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IN VITRO CONTROL OF TOMATO (*Solanum lycopersicon* L.) FRUIT ROT CAUSED BY FUNGI USING TWO PLANT EXTRACTS.

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Abstract. The inhibitory properties of the ethanolic and methanolic leaf extracts of *Vernonia amygdalina* and *Cola acuminata* on the fungal pathogens isolated from infected tomato fruits were investigated. The pathogens were *Fusarium moniliformes* and *Rhizopus stolonifer*. Various concentrations of the extracts ranging from 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% were separately added to PDA media. The fungal pathogens were separately inoculated into the media and incubated for seven days. Antifungal effects of these extracts on the mycelia growth of the pathogens were significant at $P<0.05$ for all treatments at higher concentrations. At 10-50% concentration, ethanolic and methanolic extracts of *Vernonia amygdalina* and *Cola acuminata* had no significant effect on the mycelia growth of *Fusarium moniliformes* and *Rhizopus stolonifer* after seven days observation period. At 60-100% concentrations, the two pathogens were completely inhibited by ethanolic extracts of *Vernonia amygdalina* and *Cola acuminata*. Methanolic extracts of *Vernonia amygdalina* and *Cola acuminata* inhibited completely *Fusarium moniliformes* and *Rhizopus stolonifer* at 80-100% concentrations. The in vitro inhibitory effects of these extracts at higher concentrations indicated that they can be used for the control of tomato fruit rot. It may be necessary to use them in prolonging the shelf-life of fresh tomato fruit and some other fruits.

Introduction

The tomato is the edible, often red fruit of the plant *Solanum lycopersicon* L. commonly known as the tomato plant [18]. The species originated in the South American Andes [13], and its use as food originated in Mexico, and spread throughout the world following the Spanish colonization of the Americans. Its many varieties are now widely grown, sometimes in green houses and cooler climates. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads and drinks. The fruit is rich in lycopene, which have beneficial health effects [25].

The tomato belongs to the night-shade family *Solanaceae* [16, 3]. The plants typically grow to 1-3meters/3-10ft) in height and have a weak stem that often sprawls over the ground and often vines over other plants. It is a perennial in its native habitat, although often grown outdoors in temperate climates as an annual. An average common tomato weighs approximately 100grams [20]. The tomato is now grown worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types and for optimum growth in differing growing conditions. Cultivated tomatoes vary in size, from tom-berries about 5mm in diameter, through cherry tomatoes about the same 1-2cm (0.40-0.8inches) size as the wild tomato, up to beefsteak tomatoes 10cm (1 inch) or more in diameter. The most widely grown commercial tomatoes tend to be in the 5-6cm (2.0-2.4inch) diameter range. Most cultivars produce red fruit, but a number of cultivars with yellow, orange, pink, purple, green, black or white fruit are also available [19]. Tomato fruit is classified as a berry. As a true, fruit, it develops from the ovary of the plant after fertilization, its flesh comprising the pericarp walls. The fruit contains hollow spaces full of seeds and moisture, called locular cavities. These vary among cultivated species, and according to type [19].

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Tomatoes are now eaten freely all over the world. They contain the carotene lycopene, one of the most powerful natural antioxidants. In some studies, lycopene especially in cooked tomatoes has been found to help prevent prostate cancer [12]. But other research contradicts this claim [26]. Lycopene has also been shown to improve the skins ability to protect against harmful UV rays [17]. A study done by researchers at Manchester and Newcastle Universities revealed that tomato can protect against sunburn and help keeping the skin looking youthful [27]. Natural genetic variation in tomatoes and their wild relatives has given a genetic plethora of genes that produce lycopene, carotene, anthocyanin, and other antioxidants. Tomato varieties are available with double the normal vitamin C (Double rich), 40 times normal vitamin A (97L97), high levels of anthocyanin (resulting in blue tomatoes) and from four times the normal amount of lycopene. Lycopene has also been shown to protect against oxidative damage in many epidemiological and experimental studies. In addition to its antioxidant activity other metabolic effects of lycopene have also been demonstrated. The richest source of lycopene in the diet is tomato and tomato derived products [20]. Tomato consumption has been associated with decreased risk of breast cancer [30] head and neck cancers [11] and might be strongly protective against neurodegenerative diseases [12, 26].Tomato sauces and puree are said to help lower urinary tract symptoms (BPH) and may have anticancer properties [28]. Tomato consumption might be beneficial for reducing cardiovascular risk associated with type 2 Diabetes [24].

