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Post-harvest fungal rot of green pepper fruits (*Capscium annuum*): Methanolic extracts of tropical spices as bio rescue agents

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ABSTRACT: This study aimed at evaluating the control potential of some essential oils on green pepper fruit (*Capscium annuum*) rot. Samples of green pepper fruits (infected and healthy) and spices were obtained from Ado-Ekiti market, fungal rot pathogens were isolated from infected green pepper fruit and pathogenicity test was carried out to authenticate the pathogenic status of the fungal isolates. The fungi isolated from the green pepper fruits were: *Aspergillus spp, Cladosprium spp. Fusarium solani, Rhizopus stolonifer, Mucor spp. Alternaria spp.* The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation. Extracts of clove, ginger, and garlic were obtained using established standards. The fungal inoculum was prepared from 5-days old culture grown on potato dextrose agar and the effects of the essential oils were determined on the pepper fruit rot. The sensitivity of the fungal isolates to the extracts was found by assessing the diameter of the zone of inhibition in which significant susceptibility was taken as 25 mm in diameter. The effects of various rot fungi on the carbohydrate content of green pepper showed values of 32.85, 33.57, 33.12, 33.22, 32.78, and 33.57% by *Aspergillus spp*, *Cladosprium spp*, *Fusarium solani*, *Rhizopus stolonifer*, *Mucor spp* and *Alternaria* spp respectively. The effect of various rot fungi on the moisture content of green pepper showed 19.68% for *Cladosporium* spp, followed by *Fusarium solani* (19. 24%) while the least was *Aspergillus* spp (18.59%). The effects of various rot fungi on the total ash of green pepper showed *Mucor* spp (18.87%), followed by *Cladosporium* (18.81%) and *Aspergillus* spp (18.61%). The effects of various rot fungi on the crude fat content of green pepper showed *Fusarium solani* and Mucor 9.97% while the least was *Aspergillus* spp (9.35%). The effects of various rot fungi on the protein content of green pepper showed a value of 15.96% for *Cladosporium* spp while the least effect was from *Alternaria* spp (15.01%). Rot fungi exhibited various degrees of effects on the nutritional contents of infected green pepper fruits. Therefore, various biological controls of fungal fruits rot, such as the application of powder extracts of clove, ginger and garlic can be adopted, these applications have been established for controlling fruit rot of green pepper fruits in this research.

Keywords: Bio agents, green pepper, post-harvest, tropical spices.

INTRODUCTION

Peppers are native to Mexico, Central America, and northern South America. Pepper seeds were imported to Spain in 1493 and then spread through Europe and Asia. The mild bell pepper cultivar was developed in the 1920s, in Szeged, Hungary (Sasvari, 2005). Pepper (*Capsicum annum*) is a genus of flowering plants in the nightshade family, Solanaceae. *Pepper Capsicum* consists of approximately 20-27 species out of which *Capsicum* *annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens* and *Capsicum pubescens* were domesticated (Mason, 2010). In low income Sub-Saharan African countries, the incidences of post-harvest losses (from farm to fork) are the main causes of food insecurity (Zorya *et al*., 2011). Rot fungi are ubiquitous biological agents that are able to infect fruits because of their ability to produce a wide range of hydrolytic enzymes. Mould infection depends on many factors such as pH, water activity, temperature, atmosphere, time, etc (Magan and Aldred, 2007). Fungal infections can generate quality loss and health hazards through the production of toxins (Parveen *et al*., 2016).

Moreover, pathogenic infection in the field or in post-harvest storage can affect the health of humans and livestock, especially if the pathogen produces toxins in or on consumable products (Menzler-Hokkanen, 2006). These reduce their yield and market values before and after harvest as fungi make way into plant host tissue through natural openings such as lenticels, stomata and through the unbroken epidermis by means of appressorium or germ tube (Ademoh *et al.,* 2017). Various methods, strategies, and approaches were used in the management of plant diseases. These approaches have contributed significantly to the remarkable improvements in crop productivity and the quality of crops produced (Chandrashekara *et al*., 2012).Therefore, there is a need to isolate and identify fungi associated with postharvest rot in pepper (*Capsicum annum)* in order to control the pathogens.

