the development of the causal pathogen throughout the year (Hussain *et al.*, 1997).

Onions are an attractive cash crop for small farmers and provide potential sources of employment to many rural and urban Nigerians. Despite the human need for onion bulbs, damage as a result of postharvest spoilage caused by fungal pathogens has been of serious concern. Postharvest onion bulb decay as a result of fungal pathogens is one of the main factors that determine loss and compromise the quality of the produce and export potential of the crop that significantly causes the economic loss (Mishra *et al.*, 2014). In view of the aforementioned problems, there is the need to identify and control those pathogens responsible for the

postharvest spoilage of onion bulbs, as this will undoubtedly help in planning strategic disease control in a way that will bring about an increase in onion production, prevent extensive loss of onion bulbs due to postharvest spoilage caused by fungal pathogens, and improve the well-being and livelihood options of onion farmers, onion marketers, as well as consumers.

The specific objectives of this research were to

- i) To isolate and identify fungal pathogens associated with post-harvest spoilage of onions
- ii) To evaluate the efficacy of some plant extracts in the management of fungal pathogens associated with spoilage of onions.

2. MATERIALS AND METHODS

2. 1. Experimental site

The experiment was carried out at the Research and Teaching Laboratory of the Department of Crop Protection, Faculty of Agriculture, Bayero University, Kano (11° 58" N, 8° 25" E, and 470 m above sea level) in Northwestern Nigeria.

2. 2. Source of Experimental Materials

Onion bulb samples with the sign of spoilage were identified by physical examination and then collected randomly from the two local markets in Kano metropolitan area, which include Yankaba Market in Nassarawa Local Government Area and Kwanar Ungogo Market in Ungogo Local Government Area. Healthy onion bulbs and the spoiled ones were also brought from the same markets in paper envelope bags and transported to the laboratory for isolation of the pathogens, identification, and further studies.

2. 3. Isolation of Pathogen

The pathogen isolation was carried out under aseptic conditions. All the glassware used was properly washed, dried, and sterilized. The entire working surface was also disinfected with ethanol to reduce contamination. A small section of an advanced margin of the lesion was cut with a sterilized pair of scissors. The samples were then sterilized with 1% sodium hypochlorite (NaOCl) for 3 minutes and then rinsed with distilled water three times and dried with sterile tissue (Larone, 1995). The samples were aseptically transferred into petri dishes containing 20 mL of molten Potato Dextrose Agar (PDA) amended with streptomycin to prevent unwanted growth of bacteria and other microorganisms. The plates were incubated for 7 days at room temperature (27 ±2 °C). Distinct mycelia present on the plate was subcultured for another 7 days to get pure culture.

2. 3. 1. Identification of Pathogen

Fungal identification was carried out using morphological characteristics and compared with established keys (Barnnet and Hunter, 1998). The fungal pathogen was subjected to microscopic examination, during which its morphological features were observed and recorded and found to be *Fusarium oxysporum* f.sp. *cepae*. Identification of the fungi was based on growth patterns, color of mycelia, and microscopic examination of vegetative reproductive structures.

2. 3. 2. Preparation of plant crude extracts for in-vitro evaluation

Three plant materials, Eucalyptus (*Eucalyptus camaldulensis* Dehnh) leaves, Moringa (*Moringa oleifera* Lam) leaves, and Ginger (*Zingiber officinale* Roscoe), were purchased from Rimi Market in Kano State and brought to the Phytopathology Laboratory of the Department of Crop Protection, Bayero University Kano. The Eucalyptus, Ginger, and Moringa were washed under tap water, rinsed in three changes of distilled water, and air-dried under shade. The eucalyptus, moringa leaves, and ginger were then grinded into powder by the use of a sterile blending machine so as to rupture leaf tissue and cell structures to release the active cell contents.

2. 3. 3. Preparation of crude extract

Twenty grams (20 g) of the powder from each of the plant extracts was added to 400 mL of distilled water in a 1000 mL capacity flat bottom flask. The suspension was allowed to stand overnight, and the solution was filtered using sterile muslin clotting and kept in a glass bottle. The extract at different concentrations was prepared by mixing 25 mL, 50 mL, and 75 mL of stock solution with distilled water to give the final concentration of the extract at 25%, 50%, 75%, and 100%, respectively.