In spite of the numerous beneficial effects of tomato, Tomato plants are vulnerable to infection by fungal pathogens which cause severe diseases such as *Fusarium* wilt caused by (*Fusarium oxysporum*), early blight disease (*Alternaria solani*) and tomato fruit rot caused by *Fusarium sp* and a host of other fungal pathogens [1].

These diseases are controlled mainly by the application of agrochemicals. However, the worldwide trend towards environmentally safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. In an attempt to modify this condition, some alternative methods of the control have been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases. Natural plant products are important sources of new agrochemicals for the control of plant diseases [15]. Furthermore, biocides of plant origin are non-phytotoxic, systemic and easily biodegradable [1]. It is now known that various plant products can reduce populations of folia pathogens and control the disease development, and then these plant extracts have a potential as environmentally safe alternatives and as components in integrated pest management programs [7]. Due to the menace caused by fungal pathogens on tomato fruits pre harvest and post harvest, the main aim of this study was to isolate and identify the fungal pathogens associated with fruit rot of tomato and the evaluation of ethanolic and methanolic leaf extracts of *Venonia amygdalina* and *Cola acuminata* in controlling tomato fruit rot fungi in vitro.

Materials and Methods

Sources of Materials

Infected and uninfected tomato fruits were obtained from the Research farm of the Department of Crop Science, University of Calabar and Watt and Marian markets in Calabar Metropolis of Cross River State, Nigeria and wrapped in sterile cellophane bags and transported to the Laboratory. *Venonia amygdalina* and *Cola acuminata* leaves were obtained from the Botanic Garden of the Department of Botany, University of Calabar, Cross River state, Nigeria.

Isolation of fungal pathogens and Morphological Identification

The fungal pathogens used in this research work were isolated from diseased tomato fruits. To isolate the fungal pathogens, cut sections of the diseased assay fruits were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile

distilled water, blotted dry on Whatman's No. 1filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungal growth was noticed. After 5 days, the different isolates were sub cultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Philippines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of [6, 10].

Koch's postulates and Pathogenicity test

Pathogenicity tests were carried out using the techniques of [23]. Healthy tomato fruits were washed in sterile distilled water and surface sterilized with 1% sodium hypochlorite solution. A 5mm diameter cork borer was used to cut discs from the fruits (three discs per fruit) and cultures of the isolates discs were introduced into holes and replaced with the discs. They were kept for 24-48hours. The inoculated fruits established symptoms on the second day and tissue segments from the infected fruits were excised and cultured on freshly prepared PDA and incubated at 28 ± 1˚C for seven days.

Preparation of Extracts

Leaves of *Venonia amygdalina* and *Cola acuminata* obtained were washed with distilled water and oven dried at a temperature of 80°C for 24 hours, grounded into fine powder and extracted separately using 100ml of 95% concentration of ethanol and methanol.

Susceptibility Test

The extracts percentage concentrations were prepared at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% with different solvents of ethanol and methanol.

Dilution Test Procedure

1ml of each concentration was first poured into different Petri dishes using sterile syringes. The sterilized Potato Dextrose Agar (PDA) was also poured into the plates containing the solvent extracts after which the plates were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized No.2 cork borer of 5.5mm in diameter, a disc of the matured culture was punched out, inoculated at the centre of plates and incubated at room temperature of 28 ±1°C. As a control, the dishes were inoculated in distilled water-agar mix instead of solvent extracts-agar mix. Two (2) control plates were prepared for each solvent extracts. For positive control, no solvent extracts-agar mix or distilled water-agar mix was introduced into the plates. Growth measurement of the mycelia in diameter was done daily for seven days [29].

