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Biotreatments of the Phytopathogenic Fungi in Water Melon (Post-Harvest) Using *Chromolaena Odorata* and *Azadirachta Indica* Leaves Extracts

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Abstract

In a hungry world with an ever-increasing population, efforts are made at reducing crop loss due to pathogen infestation. In recent time, the application of synthetic fungicide proved effective in inhibiting growth of plant-pathogenic fungi and this increases the use tremendously, thereby causing a lot of harmful effects such as health and environmental problems. In the attempt to combat this, many countries have made rules and regulations in the use of fungicides. The uses of phytochemicals in the treatment of plant microbes have been found to be the better alternative. Therefore, there is need to isolate and identify the pathogenic fungi associated with the deterioration of watermelon fruits (post-harvest) and the of use different plant leaves extraction in the treatment of the phytopathogens is inevitable. The highest activity of the plant extracts was found mostly in extracts of high concentrations; showing the effects of concentration on the susceptible organisms. Thus, *C. odorata* and *A. indica* could serve as a natural fungicide in inhibiting the growth of plant-pathogenic fungi due to the presence of phytochemicals in the plants. Therefore, could serve as potential raw materials in chemical industry for producing fungicides.

Keywords:* *Agrochemicals; Fungicides; Pathogenic organisms; Phytopathogenic; Microbes

Introduction

Plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, flavones, flavonoids, flavonols, tannins and coumarins [1]. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the pathogen. These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms [2].

Citrullus lanatus is a Leguminosae plant native to Nigeria, Ghana, India and many part of the world and from recent research, it was discovered to contain high water content, attractive look with different shapes (sizes) and rind pattern [3]. The edible part of the fruit (fleshly part) is known to be nutritious and medicinal; therefore, this makes the seed to be regarded as waste. The seed contains phytochemicals such as carbohydrate, phenol, flavonoids, protein, fibre, phosphorus and iron at different proportions [4]. The Proximate analysis of the seed was discovered to contain fat content (47.9%), protein (27.4%) and carbohydrate (9.9%) [5]. The presence of these phytochemicals helps the fruit to relieve inflammation, and increases digestive ability of the body system [6].

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There has been a significant increase in the consumption of sliced water melon because they are nutritious, convenient, easy to obtain and they are cheaper than the whole fruits. Watermelon is usually produced in Northern states of Nigeria and some part of Africa, Asia and Europe. Due to some factors such as poor handling, climate, insect attack and others, fruits contamination by pathogens is increasing on daily basis. In many unhygienic environments, fruits remain hygienically poor or contaminated since bacterial loads are moderately high [7].

Phytopathogenic fungi are generally controlled by synthetic fungicides. There have been strict regulations to the use of fungicides due to the problems they pose to man and his environment; despite the continual usage, these pathogenic fungi in plants have developed a strong resistance, therefore, to overcome this, a control measure must be implemented. Phytochemicals have gained attention of microbiologists and plant scientists to these phytochemicals extracted from plant materials against microbes as these products have been found to be degradable and safe for human health [8]. Therefore, the isolation and identification of pathogenic fungi associated with the deterioration of watermelon fruits after harvest and the use of different plant leaves extraction in the treatment of the phytopathogens is inevitable.

Materials and Methods

Sample collection

Matured ripe watermelon fruits were bought and collected from Adenike area, Ogbomosho, Oyo state, Nigeria. The fruits were rinsed with distilled water and were kept in a clean polythene bag. The leaves of *Chromolaena odorata* and *Azadirachta indica* were collected from Agricultural Science Farm, Lautech, Ogbomosho and were identified and authenticated at the herbarium unit of the Department of Pure and Applied Biology, LAUTECH, Ogbomosho, Nigeria.

Preparation and extraction of *C. odorata* and *A. indica* leaves

Fresh *C. odorata* and *A. indica* leaves were rinsed and stored in the oven for 5 days at 15°C. The dried leaves were pulverized and sieved through mesh size of 20 mm. About 50 g of *C. odorata* leafs were accurately weighed and extracted using the method of Adelowo and Oladeji [9] with little modification. The extract obtained was concentrated using rotary evaporator. The concentrated extract was stored at 4°C in a sample bottle. The method was repeated for *A. indica* leaves.

