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Phytochemical Composition and Biocontrol Potential of *Chromolaena odorata* Extracts against Brown Rot in Amber Sweet Orange Fruits During Ambient Storage

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ABSTRACT
Background and Objective: Brown rot, a significant postharvest disease in tropical fruits, is increasingly attributed to the fungus *Lasiodiplodia* sp., which is notorious for causing fruit decay in citrus. Thus, the objective of this study was to assess the phytochemical compositions and biofungicide efficacy of *Chromolaena odorata* in controlling brown rot of orange fruits. **Materials and Methods:** Orange fruits infected with *Lasiodiplodia* were dipped into several concentrations of *C. odorata* extracts and disease severity was determined during ambient storage. Data collected on disease severity were subjected to Analysis of Variance and means of significant treatments were separated using Tukey's Test at $p<0.05$. **Results:** Among the tested concentrations, only 10% of *C. odorata* extracts proved effective against the brown rot pathogen, with none of the fruit showing any sign of infection by day 30, while n-Hexadecanoic acid, Benzaldehyde 4-ethyl-, Hexanoic acid, 2, 3-Butanediol and Oleic acid occurred as the most abundant components in the extract. **Conclusion:** The findings of this study revealed that the aqueous extracts of *C. odorata* could be explored as natural, safe bio fungicide in citrus fruit preservation.

KEYWORDS
Chromolaena odorata, aqueous extract, phytochemicals, brown rot, disease severity, GC-MS, storage

INTRODUCTION
Citrus fruits are hesperidium fruit type which belongs to the genus *Citrus*in the family Rutaceae. As a matter of fact, citrus production far exceeds that of any other fruit crop and it is widely grown in Nigeria and many other tropical and sub tropical regions[1]. Meanwhile, sweet orange is the most commercially planted citrus in Nigeria with a total production of 2.9 million tonnes in the year 2019[2]. In fact, citrus especially sweet orange is a good source of vitamin C, folic acid and fibres. In fact, Turner and Burri[3] reported that citrus fruits contain variety of vitamins, minerals, fibres anf phytochemicals such as carotenoids, flavonoids and limonoids which appera to have biological activities and health benefits. However, one of the greatest problems encountered during storage of sweet orange fruits is pathological deterioration. Thus, the potential foreign exchange earnings through the export of citrus fruits (sweet oranges) are under serious threat due to post-harvest infections. Regrettably, more than 50% of fruits grown in Nigeria are lost while moving from farms to major city markets[4].

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The two most important postharvest diseases in all citrus production are green rots caused by *Penicillium digitatum* and blue rots caused *Penicillium italicum*[5]. However, most losses associated with citrus production are caused by the green rot which accounts for up to 90% of the total citrus losses, especially in arid zones and subtropical climates[6,7]. However, *Lasiodiplodia* is a cosmopolitan fungus that is polyphagous and widespread, unspecialized virulent rot pathogen, found in the tropics and subtropics on a variety of host plants ranging from tree crops like kola, cocoa, to vegetables and fruits like orange, avocado, mango and cherry. In fact, Oladele and Aborisade[8] reported *Lasiodiplodia* sp. IMI503248 as a major post-harvest pathogen of orange fruits in Akure, Nigeria, accounting for over 75% disease incidence of the total rots in their study. The fungus was identified by ITS rDNA sequence analysis using the FASTA algorithm with the fungus database from the European Molecular Biology Laboratory (EMBL) and the sequence showed 100% identity to numerous ITS sequences described from different *Lasiodiplodia* species or their *Botryosphaeria* teleomorphs. Best matches with the fungus included sequences of *Lasiodiplodia* species reported in the peer-reviewed literature[9] and all the known species are associated with various symptoms such as dieback, root rot, fruit rot and leaf spots among many others[10]. Initial infection shows as light brown discolouration on any area of the fruit surface. As the decay develops, the lesion becomes more brown, firm and slippery[11].

