

## INHIBITORY POTENTIAL OF SORGHUM LEAF AND ROOT EXTRACTS AGAINST *FUSARIUM OXYSPORUM* F.SP *LYCOPERSICI*

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### ABSTRACT

*A study was conducted to determine the efficacies of aqueous and methanolic extracts of sorghum leaf and root on mycelia inhibition of Fusarium oxysporum f.sp lycopersici (FOL). Sorghum leaves and root parts were collected at harvest, air dried at 28–2°C for 21 days and blended into powder. Aqueous extract was prepared by adding 100 ml of sterile distilled water to 10 g of ground tissue while methanolic extract was obtained with 90% methanol in a Soxhlet extractor to obtain stock. Preliminary phytochemical analysis of the extracts was carried out following a standard procedure. Fungitoxicity was determined by adding 15 ml Potato dextrose agar (PDA) to 1 ml extract in 9 cm Petri dish while PDA without extract served as control. Each plate was inoculated with 3mm of a 7-day-old fungal culture and incubated at room temperature (28–2°C) for 7 days. Data collected were subjected to statistical analysis. Phytochemical screening revealed the presence of saponins and cardiac glycosides in both extracts. Leaf and root methanolic extracts significantly ( $P \leq 0.05$ ) reduced radial mycelial growth of FOL compared to the aqueous extract. Mycelia growth inhibited by methanolic leaf and root extracts at 10 mg/ml ranged between (53 – 57 %) and significantly higher than inhibition by aqueous extract (29 – 40 %). Summarily, both leaf and root methanolic extracts at 10 mg/ml had the highest mycelial growth inhibition of 57.28 and 53.06 %, respectively. It is concluded that sorghum leaf and root extracts possess fungicidal potentials that could be explored in the management of tomato Fusarium wilt.*

**Keywords:** *Aqueous extraction, botanicals, Fusarium wilt, methanolic extraction, sorghum*

### INTRODUCTION

Tomato (*Lycopersicon esculentus* Mill.), a short-duration crop, is one of the most important vegetables grown worldwide. It belongs to the family “Solanaceae”.

Nutritionally, tomatoes are rich in minerals, vitamins, essential amino acids, sugars, and dietary fibers. It is a good source of vitamins B and C, iron, and phosphorus which makes it a contributor to a healthy and well-balanced diet. Tomatoes contain lycopene, an antioxidant that may contribute to protection against carcinogenic substances (Wang *et al.*,

2016). It also contains carotenoids which are well-known as natural compounds involved in reducing the risk of development of several diseases such as diabetes, gastrointestinal and cardiovascular diseases, degenerative diseases such as blindness, xerophthalmia, and degeneration of muscles. Globally, tomatoes are an important food component. Tomato is rated second largest vegetable both in terms of production and consumption and the second most consumed fresh vegetable with 6 kg/person in 2017 (USDA, 2016).

In Nigeria, tomato is widely grown by smallholder farmers which account for 90% of production while the balance is contributed by commercial producers (Sahel research,

Didwania, 2019). Soil treatment with broadspectrum fumigants such as methyl bromide, chloropicrin, or methyl isothiocyanate successfully controlled the

2015). Nigeria has the largest area cultivated for fresh tomatoes in Africa with 3.58 million tons and second to Egypt with 6.25 million metric tons FAOSTAT, (2021). Despite the large cultivation figure, Nigeria generates the lowest yield in Africa at 4.2 MT/Ha which is significantly lower than Egypt with 41.6 MT/Ha and South Africa with yields of 78.7MT/Ha (FAOSTAT, 2021). This low yield could be attributed to factors such as poor production practices, low soil fertility, and pest and disease attacks (Sahel research, 2015).

