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Fungicidal effect of pawpaw (*Carica papaya* L.) leaf extracts on *Fusarium verticilliodes*

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ABSTRACT: The *in vitro* effect of 10, 30 and 50% (w/v) concentrations of the aqueous and ethanolic extracts of pawpaw (*Carica papaya* L.) leaf on the radial growth of *Fusarium verticilliodes* was studied using the pour plate method. The diameters of radial growth of the mycelia of *F. verticilliodes* were measured at 24 hours intervals for five days. Both extracts retarded the radial growth of *F. verticilliodes* compared to that of the control, with the ethanolic extract having a greater fungistatic effect at the concentrations tested in this study.

Key Words: Pawpaw; *Carica papaya*; Fungicidal properties; *Fusarium verticilliodes.*

Introduction

Pawpaw (*Carica papaya* L.) belong to the family Caricaceae, a small group of four genera of trees or shrubs with their leaves in terminal clusters and latex vessels throughout their tissues (Cobley and Steele, 1976). About 40 species of the genus *Carica* are known in the Americal tropics and sub-tropics, and pawpaw probably originated in Central America as a hybrid between other species. It is not known to occur in the wild, but it is easy to propagate from the seed.

The fruits are eaten fresh, or may be used in drinks or for flavouring desserts. The ripe fruit is canned, and immature fruits can be cooked and eaten as a vegetable. When tapped, pawpaw yields a latex containing the proteolytic enzyme papain. *C. papaya* thrives on a variety of soils but will not tolerate waterlogging and though they are not demanding in their environmental requirements the best fruits are produced only in warm climates. Pawpaws are usually dioecious, though hermaphrodite plants and even hermaphrodite cultivars ('Solo') do occur (Cobley and Steele, 1976).

Fusarium verticilliodes belongs to the fungal division Eumycota, sub-division Deuteromycotina, class Hyphomycetes and genus *Fusarium* (Dube, 1982). It is a notorious pathogen of several crop plants including those of the following families: Gramineae, Amaranthaceae, Moraceae, Coniferae, Cruciferae, Euphorbiaceae and Cucurbitaceae. The genus may cause seedling blight, scorch, foot rot, stunting and hypertrophy (Booth, 1971) in any of its hosts. It also causes bakanae disease of rice, seedling blight, root rot and pink boll diseases of cotton. It attacks flowers and fruits of banana, and is associated with the storage rot of pineapple and tomato. *Fusarium* species are both seed- and soil-borne.

According to Emeruwa (1982), pawpaw is a medicinal plant. However, only little work has been done to assess the *in vitro* effect of extracts from various parts of this plant on plant pathogenic fungi. This study is expected to awaken the interest of researchers towards the pawpaw plant and is aimed at studying the in vitro effect of aqueous and methanolic extracts of pawpaw leaf at different concentrations on the radial growth of *F. verticilliodes*.

Materials and Methods

The test organism (*Fusarium verticilliodes*) was obtained from the Plant Pathology Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The organism was maintained on potato dextrose agar (PDA) medium in bottle slants. The medium was prepared routinely with a few drops of Streptomycin added to discourage bacterial growth. The plant materials used were fresh pawpaw leaves detached from a pawpaw plant in front of Chemistry Department, University of Ilorin, Nigeria.

Preparation of Extracts

Pawpaw leaf extracts were obtained in aqueous and ethanolic (70% v/v) solutions over a period of 24 hours. Three concentrations of the extracts 10, 30 and 50% (w/v) were prepared according to Adekunle (2001). The extracts were filter-sterilized (Nester et al., 1998) and then tested for sterility against fungal and bacterial contaminants and subsequently stored at 25^{o}C in a refrigerator prior to use.

Effect of extracts on F. verticilliodes

The pour plate method (Adekunle, 2001) was used to determine the *in vitro* effect of the extracts on the test organism. Seven sterile, disposable Petri dishes were arranged in a laminar air-flow chamber. One millilitre (1 ml) of each of the concentrations of the extracts was dispensed into each dish (Fig. 1).