Meanwhile, postharvest diseases in fruits have been controlled mainly by synthetic fungicides either as dips, sprays, fumigants, treated wraps and box liners or in waxes and coatings. However, synthetic preservatives have raised concerns regarding their potential health risks and environmental impact, underscoring the need for innovative and eco-friendly solutions[12]. Besides, export markets are increasingly more sensitive to the use of chemicals for disease control coupled with the fact that most chemicals are expensive and inaccessible to local farmers who are the major bulk producers of this fruit in Nigeria. Development of alternative methods has become urgent in recent years due to the increasing regulatory restrictions on the use of fungicides[13]. Thus, promising alternatives include natural antimicrobials extracted from plants, animals or microorganisms that can suppress bacteria and fungi growth[14].

Chromolaena odorata, commonly known as 'ewe Awolowo' and 'obu inenawa' by respective Yoruba and Ibo major tribes in Nigeria, has been reported in tradition medicine as antispasmodic, antiprotozoal, anti-trypanosomal, antibacterial, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic and hepatotropic agent[15]. Despite its invasive nature, the plant has been found to have various traditional medicinal uses in different cultures, including wound healing, anti-inflammatory, and antimicrobial applications[16].

Therefore the main objective of this study was to assess the effectiveness of *C. odorata* in controlling brown rot of orange fruits during ambient storage and its potential as a suitable alternative to synthetic preservatives.

MATERIALS AND METHODS

Source of fruits: Mature, green healthy orange fruits were harvested from a commercial orchard in September 2023 from a citrus farm in Igbatoro, Akure North, Nigeria. Fruits of uniform size and colour were selected. Before treatment, the fruits were washed with clean water, disinfected for 10 min in 10% sodium hypochlorite and allowed to air-dry at room temperature.

Preparation of spore suspension: A ten day old agar slant culture of *Lasiodiplodia* sp. (IMI Number: 503248) on malt extract agar (MEA) was used to prepare spore suspension. Sterile water was poured into the slant and shaken vigorously to dislodge the spores from the vegetative hyphae. The wash water was collected in a sterilized beaker. One milliliter of the suspension was spread on an area of 1 cm^2 and allowed to dry on a clean microscope slide before counting spores using the formula of Breed Direct Counting Technique[17] under the high dry ×40 objective microscope (Olympus).

Pathogenicity test: Spore suspension of *Lasiodiplodia* was used to inoculate fresh fruits 1 mm deep at the equator and incubated at 28±2°C and 75% relative humidity inside sterilized desiccators. The disease symptoms were noted and re-isolation from infected fruit tissue was performed on fresh sterile MEA plate and its cultural characteristics were compared with the original isolate.

Preparation of extract of *Chromolaena odorata*: Detached *Chromolaena odorata*leaves were air dried for 14 days. The dried leaves were pulverized with a blender to a smooth powdery form. About 28 g of the pulverized leaves were dissolved in 1 L of water, thoroughly shaken together and later filtered with a muslin cloth to obtain the crude/stock solution. Varying concentrations (5, 10, 15 and 100%) of the aqueous extract were then prepared from the stock/crude extract with appropriate volumes of water.

Inoculation and treatment of orange fruits with *Chromolaena odorata* extract: The orange fruits were artificially infected with spores (6.50×10^4 CFU/mL) of *Lasiodiplodia* and later dipped separately into each concentration (5, 10, 15 and 100%) of the prepared extract for 5 min while benlated (fungicide) and untreated orange fruits served as positive and negative control, respectively. Each set up consisted of 3 fruits. After treatment, the fruits were then placed inside a sterilized Petri dish and transferred into desiccators, and stored at 28±2 and 75% relative humidity and assessed daily for disease severity.

Assessment of rot severity: Assessment of disease severity was done using the scale of Miller *et al.*[18] but with slight modification and observed as follows:

- Disease free = 1
- Slight rot/decay up to 10 % of the fruit = 2
- Moderate rot/decay up to 25 % of the fruit = 3
- Severe rots/decay ≥35 % of the fruit surface = 4

Rot/decay was recognized by light brown discolouration on the fruit or by appearance of mycelium on the fruit surface.

Determination of *in vitro* activity of the most effective concentration of *Chromolaena odorata* extract on spore germination of *Lasiodiplodia* sp.: Two millimeters of the spore suspension of the *Lasiodiplodia* sp. was collected into a beaker, followed by the addition of 2 mL of the most effective concentration (10%) of the *Chromolaena odorata* extract while spore suspension without extract (0%) served as control. After 12 hrs of incubation at 28±2°C, the mixed suspension was spread on an area of 1 cm^2 and allowed to air-dry on a clean microscope slide containing solidified MEA in triplicates before viewing under ×10 objective binocular microscope (N-300M) to count the number of germinated and un-germinated spores.

