Efficacy of Paw paw *(Carica Papaya)* Leaf Extracts on Growth of Fusarium Wilt of coffee.

Chidoko Pardon

Department of Soil and Plant Sciences, Great Zimbabwe University, P O Box 1235, Masvingo, Zimbabwe

Corresponding author: pchidoko@gzu.ac.zw

Abstract

Fusarium wilt, caused by the pathogen *Fusatium lateritium* of coffee is one of the most problematic diseases with no registered fungicide for its management. A trial was established to evaluate the efficacy of paw paw leaf extracts on Fusarium growth under laboratory conditions. Six treatments were used, which were *C. papaya* at 12.5%, 25%, 50% and 100%, the control (distilled water) and the standard Copper Oxychloride 85%WP. The trial was laid out in a completely randomized design replicated three times. There were significant differences in growth inhibition of *F. lateritium* after 10 days after incubation, (*p=0.002*). *C papaya* at concentrations of more than 25% performed equally the same as the standard chemical Copper Oxychloride 85%WP. More growth (0.31cm/day) was recorded in the control. *C. papaya* concentrations had a growth range of 0 to 0.18 cm/day, with no growth in the standard treatment. There was a strong positive correlation (r = 0.98) between the concentration of *C. papaya* and the percentage growth inhibition of *F. lateritium*. The results showed that *C papaya* possesses essential elements which can be harnessed in the management of Fusarium wilt of coffee.

Keywords: Fusarium, Efficacy, pawpaw, growth inhibition

Introduction

Coffee production in Zimbabwe is limited by the availability of diseases which include Coffee Leaf Rust, Cercospora and Fusarium Bark Disease caused by *Fusarium lateritium*. Fusarium bark disease causes significant economic losses in the Eastern districts of Zimbabwe from time immemorial (CoRI, 2020; W. J. C. Logan *et al.*, 1987). The presence of major pests such as White Stem borer and Antestia bugs exacerbates the spread of the disease. *F. lateritium* is considered a weak pathogen that can only get into the plant through openings (Wrigley, 1988). The disease affects almost all above-ground parts of Arabica coffee and the type of symptoms manifested depends on the part affected (Bakkali *et al.*, 2008). Currently, Fusarium Bark Disease does not have curative fungicides since the withdrawal of

Captafol from the market due to its long residual effect in the final product and suspected carcinogenicity. Farmers are currently relying on prophylactic preventative sprays of Copper Oxychloride 85%WP, cultural practices and the burning of infected plants (CoRI, 2020; Hillocks *et al.*, 1999). The continuous use of copper as a preventative spray have resulted in negative effects in humans, animal health and agroecosystems, in addition to residues in food and beverages (Alkolaly *et al.*, 2017). Today, there is growing interest in the use of pesticides from plant origin. Eco-friendly plant extracts and organic materials, which can act directly on plant pathogens or indirectly by inducing resistance in plants have gained considerable attention as alternative options to synthetics (Oparaeke *et al.*, 2005), (Subramani *et al.*, 2012), but have limited use in Zimbabwe, especially coffee production. Coffee is a perennial crop which remains in the field for at least 10 years up to 100 years. Intercropping is mainly done in the first five years, and grown as a monocrop in the preceding years. Monocropping with fungicide use in coffee has also resulted in fungicide resistance, environmental pollution and destruction of parasitoids, which are an important component in biological control (Kutywayo, 1989).

Plant extracts such as Lantana (*Lantana camara)* on coffee leaf rust (Chidoko *et al.*, 2013b), paw paw (*Carica papaya)* on coffee leaf rust (Doughari *et al.*, 2007; Kutywayo *et al.*, 2008), pepper (*Mentha piperita L.*), (Mahshid *et al.*, 2013) , Wild mint (*Mentha arvensis)* and garlic (*Allium spp)*. (Chidoko *et al.*, 2013a, 2013b; Oparaeke *et al.*, 2005) were utilized in the inhibition of plant pathogen growth successfully. Most of the plant extracts were found to have chemical volatiles which function in chemical defence, acting as insecticides, and acaricides, avoiding bacterial or fungi phytopathogen colonization and attracting natural enemies (Bakkali *et al.*, 2008; Doughari *et al.*, 2007; Flamini, 2003).