MATERIALS AND METHODS

Collection of plant materials

Three plants (*Syzygium aromaticum*, *zingiber officinale* and *Allium sativum)* used in this study were collected from a garden at bank road, Ado Ekiti. The identity of the plant samples was authenticated using the herbarium specimens of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria.

Preparation of plant extracts

The plant samples were washed in tap water and cut into small pieces of about 2 mm x 2 mm in size. The sample pieces were air-dried for 15 days at room temperature (28°C). These were separately pulverized using an electric blender (Model M 20 IKA Universal Mill, IKA Group Japan). About 1000 g of each of the finely ground samples were carefully weighed and separately soaked in 1000 ml of 95% methanol. The plant materials were soaked for 7 days. The supernatant was decanted into a clean labelled flask and adequately corked. About 850 ml of methanol was further added to each of the plant samples left to stand for 3 days. The resulting supernatant solution was separately decanted and added to the first, the crude extracts were filtered with sterile filter paper into a labelled conical flask. The filtrates were concentrated in a rotary evaporator (Optional Oil Bath Model RE-3020 America (ambient to 180°C). The plant extract obtained was transferred into a labeled beaker and allowed to stand at room temperature for 24 hours to permit evaporation of the residual solvent. The extracts were kept in a refrigerator at 4°C until used (Fagbohun and Bamikole, 2019).

Preparation of media

Thirty nine grams (39g) of potato dextrose agar (PDA) was measured using a weighing balance, this was diluted with 1000ml of distilled water in a conical flask and autoclaved for 60 minutes at 110°C. After heating, the media was allowed to cool for 15 minutes, 8g of streptomycin was added to guide against bacterial growth and the media was poured into sterilized Petri dishes and left to solidify under aseptic conditions prior to inoculation.

Isolation and identification of the fungal organisms

Diseased portions of the pepper fruits were cut under aseptic conditions into small bits of 5 mm into a sterile dish with the aid of scissors which was flamed over a Bunsen's burner flame and dipped inside methylated spirit (Fawole and Oso, 1995; Ijato *et al*., 2022a,b). The cut diseased bits sterilized with 70% ethanol were then placed centrally on Petri dishes containing solidified potato dextrose agar (PDA). The solidified plates were incubated at room temperature (28 + 2°C) in the dark for 72 hours. The fungal colonies growing from the incubated plates were sub-cultured into fresh medium until the pure culture was obtained. Microscopic examination was used after examining the colony characteristics to establish the identity of fungi.

A sterile needle was used in taking a little portion of the hyphae containing spores and placed on the sterile glass slide, then stained with lactophenol cotton blue and examined under the microscope for fungal structures. The morphology and culture characteristics observed were compared with structures in (Snowdon, 1990). Forty grams (40 g) of PDA powder was placed in five litre conical flask. One hundred millilitres (100 mL) of distilled water was added and boiled to completely dissolve the powder. To prevent bacterial growth, 0.2 g of streptomycin was added to the potato dextrose broth. The supernatant was carefully transferred into sterile conical flasks and autoclaved at 120°C for 15 minutes at 101 lbf pressure and poured into Petri dishes for solidification.

Identification of isolates

The morphology of the fungal isolates was macroscopically studied by observing the colony features (colour, shape and size) (Cheesbrough, 2000), and microscopically studied by staining slide mounted with a small portion of the mycelium with a lactophenol cotton blue and examined under the compound microscope using x40 objective (Alsohaili and Bani-Hasan, 2018).

Pathogenicity test

The approach of Balogun *et al.* (2005) was employed to determine the pathogenicity of the various fungal isolates. Apparently, healthy and matured pepper fruits were surfaced-sterilized with 0.5% sodium hypochlorite for 30 seconds and then rinsed in three changes of sterile distilled water. Five (5) mm diameter flame-sterilized cork borer was used to remove cylindrical cores from each fruit and were then inoculated aseptically with a 5 mm diameter disc from the advancing edge of the 7-day-old fungal culture of any one isolate.

