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EFFECT OF VARIOUS SOLVENT AND CONCENTRATION OF BAOBAB AND *Lantana* PLANT EXTRACTS ON RADIAL GROWTH OF TOMATO *Fusarium* WILT (*Fusarium oxysporium F. sp lycopersici*)

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ABSTRACT

This study investigated the antifungal potential of Baobab and *Lantana camara* leave and root (aqueous, ethanol and methanol extracts), 40g, 60g and 80g/250mL where evaluated for radial growth inhibition of *Fusarium oxysporium*. Mancozeb and sterile distilled water served as positive and negative controls and was laid out in Completely Randomize Design (CRD). Two vertical lines were drawn below each petri-dishes, dividing each dish into four equal sections. A 5mm piece of a 7-day-old FOL culture was inoculated at the center of each petri dish using a sterile 5mm cork borer. The petri dishes were then incubated at 28 ± 2°C for six days, during which data on radial growth inhibition was collected. Results showed that 80g/250ml ethanol recorded the lowest (13.67mm) radial growth and was statistically comparable to mancozeb that recorded lower (12.67mm) at 6 DAI which were better than other treatments and the negative control that recorded the highest radial growth (55.67%). Similar trend was observed under lantana methanol root extract at 80g/250ml concentration recording lower radial growth (8.67%) statistically similar to the mancozeb that recorded the lowest (7.00%) percent radial growth related to negative control that recorded the highest (51.33%) radial growth at 6 DAI. The study highlights the significance of the solvent choice and concentration as antifungal efficacy. These findings suggests that Baobab and *Lantana* plant extracts have potential as eco-friendly alternative for managing tomato *Fusarium* wilt. The optimal solvent and concentration combination can be exploited for developing novel sustainable antifungal agents.

Keywords: Baobab, *Lantana*, *Fusarium* wilt, radial growth inhibition, antifungal agent.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) serves as one of Nigeria's primary vegetable crops, being extensively cultivated and playing a vital role in daily diets. The crop belongs to the *Solaniceae* family (Aremu *et al*., 2016; Akaeze *et al*, 2017), and is world widely grown vegetable. Tomato ranks one of the most economically imperative vegetal crops globally and is extensively cultivated in Nigeria, ranking sixth in importance after cereals, root, and tuber crops.

Tomato (*Solanum lycopersicum*) global annual production volume is approximately 187 million tons on a cultivated area of over 5 million hectares (FAOSTAT, 2022). Nigeria was estimated to produce 4.1 million tons of tomatoes in 2021, which places her as the second largest producer of the fruit in Africa, after Egypt (FAOSTAT, 2022). Smallholder farmers are responsible for 90 % production of tomatoes in Nigeria because of their wide scale of consumption and acceptance (Osunmuyiwa *et al*., 2021).

Nigeria is the second-largest tomato producer in Africa and a key exporter of tomato derivatives. Nevertheless, only about 20% of tomato production in Nigeria is processed, mainly due to a lack of modern processing infrastructure. Additionally, post-harvest losses in the tomato value chain in Nigeria can reach up to 50%, there is limited access to finance from smallholder producers, and non-consistent quality standards for export Investments for the Tomato value chain add up to 869 million USD, aiming to benefit 72 thousand hectares and more than 36 thousand beneficiaries by supporting access to inputs and technology, installation of processing unit plants, establishment of multiplication centres, and R&D development. The IRR is 12.5% with a net present value of 171 million USD (FAOSTAT, 2024). Tomatoes are packed with vitamins and minerals, and they contain several key carotenoids, including lycopene, zetacarotene, gamma-carotene, beta-carotene, eurosporene, and lutein (Etebu *et al*., 2013).

The Federal Government of Nigeria developed a new tomato sector policy driven by the country's large population and high consumption of tomatoes. This policy is designed to cut down on post-harvest losses, curb the import of tomato concentrates through increased imports tariff from 5 percent to 50 percent and an additional $1500 levy per metric Tonnes. (Agriculture and Rural Development of Nigeria, 2022).