Results

Fungal isolates

The fungal pathogens isolated and identified as the causative agent of fruit rot of tomato from this study were *Fusarium moniliformes* and *Rhizopus stolonifer*.

Koch's postulates and Pathogenicity test

Results from pathogenicity revealed that the fungi *Fusarium moniliformes* and *Rhizopus stolonifer* were responsible for the post harvest rot of tomato fruits obtained in Calabar Metropolis. Pathogenicity was established 12-24 hours after inoculation. The *F. moniliformes* and *R. stolonifer* isolates were pathogenic on the healthy tomato fruits used for each pathogen for the test. Symptoms of decay (rot) caused by *F. moniliformes* was seen as soft black rot while *R. stolonifer* produced soft rot symptoms. On re- isolation, the two isolates exhibited similar patterns of growth as observed in the original isolates.

Antifungal effect of *V. amygdalina* and *C. acuminata* extracts on *F. moniliformes* and *R. stolonifer* at the different concentrations.

The inhibitory effects of *V.amygdalina* and *C. acuminata* on the isolated pathogens are shown in (Figures1- 4). The efficacy of the two plant extracts against the tomato fruit rot fungi was tested *in vitro.* The results showed that, the extracts significantly ($P < 0.05$) inhibited the mycelia growth of the fungal pathogens at the higher different concentrations tested and the rate of inhibition differed from one extract to the other.

Results from percentage inhibition of the plant extracts on each fungus showed that at 10-30% concentrations, *V. amygdalina* and *C. acuminata* extracts of ethanol and methanol had no significant effect on the mycelia growth of *Fusarium moniliformes* and *Rhizopus stolonifer* after seven days observation period (Figures 1-4). At 40-60% concentrations, ethanolic extracts of *V. amygdalina* and *C. acuminata* slightly inhibited *F. moniliformes* and *R. stolonifer*, while methanolic extract of *V. amygdalina* and *C. acuminata* showed no inhibition at 10-70% concentrations (Figure 2 and 4).

Ethanolic extracts of *V. amygdalina* and *C. acuminata* was more fungitoxic to these pathogens than methanolic extracts at concentrations of 50-100%. The inhibitory effect of the plant extracts at 10-50% concentration as shown in Figures 2 and 4 has methnolic extract of *V. amygdalina* and *C. acuminata* with the least percentage of inhibition of all the tested organisms, while ethanolic extracts of *V. amygdalina* and *C. acuminata* showed the highest percentage of inhibition (Figures 1 and 3). At 10-60% concentration of ethanolic and methanolic extracts, R. *stolonifer* was the most inhibited by the plant extracts, while *F. moniliformes* was the least inhibited (Figures 1-4). However, percentage inhibition of the mycelia growth of all the tested pathogens took a similar trend in all the plant extracts. Increase in antifungal activity was observed with the corresponding increase in the concentrations of all the plant extracts (Figures 1-4). The differences in the fungitoxic potentials between these plant extracts may be attributed to the susceptibility of each of the fungal pathogens to the different concentrations of the extracts.

