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FUNGICIDAL EFFECT OF TEAK (*TECTONA GRANDIS* L.) LEAF EXTRACTS ON *FUSARIUM OXYSPORUM*

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ABSTRACT
Fusarium oxysporum f. sp. lycopersici is the pathogen of tomato wilt, a disease of great economic importance worldwide. Although application of synthetic fungicides such as thiophanate methyl and mancozeb can prevent the occurrence of this disease, their effects on the physical environment especially, is a limiting factor. Botanicals, i.e. plant-based fungicides are now being preffered for controlling fungal pathogens because they have minimal environmental impact and are less dangerous to consumers in contrast to synthetic fungicides. Teak leaves have been reported to possess a very great antimicrobial activity because of their high content of phytochemicals. In an effort to develop eco-friendly chemical strategy for control of Fusarium wilt disease of tomato plants, in vitro effect of 10, 30 and 50% (w/v) concentrations of the aqueous and ethanolic leaf extracts of teak (Tectona grandis L.) on the radial growth of the mycelia of Fusarium oxysporum f. sp. lycopersici was investigated using the pour plate method. Results of the study revealed that both extracts retarded the radial growth of mycelia of F. oxysporum f. sp. lycopersici compared to that of the control, with the ethanolic extract having a greater effect at the concentrations tested in this study. It is therefore recommended that an in vivo study of effects of the same leaf extracts on wilt – infected tomato plants be conducted.
KEY WORDS: *botanicals, in vitro, pathogen, teak, tomato, wilt.*

INTRODUCTION

The tomato plant, *Solanum lycopersicum* L. is susceptible to a number of bacterial and fungal diseases, which severely reduce its yield, one of which is Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) (Ma *et al*., 2023). Symptoms of the disease include wilting as well as droopiness and yellowing of the affected plant (Koo *et al*., 2023). Although synthetic pesticides are available for protection of crop plants from pathogens and prevent disease occurences, the use of plant materials such as fresh extracts from various medicinal plant parts generally referred to

as botanicals, is becoming very popular and better alternatives in recent time due to the high cost of some of the synthetic pesticides and their potentially dangerous side effects on the environment and living things (Boboescu et al, 2020; Drăgoi & Ianovici, 2021; Almășan & Ianovici, 2022; Ayilara *et al*., 2023).

Some medicinal plants have been reported to possess antimicrobial activity against some plant pathogens *(Lee et al*., 2022). The antimicrobial activity is attributed to the presence of phytochemicals (secondary metabolites) such as flavonoids, alkaloids, terpenoids, tannins, phenols, etc in the medicinal plants (Alexan & Ianovici, 2018). Their extracts can, therefore be utilized to create new, environmentally friendly, and safer non synthetic pesticides for controlling plant pathogens as well as the diseases they cause. Popular amongs these medicinal plants are neem, pawpaw, mango, guava, bitterleaf, khaya, mahogany, aloe vera, teak, orange, teak, etc.(Asdaq *et al*., 2022). The volume of studies conducted on *in vitro* antimicrobial activity of teak leaf extracts is less compared to the other medicinal plants listed above, hence the justification of this study, which was aimed at determining the *in vitro* antifungal aactivity of teak leaf extracts against *Fusarium oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Fusarium oxyspororum f. sp. *Lycopersici* (CRIN/PPL/FO/08-03-23), the test organism was acquired from the Plant Pathology Laboratory of Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. The organism was kept alive on streptomycin – amended Potato Dextrose Agar (PDA) media in bottle slants. The plant materials used were fresh teak leaves which were also collected from a teak tree within the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Oyo State, Nigeria.

Preparation of the Aqueous and Ethanolic Teak Leaf Extracts. Aqueous and ethanolic (70% v/v) teak leaf extracts were prepared in 10, 30, and 50% (w/v) concentrations following the methods of Ekhuemelo & Eigege (2017). The extracts were filter-sterilized, and kept at 25^{o}C in a refrigerator until they were ready for use.

Preparation of Potato Dextrose Agar (PDA) Culture Medium. Potato Dextrose Agar (PDA) culture medium was prepared following the Manufacturer's instructions. Nineteen grams of the powder was weighed into 500 ml of sterile distilled water inside a conical flask. It was allowed to dissolve on a hot plate and then autoclaved for 15 minutes at 121 ℃ and 15 lb/inch2 of pressure. After cooling, Streptomycin was added to it at 1 ml to prevent the growth of bacteria.