Clean up

The column used was made of Pyrex glass, and have small diameter so as to have effective separation and obtain distinctive bands. The cleanup method was carried out according to the method of Adelowo and Oladeji [9].

Preparation of the media

About 39 g of Potato Dextrose Agar was weighed and dissolved in 1000 ml of distilled water. Inhibition of bacterial growth was done by aseptically adding about 1 ml of tetracycline to 1000 ml of the sterile medium; this was then poured into sterile petri dishes. The PDA media was prepared by sterilizing in autoclave for 15 min at 121°C. The infected fruit portion was sterilized using 70% ethanol then sterile distilled water. Portions of the infested fruits were obtained using sterile scalpel and then transferred aseptically onto the PDA media prepared; these were incubated at 30°C for 72 h to 96 h.

Pure culturing

Identification and quantification of the colonies were investigated and observed at the end of incubation. This was followed by the selection of representative colonies and sub-cultured. This continues until pure culture of isolates were obtained and maintained on PDA slants at 4°C.

Characterization and identification of pure isolates

The characterization of the pure isolates was carried out based on their colonial and cellular morphology (observed macroscopically; colour of mycelia and spores, shape and surface texture were noted). They are observed microscopically using wet mounting method under light microscope for their cellular morphology. On a drop of distilled water on a glass slide a small mycelial portion was introduced with an inoculating needle and then teased with the needle. Two drops of methylene blue were added, covered with a cover slip and observed under 40X objective lens for hyphal nature and disposition of mature fruiting structures and different characteristics of the fungal isolates observed were recorded. However, the various fungal isolates were compared with those of fungi in a compendium of pathogenic fungus by Barnnet and Hunter [10].

Inocula preparations of the pure fungi isolates

Suspension of each of the pure fungi isolate was prepared by dissolving 0.2 g of yeast extract and 1 g of sucrose in 100 ml of distilled water. The PDA media were agitated and sterilized by autoclaving at 121°C for 15 min. After they were cooled, a loopful of each of the fungal isolate was inoculated into the sterile bottles and incubated in a mechanical shaker at 25°C for 48 h.

Pathogenicity test

Fresh water melon rinsed with distilled water and sterilized with 70% ethanol. Columns of 4 mm diameter were obtained from the fruits with a sterile cork borer and the fungal mycelia (pure culture of the isolates). The discs were removed using sterile inoculating loop and placed in fruit columns (a fruit column for a disc column) this was covered with fruit skin columns and then sealed up with Vaseline gel to prevent contamination by other organisms. This was then incubated at 30°C.

Koch postulate

The pathogens were reisolated from the inoculated fruit samples; this was done by obtaining small portion of spoilt fruit sample with sterile scapel and inoculated directly onto sterile PDA plates; this was then followed by incubation. A pure culture was obtained; the isolates were reexamined macroscopically and microscopically.

Preparation of sensitivity disc and standardization of inoculums

Sensitivity discs of 6.0 mm in diameter were sterilized at 160°C for 15 min. Each culture of the fungal isolates was standardized for 96 h at 30°C in PDA media. After 72 h, they were sub cultured in yeast extract broth to achieve turbidity of desirable standard. They were kept on incubator shaker for 48 h.

Antimicrobial susceptibility

The plants extracts were screened for their fungi toxicity using disc diffusion method using PDA plates as inoculums. The test organisms were swabbed evenly on the media and impregnated sensitivity discs immersed in the extracts of different concentrations (250 mg/discs, 100 mg/discs, 50 mg/disc, 25 mg/disc and 5 mg/disc); the control disc was placed at the centre. The media were incubated at 30°C for 48 h. The degree of sensitivity was determined according to Stockes and Ridgeway [11].