2. 3. 4. Culture media Preparation and Inoculation of the Pathogen

Two hundred grams (200 g) of the peeled Irish potato were weighed and poured into 1000 mL of distilled water in a laboratory pot and boiled till it became soft. The softened potato was filtered using muslin cloth, and 20 g of glucose and 18 g of agar were added to the supernatant. The media is then sterilized by autoclaving it at 121°C and 15 lbs pressure for 15 minutes, after which it was removed and allowed to cool. Streptomycin (100µg/mL) was added to avoid unwanted growth of bacteria. About 15 mL of molten PDA was poured aseptically onto the petri dishes. Prior to the pouring, the media was amended with different concentrations of the plant extract and allowed to solidify.

2. 3. 5. In-vitro Assessment of Antifungal Potential of the Plant Extracts

A mycelia plug of 5 mm diameter from a 3-day-old *Fusarium oxysporum* isolated from the onions was taken using a 5 mm sterile cork borer and placed at the center of the petri dishes containing the plant extracts amended PDAs. The *in-vitro* experiment was laid out in a complete randomized design (CRD) consisting of 5 treatments replicated 3 times. Eucalyptus extract labeled as T1, Ginger extract as T2, Moringa extract as T3, synthetic fungicide as check labeled as T4, and control as T5. The control, which is used as a comparison, had no extracts and no synthetic fungicide. Four concentrations of the plant extracts (25%, 50%, 75%, and 100%) were used in the experiment. The radial growth diameter of the pathogen was measured using a meter rule. Fungal growth was measured on the second, fourth, sixth, and eighth days after inoculation. Percentage reduction on mycelia or zone inhibition was determined according to the formula described by He et al. (2020)

$$\% \text{ Inhibition} = \frac{C-T}{C} \times 100 \quad (\text{Eq. 1})$$

where: C = Radial growth of fungus in control, T = Radial growth of fungus in treatment

2. 4. Data Analysis

Data on mycelial growth and percentage inhibition from each treatment were taken and subjected to analysis of variance (ANOVA) Using Genstat version 17, means were separated using Student-Newman-Keuls (SNK) at 5% level of significance.

3. RESULTS AND DISCUSSION

3. 1. Identification of the Fungus and Pathogenicity Test

The result obtained from the identification of the pathogen isolated from the spoiled onions showed a white aerial mycelium that later produced a light pink pigment on Potato Dextrose Agar (PDA). Based on the fungal morphology by Barneet and Hunter (1998), it's a typical colony characteristic of *Fusarium oxysporum*. The pathogenicity test confirmed the fungus as a causal organism for fusarium basal rot of onions.

3. 2. Effect of plant extracts on mycelial growth of *Fusarium oxysporum* 2-8 days after inoculation

As shown in Table 1, significant differences ($P \leq 0.05$) were observed in the mycelial growth of *F. oxysporum* among the plant extracts in all the inoculation days. The result shows moringa had the lowest mycelial growth of *Fusarium oxysporum* at all the inoculation days, followed by ginger (Table 1), while Eucalyptus recorded the highest mycelial growth in all the inoculation days.

A significant difference was also observed among the concentrations of the plant extracts on mycelial growth of the fungus among all the inoculation days (Table 1). Least mycelial growth was observed in 100%, followed by 75% concentrations in all the plant extracts across the inoculation days compared to control.

Table 1. Effect of plant extracts on mycelial growth of *Fusarium oxysporum* 2-8 days after inoculation.

Treatment	Days after inoculation (DAI)			
	2	4	6	8
Plant extract (P)				
Eucalyptus	2.14 ^a	3.83 ^a	4.64 ^a	4.79 ^a
Ginger	2.01 ^{ab}	2.95 ^b	3.56 ^b	3.76 ^b
Moringa	1.94 ^b	2.89 ^b	3.40 ^b	3.66 ^b
LSD (0.05)	0.06	0.01	0.01	0.01
Concentrations (C) [%]				
25	2.12 ^{bc}	3.38 ^b	3.77 ^b	3.86 ^b