Like other fruits and vegetables, tomato is affected by various diseases during production and at harvest. *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f.sp *lycopersici* is one of the most important diseases that affect tomato plants at all stages of production. It affects the whole plant parts both leaves and stems (Ravindra *et al.*, 2015). Infection by the fungus is characterized by wilted plants, yellowed leaves, and crop loss of about 30 to 40% which may go up to 80% under favorable weather conditions (Njiru, 2012). The most practical, cost-effective, and environmentally safe method of managing *Fusarium* wilt is the use of resistant cultivars but this could be limited by several factors such as resistance breakdown, evolutionary pattern of the pathogen, and development of new races of the pathogen (Sadana and

*Fusarium* wilt of tomatoes and increased crop yield (Adedeji and Aduramigba-Modupe, 2016) however, these chemicals have a negative impact on human health and are hazardous to the environment.

Moreover, pathogen resistance could emerge against these fungicides under certain circumstances and the residue of many fungicides could damage the ecosystem and negatively affect human life (Sadana and Didwania, 2019). Over the years, plants have been good sources of metabolites. These metabolites pose a wide range of biological activities and are also biodegradable. As plants absorb the sunlight they produce high levels of oxygen and secondary metabolites by photosynthesis, which results in medicinal components being produced and stored in plant leaves. Plants are also rich in antioxidants which are specific compounds that protect humans, animals, and plants against the damaging effects of free radicals or reactive oxygen species (Ayuba *et al.*, 2014). Sorghum *Sorghum bicolor* (L.) is the fifth most-produced cereal in the world, with Africa being the major producing region (>40% of world production (FAOSTAT, 2019). Sorghum is well-suited for cultivation in the semiarid and subtropical regions of Africa as it is one of the most droughttolerant cereal crops (Pontieri and Del Giudice, 2016). The sorghum plant (*Sorghum bicolor*) is a rich

and diverse source of phenolic compounds, particularly phenolic acids and flavonoids which were significantly higher in sorghum compared to most cereals and even fruits and vegetables (Barros *et al.*, 2012).

Anthocyanidins are the primary flavonoids found in sorghum and these flavonoids are believed to contribute to high antioxidant capacity in plants which provides overall disease protection through anti-oxidative mechanisms. This uncommonly high level of flavonoid accumulation in the sorghum makes it an interesting source of bioactive compounds (Carbonneau *et al.*, 2014). Biological activities of sorghum plant parts have been reported and documented by several authors such as the antimicrobial of its stem extract (Adetuyi *et al.*, 2007), *in vitro* anti-inflammatory and immune-modulating properties of leaf sheath (Beson *et al.*, 2013), antibacterial activities of leaves against bacteria strains *in vitro* (Ali *et al.*, 2020) and in various *in vivo* studies on rats using sorghum leaves, leaf base, and leaf sheath extracts (Salawu and Salimon, 2014). However, information on the antifungal potential of sorghum extract on fungal diseases of tomatoes is limited in the literature. Thus, this study was carried out to explore the antifungal activity of sorghum leaf and root extracts in the management of tomato Fusarium wilt in order to promote sustainability and increase food production while maintaining high levels of disease control.

## MATERIALS AND METHODS

**Source of seeds and isolate** Leaves and roots of sorghum at maturity were collected from an established plot at NIHORT experimental

field Ibadan between 6 and 7 a.m. throughout the study period. The leaves and root were air dried at 28–2°C in the laboratory for 21 days, blended into powder with an electric blender, and kept at room temperature (28–2°C) in clean bottles until needed. Isolate of *Fusarium oxysporum* f.sp. *lycopersici* was obtained from the pathological unit of Horticultural Research Institute, Ibadan.

### Extracts Preparation

Aqueous extract of leaf and root was prepared by adding 100 ml of sterile distilled water to 10 g of ground tissues in a 250 ml beaker. The mixture was stirred vigorously, allowed to settle for 4 hr, and then filtered through 2 mm-diameter sterile filter paper (Whatman No.42 110 mm) to obtain the stock. Lower concentrations of the extracts were prepared from the stock. The residue was oven-dried at 80°C for 2 hrs. The weight of plant material that dissolved in cold water was calculated using the formula of Enikuomihin and Oyedeji (2010):

$$W_s = W_1 - (W_2 + W_3)$$

Where:

$W_s$  = weight of dissolved samples  $W_1$  = initial weight of the sample before oven-dried drying  $W_2$  = weight of the oven-dried sample  $W_3$  = weight of filter paper

The methanolic extract was obtained with 90% methanol in a soxhlet extractor. The extract was concentrated by steam distillation, stored in clean bottles, and kept in the refrigerator at 4 °C until needed. A stock (10 mg/ml) was prepared with 20% propylene glycol and lower concentrations (8, 6, 4, and 2 mg/ml) were prepared from the stock.