Results

From the isolation of pure isolates from the infected watermelon fruits, three fungal phytopathogenic organisms were isolated and identified: *Aspergillus flavus*, *Streptomyces* spp. and *Fusarium oxysporum* (TABLE 1). The authenticity of these pathogenic organisms was carried out using pathogenicity test. The results on the effect of ethanolic plant extracts at different concentrations (i.e. 250, 100, 50, 25, 5 mg/ml) and the control against pure isolates of *Streptomyces* spp. is given in TABLE 2. The inhibitory

effects of the ethanolic plant extracts on the mycelial growth of *F. oxysporum* are shown in the TABLE 3 and the effect of ethanolic plant extracts on *in vitro* inhibition of *A. flavus* was given in TABLE 4.

TABLE 1. **Macro and micro identifications of fungi.**

S.No.	Characteristics	Probable fungus
S-1	It has felt of yellowish-green becoming dark yellow-green conidiophores. Conidial heads typically radiate, later splitting into several loose columns, yellow-green becoming dark yellow-green. Conidiophores hyaline, coarsely roughened. Conidia globose to subglobose.	*Aspergillus flavus*
S-2	Aerial mycelium sparse or floccose, becoming felty, with whitish or peach, usually with a purple tinge, more intense near the medium surface. Micro-conidia septate, borne on lateral, simple (often reduced) phialides or on short branched conidiophores variable in shape and size, ovoid-ellipsoidal to cylindrical, straight or slightly curved.	*Fusarium oxysporum*
S-3	Aerial mycelium sparse or dense and floccose, sometimes leathery, greyish white, cream to buff, conidial slime formed in sporodochia or pionnotes. Micro-conidia usually abundant, ovoid or oblong. Chlamydospores hyaline, smooth-or rough-walled, globose to ovoid.	*Streptomyces* spp.

Where S-1, S-2, and S-3 represents watermelon samples.

TABLE 2. **Effect of ethanolic plant extracts on *in vitro* inhibition of *Streptomyces* spp.**

Cn (mg/ml)	A1 (mm)	A2 (mm)
250	16	18
100	15	17
50	14	14
25	13	13
5	13	13
C	7	8

Cn=Concentrations; C=Control; A1=*C. Odorata*; A2=*A. indica*.

TABLE 3. **Effect of ethanolic plant extracts on *in vitro* inhibition of *Fusarium oxysporum*.**

Cn (mg/ml)	A1 (mm)	A2 (mm)
250	22	19
100	19	18
50	17	15
25	17	13
5	14	13
C	10	9

Cn=Concentrations; C=Control; A1=*C. Odorata*; A2=*A. indica*.

TABLE 4. **Effect of ethanolic plant extracts on *in vitro* inhibition of *Aspergillus flavus.***

Cn (mg/ml)	A1 (mm)	A2 (mm)
250	18	18
100	15	16
50	14	13
25	11	12
5	9	12
C	8	9

Cn=Concentrations; C=Control; A1=*C. Odorata*; A2=*A. indica*.

C. odorata leaf extract gave the highest inhibitory effect of 16 mm at 250 mg/ml and least inhibition of 13 mm at 5 mg/ml while *A. indica* leaf extract gave the highest inhibitory effect of 18 mm at 250 mg/ml and the least inhibition of 5 mm at 5 mg/ml. The inhibitory effect of the two plants extracts i.e., *C. odorata* and *A. indica* extracts on the mycelial growth of *Streptomyces* spp. was significantly different at the various concentrations tested FIG. 1 and 2. From TABLE 3, *A. indica* leaf extract gave the highest inhibitory effect of 22 mm at 250 mg/ml and 19 mm at 250 mg/ml for *C. odorata* and *A. indica* against *Fusarium oxysporum* while the least activity was observed at 5 mg/ml for both plant extracts. From TABLE 4, the inhibition effects of 18 mm was observed in both plant extracts and the lowest inhibition of 9 mm and 12 mm was observed for the plant extracts against *A. flavus.*