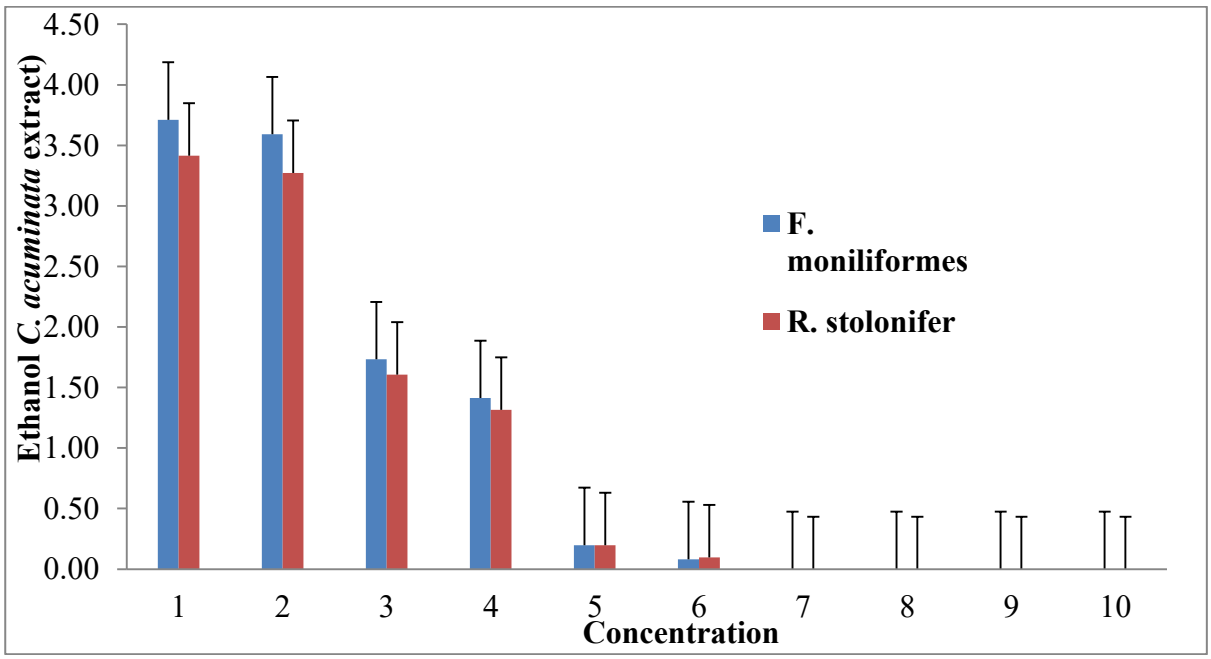


Figure 1: Effect of *C. acuminata* extract (ethanol) on mycelia growth of *F. moniliformes* and *R. stolonifer* at different concentrations.