Petroleum jelly was smeared to completely seal the surface of each of the inoculated pepper fruit to prevent external infection before incubating for 10 days in three replicates. The controls were inoculated with a disc of solidified potato dextrose agar medium. Fruits were inoculated in three replicates. Rot symptoms developed with different fungal isolates were compared to the natural original rot. The pathogens were re-isolated and identified using the same procedures described earlier in this finding.

Determination of antifungal activities of test plants

About 1 mL each of varying concentrations viz: 0.25, 0.50, 0.75 and 1.0% of crude extracts were dispensed separately onto the 15 mL molten medium in Petri dishes with the help of a syringe. These were thoroughly mixed and allowed to solidify. A portion of fungal growth from the periphery was cut with the help of a sterilized cork borer of 6 mm in diameter and placed onto the middle on each Petri dish and incubated at room temperature. A week after incubation, measurement of the colony was taken directly with the help of a scale. The control experiment was set up without adding any plant extract.

The difference in the measurement between the mycelia growth of the fungi and that of the cork borer gives the growth of the colony and the effect of different concentrations of extraction on the growth of the species. Fungi toxicity was recorded in terms of percentage mycelia growth inhibition which was calculated using the formula described by Singh and Singh (2002) and Ijato *et al.* (2022a, b).

$$I = \frac{DC - DT}{DC} x\ 100$$

Where: I = Percentage inhibition, DC = Average diameter of control (8 cm), DT = Average diameter of growth with treatment.

Proximate analysis

The proximate composition was determined according to AOAC (2000).

Moisture content determination

Two grams (2.0 g) of each sample was placed in an oven maintained at 100 to 103°C for 16 hours with the weight of the wet sample and the weight after drying noted. The drying was repeated until a constant weight was obtained. The moisture content was expressed in terms of loss in weight of the wet sample.

$$\%\ \text{Moisture content} = \frac{\text{weight of moisture}}{\text{weight of sample}} x\ 100$$

Ash content determination

Two grams (2.0g) of each of the oven-dried samples in powder form were accurately weighed and placed in a crucible of known weight. These were ignited in a muffle furnace and ashed for 8 hours at 550°C. The crucible containing the ash was then removed, cooled in a desiccator and weighed and the ash content was expressed in terms of the oven-dried weight of the sample.

$$\%\ \text{Ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} x\ 100$$

Protein content determination

The protein nitrogen in 1 g of the dried samples was converted to ammonium sulphate by digesting with concentrated H_2SO_4 in the presence of $CuSO_4$ and Na_2SO_4. These were heated and the ammonia evolved was steam distilled into a boric acid solution. The nitrogen from ammonia was deduced from the titration of the trapped ammonia with 0.1M HCl with Tashirus indicator (double indicator) until a purplish pink colour was obtained. Crude protein was calculated by multiplying the value of the deduced nitrogen by the factor of 6.25 mg.

Crude fibre content determination

A 2.0 g of each sample was weighed into separate beakers, the samples were then extracted with petroleum ether by stirring, settling and decanting 3 times. The samples were then air-dried and transferred into a dried 100 ml conical flask. 200 cm^3 of 0.127M sulphuric acid solution was added at room temperature to the samples. The first 40 cm^3 of the acid was used to disperse the sample. This was heated gently to boiling point and boiled for 30 minutes. The contents were filtered to remove insoluble materials, which were then washed with distilled water, then with 1% HCl, next with twice ethanol and finally

Table 1. Percentage frequency of fungi isolates from stored green pepper fruits.

Fungi species	Number of isolates	Frequency (%)
Aspergillus spp	21	29.6
Cladosprium spp	14	19.7
Fusarium solani	8	11.3
Rhizopus stolonifer	17	23.9
Mucor spp	2	2.8
Alternaria spp	9	12.7
Total	71	100

Table 2. Antifungal activity of clove (*Syzygium aromaticum*) methanol extract on fungal pathogens of green pepper.