50	2.37 ^{ab}	3.40 ^b	3.72 ^b	3.92 ^b
75	2.20 ^{bc}	3.10 ^c	3.39 ^c	3.63 ^{bc}
100	1.93 ^c	3.33 ^d	3.37 ^d	3.68 ^c
0 (Control)	2.58 ^a	5.65 ^a	7.60 ^a	8.18 ^a
Ridomil Gold	1.00 ^d	1.47 ^e	2.15 ^e	2.38 ^{bc}
LSD (0.05)	0.01	0.01	0.01	0.01
Interaction				
P × C	NS	**	**	**

Means with a superscript of same letters within a column are statistically similar at 5% level of significant ($P \leq 0.05$) using Student Newman-Keuls (SNK) test. Ridomil Gold (Metalaxyl-M + Mancozeb). NS = Not significant ** = Significant difference

Interaction between plant extracts and concentration on the mycelial growth of *Fusarium oxysporum* at 4, 6 and 8 days after inoculation

A significant difference was witnessed in the interaction between plant extracts and their different concentrations on the mycelial growth of *Fusarium oxysporum* at 4 days after inoculation (Figure 1). The interaction shows that 75% and 100% of a ginger had the lowest mycelial growth, which effectively reduced the growth of the fungus compared with the 50%, 25%, and 0% concentrations that served as controls. Another significant difference in the interaction effects was also observed at 6 and 8 days after inoculation (Figures 2 and 3). It was observed that 75% and 100% concentrations of ginger similarly had the lowest mycelial growth compared with 50%, 25%, and 0% concentrations, respectively.

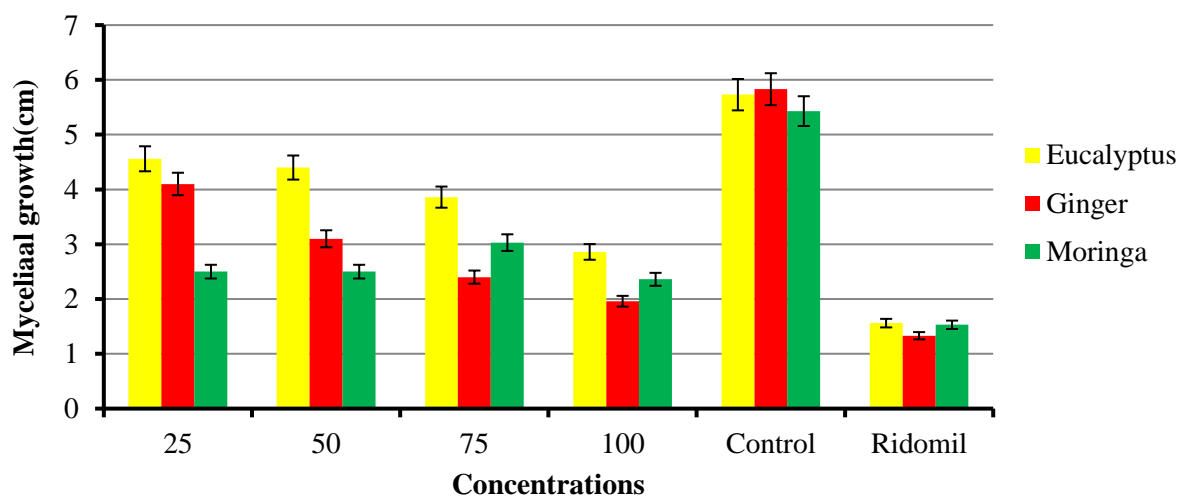


Figure 1. Interaction between plant extract and concentrations on *F. oxysporum* mycelial growth at 4 days after inoculation.