Carica papaya L. belongs to the plant family *Caricaceae*. Work carried out on the extracts derived from papaya leaves, fruits, seeds and roots have indicated the presence of biologically active compounds which can be used for microbial growth inhibition (Kavimandan *et al.*, 2016). In human healthy, leaves of papaya have been used in the management of malaria, jaundice, and antiviral activities (Yogiraj *et al.*, 2014). One of the most important constituents in paw paw leaf extracts is papain, which has strong antimicrobial properties, and have been useful in beer brewing, winemaking, tannin and the textile industry. Studies have also revealed the important anticancer properties and antitumor growth inhibition by papaya (Yogiraj *et al.*, 2014). Paw paw extracts have therefore been used in various ways in managing human and plant diseases. In plants, paw paw leaf extracts were used in the management of coffee leaf rust, a biotroph fungus. The leaf extracts at 50% concentration effectively inhibited spore germination (Kutywayo *et al.*, 2008), giving a positive clue that active

ingredients in the extracts may be exploited for the management of the problematic fungus in coffee. Similar work was also carried out by Doughari *et al.* (2007).

Considering the limited options in the management of Fusarium wilt of coffee in Zimbabwe, and the demand for organically produced coffee products, it is imperative to evaluate different plant options which can be harnessed in managing fusarium. The objective of the study was to determine the effectiveness of paw paw leaf extracts on the growth and growth inhibition rate of *F. lateritium invitro*.

Materials and Methods

Study site

This work was conducted at Coffee Research Institute ($20^{0}21^{1}$S and $32^{0}37^{1}$E), situated 8km South-West of Chipinge town, in the plant pathology laboratory.

Preparation Plant extracts

Paw paw leaves were obtained from trees and plant extracts were prepared by crushing fresh plant leaves using a pestle and mortar. The crushed plant parts were measured to obtain 100 g and soaked in 100 ml water (aqueous) for 16 hours. After 16 hours the soaked extracts were filtered through a mutton cloth, centrifuged at 5000rpm for 10 minutes and considered to be the standard solution (100% neat extract). The neat extract was diluted using sterile distilled water into different concentrations of 50%, 25% and 12.5%before use in the bioassays.

Fusarium lateritium spore preparation

Stems, branches and berries of coffee exhibiting symptoms of *F. lateritium* were collected from the field at Coffee Research Institute. The diseased plant parts were brought to the laboratory for culturing before microscopic examination and isolation. The diseased samples of *Coffea arabica* showing different types of typical symptoms were thoroughly washed under running water and separately cut into small pieces of about half the length of a centimetre in size using sterilized blades. The pieces were washed under running tap water and then surface sterilized with 70% sodium hypochlorite solution for 40- 60 seconds, followed by 3 – 4 washings with sterilized distilled water and then dried on laminar airflow. The pieces were aseptically transferred into Petri dishes containing Potato Dextrose Agar (PDA) medium and incubated at 24 ± 2^{o}C. A new PDA solution was prepared and 9 ml of the media was poured into the test tubes and 1ml of the test treatment was then added. The mixture

was shaken well before pouring into the Petri dishes. Incubated *F. lateritium* spores were sub-cultured on fresh Petri-dishes containing PDA and 1 ml of the test solution and then incubated for 48-72 hours.

Experimental Design

The trial was laid out in a Completely Randomized Design. There were six treatments made up of four *Carica papaya* concentrations at 12.5%, 25%, 50%, 100%, the standard chemical Copper Oxychloride 85%WP and distilled water as the control.

Data collection and analysis

Observations in the laboratory were recorded daily from two days after incubation up to ten days after incubation. Data was collected on radial growth in Petri dishes for each treatment. Percentage growth Inhibition due to various treatments was computed as follows:

$$GI\% = \frac{GC-GT}{GC} * 100\% \quad [1]$$

Where

GI% = Growth inhibition percentage

GT = Growth in the treatment

GC = Growth in the control

Growth Inhibition data were subjected to statistical transformation before analysis of variance (ANOVA) using a statistical package, Genstat 14th edition for windows (VSN International). Graphs on radial growth over time were also developed in Microsoft Excel.