Effects of the Leaf Extracts on the Test Organism. The *in vitro* effects of the leaf extracts on the test organism were assessed using the pour plate method as described

by Gonelimali *et al*. (2018). In a laminar air-flow chamber, 7 disposable, sterile Petri dishes were set up (Fig. 1). One milliliter (1 ml) of different concentrations of the aqueous and ethanolic teak leaf extracts was pipetted into 6 of the Petri dishes as labeled in Fig. 1, after which 10 ml of the streptomycin amended molten PDA was aseptically poured into each of the labeled Petri dishes and the 7th Petri dish labeled as control. Each plate was gently swirled to allow the teak leaf extracts and the culture medium mix up uniformly. At the centre of the cover of each of plate, two parallel lines were drawn, crossing each other. Seven inoculi (of 1m mm diameter each) were cut out from the actively growing edge of the test organism (*F. oxysporum* f. sp. *lycopersici* (CRIN/PPL/FO/08-03-23)) using a sterile 10 mm cork borer into a sterile Petri dish. Each of these was inoculated at the centre of each plate using a sterile forcep. The above set up was replicated three times within the laminar air – flow chamber. The plates were incubated at 25°C for five days, with radial growth of the inoculim (D) being measured according to Olahan *et al.* (2022), using the formula:

$$D_{(mm)} = \frac{dx+dy}{2}$$

where dx is the diameter of the mycelium along the horizontal line and dy is the diameter of the mycelium along the vertical line on each of the cover of the plates.

The Precentage of Growth Inhibition by the teak leaf extracts was determined using the formula proposed by Okigbo and Nneka (2013), i.e.

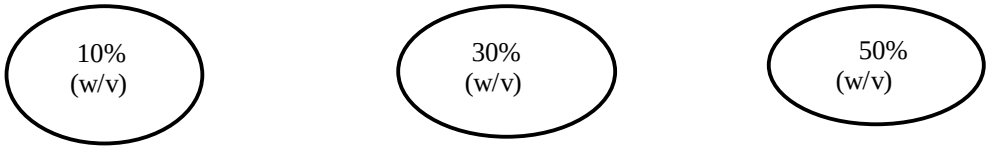
$$\text{PGI (\%)} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

Where, PGI (%) = Percentage Growth Inhibition;

DC = Diameter of the test organism on the Control plates; and

DT = Diameter of the test organism on the treated plates.

Analysis of Variance of the mean percentage growth inhibition of the leaf extracts at $p<0.05$ and the differences between the means were determined using Fisher's LSD test.



Aqueous Extracts

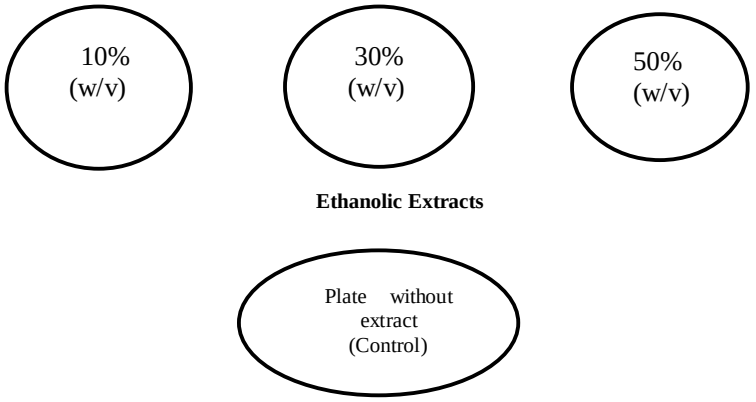


FIG. 1: Experimental Design (Olahan and Amadi, 2006)

RESULTS AND DISCUSSIONS

The results obtained revealed that the three concentrations of the extracts used in this study retarded the radial growth of *Fusarium oxysporum* f.sp. *lycopersicum* on PDA plates compared to the control. At 24 Hours After Incubation (HAI), the Percentage Growth Inhibition (PGI) induced by the 10, 30 and 50%(w/v) concentrations of the aqueous leaf extracts were 33.54, 36.16 and 33.88 respectively, while the PGI for the 3 concentrations of the ethanolic leaf extracts was the same (30.38). The ALE at the 3 concentrations were more potent than the same concentrations of the ELE. Results of the analysis of variance showed that there was significant difference between the PGIs induced by 10 and 50% of ALE, and no significant difference between the 3 concentrations of ELE at ($P<0.05$) (Table 1).

At 48 HAI, the PGI for 10% (w/v) ALE was 38.08, while those for the 30 and 50% (w/v) were 32.16 and 32.29 respectively. There was no significant difference between the PGI for the 3 concentrations of ALE at ($P<0.05$) (Table 1). The scenarios were the same for the 3 concentrations of the ELE, with the PGIs being 61.23 for 10%(w/v), 58.33 for 30% (w/v) and 55.44 for 55% (w/v). The ELE was more potent than the ALE, in contrast to what was observed at 24 HAI. At 72 HAI, there was no significant difference at ($P<0.05$) between the PGIs induced by 30 and 50% (w/v) concentrations of the ALE (25.04 and 22.23 respectively), but there exist a significant between these PGIs and the PGI induced by 10% (w/v) concentration (36.71) at ($P<0.05$) (Table 1). The ELE was again more potent than the ALE. There was no significant

difference at (P<0.05) between the PGIs induced by the 10 and 30% (w/v) concentrations of ALE (27.36 and 27.03, respectively) at 96 HAI, while there exist a significant difference at (P<0.05) between the above PGIs and the PGI for 50% (w/v) concentration (17.84) for the ALE (Table 1). For the ELE, there was no significant difference at (P<0.05) between the 3 concentrations tested, i.e. 52.25 by the 10% (w/v), 58.26 by the 30% (w/v) and 59.16 by the 50% (w/v) (Table 1). The ELE was again more potent than the ALE.