Determination of *in vitro* activity of the most effective concentration of *Chromolaena odorata* extract on mycelia growth of *Lasiodiplodia* sp.: The effect of *Chromolaena odorata* extract on mycelia growth of *Lasiodiolodia* sp. was carried out using agar diffusion method. With 6mm cork-borer, two wells were bored at 2 cm apart on already solidified MEA. One well contained the most effective concentration (10%) of the extract while the other well contained 6 mm mycelia plug of the *Lasiodiolodia* sp. The set up was incubated at 28±2°C for 3 days. Each setup was in triplicates. After incubation, the mycelia growth was measured along the transect in two directions at right angle to each other.

Phytochemical screening of the aqueous extract of *Chromolaena odorata*: The phytochemical screening of the aqueous extract was done according to the method described by Harborne[19]. The phytochemicals screened for were tannin, saponin, phlobatinnin, flavoniod, alkaloid and cardiac glycosides.

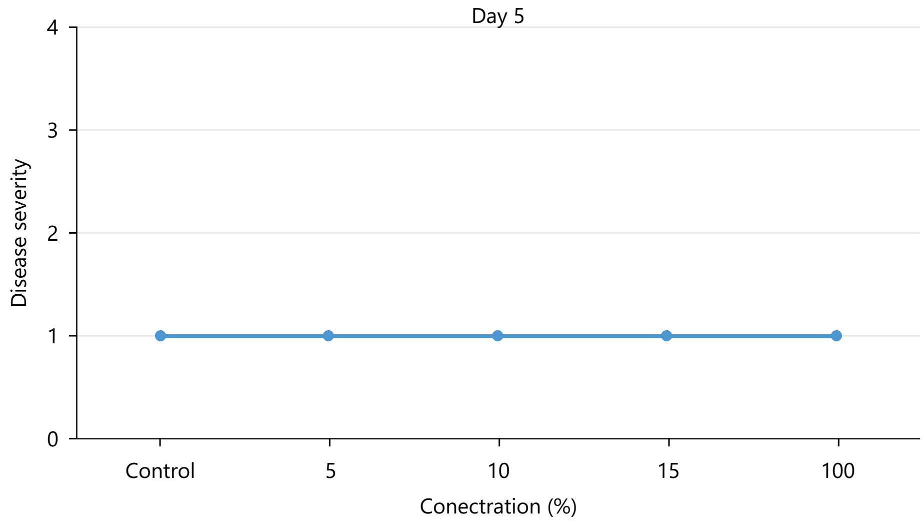

Fig. 1: Disease severity of orange fruits infected with *Lasiodiplodia* sp. following treatment with different concentrations of *Chromolaena odorata* and stored for 5 days under ambient temperature

Gas chromatography-mass spectrophotometry (GC-MS) of *Chromolaena odorata* extract: The *Chromolaena* extract was subjected to chromatographic analysis using a Varian 3800/4000 gas chromatograph mass spectrometer equipped with an Agilent Technologies and BP5 (30 m×0.25 mm×0.25 microns) capillary column. Nitrogen was used as a gas carrier. A 1.0 μL volumes of the extract were injected using a splitless mode at an injector temperature of 270°C. The oven temperature was ramped from 80 to 200°C (1 min hold) at a rate of 5°C/min. The oven temperature was held at 280°C for 6 min following each analysis. The total run time for each sample was approximately 45 min. The GC-MS interface temperature was set to 280°C. The peaks of the organic compounds in the samples were identified in Wiley's NIST 08 Mass Spectral Library and expressed in terms of its balance and retention time.

Statistical analysis: The data obtained for the disease severity, spore germination and mycelia growth were subjected to analysis of variance and where significant, the means were compared at 5% level of probability using Tukey's Test (SPSS version 20). The values are expressed as Mean±Standard Deviation.