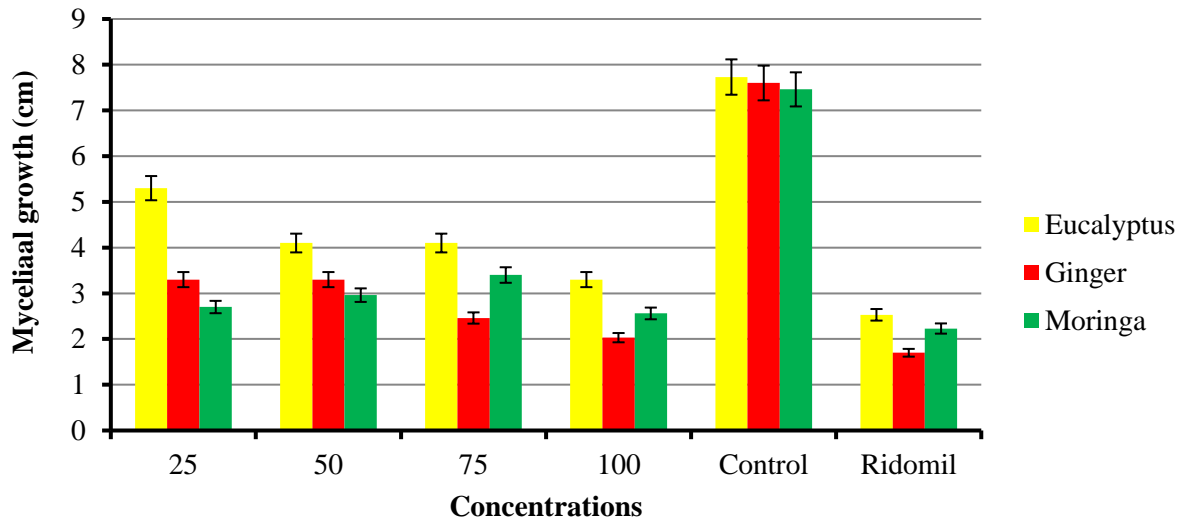


Figure 2. Interaction between plant extract and concentrations on *F. oxysporum* mycelial growth at 6 days after inoculation.

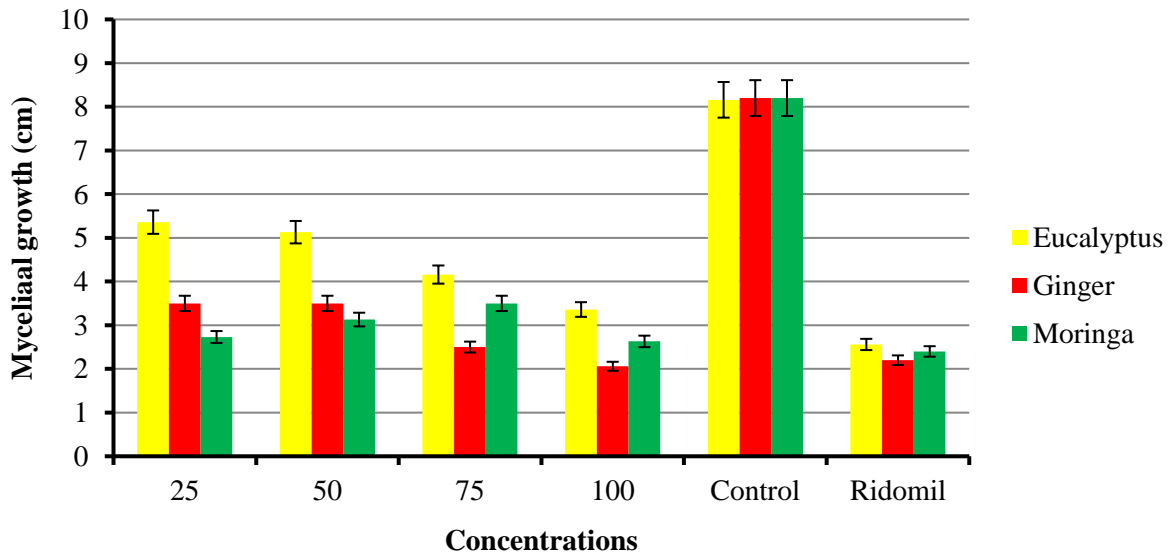


Figure 3. Interaction between plant extract and concentrations on *F. oxysporum* mycelial growth at 8 days after inoculation.

3. 3. Effect of plant extracts on percent inhibition of *Fusarium oxysporum* growth 2-8 days after inoculation

As shown in Table 2, significant differences ($P \leq 0.05$) were observed in the percentage growth inhibition of *F. oxysporum* among the plant extracts in all the inoculation days. The results indicate that Ginger had the highest percent growth inhibition, followed by Moringa, in all the inoculation days (Table 2). A significant difference was also observed among the concentrations of the plant extracts on percent growth inhibition of *F. oxysporum* among all the inoculation days (Table 2). The highest percent growth inhibition was observed in 100%,

followed by 75% concentrations in all the plant extracts across the inoculation days compared to control.

