ISSN:
Print - 2277 - 0593
Online - 2315 - 7461
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Journal of Natural Science, Engineering and Technology

# ANTAGONISTIC ACTIVITY OF SOME BACTERIAL SPECIES AGAINST FUNGI IMPLICATED IN POST-HARVEST DISEASES OF CHILLI PEPPER (*CAPSICUM CHINENSE*) FRUITS

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## ABSTRACT

Various fungal species have been implicated in post-harvest diseases of chilli pepper reducing its qualities worldwide. The use of pesticides for the prevention of plant diseases could lead to increased pathogen-resistance and accumulation of the chemical constituents of the pesticides in the environment. This study was therefore undertaken to investigate the *in-vitro* antagonistic potentials of some bacterial species against phytopathogenic fungi implicated in post-harvest diseases of chilli pepper fruits. Fungi were isolated from diseased chilli pepper fruits and tested for their pathogenicity on healthy chilli pepper fruits. The pathogenic fungi were re-isolated, and identified by colonial and morphological characterization methods. The *in-vitro* antagonistic activity of *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas fluorescens* and *Lactobacillus plantarum* on phytopathogenic fungal isolates was conducted using dual plate culture method. Eight different fungi were isolated from diseased fruits but only four showed visible disease symptoms like fruit rot, black rot, sunken spots and brown discoloration on the healthy pepper fruits with disease severity ranging from 14.0% to 35.0%.These phytopathogenic fungi were identified as strains of *Aspergillus niger, Aspergillus terrus, Geotrichum candidum* and *Fusarium* species. The results of *in-vitro* antagonistic activity showed that *Bacillus subtilis, Lactobacillus plantarum, Bacillus cereus* and *Pseudomonas fluorescens inhibited the growth of the tested phytopathogens with g*rowth inhibition ranging from 20.0% to 57.1%. Bacillus *subtilis* generally showed maximum growth inhibition of all pathogens, closely followed by *Pseudomonas fluorescens*. The study therefore showed that *B. subtilis* and P. fluorescens could be used as biocontrol agents to reduce post-harvest diseases of chilli pepper fruits.

**Keywords**: Biocontrol, chilli pepper, phytopathogenic fungi, post-harvest

## INTRODUCTION

Chilli pepper (*Capsicum chinense),* a member of *Solanaceae* family, is an important vegetable cultivated in most parts of the world for its culinary purposes and seasonings (Idowu – Agida *et al*., 2010). It is an important spice that is used in the preparation of meals and chilli sauce in various parts of the world (Pawar *et al*., 2011). Nutritionally, chilli pepper fruit possesses all the properties for which it is considered a food. It is very rich in vitamin C (about twice that of citrus fruit) of which only about 30 percent is lost after cooking (Martin *et al.,* 2014). Dried chilli fruit is very high in vitamin A (Syeda, 2015). It is also an essential source of β-carotene, thiamine, niacin and riboflavin (Ekwere and Udoh, 2018). *Capsicum chinense* also contains a complex mixture of essential oils, waxes and coloured materials (mainly capsanthin, capsorubin, zeaxanthin, cryptoxanthin and lutein) as well as several capsaicinoids which are responsible for its pungency (Syeda, 2015; Garcia-Gaytan*et al.*, 2017).

Chilli pepper can be used as a condiment in the form of cayenne pepper. It is extensively used as a spice in all types of curried species in various parts of the world. Grinding roasted dry chilli with other condiments such as coriander, cumin, turmeric and farinaceous matter makes curry powder (Ekwere and Udoh, 2018). It is also used for seasoning of egg, fish and meat preparations, sauces, chutneys, pickles, frankfurters and sausages. It is also used as a counter-irritant in the form of ointment, plaster, medicated wool and also for the relief of rheumatism and lumbago (Garcia-Gaytan *et al.*, 2017). Capsaicin creams are made in some countries for the relief of pain in osteoarthritis, post-herpetic neuralgia and painful diabetic neuropathy (Omolo *et al.,* 2014). According to Siwei *et al.* (2015), capsaicin could help to prevent cholesterol and associated heart diseases such as arteriosclerosis. Syeda (2015) also reported that when powdered red chilli is applied to the part affected by a dog bite, it immediately minimizes the effect of the poison. It could also act as an antiseptic by preventing the formation of puss in the wound (Omolo *et al.,* 2014). Chilli pepper is suitable in diets of the obese and useful in the control of cancer of the stomach and colon (Syeda, 2015). It also improves circulation, stimulates the appetite, reduces inflammation and aids digestion by stimulating gastric juices (Siwei *et al.,* 2015).