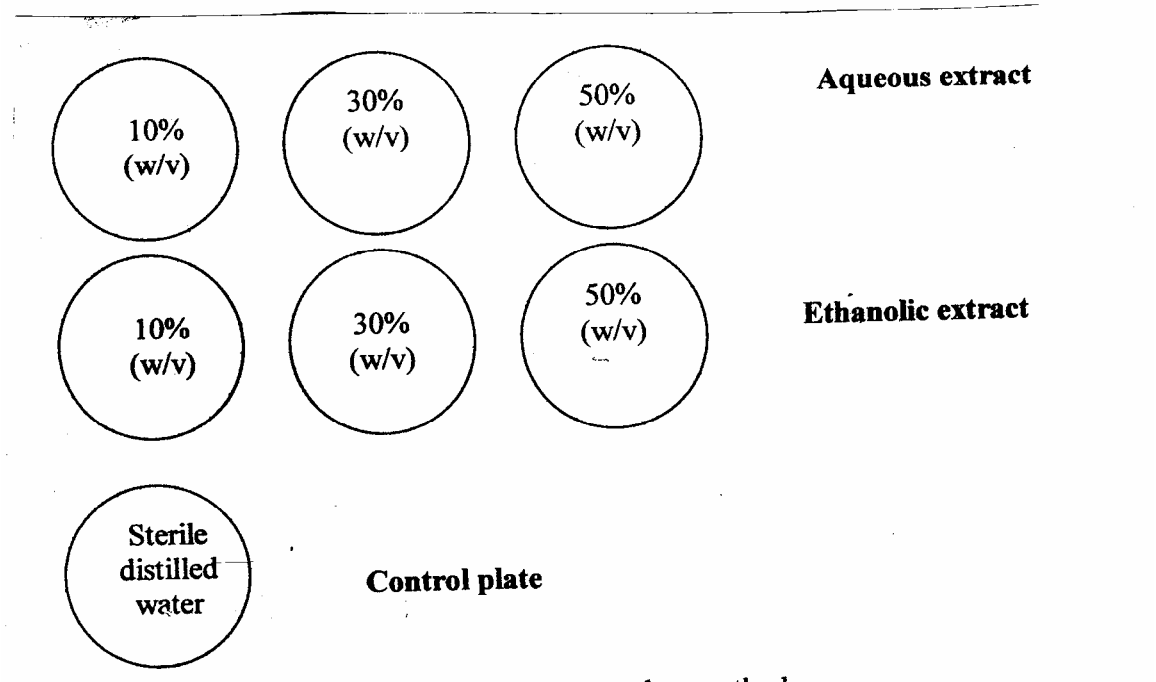

Figure 1: Experimental design for the pour plate method.

Ten millilitres (10 ml) of sterile agar was aseptically added into each plate. All the plates were swirled gently to mix the extracts and the agar medium. Two perpendicular lines were drawn at the bottom of each plate crossing each other at the centre. The Petri dishes were inoculated at the centre with inoculum plugs (10mm diameter) cut out from the edges of actively growing culture of *F. verticilliodes*. All the plates were incubated at 25°C and growth measured along the perpendicular lines for 5 days. This experimental set up was replicated three times.

At 24 hr intervals, the diameters of radial growth of the mycelia on each plate were measured along the lines on the bottom of the plates. The diameter of the inoculum plug was subtracted from the measurements obtained and a mean radial growth was calculated as follows:

$$D_1 \text{ (mm)} = \frac{(dx + dy)}{2}$$

$$D_2 \text{ (mm)} = \frac{(dx + dy)}{2}$$

$$D_3 \text{ (mm)} = \frac{(dx + dy)}{2}$$

where

D_1 = diameter of the control plate.

D_2 = diameter of growth with the aqueous extract.

D_3 = diameter of growth with the ethanolic extract.

dx = diameter of growth along the horizontal line.

dy = diameter of growth along the vertical line.

The results were analysed using the paired sample test (P-test).