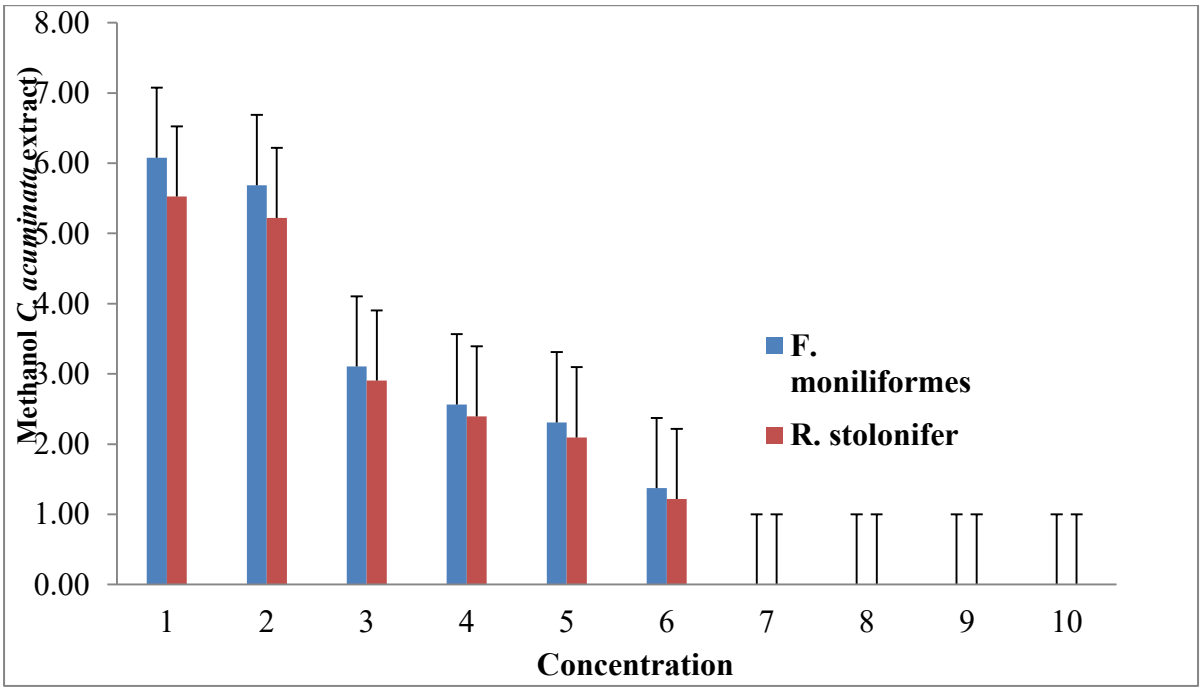


Figure 2: Effect of *C. acuminata* extract (methanol) on mycelia growth of *F. moniliformes* and *R. stolonifer* at different concentrations.

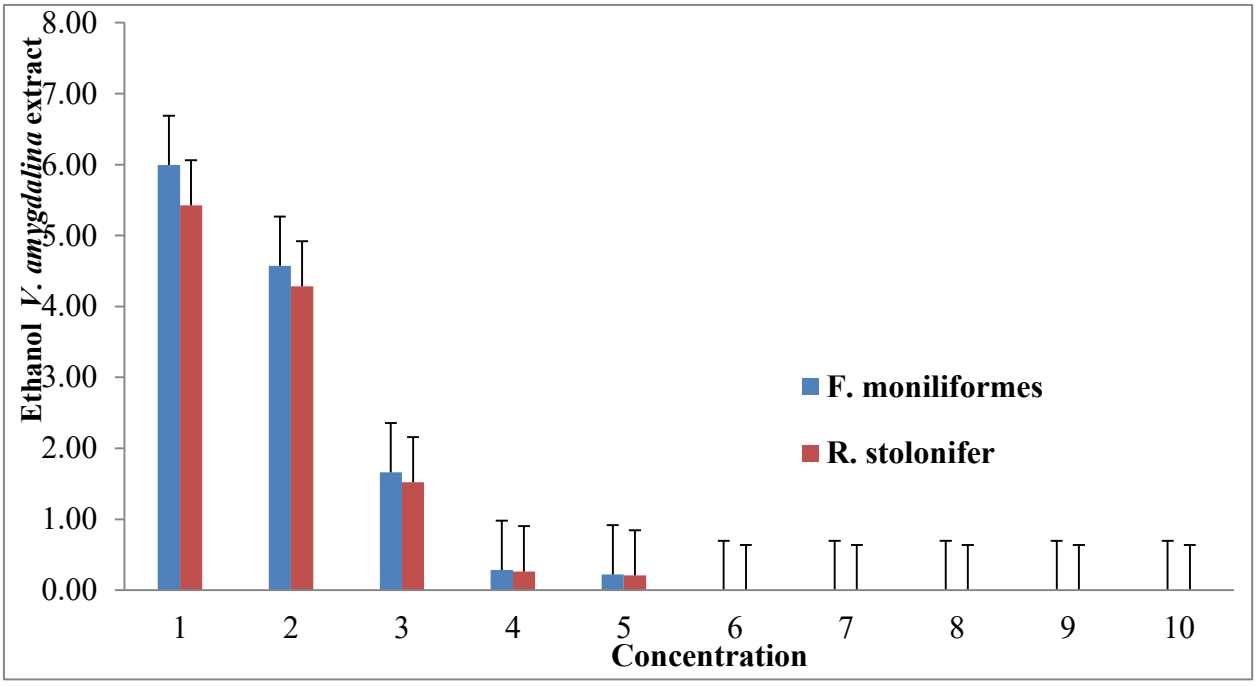


Figure 3: Effect of *V. amygdalina* extract (ethanol) on mycelia growth of *F. moniliformes* and *R. stolonifer* at different concentrations.