Results

Effects on growth inhibition

There were significant differences ($p<0.002$) in percentage growth inhibition of *F. lateritium* due to the effects of *C. papaya* at 10 Days after Incubation (DAI). There was complete germination inhibition in the standard chemical Copper Oxychloride 85%WP and the neat concentration (100%) of *C. papaya*, which were not significantly different from the 50% and the 25% concentrations at 10(DAI) (Table 1). The control (distilled water) was significantly different from the rest of the other treatments at 10 DAI. Distilled water did not inhibit the growth of *F. lateritium* in the petri dishes at all.

Table 1. Effects of *C. papaya* on growth inhibition of *F. lateritium* at 10 DAI *in vitro*

Treatments	**10 DAI**
Control	0[a]
C.Papaya 12.5%	47.31[b]
C.Papaya 25%	64.52[bc]
C.Papaya 50%	74.19[bc]
C.Papaya 100%	100.00[c]
Copper Oxychloride 85%WP	100.00[c]
Grand mean	65.80
CV%	31.7
p	*0.002*

*Means with the same letter are not different according to the LSD test at 5% significance.

Radial growth curves of *F. lateritium* in PDA

Examination of the radial growth of *F. lateritium* on PDA indicated differences in growth over time. More growth was observed in the control which was growing at a rate of an average of 0.31cm/day. The other concentrations of *C. papaya* had a growth range of 0 to 0.18 cm/day (Figure 1). There was a highly significant strong correlation (r^2= 0.98) between the concentration of paw paw leaf extracts and the growth inhibition of Fusarium pathogen under laboratory conditions. There was a progressive increase in pathogen growth inhibition with an increase in concentration of the plant extract. The Lethal dose 50 (LD50) managed to restrict 74% of the pathogen from growing, while 100% concentration also managed to inhibit all the pathogens from growing.

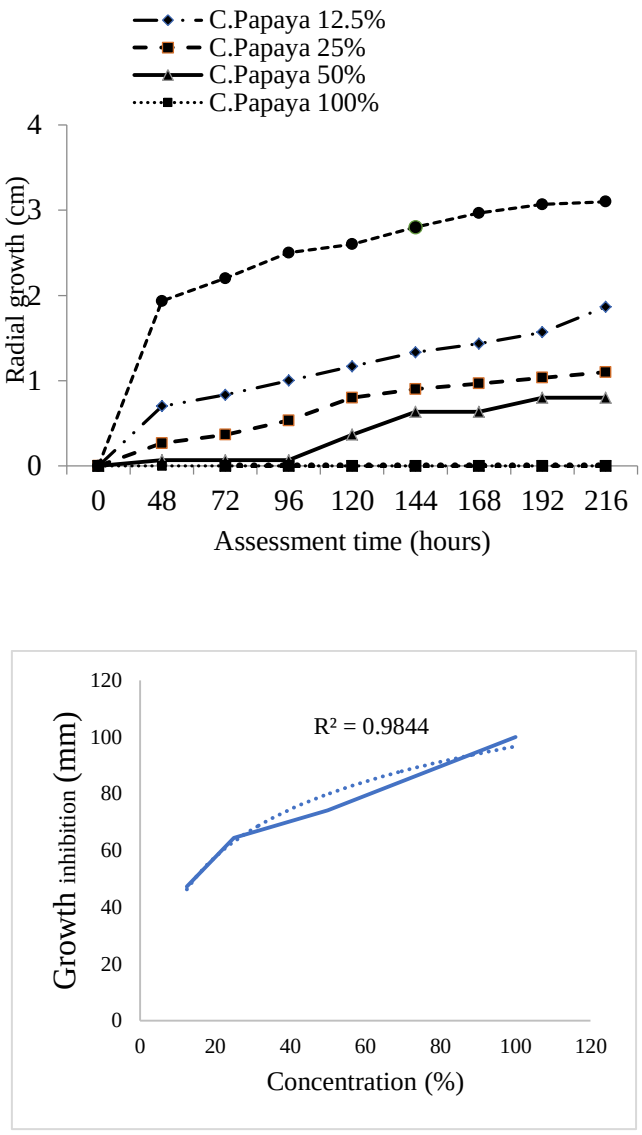

Figure 1 (a). Effect of *Carica papaya* on radial growth of *F. lateritium* in PDA medium under laboratory conditions (b) Effect of paw paw leaf extract concentration on growth inhibition of *F. lateritium*