RESULTS

Disease severity of orange fruits infected with *Lasiodiplodia* sp. following treatment with different concentrations of *Chromolaena odorata* extracts: The observed results shown in Fig. 1 revealed that both the control (untreated) fruits and all the treated fruits with different concentrations (5, 10, 15, 100%) of the *Chromolaena* extract had the same disease severity (1.00±0.00) by day 5 of storage, indicating that they were all disease-free. As storage duration progressed till day 10, Fig. 2 showed that all treated fruits were still disease free, except the control and fruits treated with 15% extracts of *C. odorata* that had shown slight rottenness as evident by their mean disease severity values of 2.00±0.00. Even by days 15 and 20 in storage as observed in Fig. 3-4, respectively, fruits treated with 10%extractsstill maintained their diseased free status (1.00±0.00) when compared with fruits treated with 5% and 15% extract of *C. odorata* that showed moderate rottenness (3.00±0.03) while control and fruits treated with 100% *C. odorata* demonstrated complete rottenness (4.00±0.00). Likewise, as the storage durations became further extended to days 25 and 30, only fruits treated with 10% of *C. odorata* as shown in Plate 1 retained their non-diseased status (wholesomeness) of the orange fruits while Fig. 5-6 revealed that all other fruits (both control and treated fruits) had completely rotten.

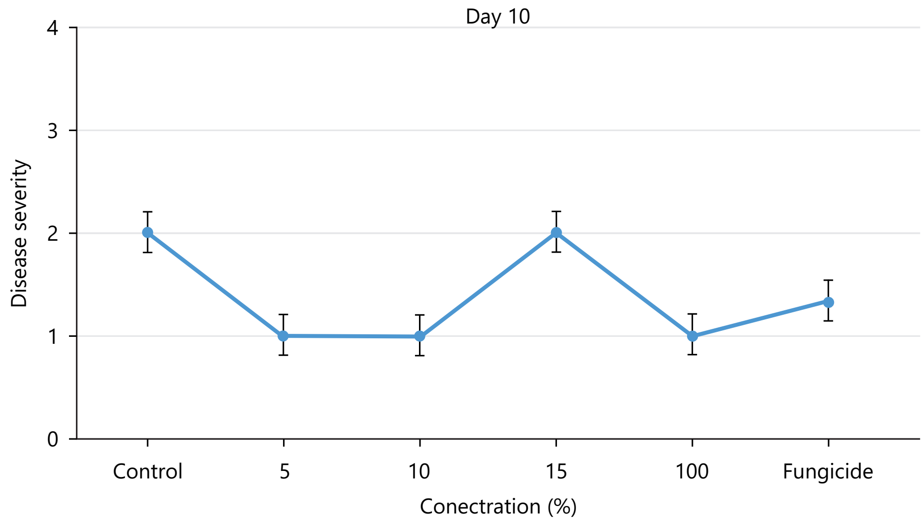

Fig. 2: Disease severity of orange fruits infected with *Lasiodiplodia* sp. following treatment with different concentrations of *Chromolaena odorata* and stored for 10 days under ambient temperature

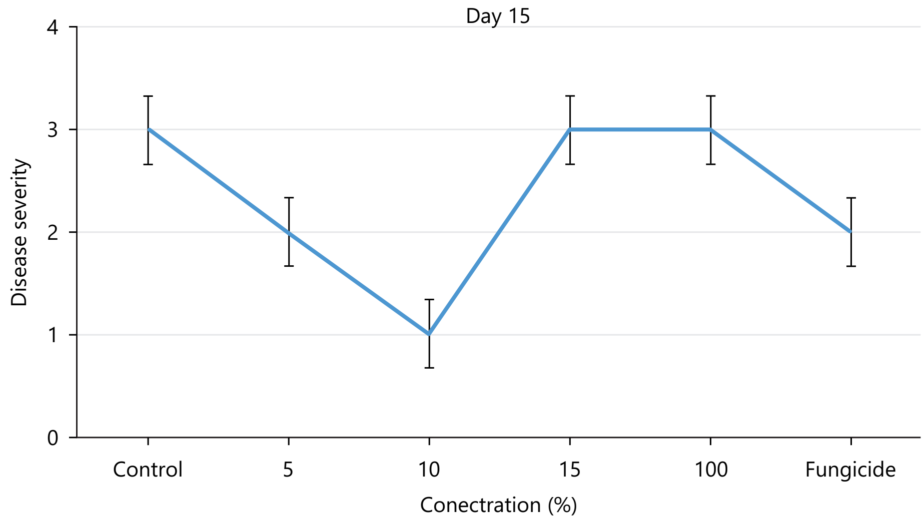

Fig. 3: Disease severity of orange fruits infected with *Lasiodiplodia* sp. following treatment with different concentrations of *Chromolaena odorata* and stored for 15 days under ambient temperature