Table 2. Effect of plant extracts on percent inhibition of *Fusarium oxysporum* growth 2-8 days after inoculation

Treatments	Days after inoculation (DAI)			
	2	4	6	8
Plant extract (P)				
Eucalyptus	23.9 ^b	33.4 ^b	39.9 ^c	41.3 ^b
Ginger	28.4 ^{ab}	46.6 ^a	35.6 ^b	54.1 ^a
Moringa	29.9 ^a	48.9 ^a	55.1 ^a	55.4 ^a
LSD (0.05)	0.48	0.01	0.01	0.01
Concentrations (C) [%]				
25	25.9 ^{bc}	40.6 ^d	50.4 ^d	52.7 ^d
50	17.8 ^c	41.2 ^d	51.1 ^d	52.1 ^d
75	23.2 ^c	45.1 ^c	56.3 ^c	58.6 ^c
100	32.5 ^b	57.5 ^b	65.4 ^b	66.7 ^b
0 (Control)	0.0 ^d	0.0 ^d	0.0 ^d	0.0 ^d
Ridomil Gold	65.0 ^a	73.7 ^a	71.6 ^a	70.8 ^a
LSD (0.05)	0.08	0.01	0.01	0.01
Interaction				
P × C	NS	**	**	**

Means with a superscript of same letters within a column are statistically similar at 5% level of significant ($P \leq 0.05$) using Student Newman-Keuls (SNK) test. Ridomil Gold (Metalaxyl-M + Mancozeb). NS = Not significant, ** = Significant difference

Interaction between plant extracts and concentration on the percent growth inhibition of *Fusarium oxysporum* at 4, 6 and 8 days after inoculation

A significant difference was seen in the interaction between plant extract and their different concentrations on the percent growth inhibition of *Fusarium oxysporum* at 4 days after inoculation (Figure 4). The interaction shows that 75% and 100% concentrations of ginger had the highest percent inhibition growth, which efficiently inhibits the growth of the fungus

compared with the 50%, 25%, and 0% concentrations, which served as controls. Another significant difference in the interaction effects was also observed at 6 and 8 days after inoculation (Figures 5 and 6). It was observed that 75% and 100% concentrations of ginger similarly had the highest percent growth inhibition compared with 50%, 25%, and 0% concentrations, respectively.