Despite the health benefits of chilli pepper, several constraints such as pests and diseases have affected its production in most African countries. Among the diseases that reduce the qualities of chilli pepper fruits are post-harvest diseases such as black mould and sour rot caused by different species of fungi which results in economic losses to the farmers (Onuorah and Orji, 2015). Chemical fungicides are being used extensively for postharvest disease control of fruits and vegetables. Postharvest fungicides are applied to control infections already established on the surface tissues of produce to protect against infections which may occur during storage and handling, or to interrupt pathogen development (Abada and Ahmed, 2014;Onuorah and Orji, 2015). The success of these fungicides depends largely on the extent to which infection has developed at the time of fungicide application and how effectively the fungicide penetrates the host tissue (Wenyue *et al.*, 2017). However, the use of chemical fungicides for the control of postharvest diseases of chilli pepper could lead to increased pathogen resistance. It could also lead to the accumulation of the chemical constituents of the fungicides in the fruits, resulting to increased health hazards to the consumers, hence, the search for alternative methods of controlling postharvest diseases of chilli pepper fruits. The use of biological control agents has been suggested as a safe method of controlling various plant diseases. This study was therefore undertaken to investigate the *in-vitro* antagonistic potentials of some bacterial species against phytopathogenic fungi implicated in post-harvest diseases of chilli pepper fruits.

## MATERIALS AND METHODS

***Sample collection*** Infected chilli pepper (*Capsicum chinense*) fruits were obtained from Alabata farm, and Osiele market, Abeokuta, Ogun-State. Healthy chilli fruit samples were also collected for pathogenicity test. All the samples were collected in different sterile Ziploc bags, labeled appropriately and transported to the laboratory for necessary investigation.

The bacterial cultures (*Lactobacillus plantarum*, *Pseudomonas fluorescens*, *Bacillus cereus and Bacillus subtilis*) were obtained from the Department of Microbiology, Federal University of Agriculture, Abeokuta and confirmed by subjecting them to several biochemical tests.

***Isolation of fungi associated with diseased chilli pepper fruits***

The fungi were isolated from the diseased pepper fruits using the method described by Liu *et al.* (2016).Tissues of approximately 5.00 mm in diameter were collected from the edges of lesions, surface-sterilized with 70% ethanol for 30 seconds and 3.5% Sodium hypochlorite for 15 seconds, washed three times with sterile distilled water and then dried on sterile filter paper. The tissues were inoculated on Potato Dextrose Agar plates supplemented with 50.0 mg/1000ml streptomycin. The plates were then incubated at 27°C for 5 days. Pure cultures of the fungal isolates were obtained by a series of sub-culturing on the agar plates.

***Pathogenicity test***

Pathogenicity test was carried out on the fungal isolates as described by Onuorah and Orji (2015). Each fungal isolate was tested on healthy pepper fruits for its ability to show characteristic disease symptoms on pepper fruits. Clean mature healthy fruits were rinsed with distilled water, followed by surface sterilization with 70% ethanol, washed twice with sterile distilled water and then air-dried. A sterile cork borer was used to bore holes into healthy fruits and colonies of five-day-old fungal isolate (from each pure culture) were inoculated on the fruits and the cores of the fruit were replaced. The point of inoculation was sealed with petroleum jelly to prevent contamination. Controls of pepper fruits were wounded with sterilized cork borer but not inoculated. The inoculated fruits and the controls were incubated at 25°C. After 48 hours, the inoculated fruits were closely observed for symptom development. Observation for the level of fungal growth and fruit rot was made daily for four days and the percentage rot severity was determined using the method of Zakari- *et al.* (2015). The growth and pathogenicity were rated as follows: low (rot covered less than 25% of the fruit surface); medium (covered 25-50% of the fruit surface); high (51-75% covered) and very high (covered 75% and above). Percentage rot severity was determined using the formula below:

Percentage rot severity = $\frac{Diameterofrotcovered \times 100}{Diameteroffruitsurface}$

The causal agents were re-isolated from infected pepper fruits, compared with the original isolates and characterized.