Tomato production encounters several challenges, including high rainfall, temperature fluctuations diseases and insect pests. A among these wilt diseases are a significant issue affecting tomatoes worldwide, cause by bacterial, nematodes, environmental factors and fungi. Specifically, the wilt, caused by fungus *fusariunm oxysforum* characterized by tint and wilting of the lower leaves, which then spread upward as the fungus further invades the plant (Agbenin and Erinje, 2001).

. Effective disease management often relies on the consistent use of synthetic chemical pesticides and copper compounds.

This practice helps prevent significant losses in both yield and quality. These have consequences for human health, environment and development of resistant pathogen strains, due to continuous use of the same available chemicals (Adedeji and Aduramigba, 2016; Akaeze and Aduranigba, 2017).

Bio fungicides have become a primary alternative to traditional fungicides, with the use of products derived from plants showing promise in managing diseases effectively (Pusztahelyi *et al*., 2015; Aduramigba-Modupe *et al*., 2021). Natural plant products and their analogues have been found as important sources of agricultural biopesticides.

Certain plant extracts have demonstrated the presence of various antimicrobial compounds that exhibit potential in controlling plant pathogens (Tijjani *et al*., 2014; Okey *et al*., 2016; Terna *et al*., 2017; Akaeze *et al*., 2017; Gurama and Haruna, 2018; Giordani *et al*., 2020). Therefore, this study was conducted to investigate the antifungal properties of both leaves and roots of Baobab and *Lantana camara* for the control of *Fusarium* wilt disease associated with tomato plant. These methods aim to provide eco-friendly, effective, and affordable alternatives to synthetic chemicals for controlling the disease in tomato production.

MATERIALS AND METHODS

Study area

This experiment was conducted at the Department of Microbiology laboratory, Faculty of Science, Abubakar Tafawa Balewa University, Bauchi (ATBU), Bauchi, under an ambient temperature of 28^0C to 32^0C (room temperature) and relative humidity of 55% to 75%. Bauchi town is located on the latitude $10^0$74`N and longitude $90^0$49`E situated at 609.37 above sea level, lies in the Northern Guinea savannah ecological zone of Nigeria.

Sources of Materials

Baobab leaves and roots were collected from Kari in Darazo local government area of Bauchi state. While *Lantana camara* leaves and roots were collected from College of Forestry, Jos, Plateau State. The plant parts (leaves and roots) was collected in the morning was then wash with sterile distilled water and packed in a sterile black polytene bags already lined with soft tissue paper and transported to the laboratory for further use. The botanical identity of the two plants was authenticated by the Herbarium unit of the Department of Biological Sciences of Abubakar Tafawa Balewa University (ATBU) Bauchi, with the following codes DBS 2679 for Boabab plant and DBS 2578 for lantana plant.

Tomato plants exhibiting wilt symptoms were collected randomly from a farm in Gubi campus and transferred to the microbiology laboratory at Abubakar Tafawa Balewa University (ATBU) in Bauchi State, Nigeria.

Isolation and Identification of Fungi

Isolation of the pathogen was performed using the direct inoculation method described by Okhuoya *et al*., (2012). Plant parts exhibiting wilt symptoms were first cleaned under sterile running water. These fragments were excised, surface-sterilized with a 0.5% sodium hypochlorite solution for one minute, and then rinsed three times with sterile distilled water. The fragments were dried on sterile filter paper to eliminate excess moisture and then cultivated on acidified potato dextrose agar in sterile petri dishes. The dishes were incubated at 28°C for 3–5 days. After incubation, the plates were observed for fungal growth. The isolated fungi were sub-cultured to achieve pure cultures, and their morphological characteristics were analyzed and compared to the standard descriptions provided by Barnett and Hunter (1988).

Preparation of Plant Extracts

The leaves and roots were rinsed three times with distilled water, then left to air dry at room temperature (28°C) for two weeks. After drying, the materials were ground into a fine powder using a mechanical grinder and stored in airtight containers for future use. For extraction, 40g, 60g, and 80g portions of the ground powder from the leaves and roots were weighed and each mixed with 250ml of distilled water, ethanol, and methanol in separate conical flasks respectively. The mixtures were soaked in an electric soaker for 48 hours. The solvent suspensions were then filtered through Whatman No. 1 filter paper. The filtrates were evaporated using a vacuum evaporator with a water bath to obtain the crude extracts, which were stored in amber bottles for later use. Mancozeb 63% wp (3g per 250ml) (Gondai *et al*., 2012) and sterile distilled water (SDW) were used as positive and negative controls, respectively.