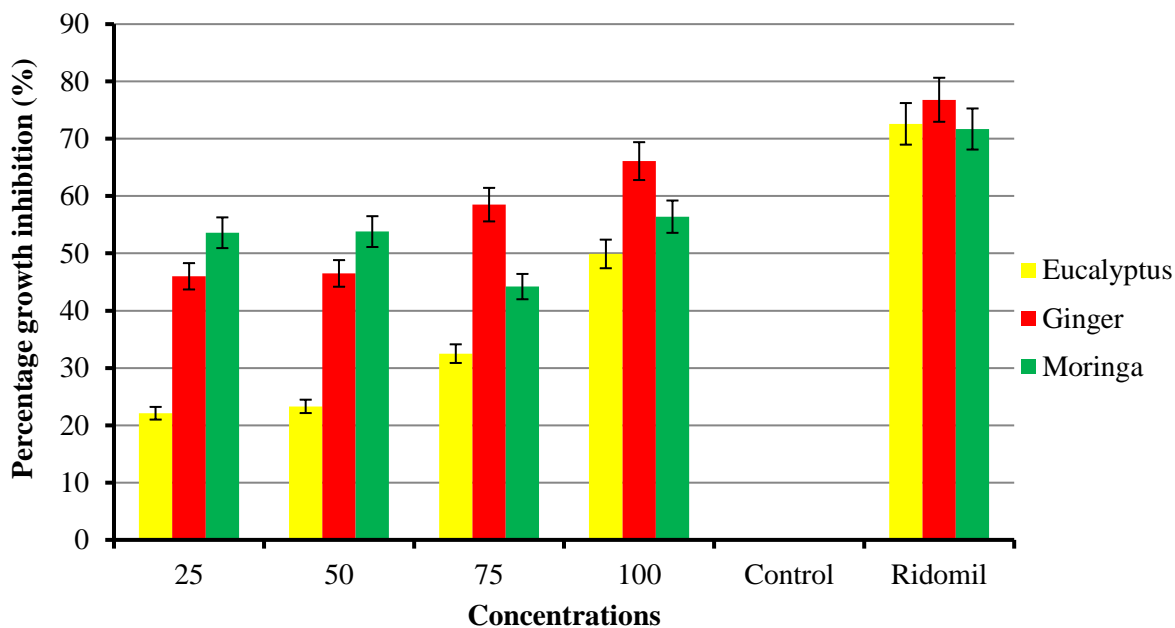


Figure 4. Interaction between plant extracts and concentrations on *F. oxysporum* percent growth inhibition at 4 days after inoculation.

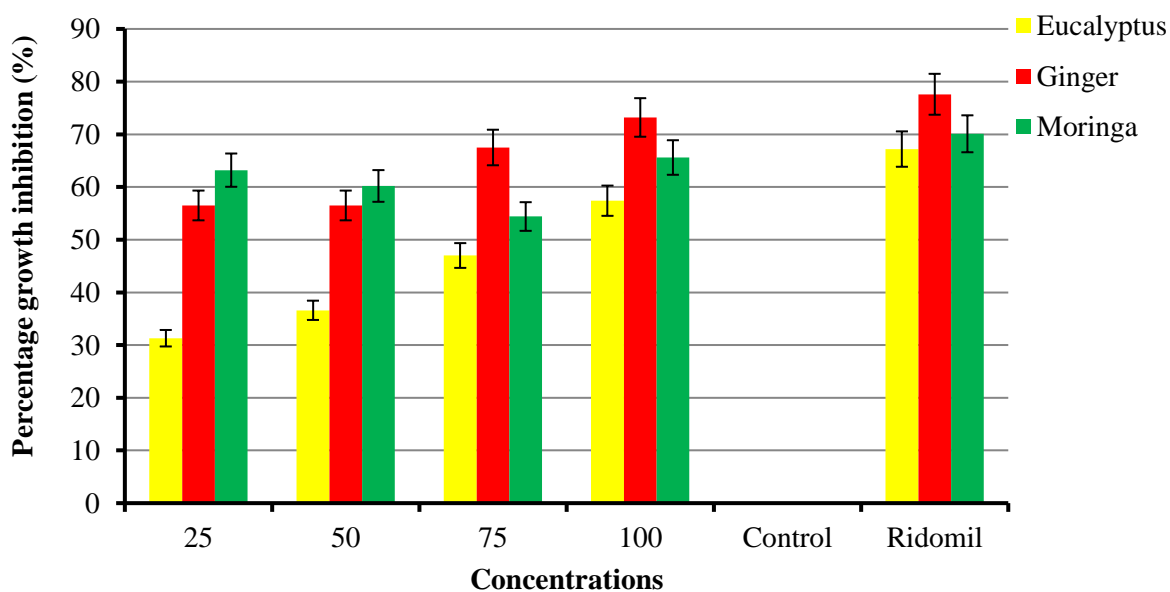


Figure 5. Interaction between plant extracts and concentrations on *F. oxysporum* percent growth inhibition at 6 days after inoculation.