At 120 HAI, the scenario observed for ALE were the same as reported for ALE at 96 HAI (Table 1). For the ELE, there was a significant difference at (P<0.05) between the PGIs induced by the 3 concentrations tested in this study, with the 50% (w/v) concentration inducing the highest PGI (61.80), followed by the 30% (w/v) with PGI of 58.39 and the 10% (w/v) with PGI of 53.53 (Table 1). The PGI for ALE were 33.09, 37.69 and 16.79, respectively at the 10, 30 and 50% (w/v) concentrations.These scenario are the same with what was observed at 96 HAI (Table 1). The ELE was again, more potent than the ALE at all the concentrations tested.

TABLE 1: Percentage of Growth Inhibition inhibition of Teak leaf extracts on the radial growth of *Fusarium oxysporum* f.sp. *lycopersicum* at different concentrations after 120 hours of incubation and are statistically significant (p<0.05).

Concentrations of	Percentage growth Inhibition (%)				
The Extracts	24HAI	48HAI	72HAI	96HAI	120HAI
(%w/v)					
ALE					
10	33.54^{ab}	38.08^{b}	36.71^{c}	27.36^{b}	33.093^{c}
30	36.16^{a}	32.16^{b}	25.04^{d}	27.03b	37.69c
50	33.88^{ab}	32.29^{b}	22.23^{d}	17.84^{bc}	16.79^{d}
ELE					
10	30.38^{b}	61.23^{a}	56.79b	52.25a	53.53^{b}
30	30.38^{b}	58.33^{a}	61.36^{a}	58.26^{a}	58.39^{ab}
50	30.38^{b}	55.44^{a}	55.973^{b}	59.16^{a}	61.797^{a}

Mean values with same letters in the same column are not significantly different at P<0.05 using Fisher's LSD Test.
Keys: ALE: Aqueous Leaf Extract; ELE: Ethanolic Leaf Extract; HAI: Hours After Incubation and PGI: Percentage Growth Inhibition.

This study was conducted to test the *in vitro* antifungal activity of aqueous and ethanolic leaf extracts of teak at 10, 30 aand 50% (w/v) using Pour Plate Method on *F.*

oxysporum f.sp. *lycopersici*. This was because over the years, there has been a lot of interest in the possibility of using plant-derived compounds as effective agents for chemical control of plant diseases (Lahlali *et al*., 2022). The importance of *Tectona grandis* (teak) leaves as a therapeutic herb containing bioactive/antimicrobial substances have earlier been reported by Vaou *et al*. (2021) and Asdaq et al. (2022). Findings from this study showed that both the ethanolic and aqueous teak leaf extracts inhibited the radial growth of *F. oxysporum* f.sp. *lycopersici*, with the ethanolic extract being more potent. This may be partly due to the effectiveness of ethanol for extraction as well as a chemical sterilant (Olahan & Amadi, 2006). According to Altemimi *et al*. (2017), extracts made with alcohol increase the presence of bioactive molecules that possess stronger antifungal activity. Stéphane *et al*. (2022) and Mogana *et al*. (2020) differently reported that alcohol-based extractants have higher volatility water, and tend to extract more kinds of antimicrobial substances from plant materials. These reports seem to explain why the ethanolic and aqueous leaf extracts of *Tectona grandis* had different antimicrobial effects on *F. oxysporum* f.sp. *lycopersici* in this study.

Olahan and Amadi (2006) investigated *in-vitro* effect of various concentrations of pawpaw (*Carica papaya* L.) leaf extracts on the radial growth of *Fusarium verticilloides* and reported that the aqueous and ethanolic leaf extracts retarded radial growth of the test microorganism compared to the control at the tested concentrations. Similarly, the aqueous extracts of *Polystichum squarrosum*, *Adiantum venustum*, *Parthenium hysterophorus*, *Urtica dioeca* and *Cannabis sativa* leaves exhibited antifungal activity against *R. solani*, *F. oxysporum* and *A. solani* but with a lower effectiveness compared to *E. hirta* ethanolic extracts (Tapwall *et al*., 2011). Also, Mekam *et al*. (2019) reported that ethanolic extracts of *Oxalis barrelieri* L. (Oxalidaceae), *Stachytarpheta cayennensis* L. (Verbenaceae) and *Euphorbia hirta* L. (Euphorbiaceae) had a higher antifungal activity compared to the aqueous extracts on the cultures of *Fusarium solani*, *Rhizoctonia solani* and *Alternaria solani*, using the pour plate and agar diffusion techniques. The aqueous extract of *Picralima nitida* fruits proved less effective than its ethanolic extract at suppressing the growth of *Fusarium oxysporum* (Akabassi *et al*., 2022). Also, Olahan *et al*. (2020) reported the effectivess of ethanolic extracts against *Colletotrichum falcatum* compared to aqueous extract.

CONCLUSIONS

The aqueous and ethanolic leaf extracts of *Tectona grandis* have *in vitro* antifungal efficacy against *F. oxysporum* f.sp *lycopersici*, with the ethanolic leaf being more potent at the concentrations tested.

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