### **Phytochemical Screening of Extracts**

Phytochemical analysis for some major phytoconstituents of the plant extracts was determined following the method of Harborne (1998). Plant extracts were screened for the presence of biologically active compounds such as saponins, tannins, cardiac glycosides and Anthraquinones.

### **Antifungal Screening of Plant Extracts**

The fungitoxic effect of extracts on fungi mycelial growth was evaluated using the method of Amadioha and Obi (1998). Four equal sections were created on the Petri dish containing PDA by drawing two perpendicular lines at the reverse side of each plate. One millilitre of aqueous or methanolic extracts of sorghum leaf or root at varying concentrations was dispensed in 9 cm - diameter Petri dishes while PDA without extract served as control. Fifteen milliliters of PDA containing (5 mg/L streptomycin sulfate) was dispensed, gently swirled and allowed to solidify. Three millimeters of the mycelial disc of the fungal species obtained from the edge of a 7-day-old culture were placed upside down at the point of intersection which indicates the centre of the plate. Plates were incubated at room temperature (28–2°C) for 7 days. Radial growth was measured daily as the mean growth along the two pre-drawn perpendicular lines on the reverse side of the plate. Fungitoxicity was expressed as percentage inhibition of mycelia growth using the formula of Awuah (1989);

$$M_P = \frac{M_1 - M_2}{M_1} \times 100$$

Where:

$M_P$  = Percentage inhibition of mycelia growth  
 $M_1$  = Mycelial growth in the control plate  
 $M_2$  = Mycelial growth in a plate containing plant extracts

Data collected were subjected to analysis of variance and the means were separated using Duncan Multiple Range Test

## **RESULTS**

The qualitative phytochemical screening of sorghum leaf and root extracts revealed the presence of cardiac glycoside and saponins. However, anthraquinone and alkaloids were absent both in leaf and root extracts of sorghum (Table 1). Methanolic extracts of both sorghum leaf and root significantly ( $P \leq 0.05$ ) reduced the mycelial growth of *F. oxysporum* f. sp. *lycopersici* compared to the aqueous extract. Sorghum leaf methanolic extract at 10 mg/ml had the least (37.0 mm) mycelial growth with mycelium inhibition of (57.3 %). This was significantly ( $P \leq 0.05$ ) lower than mycelia growth recorded against other concentrations.

Mycelia growth inhibition due to 6 and 4 mg/ml concentrations of sorghum leaf were at par. Of all the concentrations of sorghum leaf methanolic extract, 2 mg/ml had the least mycelia growth inhibition (23.9 %). No significant difference was observed in radial mycelial growth among all the concentrations due to sorghum leaf aqueous extract except at 10 mg/ml where a significantly ( $P \leq 0.05$ ) higher (40.8 %) mycelia inhibition was recorded (Table 2). While the lowest mycelia inhibition (13.2 %) was recorded against 2 mg/ml sorghum root aqueous extract, other concentrations exhibited no difference in terms of mycelia growth inhibition.

However, a similar trend was observed in the methanolic extract of sorghum root where 2 mg/ml induced the least (22.9 %) mycelial inhibition while the highest inhibition (53.1 %) was recorded at 10 mg/ml (Table 2).

Other concentrations of sorghum root methanolic extract had a range of between 32.6 and 43.5 % mycelial inhibition against *F. oxysporumf.splycopersici* (Table 2).