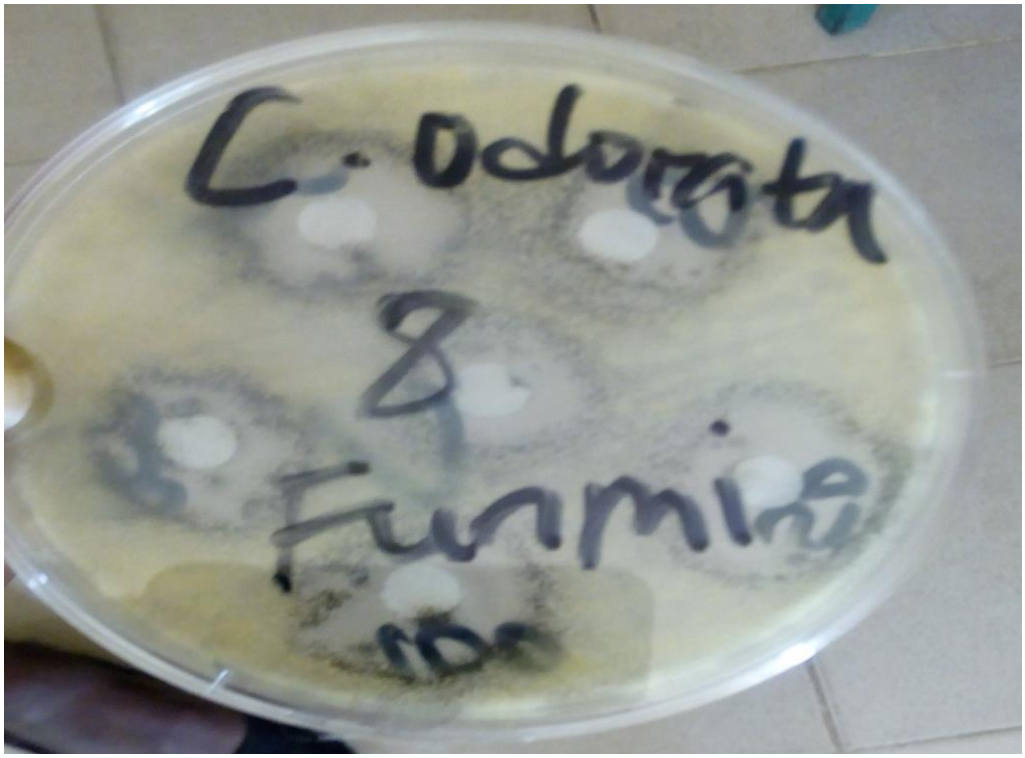

FIG. 1. **The effect of ethanolic plant extract of *C. odorata* on *in vitro* inhibition of mycelial growth of *Streptomyces* spp. at different concentrations.**