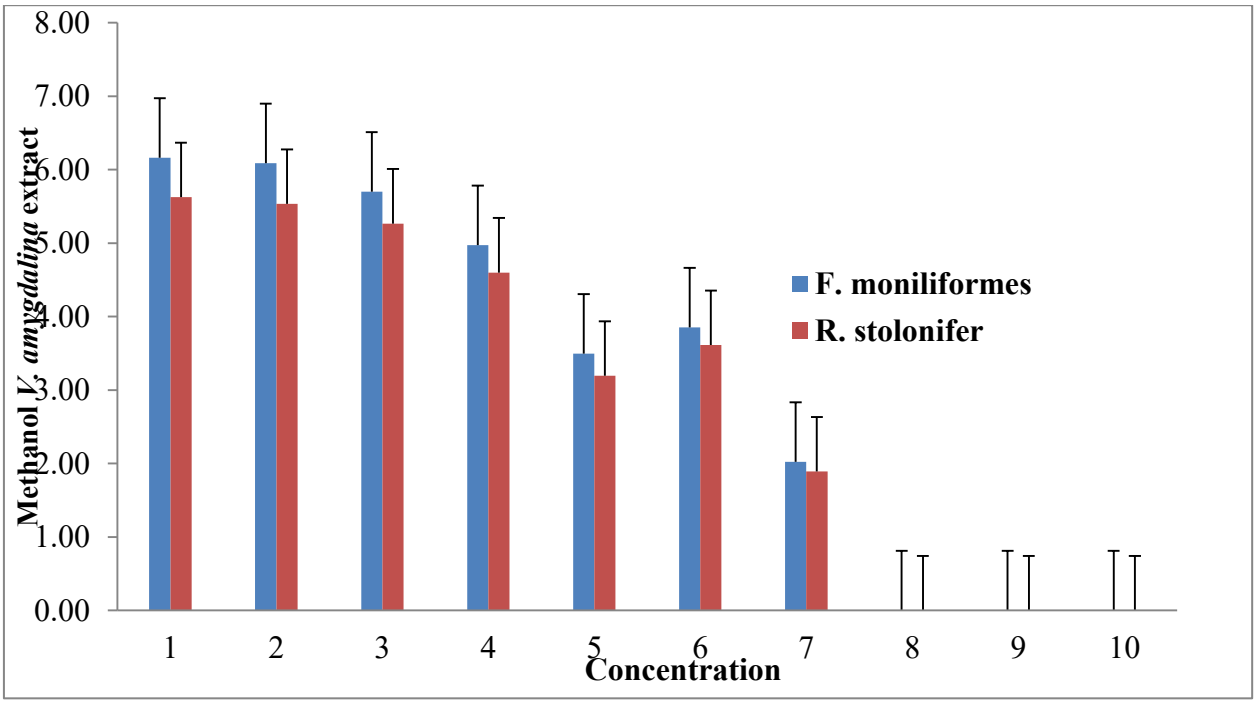


Figure 4: Effect of *V. amygdalina* extract (methanol) on mycelia growth of *F. moniliformes* and *R. stolonifer* at different concentrations.