Summarily, both sorghum leaf and root methanolic extracts at 10 mg/ml had the highest mycelial growth inhibition of 57.28 and 53.06 % respectively plate (Fig. 2 and 4). This was followed by sorghum leaf aqueous extract at 10 mg/ml with mycelial growth inhibition of 40.80 % (Fig. 1). Sorghum root aqueous extract at 2 mg/ ml had the least (13.17 %) mycelial growth inhibition (Fig. 3).

**Table 1: Occurrence of some phytochemicals in extracts of the test plant**

Extracts	Tannins	Cardiac glycosides	Anthraquinones	Saponins	Alkaloids
Sorghum leaf	-	++	-	++	-
Sorghum root	-	++	-	++	-

+ = present, - = absent

**Table 2: Effect of the cold water and methanolic extracts of sorghum leaf and root on mycelia growth of *Fusarium oxysporumf.sp. lycopersici***

Extract	Concentration (mg/ml)	Radial growth (mm)	
		Sorghum leaf	Sorghum root
Cold water	10	51.50ef (40.80)	61.83cde (28.93)
	8	60.00c (31.03)	62.00cde (28.73)
	6	60.83c (30.08)	61.50cde (29.31)
	4	60.33c (30.08)	65.33cd (24.90)
	2	59.00cd (31.18)	73.83b (13.17)
Methanolic	10	37.16g (57.28)	40.83g (53.06)
	8	49.33f (43.29)	49.16f (43.49)
	6	54.50de (37.35)	56.16e (35.44)
	4	54.83de (36.97)	58.67de (32.56)
	2	66.17b (23.94)	67.06bc (22.91)
Control	-	87.00a (0.00)	87.00a (0.00)

Values in parentheses are percentage reductions in radial length. Values are obtained from mean of 4 replicate plates of fungal pathogen per treatment. Means with the same alphabet in the same column are not significantly different ( $P \leq 0.05$ ) Duncan's multiple range test



Figure 1: Growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* by different concentrations of Sorghum leaf aqueous extract after 7 days incubation at  $28 - 2^{\circ}\text{C}$ . A: 10 mg/ml; B: 8 mg/ml; C: 6 mg/ml; D: 4 mg/ml; E: 2 mg/ml; F: control (No extract)



Figure 2: Growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* by different concentrations of Sorghum leaf methanolic extract after 7 days incubation at  $28 - 2^{\circ}\text{C}$ . A: 10 mg/ml; B: 8 mg/ml; C: 6 mg/ml; D: 4 mg/ml; E: 2 mg/ml; F: control (No extract)

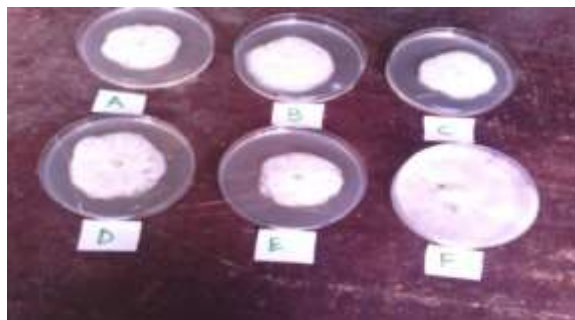


Figure 3: Growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* by different concentrations of sorghum root aqueous extract after 7 days incubation at 28 – 2 °C.

A: 10 mg/ml; B: 8 mg/ml; C: 6 mg/ml; D: 4 mg/ml; E: 2 mg/ml; F: control (No extract)

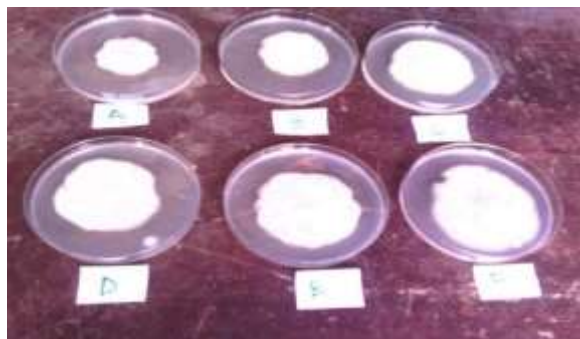


Figure 4: Growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* by different concentrations of sorghum root methanolic extract after 7 days of incubation at 28 – 2 °C. A: 10 mg/ml; B: 8 mg/ml; C: 6 mg/ml; D: 4 mg/ml; E: 2 mg/ml; F: control (No extract)