***Identification of phytopathogenic fungal isolates***

Mycelial discs (5.00 mm diameter) were collected from actively growing areas near the growing edges of the 5-day-old culture of each phytopathogenic fungal isolate, transferred to potato dextrose agar plates and incubated at 27°C for 10 days. The colony characteristics were recorded while the morphology of spores and mycelia of the fungal isolates were examined under the microscope at × 40 magnifications after staining with lacto phenol cotton blue. The identification was then done by comparing the characteristics of the isolates with mycological identification keys and taxonomic descriptions (Kidd *et al*., 2016).

### *In-vitro activity of some bacterial strains on phytopathogenic fungal isolates*

*In-vitro* study of antifungal activity of *Lactobacillus plantarum*, *Pseudomonas fluorescens*, *Bacillus cereus and Bacillus subtilis* against fungal pathogens isolated from diseased pepper fruits was conducted using the dual plate culture method as described by Oloyede *et al.* (2019). *Bacillus subtilis, B. cereus* and *Pseudomonas fluorescens* were grown on nutrient agar and incubated at 35±2°C for 24 hours while *Lactobacillus plantarum* was grown on de Mann Rogosa (MRS) agar in an anaerobic condition at 35±2°C for 24 hours.

Each fungal isolate was inoculated on PDA plate and incubated at room temperature (25±2°C) for seven days. After incubation, small fragments of the mycelia were cut and placed at the centre of PDA plates. On the opposite side, about 3.0 cm away from the fungus, a loop full of 24-hour old culture of each bacterium was streaked vertically. Three replicates per bacterial strain were prepared. For the control, bacterial strains were replaced with sterile distilled water. The inoculated plates were incubated at room temperature (25±2°C) for three days and the radial growths of fungal mycelia in treated and control plates were measured and recorded. The percentage growth inhibition was calculated using the formula:

Percentage growth inhibition $= 1 - (a/b) \times 100\%$ (Oloyede *et al*., 2022)

a: radial growth of fungus interacting with antagonistic bacteria

b: radial growth of fungus in control plate.

## RESULTS

### *Phytopathogenic fungi associated with diseased chilli pepper fruits*

In this study, eight (8) different fungal strains were isolated from the diseased chilli pepper fruits and tested for their pathogenicity on healthy pepper fruits. Four of the isolates were observed to induce visible disease symptoms on the healthy pepper fruits after 4 days of inoculation, implying that these isolates are pathogenic. These phytopathogenic fungi were re-isolated and identified as *Aspergillus niger, Aspergillus terreus, Geotrichum candidum* and *Fusarium* species based on their macroscopic and microscopic features (Table 1).

### *Pathogenicity of the fungi associated with diseased chilli pepper fruits*

The pathogenicity study showed that *Aspergillus niger, Aspergillus terreus, Geotrichum candidum* and *Fusarium* species had obvious effects on the quality of chilli pepper fruits (Table 2). The visible disease symptoms observed at 4 days after inoculation included dark mould growths on chilli pepper fruits and irregular, light brown to dark brown sunken spots or lesions, with fruits becoming spongy (Table 2; Plate 1). No visible symptoms were observed in un-inoculated (control) pepper fruits. The percentage disease severity of the fungal isolates ranged from 14.0% to 35.0% (Figure 1). *Aspergillus niger* had the highest rot severity on chilli pepper fruits, closely followed by *A. terreus* while *Fusarium* spp. had the lowest severity.

**Table 1: Macroscopic and microscopic characteristics of fungi associated with diseased pepper fruit**

| **Fungal isolates** | **Macroscopic characteristics** | **Microscopic characteristics** |
|---|---|---|
| *Aspergillus niger* | The colonies were black with smooth wide edges, white to pale in colour on reverse | Presence of conidiospore and septate hyphae |
| *Aspergillus terreus* | Rapid growing powdery colonies with a cinnamon brown colour on the surface. Reverse remained yellow to beige brown | Presence of conidiospore and septate hyphae |
| *Geotrichum candidum* | The colony was cottony white turning purple background colour. Reverse was white. | Presence of arthrospore and septate hyphae |
| *Fusarium spp.* | The colony was whitish–cream. Reverse was pale to bluish violet | Presence of conidiospores with septate and branched hyphae |

**Table 2: Visible symptoms on pepper fruits inoculated with phytopathogenic fungal isolates**

| Fungal isolates | Visible Symptoms |
|---|---|
| *Aspergillus niger* | Black rot with black discoloration, sunken spots, |
| *Aspergillus terreus* | Fruit rot with light brown colouration |
| *Geotrichum candidum* | Geotrichum sour rot on the surface of pepper with |
| *Fusarium* spp. | Black colouration causing rots inside out |
| Un-inoculated pepper fruits | No visible symptoms |