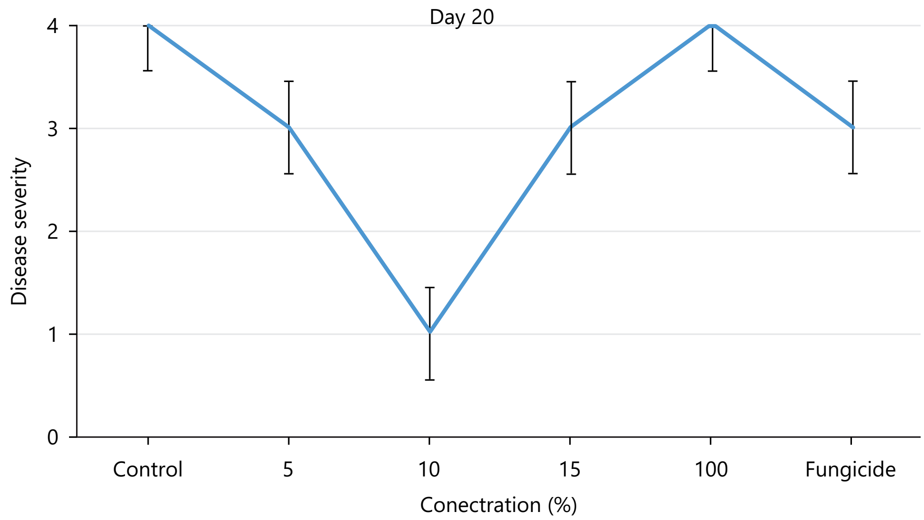

Fig. 4: Disease severity of orange fruits infected with *Lasiodiplodia* sp following treatment with different concentrations of *Chromolaena odorata* and stored for 20 days under ambient temperature

Plate 1: Orange fruits infected with *Lasiodiplodia* sp and treated with 10% extract of *C. odorata,* showing no sign of brown rottenness following ambient storage for 30 days

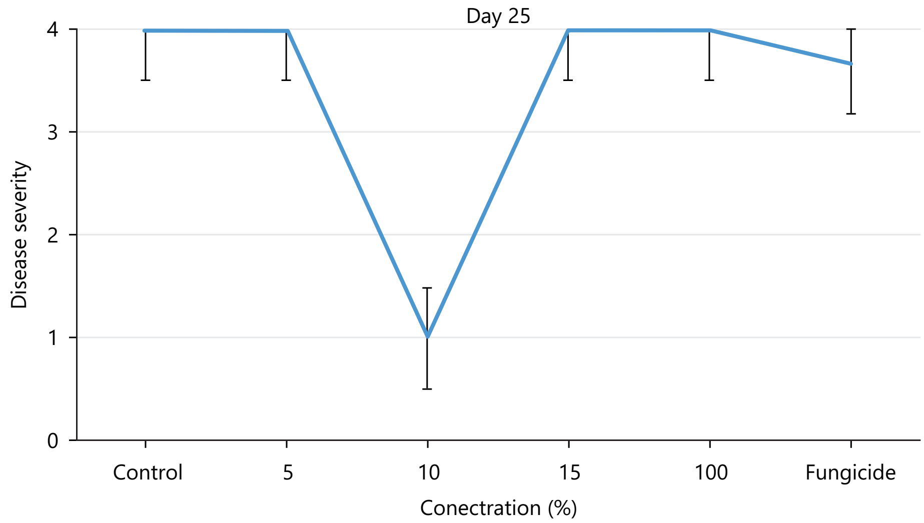

Fig. 5: Disease severity of orange fruits infected with *Lasiodiplodia* sp following treatment with different concentrations of *Chromolaena odorata* and stored for 25 days under ambient temperature