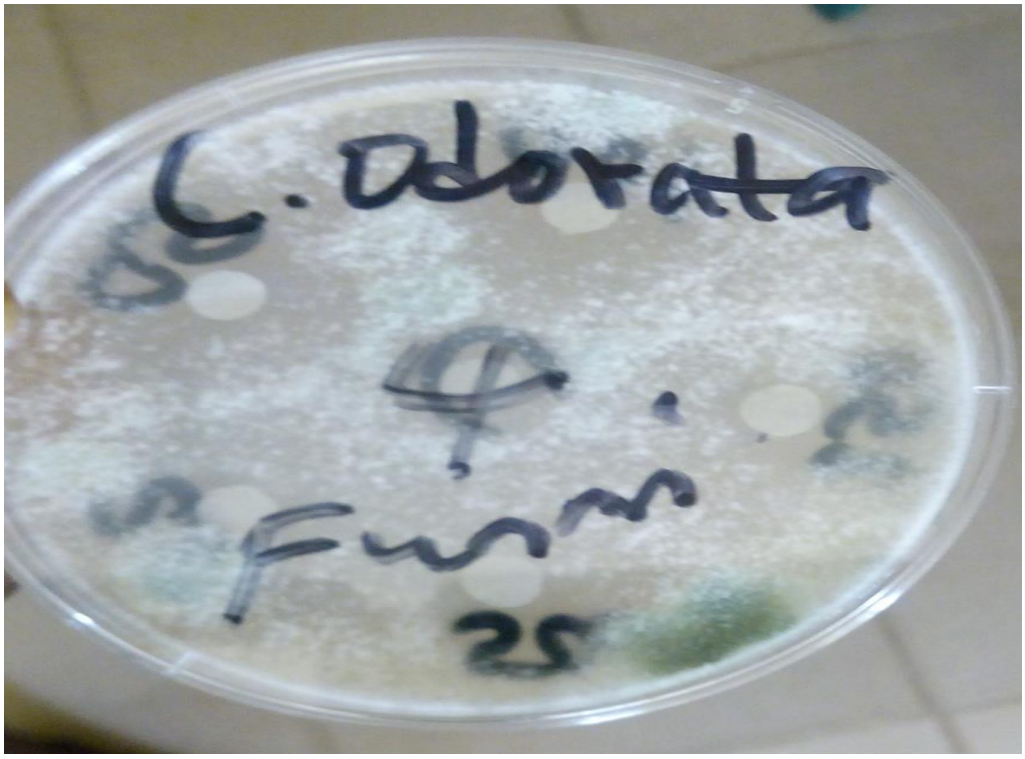

FIG. 2. **The effect of ethanolic plant extract of *C. odorata* on *in vitro* inhibition of mycelial growth of *Streptomyces* spp. at different concentrations.**

Discussion

Pathogens are known to be paramount in living things, in recent times, different researches have shown the negative or side effects of pathogens. Water melon is known to contain high moisture content and fiber; this makes the growth of organisms to be fast most especially under certain conditions like poor handling, storage methods and others. From the sample analyzed, three major pathogenic fungi were isolated to cause post-harvest rot in water melon; the pathogens are *A. flavus*, *Streptomyces* spp. and *F. oxysporum*. The ability of these pathogenic organisms to cause these post-harvest rot suggested that they are ubiquitous within short period of time. Phytopathogenic infection by fungi are often favoured by some factors such as poor production practices in the field (like excessive irrigation and nitrogen fertilization, lack of crop rotation and poor soil drainage) and wounds created during harvest, storage and packaging [12]; these pathogenic fungi are known to produce mycotoxins, which when ingested may cause mycotoxicosis leading to an acute or chronic disease episode like cancer [7]. Fungal genera such as *Candida albicans*, *Aspergillus niger*, *Streptomyces* spp., *A. flavus*, *Alternaria* spp., *Cladosporium* spp. and *Penicillium* spp. are capable of causing hypersensitivity in humans. In the view of this, consumption of unclean watermelon can be hazardous and harmful. There have been records of the effects of pathogenic fungi on watermelon. These fungi can cause up to 10% to 30% reduction in the yield of major food and cash crops [13], this necessitates pre- and post- harvest technologies to control them [14].

From the present study, ethanolic extracts of *C. odorata* and *A. indica* significantly reduced the mycelial growth of the fungal isolates; this may be due to the presence of bioactive components such as flavonoids, tannins, lignins, saponins, steroids [15]. The inhibitory effects of the plant extracts in the study agree with the research report of Omigie and Agoreyo [16]; they discovered the ability of the plant extracts to inhibit the growth and development of plant pathogenic fungi. A similar report of

the application of phytopathogenic agents in inhibiting growth of organism was discovered by Eunice and Osuji [17]; they control *Sclerotium rolfsii* causing cocoyam cormel rot with extracts of *Cassia alata* and *Dennetia tripetala.* The present study revealed the inhibitory effects of the plant extracts revealed was effective against the pathogenic organism isolated from watermelon fruits. This indicates that the extract is a potent antifungal agent against diverse fungal pathogens. This study also showed that *A. indica* is more potent antifungal than *C. Odorata.*

Conclusion

To overcome this alarming problem of post-harvest infections on fruits, the discovery of phytochemicals against new targets is a matter of urgency. Thus, *C. odorata* and *A. indica* could become promising natural antifungal agents with potential applications in pharmaceutical industry for controlling the pathogenic fungi. The plant extracts in the study (*C. odorata* and *A. indica*) could be used to inhibit post-harvest rot fungi of watermelon fruit, their use should be encouraged since they are safer, readily available, environmental friendly and nontoxic to man.

Conflict of Interest

The authors declare that they have no conflict of interest.

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