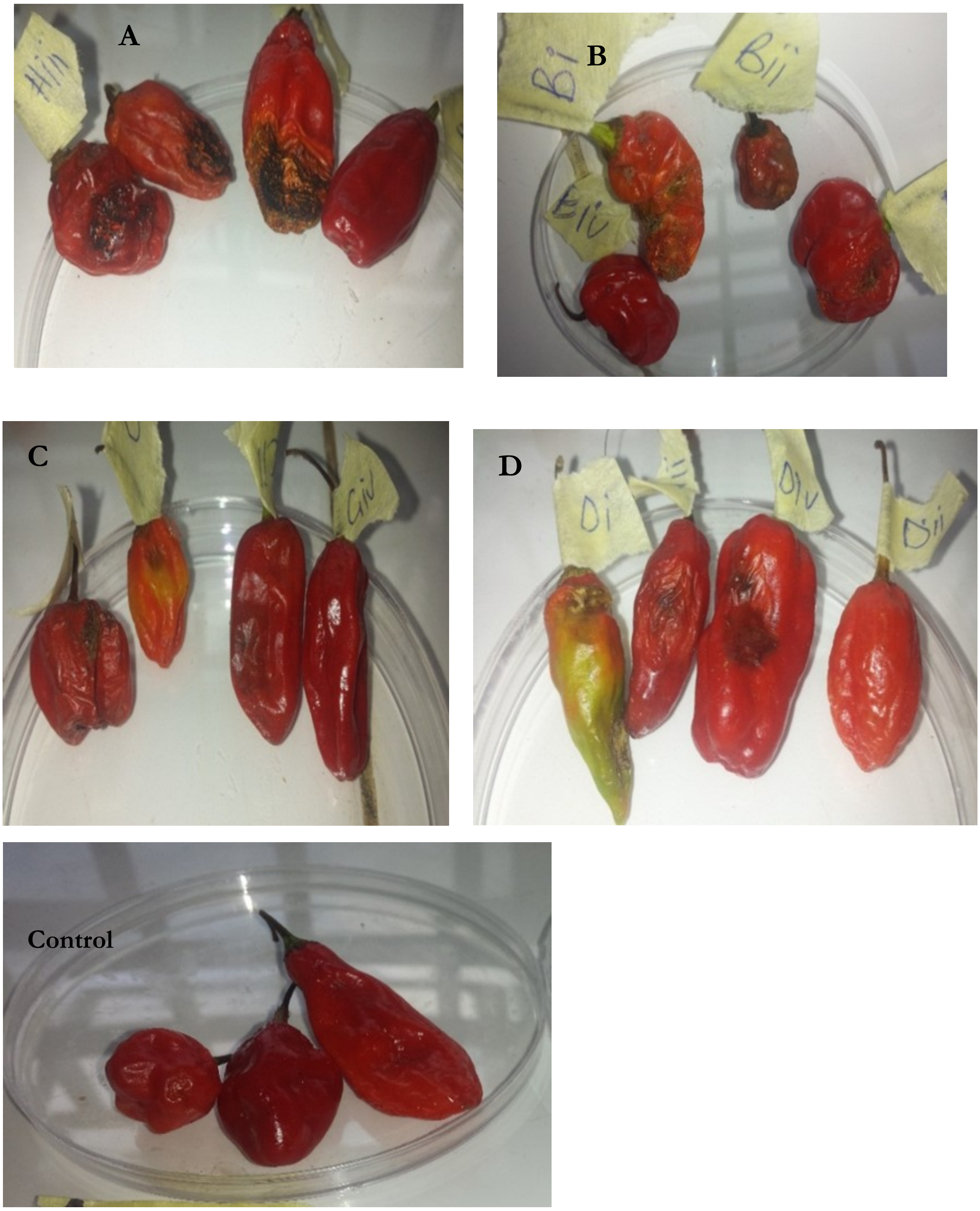


**Plate 1: Visible disease symptoms induced by fungal isolates at 4 days after inoculation. A: *Aspergillus niger*, B: *A. terreus*, C: *Geotrichum candidum*, D: *Fusarium* species, E: Un-inoculated fruits**