## DISCUSSION

Plants are rich in a wide variety of naturally occurring secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, quinone, and essential oils to mention but a few. These secondary metabolites are known to possess antifungal and antibacterial properties (Moses *et al.*, 2014). In this study, results from phytochemical analysis revealed the presence of cardiac glycosides and saponins in sorghum leaf and root extracts.

Previous studies have shown that phytochemicals such as saponins and cardiac glycosides demonstrated antimicrobial properties, particularly against fungi, bacteria, and protozoa (Matušinský *et al.*, 2015). Saponins have been involved in plant

defense responses essential for plant protection against microbial or pest attacks (da Cruz Cabral *et al.*, 2013). Saponins have a broad spectrum of activities in living organisms. They are generally antimicrobial against bacteria and fungi invading plants (Moses *et al.*, 2014). In addition, the insecticidal, antiviral, and molluscicidal activities of saponins have been documented (Singh and Kaur, 2018). Saponins are mainly considered to comprise a part of plants' antimicrobial defense system. The underlying mechanisms of their activity are understood to be based on their ability to form complexes with sterols present in the membrane of microorganisms and consequently to cause membrane perturbation (Sreij *et al.*, 2019). The antifungal activity of saponins has been

known for decades and their activity against fungal plant pathogens of crops such as soilborne and air-borne fungal pathogens and *Colletotrichum gloeosporioides* have been reported by Teshima *et al.* (2013).

In addition, the antifungal activity of sorghum leaf and root extracts in this study could also be traced to the presence of cardiac glycosides. About 15-18 types of cardiac glycosides are present in different parts of the plants (Nesy and Mathew, 2015). Cardiac glycosides and flavonoids showed broadspectrum antimicrobial activity which is quite encouraging and this agrees with studies on *Moringa oleifera* by Arora and Onsare (2014). Aboaba *et al.* (2006), in their findings, reported that many plants contain toxic glycosides which are toxic to pathogens. The effectiveness of methanolic extract over aqueous in inhibiting the mycelia growth of *Fusarium oxysporum* f.sp

*lycopersici* observed in this study could be attributed to the difference in the relative solubility of different phytochemicals. It could also be traceable to the differences in polarities of the solvents. This corroborated the findings by Bakht *et al.* (2011) in which they reported that the type of solvent used has an important role in determining the activity of the extract. Sidawi, *et al.* (2015) evaluated the efficacies of methanolic extracts of fig leaves (*Ficus carica*), leaves and stem of myrtle (*Myrtus communis*), and leaves, flowers, and roots of the marigold plant (*Tagetes patula*) separately to control Fusarium wilt and root rot disease on tomato plants. Their results showed that both extracts of marigold stem and leaves at 6%

concentration significantly reduced the wilt incidence.

Lower inhibitory properties exhibited by aqueous extract against *F. oxysporum* f.sp *lycopersici* in this study could be traced to the inability of the water to extract some phytochemical constituents. This is in agreement with the works of Shittu *et al.* (2006) who reported that an aqueous extract of *S. radiatum* showed no inhibitory effect on *S. pneumoniae* and *C. albicans* while ethanolic extract had a mild inhibitory effect on the same organisms at the same concentration.

## CONCLUSION

The use of botanicals to control soil-borne disease is a viable option due to the fact that it provides several advantages to growers such as a reduction in the use of pesticides thereby providing both economic and environmental benefit. Sorghum leaf and root extracts at 10 mg/ml in this study excellently inhibited the mycelia growth of *F. oxysporum* f.sp *lycopersici* *in vitro*. This suggested that sorghum leaf and root extract could be a potential alternative to synthetic pesticides in the management of tomato Fusarium wilt.

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