Preparation of media

Prepared 9.8 grams of PDA was weighed and poured into a clean sterile 250 millilitres conical flask, 250ml of distilled water was added, a cork inserted into the conical flask and sealed with masking tape. The medium then stirred vigorously and placed on a hot plate, heated to boiling to ensure the medium was completely homogenised. The culture medium was sterilized by autoclaving at 121°C for 15 minutes and then cooled at room temperature.

In-vitro Evaluation of Plant Extracts against *Fusarium oxysporum f.* sp. *Lycopersici*

The experiment employed a completely randomized design with three replicates. Following the method of Amadioha and Obi (1999), this study was conducted to assessed the effect of plant extracts on the radial growth of *Fusarium oxysporum f. sp. Lycopersici*. The experimental layout and methodology were designed to evaluate the effect of plant extracts on the fungal pathogen.

At the bottom of each petri dish, two vertical lines were drawn, dividing the dish into four equal sections. Plant extracts at concentrations of 40g, 60g, and 80g per 250ml were dispensed into sterile petri dishes, gently swirled to ensure a homogeneous mixture, and allowed to solidify. Control petri-dishes consist of PDA with no plant extracts but sterile distilled water (SDW) and synthetic fungicide mancozeb was inoculated with the test fungus, both serve as negative and positive control. A 5mm disk of 7-day-old *Fusarium oxysporum f. sp. Lycopersici* culture was placed at the center of each petri dish (where the two lines intersected) using a 5mm cork borer. The dishes were incubated at 28 ± 2°C for six days. The colony diameter was measured as the average growth along the two perpendicular lines on the back of the petri dishes, and the percentage inhibition of *Fusarium oxysporum f. sp*. Lycopersici was calculated following the method described by Terna et al. (2016).

Data Collection

Data collected on the radial growth inhibition Days After Inoculation (DAI) of each inoculum was measured at 24-hour intervals over a six-day period using a meter rule (Ortiz and Hoyos-Carvajal, 2016; Akaeze *et al*., 2017).

Data Analysis

The data obtained were subjected to analysis of variances (ANOVA) using a GENSTAT (17th editions) statistical package and were separated by level of significance at 1% of probability

RESULTS AND DISCUSSION

Table 1 Effect of Various Solvent and Concentration of Baobab leave Extracts on Radial Growth of *Fusarium oxysporum*

Baobab Leave Extracts (Treatments)		Radial Growth Days After Inoculation (%)					
Solvent	**Concentration g/ml**	**1**	**2**	**3**	**4**	**5**	**6**
Aqueous	40	0.00^{a}	12.33^{c}	24.67^{de}	27.00^{bc}	34.00^{b}	42.00^{b}
	60	0.00^{a}	12.33^{c}	22.00^{cde}	26.67^{bc}	32.33^{b}	37.67^{b}
	80	0.00^{a}	11.33^{c}	22.00^{cde}	25.67^{bc}	28.33^{b}	36.67^{b}
Ethanol	40	0.00^{a}	10.00^{bc}	16.33^{bc}	24.00^{bc}	31.33^{b}	34.33^{b}
	60	0.00^{a}	8.67^{bc}	14.67^{bc}	21.33^{bc}	28.33^{b}	33.33^{b}
	80	0.00^{a}	5.33^{b}	11.33^{b}	19.33^{b}	26.33^{b}	32.33^{b}
Methanol	40	0.00^{a}	9.00^{b}	18.67^{bcd}	29.67^{bc}	35.00^{b}	37.67^{b}
	60	0.00^{a}	8.33b^{c}	14.67^{bcd}	25.67^{bc}	31.00^{b}	35.00^{b}
	80	0.00^{a}	6.00^{b}	13.67^{bc}	20.00^{cd}	26.00^{b}	30.67^{b}
Mancozeb		0.00^{a}	0.00^{a}	0.00^{a}	4.33^{a}	7.33^{a}	12.33^{a}
Control		7.33^{b}	18.00^{d}	29.67^{e}	36.00^{d}	50.00^{c}	55.33^{c}
LS		**	**	**	**	**	**
SE (±)		0.284	2.270	3.599	3.903	4.612	5.82