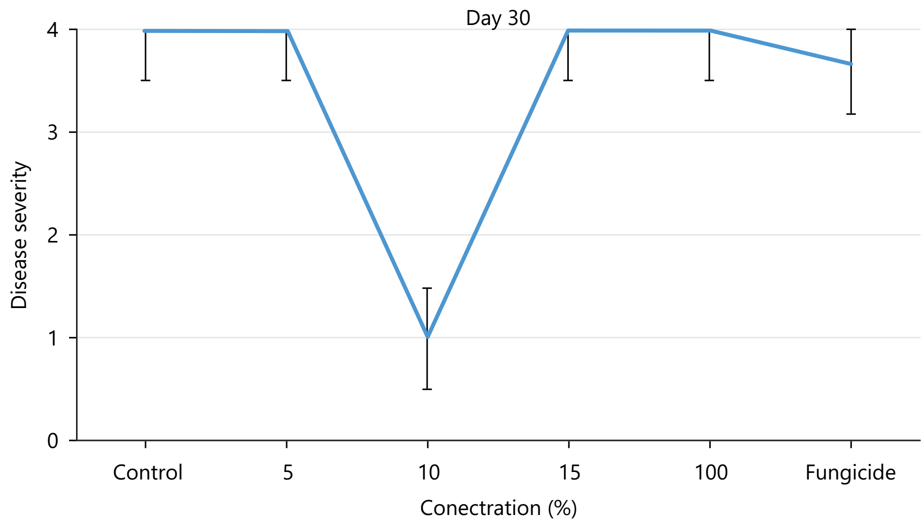

Fig. 6: Disease severity of orange fruits infected with *Lasiodiplodia* sp following treatment with different concentrations of *Chromolaena odorata* and stored for 30 days under ambient temperature

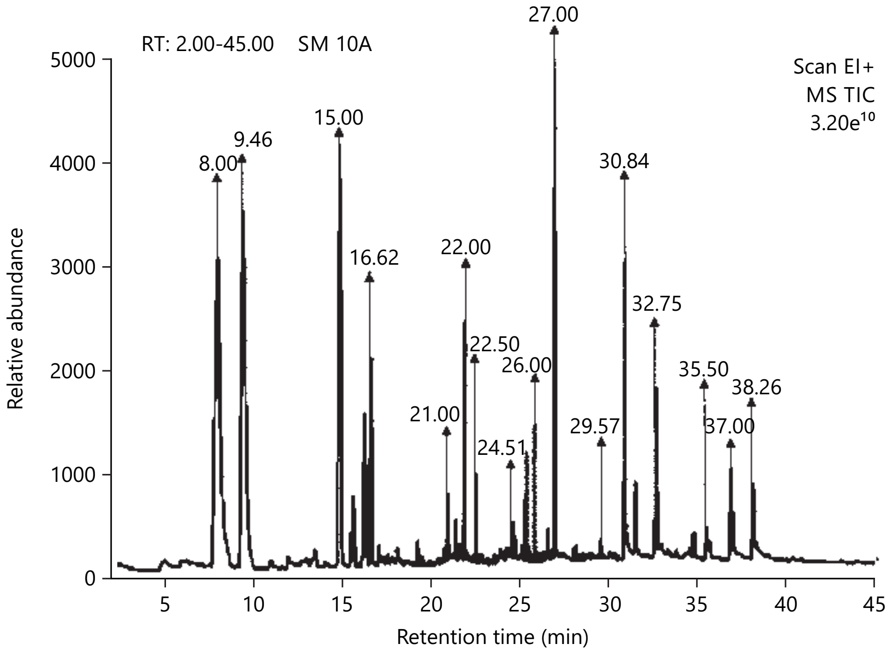

Fig. 7: Chromatogram of aqueous extract of *Chromolaena odorata*

Table 1: Effect of most effective concentrations of *Chromolaena odorata* extract on spore germination of brown rot pathogen *in vitro*

Most effective concentration of *Chromolaen aodorata* extract (%)	Germinated spores (%)	Non-germinated spores (%)
0 (control)	115.67±12.81[b]	13.33±9.61[b]
10	10.33±5.55[a]	71.67±2.33[a]

Means followed by same letters within columns are not significantly different (p>0.05) from one another using Tukey's HSD Test