Discussion

The study demonstrated the potential of *C. papaya* in the management of *F. lateritium*. The zone of inhibition increased with an increase in the concentration of *C. papaya.* More paw paw leaf extract inhibited pathogen growth, signifying its positive effect in suppressing pathogen invasion. The 25% concentration of *C. papaya* used in this experiment performed equally the same as the standard chemical Copper Oxychloride 85%WP. The results are in agreement with a study where *C. papaya* was effective in inhibiting radial growth of *Fusarium oxysporum,* a different species from the one in this study (Sherwani *et al.*, 2013).

Growth inhibition was recorded to a maximum of 15 to 20mm zone, which was a significant reduction. In another study, paw paw leaf extracts were used in the management of coffee leaf rust, a biotrophic fungus successfully, under laboratory and greenhouse conditions. Undiluted *C. papaya* extract had the least spore germination of 16.5% followed by the 50% concentration while the weakest dilution (12.5%) had the highest spore germination percentage of 69.88%. (Kutywayo *et al.*, 2008). The positive result shown by *C. papaya* in this study, confirmed by previous studies also, may give way for the identification of active compounds in the botanical and lead to possible synthesis and exploitation of the compounds.

C. papaya leaf extracts have various phytochemical components such as flavonoids, alkaloids, tannins, saponins, cardiac glycosides and some protein components as well (Ayoola *et al.*, 2010; Doughari *et al.*, 2007; Sherwani *et al.*, 2013). Papaya leaves contain carpain, acetogenin and phenolic compounds. Carpain is a chemical compound or a substance with ability to kill microorganisms that often intervene in food digestion processes (Anibijuwon *et al.*, 2009). Other studies showed that these medicinally bioactive components in papaya exert antimicrobial action through different biological mechanisms. For example, tannins cause inhibition in cell wall synthesis by forming irreversible complexes with proline rich protein. The steroids and saponins have the ability to cause leakage of proteins and certain enzymes from the cell. Flavonoids have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Rahmani *et al.*, 2016). The difference in antimicrobial activity of these extracts also depends on the quantity of chemical substances present in the plant. This was also revealed by the relationship between paw law leaf extract concentration and the level of pathogen growth inhibition shown in this study. High extract concentration resulted in high inhibitory effect. Leaves, roots, fruits, stems and seeds have different quantities of chemical compounds. In this study, leaves were used successfully inhibiting fusarium growth. Different studies also utilized leaf extracts in pathogen inhibition (Charan *et al.*, 2016; Singh *et al.*, 2020), showing the presence of theses active compounds.

Most plants were scientifically proven to be useful in disease management, cheap and readily available to smallholder farmers (Chidoko *et al.*, 2013b; Oparaeke *et al.*, 2005). *C. papaya* is a common plant in many communities in Zimbabwe, and the region as a whole, which makes it a possible solution for farmers. In addition, the plant extracts are generally user friendly as they do not destroy the environment and non-targeted species but can act as repellents or

growth inhibitors to certain microbes (Ayoola *et al.*, 2010; Flamini, 2003; Oparaeke *et al.*, 2005). Previous fungicides such as Captafol used in the management of *F. lateritium* had long residual effects and were suspended because of its carcinogenic effects (W. Logan *et al.*, 1987). Given such challenges, and the potential of paw paw extracts revealed in this study, it is worthwhile to further evaluate *C. papaya* under greenhouse and field conditions to get more information on the potential of the plant extract on coffee plants.

Conclusion

The study showed the potential of paw paw leaf extracts in the management of *F. lateritium* by inhibiting growth under laboratory conditions. Basing from the observations of this study, and reports from the success of plant extracts in managing plant pathogenic fungi and human diseases, paw leaf extracts holds promise in organic farming and in ecofriendly management of Fusarium. Therefore, the findings of this study can be used as a foundation for the use of biocontrol agents in the management of Fusarium lateritium of coffee.

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