Discussion

The fungal pathogens isolated were identified as *Fusarium moniliformes* and *Rhizopus stolonifer*. The results of this study revealed that the fungi were responsible for the post harvest rot of tomato fruits in Calabar Metropolis as evidenced by the Pathogenicity tests. The fungal spores of these pathogens could be air-borne and therefore spread by wind and may land on susceptible fruits like tomato and other plants. The pathogens (*Fusarium spp, Mucor spp,* and *P. digitatum*) were reported by [23] to cause rot of cassava. Also [18] reported *Helminthosporium* spp and *Rhizopus stolonifer* as pathogens of post harvest rot of tomato, which is in agreement with the finding of this study. The inhibitory effects of *V. amygdalina* and *C. acuminata* on the isolated pathogens are shown in (Figures1- 4). The efficacy of the two plant extracts against the tomato fruit rot fungi was tested *in vitro.* The results showed that, the extracts significantly ($P < 0.05$) inhibited the mycelia growth of the fungal pathogens at the different higher concentrations tested and the rate of inhibition differed from one extract to the other. Increase in antifungal activity was observed with the corresponding increase in the concentrations of all the plant extracts (Figures 1-4). The differences in the fungitoxic potentials between these plant extracts may be attributed to the susceptibility of each of the fungal pathogens to the different concentrations of the extracts. This agrees with the results of some workers like [4] and [21]. [14] reported that some plants contain phenolic substances and essential oils, which are inhibitory to micro-organisms. The presence of these compounds in these extracts has been reported to be responsible for their antifungal properties [2]. These antifungal properties control various pests including fungi while the extract of ginger rhizomes is specially valued for their effectiveness against fungi [2]. The plant extracts differed significantly in their potential to inhibit the growth of these fungal pathogens. Complete inhibition of the growth of all the pathogens was achieved with ethanolic *V. amygdalina and C. acuminata* extract at 60-100% concentration but not with methanolic extracts. *V. amygdalina and C. acuminata* had total inhibition of all the pathogens at 70-100% concentrations. Concentrations of the extracts had significant effects on the mycelia growth of these pathogens ($P < 0.05$). Generally, mycelia growth decreased with increased in each of the plant extract concentrations. Also all the tested extracts concentrations inhibited significantly the mycelia growth of the pathogens. It is noteworthy that at all tested concentrations, ethanolic extracts of *V. amygdalina* and *C. acuminata* were inhibitory than

methanolic extracts of *V. amygdalina and C. acuminata.* The inhibitory potency of the plant extracts may be attributed to the phytochemical compounds like tannins, alkaloids, flavonoids and saponins in them as reported by [8]. This is also in agreement with the works of [5] and [29] who reported that the high potency of plant extracts containing the same bio-active compounds could be use for the control of fungal pathogens of plants. The inhibitory effects of *V. amygdalina* and *C. acuminata* ethanolic and methanolic extracts at ten different concentrations were evaluated in order to develop the cheaper methods of controlling the rot of tomato and other fruits.

The greater efficiency of *V. amygdalina* and *C. acuminata* may be due to the high contents of alkaloids they contain [8], since alkaloids are ranked as the most efficient therapeutically significant plant substances [21]. This result is in line with the work of [9] on the use of ginger and garlic in controlling fungal spoilage of tomato. Their result revealed that the growth of the fungi was completely inhibited at a higher concentration of 3 grams/20ml of ginger powder than at the lower concentrations (1g and 2g/20ml) and garlic powder was not effective.

Conclusion

The fungal pathogens isolated and identified from this study as the causative agents of soft rot of tomato fruits obtained in Calabar Metropolis were F. *moniliformes* and R. *stolonifer*. The efficacy of the two plant extracts (*V. amygdalina* and*C. acuminata*) against the tomato fruit rot fungi was tested *in vitro.* The results showed that the extracts significantly ($P < 0.05$) inhibited the mycelia growth of the fungal pathogens at the higher different concentrations tested and the rate of inhibition differed from one extract to the other.

Preservation of fruits and vegetables is of great importance because it makes provision for delayed use and eliminates wastage. Due to the perishable nature of tomato, post harvest loss is high. Therefore, production must go hand in hand with proper preservation and storage. The inhibitory activity of these extracts suggests their fungitoxic ability on these fungal pathogens. The findings of the present investigation are pointed to the crop protection strategies against fungal pathogens. The results of this study are also important steps towards developing plant based fungicides which are eco-friendly for the management of fungal rot in fruits and the development of commercial formulations of botanicals. This investigation demonstrates the potentials of *V. amygdalina* and *C. acuminata* as potential alternatives to synthetic fungicides in the control of post harvest rot of tomato fruits. The plant extracts are highly recommended for use at the higher concentrations in controlling post harvest rot of tomato fruits.

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