LS: level of significance, SE Standard error, DAI Days after inoculation. Mean followed by the same letter in the same columns are not significantly different p ≤ 0.01

Table 1 showed the effect of different solvents and concentration of Baobab leaves extracts on the radial mycelial growth of *fusarium oxysporium* days after inoculation *(DAI).* The result showed a constant radial growth of the fusarium oxysporium at 1 DAI regardless of the solvent or its concentration but increased slowly with the increase in the period. At 6 DAI it was observed that Baobab at 80g/250ml gave the lowest radial growth (30.67mm) after the positive control that recorded 12.33. this was followed by 33.33mm observed under Baobab ethanol leaf extracts at 80g/250ml at 6 DAI. Baobab leave extracts were generally observed to be the most tolerant or

resistant to fungal infection. confirmed the finding of Latifou *et al*, (2012) who reported that the antifungal activity of extracts is more marked on the inhibition of the sporulation than on the mycelia development of the fungi. The increased inhibitory effect seen in methanol and hydroethanolic extracts may be attributed to their tannin and flavonoid content. Research indicates that flavonoids can suppress the germination of spores in plant pathogens, while tannins are known to disrupt germ-tube development, as cited by Harborne and Williams (2000).

Table 2: Effect of Various Solvent and Concentration of Baobab Root Extracts on Radial Growth of *Fusarium oxysporum*

Treatments		**Radial Growth Days After Inoculation (DAI)**					
Solvent	**Concentration g/ml**	**1**	**2**	**3**	**4**	**5**	**6**
Aqueous	40	0.00^{a}	14.67^{g}	16.67^{bf}	20.33cf	27.33^{ce}	35.00^{e}
	60	0.00^{a}	11.67^{bfg}	15.33^{bef}	20.00^{cef}	26.00^{ce}	34.00^{ce}
	80	0.00^{a}	11.00^{bcefg}	15.00^{bdef}	17.00^{cdef}	21.33^{bcde}	27.00^{bcde}
Ethanol	40	0.00^{a}	4.67^{abcde}	8.00^{abcd}	12.00^{abcd}	17.^{abcd}	21.67^{abcd}
	60	0.00^{a}	4.00^{abcd}	7.33^{ab}	11.33^{abcd}	16.67^{abcde}	21.33^{abcd}
	80	0.00^{a}	2.00^{a}	5.33^{a}	8.67^{ab}	11.00^{a}	13.67^{a}
Methanol	40	0.00a	4.67^{abcde}	9.00^{abcdef}	13.^{abcde}	16.67^{abcd}	20.67^{abcd}
	60	0.00a	5.50^{abcdef}	8.67^{abcde}	13.^{abcdef}	15.50^{ab}	17.50^{ab}
	80	0.00a	3.50^{abc}	8.50^{abcde}	10.50^{abcd}	13.50^{ab}	17.00^{ab}
Mancozeb		0.00^{a}	0.00^{a}	5.50^{a}	8.00^{a}	10.67^{a}	12.67^{a}
Control		6.00^{b}	14.67^{g}	24.67^{g}	36.00^{g}	45.67^{f}	55.67^{f}
LS		**	**	**	**	**	**
SE (±)		0.447	2.946	3.083	3.069	3.665	4.345

LS: level of significance, SE Standard error, DAI Days after inoculation. Mean followed by the same letter in the same columns are not significantly different p ≤ 0.01

The results of the effect of Baobab roots extracts on radial mycelial growth of *fusarium oxysporium* was presented in Table 2. Similar trend of constant radial growth of 0.00mm was observed at 1 days after inoculation (DAI) across the solvents and their concentration with the exception of the negative control recording 6.00mm at that day. As the concentration of the extract increases, a significant difference ($p \leq 0.01$) was observed among the treatments. At 6 DAI, the result indicated a lower (13.67mm) radial mycelial growth under ethanol Baobab root extract which was comparable to mancozeb of 3 g/250ml concentration recording (12.67mm) respectively. The control exhibited the highest radial mycelial growth, measuring 55.67mm. Keyla *et al*. (2022) explored the potential of *Adansonia digitata* L. for glycemia management through various animal models, suggesting its antidiabetic effects in vivo. The traditional use of baobab pulp has generated significant scientific interest, leading researchers to investigate its pharmacological properties. While *A. digitata* has demonstrated hypoglycemic effects, limited scientific studies have been conducted to fully understand its activity in this regard (Doan *et al*., 2015).