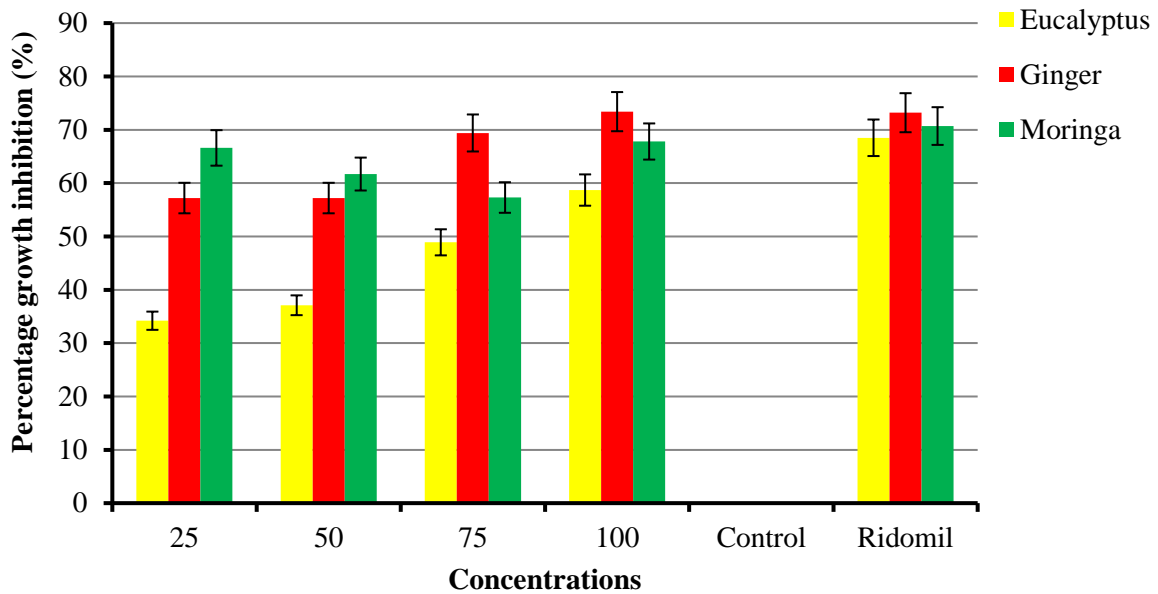


Figure 6. Interaction between plant extract and concentration on *F. oxysporum* percent growth inhibition at 8 days after inoculation.

4. DISCUSSION

In this study, we evaluated some plant extracts for the management of onion bulb rots as well as identifying the best extract for control practices to limit postharvest loss of onion due to bulb rots. Our result indicated that *Fusarium oxysporum* f.sp. *cepae* was the causal agent for the onion bulb rot. The colony formed with white aerial mycelia that later produced light pink pigment on PDA, which is one of the well-known properties of *Fusarium oxysporum* (Kelaniyangoda *et al.*, 2011). Significant differences ($P \leq 0.05$) of plant extracts on *Fusarium oxysporum* mycelial growth rate and percent growth inhibition were observed among different extracts and concentrations across all the inoculation days.

This study revealed that the lowest mean mycelial growth was observed from *Moringa oleifera*. The result is similar to that of Elad *et al.* (2016); Pinto *et al.* (2010) who reported the activity of *Moringa oleifera* extract against fungal pathogens. Ahmed *et al.* (2020) also reported that *Moringa oleifera* leaf extract has strong antifungal efficacy on mycelial growth and spore germination of *Fusarium solani*.

Furthermore, results from this study indicate that moringa (*Moringa oleifera*) had the highest percent growth inhibition, followed by *Zingiber officinale* in all the inoculation days and higher concentrations, respectively. This finding is similar to that of Banson *et al.* (1999), who reported the antifungal effects of moringa and ginger extracts were fungistatic at lower concentrations while becoming fungicidal at higher concentrations.

Additionally, our results demonstrated how moringa was found to be effective in controlling *Fusarium oxysporum*. The result is similar to that of Manasa *et al.* (2013); Munda *et al.* (2018) who attributed the activity of moringa extracts against different fungal pathogens due to the presence and abundance of bioactive compounds as well as the susceptibility of the

pathogen. In another experiment, Okigbo *et al.* (2018) also found moringa and ginger extracts to be effective against some fungal species like *Fusarium*, *Penicillium*, *Corticinium*, *Rhizoctonia*, and *Aspergillus*.

5. CONCLUSION

The results of this study revealed that *Fusarium oxysporum* f.sp. *cepae* was found to be responsible for the onion basal rot. Based on the study, the *in vitro* experiment concluded that moringa and ginger extracts contained antifungal compounds that suppressed the growth of *Fusarium oxysporum*. The findings from this study can be useful to farmers as a safer and environmentally compatible alternative to chemical fungicides and very affordable in managing fusarium basal rot in both field and storage.

ACKNOWLEDGEMENT

We thank Prof. Hassan Sule of the Crop Protection Department, Bayero University, Kano, for proofreading the manuscript and Mr. Prince Peter Olu of the Plant Pathology Laboratory, Crop Protection Department, Bayero University, for laboratory assistance.

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