Table 2: Effect of most effective concentrations of the aqueous extract of *Chromolaena odorata* on mycelia growth of the brown rot pathogen *in vitro*

Most effective concentrations of *Chromolaena odorata* extract (%)	Mycelia growth (cm)
0 (control)	1.20±0.12[b]
10	0.83±0.12[a]

Means followed by same letters within the column are not significantly different (*p*<0.05) from one another using Tukey's HSD Test

***In vitro* effect of *Chromolaena odorata* extract on spore germination and mycelia growth of the brown rot pathogen:** The *in vitro* findings revealed that the percent spores that did not germinate following treatment with 10% extract of *C. odorata* was significantly higher (71.67±2.33) when compared with non-germinated spores (13.33±9.61) of the control (spores without extract treatment) (Table 1). Likewise, the brown rot pathogen had lesser mycelia growth (0.83±0.12 cm) when treated with 10% extract of *C. odorata* and was significantly different (p<0.05) from the untreated mycelia (Table 2).

Phytochemical compositions of *Chromolaena odorata* extracts: Tannins, alkaloids, flavonoids, saponins were all detected as phytochemical constituents in the extracts with the exception of cardiac glycosides that were absent (Table 3).

Bioactive compounds detected in *Chromolaena odorata* extracts: The chromatogram of aqueous extract of *Chromolaena odorata* using GC-MS is illustrated in Fig. 7. The peak area (%) of the various identified compounds included 2,3-Butanediol (10.09), Hexanoic acid (11.72), Benzaldehyde, 4-ethyl- (12.37), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (7.16), Ethanone, 1-(2-hydroxy-5-methylphenyl)- (2.27), Methyl 4-(hydroxymethyl) Benzoate (7.82), p-Menth-3-en-9-ol (3.26), Eugenol (1.30), Acetamide, N-tetrahydrofurfuryl-2-methoxy- (4.56), n-Hexadecanoic acid (14.33), 9,12-Octadecadienoic

Table 3: Phytochemical constituents of aqueous extract of *Chromolaena odorata*

Phytochemicals	*Chromolaena odorata* extract
Tannins	+
Alkaloids	+
Cardiac glycosides	-
Saponins	+
Flavonoids	+

Table 4: Bioactive compounds of *Chromolaena odorata* extract

S/N	Retention time (Min)	Compound detected	Peak area (%)
1	8.00	2,3-Butanediol	10.09
2	9.46	Hexanoic acid	11.72
3	15.00	Benzaldehyde, 4-ethyl-	12.37
4	16.62	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.16
5	21.00	Ethanone, 1-(2 hydroxy-5-methyl phenyl)-	2.27
6	22.00	Methyl 4-(hydroxymethyl) Benzoate	7.82
7	22.50	p-Menth-3-en-9-ol	3.26
8	24.51	Eugenol	1.30
9	26.00	Acetamide, N-tetrahydrofurfuryl-2-methoxy-	4.56
10	27.00	n-Hexadecanoic acid	14.33
11	29.57	9,12-Octadecadienoic acid methyl ester	1.04
12	30.84	Oleic acid	9.77
13	32.75	Octadecanoic acid	5.93
14	35.50	Squalene	1.37
15	37.00	Stigmasterol	3.58
16	38.26	γ-sitosterol	3.41

acid methyl ester (1.04), Oleic acid (9.77), Octadecanoic acid (5.93), Squalene (1.37), Stigmasterol (3.58), and γ-Sitosterol (3.41) (Table 4). The analysis revealed the presence of n-Hexadecanoic acid (14.33%), Benzaldehyde 4-ethyl- (12.37%), Hexanoic acid (11.72%), 2, 3-Butanediol (10.09%) and Oleic acid (9.77%) as the major bioactive compounds in *Chromolaena* extract.