Table 3: Effect of Various Solvent and Concentration of *Lantana camara* Leave Extract on Radial Growth inhibition of *Fusarium oxysporum*

Treatments		Radial Growth Days After Inoculation (DAI)					
Solvent	**Concentration g/ml**	**1**	**2**	**3**	**4**	**5**	**6**
Aqueous	40	0.00^{a}	10.67^{b}	22.33^{c}	28.67^{d}	35.33^{d}	40.67^{e}
	60	0.00^{a}	7.33^{c}	19.67^{c}	28.00^{d}	19.00^{c}	38.87^{e}
	80	0.00^{a}	6.67^{b}	13.67^{b}	16.00^{c}	32.67^{d}	25.67^{d}
Ethanol	40	0.00^{a}	2.67^{a}	4.67^{a}	9.33^{b}	13.67^{bc}	20.33^{cd}
	60	0.00^{a}	0.00^{a}	4.33^{a}	6.67^{ab}	11.33^{abc}	16.67^{bcd}
	80	0.00^{a}	0.00^{a}	2.33^{a}	3.67^{ab}	7.67^{ab}	15.00^{abc}
Methanol	40	0.00^{a}	0.00^{a}	2.33^{a}	5.67^{ab}	7.00^{ab}	12.67^{abc}
	60	0.00^{a}	0.00^{a}	2.00^{a}	2.67^{ab}	7.00^{ab}	10.00^{ab}
	80	0.00^{a}	0.00^{a}	1.67^{a}	2.67^{ab}	6.00^{ab}	8.33^{ab}
Mancozeb		0.00^{a}	0.00^{a}	0.00^{a}	1.33^{a}	3.67^{a}	5.00^{a}
Control		5.00^{b}	14.67^{d}	29.33^{d}	34.00^{d}	56.00^{e}	58.33^{f}
LS		**	**	**	**	**	**
SE (±)		0.246	1.449	2.651	3.072	4.141	4.483

LS: level of significance, SE Standard error, DAI Days after inoculation. Mean followed by the same letter in the same columns are not significantly different $p \leq 0.01$

Table 3 shows the result of *Lantana camara* leaf extracts from distilled water, ethanol and methanol at 40g, 60g, and 80g/250ml days after inoculation (DAI). A constant growth of 0.00mm was observed at 1DAI and 2 DAI at 60 and 80g/250ml under ethanol and all concentrations under methanol. Although a significant difference ($p \leq 0.01$) existed among the treatments, but at 6 DAI the smallest radial growth was observed in petri plates treated with 80g/250ml methanol extract (8.33mm) followed by 60g/250ml (10.00mm). The highest mycelial growth was observed (58.33mm) under the control at 6 DAI.

This result is in agreement with Sreenu and Zacharia, (2017); Awaias *et al*, (2017); Samar *et al*, (2021) and Mbasa, (2021), (Rasa *et al*, 1993), Ghante *et al*, (2019) which indicates that the efficiency of *Lantana camara* leaf extracts on management of *F. oxysporium*. But *Lantana camara* exhibits broad antifungal activities, including control of Colletotrichum gloeosporioides penz in mango (Bashir *et al*., 2019) and *Alternaria alternata* in potato (Singh and Srivastava, 2012). These antifungal properties are attributed to the presence of bioactive and phytochemical compounds such as flavonoids, phenols, alkaloids, saponins, glycosides, tannins, naphthoquinones, coumarins, and terpenoids (Singh and Srivastava, 2012; Fayaz *et al*., 2017; Bashir *et al*., 2019).