Isolates	Diameter of zones of inhibition (mm)				
	0.2	0.4	0.6	0.8	1.0
	Concentrations (mg/mL)				
Aspergillus spp	21. 00[a]	18.00[a]	14.00[a]	10.00[a]	8.00[a]
Cladosprium spp	19.00[b]	15.00[b]	12.00[c]	9.00[b]	7.00[b]
Fusarium solani	13.00[e]	10.00[d]	8.00[e]	0.00[d]	0.00[d]
Rhizopus stolonifer	17.00[c]	15.00[b]	13.00[b]	10.00[a]	8.00[a]
Mucor spp	17.00[c]	15.00[b]	13.00[b]	10.00[a]	8.00[a]
Alternaria spp	15.00[d]	13.00[c]	10.00[d]	8.00[c]	6.00[c]

Values are mean ± standard error of the mean for bioassay conducted in triplicate.Means followed by the same letter(s) are not significantly different (multivariateanalysis, Fisher's protected LSD at p≤0.05). **Key:** Control Nil.

with diethyl ether. Finally, the oven-dried residue was ignited in a furnace at 550°C. The fibre contents were measured by the weight left after ignition and were expressed in terms of the weight of the sample before ignition.

Fat content determination

The lipid content was determined by extracting the fat from 10 g of the samples using petroleum ether in a Soxhlet apparatus. The weight of the lipid obtained after evaporating off the petroleum ether from the extract gave the weight of the crude fat in the sample.

Carbohydrate content determination

The carbohydrate content of the samples was determined as the difference obtained after subtracting the values of protein, lipid, ash and fibre from the total dry matter (AOAC, 2009).

Data analysis

Data obtained were subjected to a one-way analysis of variance while the means were compared by Duncan's New Multiple Range Test at a 95 % confidence interval using Statistical Package for Social Sciences version. Differences were considered significant at p≤0.05.

RESULTS

Table 1 shows the fungi isolated from the stored green pepper fruits: *Aspergillus spp Cladosprium spp., Fusarium solani, Rhizopus stolonifer, Mucor spp* and *Alternaria* and the frequency of occurrence of the fungal isolate from green pepper fruits. It was observed that *Aspergillus spp* had the highest occurrence of 36%, followed by *Alternaria spp* (28%), *Cladosprium spp* (16%), *Fusarium solani* (8%), *Mucor spp* (8%) and *Rhizopus stolonifer* (4%).

The antifungal effects of methanol extract of *Syzygium aromaticum*, *zingiber officinale* and *Allium sativum* on the fungal isolate from rotten green pepper fruits

It was observed that at 0.2 mg/mL, rot pathogens were inhibited thus: *Aspergillus spp* (21.00 mm), *Cladosprium spp* (19.00 mm), *Fusarium solani* (13.00 mm), *Rhizopus stolonifer* (17.00 mm), *Mucor spp* (17.00 mm), and *Alternaria sp* (15.00 mm) (Table 2).

The antifungal effects of methanolic extract of ginger (*Zingiber officinale*)

It was observed that at 1.0 mg/mL, rot pathogens were inhibited thus: *Cladosprium spp* (15.00 mm), *Rhizopus stolonifera* (17.00 mm), *Fusarium solani* (13.00 mm),

Table 3. Antifungal activity of ginger (*Zingiber officinale*) methanol extract against fungi pathogen of green pepper.

Isolates	Diameter of zones of inhibition (mm)				
	1.0	0.8	0.6	0.4	0.2
	Concentrations (mg/mL)				
Aspergillus spp	18.00[b]	0.00[d]	0.00[d]	0.00[d]	0.00[c]
Cladosprium spp	15.00[d]	12.00[b]	10.00[b]	8.00[b]	7.00[b]
Fusarium solani	13.00[f]	10.00[c]	8.00[c]	7.00[c]	0.00[c]
Rhizopus stolonifer	17.00[c]	15.00[a]	13.00[a]	10.00[a]	8.00[a]
Mucor spp	14.00[e]	12.00[b]	10.00[b]	8.00[b]	0.00[c]
Aiternaria spp	20.00[a]	10.00[c]	10.00[b]	8.00[b]	8.00[a]

Values are mean ± standard error of the mean for bioassay conducted in triplicate.Means followed by the same letter(s) are not significantly different (multivariateanalysis, Fisher's protected LSD at p≤0.05). **Key:** Control Nil.