DISCUSSION

This study has shown the efficacy of aqueous leaf extracts of *Chromolaena odorata*in controlling brown rot of orange fruits during ambient storage. Remarkably, only fruits treated with 10% extract were still disease free by day 30 under ambient storage. Thus, this showed that 10% concentration of the *Chromolaena* extract was able to compete favourably with the other concentrations by extending the shelf life of the orange fruits for 30 days at ambient storage as against the untreated that had already deteriorated by day 20 in storage. This is supported by the works of Abdullah *et al.*[20] who conducted a study highlighting the inhibitory effects of *Chromolaena odorata* extract against pathogenic fungi responsible for postharvest decay in fruits and this underscores the plant's ability to combat microbial growth, making it promising for postharvest disease control. Even, Efunwoye *et al.*[21] in their own work reported that the treatment observed to be most effective in reducing the rates of disease incidences, extending the shelf-life and preserving marketability attributes in Padma tomato fruits was by submerging the fruits inside the botanical extract of *Chromolaena odorata*.

Even the *in vitro* study further supports the antifungal efficacy of the 10% extract of *C. odorata* in controlling brown rot on orange fruits for 30 days by inhibiting spore germination and significantly reducing the pathogen's mycelial weight. This confirms the effectiveness of plant extracts in managing post harvest pathogen. This observation is in agreement with the works of Oladele[22] who reported though on garlic powder, that the different weights of garlic powder apart from the control (0 g garlic) significantly inhibited the mycelia growths of *Aspergillus, Rhizopus, Lasiodiplodia* and *Mucor* species which are the major post-harvest pathogens tested in his study.

In fact, the antimicrobial activity of *Chromolaena odorata* extract is attributed to bioactive compounds such as flavonoids and alkaloids[18]. This observation is equally in agreement with the reports of Chiejina and Onaebi[23] that *Chromolaena odorata* extracts possess alkaloids, phenols, tannins, saponins and flavonoids with antimicrobial activities against *Geotrichum candidum*. These compounds have been shown to interfere with the growth and development of pathogenic fungi, making *Chromolaena odorata* an effective natural agent for disease management. Each of these phytochemical constituents as enunciated by other authors, is connected with significant antimicrobial properties. Some of these compounds are believed to contribute to its medicinal properties, including its antifungal activity[24].

For instance, alkaloids and flavonoids when presentexhibit antimicrobial properties. These compounds inhibit the growth of spoilage-causing microorganisms, extending the shelf life of fruits[25]. These compounds possess antioxidant properties, which play a crucial role in fruit preservation by inhibiting oxidative processes and prevent the degradation of fruits by scavenging free radicals that contribute to spoilage[26]. This is further buttressed by the reports of Cerioni and Rapisarda[27] that alkaloids and flavonoids act as natural antimicrobial agents, inhibiting the growth of bacteria and fungi that contribute to fruit spoilage. Also, tannins act as natural preservatives by forming complexes with proteins and enzymes, thereby inhibiting microbial growth and enzymatic browning[28]. Recent research has focused on the plant's potential as a source of bioactive compounds, with studies identifying several flavonoids and terpenoids that may have pharmaceutical significance[29].

A lot of bioactive compounds and these natural compounds exert their effects through various mechanisms, making them effective against different types of microorganisms. Also, 9-octadecenoic acid methyl ester according to Donia and Hamann[30] possesses anti-microbial properties.

CONCLUSION

This study demonstrates the efficacy of *Chromolaena odorata* extracts in controlling brown rot in orange fruit and providing significant insights into alternative and sustainable methods for managing this fungal disease. From this study, the use of aqueous extract (10%) of *Chromolaena odorata* was able to prolong the shelf life of orange fruits at ambient storage at least for 30 days. The extracts exhibited promising antifungal activity and effectively curtailed the spread of brown rot, with a reduction in disease severity observed in treated fruits. The ability of *C. odorata* in inhibiting the growth of microorganism can be traced to the phytochemicals present in the extracts that have also been identified to exhibit bioactive properties. These extracts are biodegradable and present minimal risk to human health and the environment, positioning them as safer bio fungicide alternative to synthetic fungicides.

SIGNIFICANCE STATEMENT

This study explored the use of *Chromolaena odorata* extracts in managing brown rot disease in orange fruits. The results showed that the aqueous extracts of *C. odorata* significantly extended the shelf life of orange fruits, with 10% aqueous extracts outperforming other concentrations and the test fungicide by keeping the orange fruits healthy for at least 30 storage days. Thus, this research revealed eco-friendly, cheap, local and non-chemical approach to solving post management of plant diseases.

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