Results

The results of the present study revealed that pawpaw leaf extracts contain some active principles which could inhibit fungal growth. The extracts at the three concentrations used in this study retarded the radial growth of *F. verticilliodes* on PDA plates compared to the control. However, the activity of the ethanolic extract was more than that of the aqueous extract. After 2 days of incubation, the diameter of growth in the control plate was 16.5 mm while that in the aqueous extract was 13.5 mm for both the 10 and 30% (w/v) concentrations and 12.5 mm for the 50% concentration (Table 1). The diameter of growth with the ethanolic extract were 12.5, 11.5 and 11.0 mm, respectively, for the 10%, 30% and 50% (w/v) concentrations after 2 days.

After 5 days, however, it was observed that the diameters had increased appreciably in all the plated. For instance, the diameters in 50% (w/v) concentration of aqueous and ethanolic extracts were 22.5 and 21.0 mm from 12.5 and 11.0 mm, respectively, and 28.0 mm in the control from 16.5 mm.

Statistical analysis of the results showed that at 5% probability level, there was significant difference between the diameter of growth in the control plate and that in the aqueous extract and also between the control and the ethanolic extract at the three concentrations tested. The difference between growth in aqueous and ethanolic extracts was not significant at 5% level. The negative values of the t_{cal} (calculated value of "t") for the diameters of D_1 and D_2, and of D_1 and D_3 indicated that the aqueous and ethanolic extracts retarded the radial growth of *F. verticilliodes*.

Table 1: Effect of pawpaw leaf extracts on the radial growth of *F. verticilliodes*.

Concentrations of the extracts (% w/v)*	Incubation period (hrs)	D1 (mm)	D2 (mm)	D3 (mm)
10	24	12.0	11.0	11.0
	48	16.5	13.5	12.5
	72	20.0	18.5	17.0
	96	25.0	23.0	21.0
	120	28.0	26.0	23.0
30	24	12.0	11.0	11.0
	48	16.5	13.5	11.5
	72	20.0	17.5	16.5
	96	25.0	21.5	20.0
	120	28.0	24.5	22.5
50	24	12.0	11.0	11.0
	48	16.5	12.5	11.0
	72	20.0	17.0	16.0
	96	25.0	20.0	18.0
	120	28.0	22.5	21.0

*Radial growth significant between the control plate and the test extracts at all concentrations at 5% but not significant between the aqueous and ethanolic extracts.

Discussion

Both the aqueous and ethanolic extracts of *C. papaya* leaf retarded the radial growth of *F. verticillodes* on PDA plates. However, the ethanolic extract was a stronger antimicrobial agent. These observations agree with the findings of previous workers on the antimicrobial activity of extracts from medicinal plants.

Adekunle (2001) has demonstrated that the ethanolic extracts of *Ageratumn conyzoides* (goat weed) is more active that the aqueous extract against the culture of *F. solani* using both the pour plate and agar diffusion methods. Ebana et al. (1993) showed that the ethanolic extracts of the roots and leaves of *Strophantus hpidis* (arrow poison) and *Secamone afzeli* showed higher antibacterial activity against *Neisseria gonorrhoea, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus pyrogenes* and *Proteus mirabilis* compared to the aqueous extracts.

Both the aqueous, ethanolic and methanolic extracts of *Vernonia amygdalina* leaves shiwed antimicrobial activity against *Trychophyton rubrum* and *Microsporum canis* with the ethanolic extract producing the greatest antimicrobial activity and the aqueous extract the least (Vunci and Banbo, 2004). Sale et al. (2001) reported that the ethanolic and acetone extracts of *Zingiber officinale* (ginger) and *Allium sativum* (garlic) produced a stronger antimicrobial activity against *Salmonella typhi* and *S. protyphi* than the aqueous extract.

Various studies have shown that the ethanolic extract of medicinal plant parts compared favourably with the commercial antibiotics and systemic fungicides (Fawole and Abikoye, 2002). According to Fawole and Oso (2001) this may be due to the property of ethanol solution itself which is used as a disinfecting agent in different dilution ratios.

The antifungal activity observed in this study against *F. verticilliodes* may be due to the action of the proteolytic enzyme papain, which is the major component of pawpaw latex. This enzyme may have acted in an adverse manner on the protein components of the fungal cells thereby hindering growth and other activities of the cell. Further research is required to identify the exact principles in pawpaw extracts and the mode and site of action of such principles on microorganisms.

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