Table 4: Effect of Various Solvent and Concentration of *Lantana camara* root Extract on Radial Growth inhibition of *Fusarium oxysporum*

Treatments		Radial Growth Days After Inoculation (DAI)					
Solvent	**Concentration g/ml**	**1**	**2**	**3**	**4**	**5**	**6**
Aqueous	40	0.00^{a}	5.67^{b}	11.67^{e}	15.00^{bc}	24.67^{b}	28.69^{c}
	60	0.00^{a}	1.67^{a}	7.33^{de}	15.67^{c}	22.33^{b}	25.00^{bc}
	80	0.00^{a}	0.00^{a}	5.00^{acd}	8.33^{ab}	14.00^{a}	16.33^{ab}
Ethanol	40	0.00^{a}	1.33a	5.00^{a}	8.00^{ab}	13.00^{a}	15.33^{ab}
	60	0.00^{a}	0.00a	4.67^{abcd}	6.00^{a}	9.00^{a}	10.00^{a}
	80	0.00^{a}	0.00^{a}	0.00^{a}	2.67^{a}	7.67^{a}	15.33^{ab}
Methanol	40	0.00^{a}	0.00^{a}	2.00^{abc}	3.67^{a}	9.00^{a}	12.00^{a}
	60	0.00^{a}	0.00^{a}	1.67^{abc}	5.00^{a}	7.67^{a}	11.00^{a}
	80	0.00^{a}	0.00^{a}	0.00^{a}	2.33^{a}	6.67^{a}	8.67^{a}
Mancozeb		0.00^{a}	0.00^{a}	0.00^{a}	2.67^{a}	5.67^{a}	7.00^{a}
Control		6.67^{b}	13.33^{c}	22.67^{f}	33.67^{d}	43.00^{c}	51.33^{d}
LS		**	**	**	**	**	**
SE (±)		0.376	1.082	2.156	3.378	3.816	4.72

LS: level of significance, SE Standard error, DAI Days after inoculation. Mean followed by the same letter in the same columns are not significantly different $p \leq 0.01$

The result of the effect of *lantana camara* root extract on the radial growth of F. *oxysporium* was presented in Table 4. It was observed with the exception of 1 and 2 DAI there were significant differences ($p \leq 0.01$) among the treatments. It was also observed that from day 4, 5 and 6 after inoculation the results showed that petri plates treated with methanol root extracts *L. camara* showed lower radial growth and this were comparable to the mancozeb. At day 4, the result showed 2.33mm for plates treated with 80g/250ml of methanol root extract, mancozeb 2.67mm, at 5 day after inoculation methanol root extract 6.67mm, mancozeb 5.57; at day 6 after inoculation methanol treated Petri plates 8.67mm and mancozeb treated plates 7.00mm which were all comparable to each other across day 4, 5, and 6. Highest radial growth was observed (51.33m) under the control or untreated plates. This study's findings align with those of Rongai *et al*. (2015), who noted that the high antifungal activity of *Zizyphus spinachrist* extract is attributed to the presence of phenolic compounds. The bioactive polyphenols, whether acting individually or in combination, interfere with fungal life processes through several mechanisms: binding to proteins, acting as chelating agents, altering structural component synthesis, and disrupting or destroying cell membrane permeability, which in turn alters the cells' physiological status. These results are consistent with earlier studies. However, other researchers have found that plant extracts inhibit fungi and bacteria primarily due to their flavonoid content.

CONCLUSION

This study investigated the radial growth inhibition of *Fusarium oxysporium* pathogen exposed to aqueous, ethanol and methanol solvents of *lantana camara* and Baobab at varying concentrations (40g, 60g and 80g) over a six (6) day period.

The results provide valuable insights into the antifungal properties of Lantana and Baobab. The methanol solvent demonstrated superior radial growth inhibition, particularly at 80g concentration, indicating its potential as an effective

antifungal agent. Ethanol solvent exhibited moderate antifungal inhibition, while aqueous solvent showed least efficacy.

Those findings suggest that solvent choice and concentration significantly impact radial growth inhibition. The study outcomes have important implications for developing novel antifungal agents and optimizing solvent-based treatments for fungal control. The result also underscores the importance of considering solvent concentration and exposure duration when designing antifungal therapies.

RECOMMENDATIONS

Methanol solvent at 80g concentration is recommended for optimal radial growth inhibition of tested pathogen *Fusarium oxysporium*. Ethanol solvent at 80g concentration may be considered for moderate inhibition. Further research is needed to explore the antifungal properties of these solvents. Further research is warranted to explore the underlying mechanisms of solvent micro-organisms interaction and to investigate the efficacy of solvent combinations.

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