Table 4. Antifungal activity of garlic methanol extract on fungi pathogen of green pepper.

Isolates	Diameter of zones of inhibition (mm)				
	1.0	**0.8**	**0.6**	**0.4**	**0.2**
	Concentrations (mg/mL)				
Aspergillus spp	20.00d	10.00f	9.00d	0.00b	0.00a
Cladosprium spp	24.00b	12.70b	9.50c	0.00b	0.00a
Fusarium solani	25.70a	15.20a	8.50e	8.00a	0.00a
Rhizopus stolonifer	20.00d	12.00d	8.00f	0.00b	0.00a
Mucor spp	22.00c	11.90e	10.80a	0.00b	0.00a
Alternaria spp	19.00e	13.10d	10.30b	8.00a	6.00a

Values are mean ± standard error of the mean for bioassay conducted in triplicate.Means followed by the same letter(s) are not significantly different (multivariateanalysis, Fisher's protected LSD at p≤0.05). Key: Control Nil.

Aspergillus spp (18.00 mm), *Cladosporium spp* (15.00 mm) (Table 3).

The result of garlic (*Allium sativum*) methanolic extract against fungal isolates of green pepper fruits at 0.1mg/mL

It shows that *Aspergillus spp* (12.00 mm), *Cladosprium spp* (24.00 mm), *Fusarium solani* (25.70 mm) *Rhizopusstolonifer* (20.00 mm), *Mucor spp* (22.00 mm), *Alternaria spp* (19.00 mm), *Fusarium solani* (25.70 mm) and *Cladosprium spp* (24.00 mm) (Table 4).

The antifungal effect of methanolic extract of various spices againstrot fungi of green beans.

The antifungal effect of garlic was most effective against *Cladosporium* sp (24), *Fusarium* sp (25 mm), *Rhizopus* sp (20 mm), *Mucor* (22 mm) while clove was most active against *Aspergilus* sp (21 mm) (Table 5).

Proximate composition of infected green pepper fruits with various fungi

Moisture content of *Cladosprium spp* (19.68) infected green beans was highest, total ash was highest in Mucor sp infected green beans (18.87), crude fibre was highest in green beans infected with *Alternaria spp* (23.98), crude fat was highest in green beans infected with Mucor sp and *Fusarium solani* (9.97), crude protein was highest in green beans infected with *Cladosprium spp* (15.96), carbohydrate was highest in green beans infected with *Aspergillus spp* (32.85) (Table 6).

DISCUSSION

The findings of this study showed that *Aspergillus* spp with the highest percentage occurrence of 36%, *Alternaria* spp. (28%), *Cladosprium* spp, (16%), *Fusarium solani* (8%), *Mucor* spp(8%) and *Rhizopus stolonifer* (4%) were post-harvest rot pathogens of pepper fruits sold in major markets in Ado Ekiti, Nigeria. A number of these microbes were isolated from deteriorated mango and oranges by

Table 5. Antifungal activity of methanol extract of different spices against isolated fungi.

Isolates	Fluconazole(control) mm	Clove (100µl)mm	Ginger (100µl)mm	Garlic (100µl) mm
Aspergillus spp	22	21	18	20
Cladosprium spp	18	19	15	24
Fusarium solani	19	13	13	25
Rhizopus stolonifer	22	17	17	20
Mucor spp	28	17	14	22
Alternaria spp	29	15	20	19

Table 6. Proximate composition of infected green pepper fruits with various fungi.

Parameters (%)	Sample codes						
	Control	A	B	C	D	E	F
Moisture content	18.41^{c}	18.59^{d}	19.68^{c}	19.24^{c}	19.08^{c}	19.26^{c}	19.20^{c}
Total ash	18.41^{c}	18.61^{c}	18.81^{d}	18.85^{d}	18.77^{d}	18.87^{d}	18.71^{d}
Crude fiber	22.90^{b}	23.31^{b}	23.22^{b}	23.86^{b}	23.52^{b}	23.21^{b}	23.98^{b}
Crude fat	9.25^{e}	9.35^{f}	9.92^{f}	9.97^{f}	9.91^{f}	9.97^{f}	9.95^{f}
Crude protein	15.89^{d}	15.63^{e}	15.96^{e}	15.16^{e}	15.67^{e}	15.68^{e}	15.01^{e}
Carbohydrate	33.75^{a}	32.85^{a}	33.57^{a}	33.12^{a}	33.22^{a}	32.78^{a}	33.57^{a}

Values are mean ± standard error of the mean for bio-assay conducted in triplicate. Means followed by the same letter are not significantly different (multivariateanalysis, Fisher's protected LSD at p≤0.05). Harmonic mean sample size 6.00. **Key:** A = *Aspergillus spp*, B = *Cladosprium spp,* C = *Fusarium solani,* D = *Rhizopus stolonifera*, E = *Mucor spp*, F= *Alternaria spp.*

Ijato *et al.* (2021). Similarly, Ugwu *et al*. (2014) isolated *Candida tropicalis, Penicillium notatum, Aspergillus niger, Fusarium oxysporum, Absidia corynbifera* and *Rhizopus stolonifer* from post-harvest pepper fruits, this was similar to the result obtained in his study. Also, the isolated fungi in this study agreed with fungal species associated with post-harvest rot of common fruits pepper in Sokoto metropolis, Nigeria as reported by Salau (2012). The occurrence of *Aspergillus niger*, *Fusarium* species and *Mucor* species as identified in this study agree with the work of Mensah and Owusu (2011) who isolated *Aspergillus niger, Fusarium* species and *Mucor* species from *Capsicum annuum, Abelmoscus esculentus*, and *Lycopersicon esculentum* in Accra metropolis.

However, *Aspergillus niger* was found to be the most abundant fungus of pepper fruit in this study. This is similar to the report of Mensah and Owusu (2011) that listed *Aspergillus niger* as one of the most common fungal species found on fruits in the Accra metropolis. It also agrees with Chiejina (2008) who indicated that *Aspergillus* was isolated from 79.5% of the samples. Several studies have also reported that *Aspergillus* spp. is associated with the spoilage of tomatoes, pepper, apricot, orange, lemon, peach, apple, kiwi, mango, and banana (Rashad *et al*., 2011; Ijato et al., 2022a, b). Onuorah and Orji (2015) reported that *Aspergillus* had the highest decay diameter among other fungi associated with pepper spoilage. The fungal isolates from this study have been reported to form mycotoxins (Onuorah and Orji, 2015). Generally, fungi that cause spoilage are considered toxigenic or pathogenic (Al-Hindi *et al*., 2011). Also, some of the fungi isolated in this study have been reported to produce secondary metabolites in plant tissues which are harmful to humans and animals (Baiyewu *et al.*, 2007).

In this study, garlic was most effective compared to other spices used. Garlic has the highest inhibitory effect on *Cladosprium* spp (24 mm), *Fusarium solani* (25 mm), *Rhizopus stolonifer* (20 mm) and *Mucor* spp (22 mm). Several *in vitro* studies have shown the efficacy of certain medicinal herbs or plant extracts against *Colletotrichum* spp (Saravanakumar *et al*., 2011; Ajith *et al*., 2012). Also, Alves *et al*. (2015) reported the efficacy of 1% aqueous or 20% ethanol plant extracts to control bell pepper (*C. annuum*) anthracnose caused by *C. acutatum* after the fruits were treated with plant extracts before inoculation with *C. acutatum* conidial suspension of *C. acutatum* ($2x10^5$) conidia/ml. Garlic is more effective in this study as compared to commercial antibiotics. Ijato (2021a, b, c & d) reported the use of plant extracts as an alternative remedy for the control of various plant pathogens ranging from fungi to bacteria. Garlic is not only effective against gram-positive and gram-negative bacteria but also possesses antiviral and antifungal activities (Benavides *et al*., 2007). These studies elaborate on the effective antimicrobial quality of different spices, especially garlic. Therefore, garlic can be used as an effective anti-fungal agent against many fungal diseases and is also used as a natural food preservative.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest in this research as all the authors agreed on the procedures and results presented in this research.

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