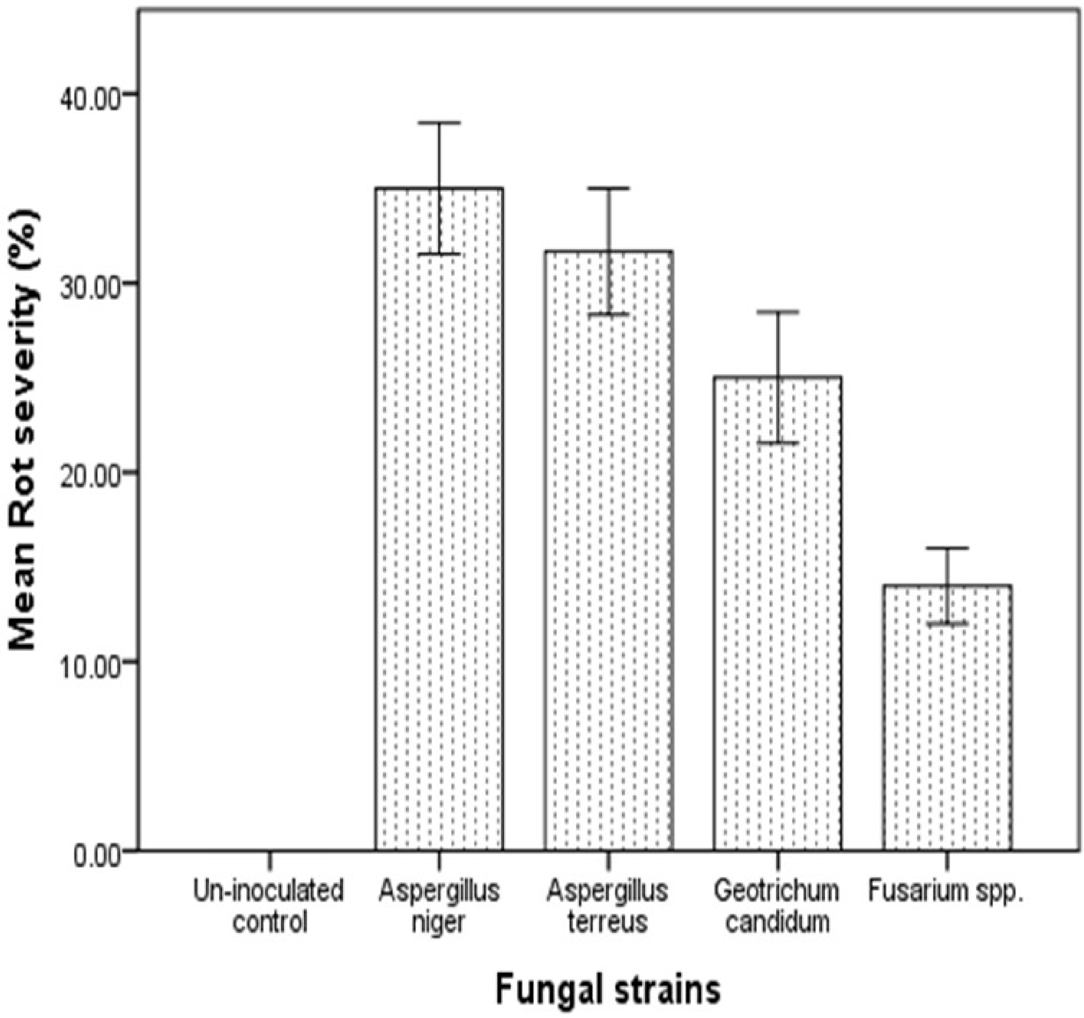


**Figure 1: Percentage disease severity of the fungal isolates on chilli pepper fruits**

***In-vitro antagonistic activity of bacterial strains against pathogenic fungi associated with diseased chilli pepper fruits***

All tested bacterial strains inhibited the growth of phytopathogenic fungi. Growth inhibition of all four fungal pathogens ranged from 20.0% to 57.1 (Figure2). *B*acillus *subtilis* generally showed maximum growth inhibition against all pathogens, closely followed by Pseudomonas fluorescens. Similarly, Aspergillus niger was mostly inhibited among the pathogens (Figure 2). The results therefore showed that Bacillus *subtilis and Pseudomonas fluorescens could be used as biological control agents of phytopathogenic fungi associated with diseased chilli pepper fruits.*

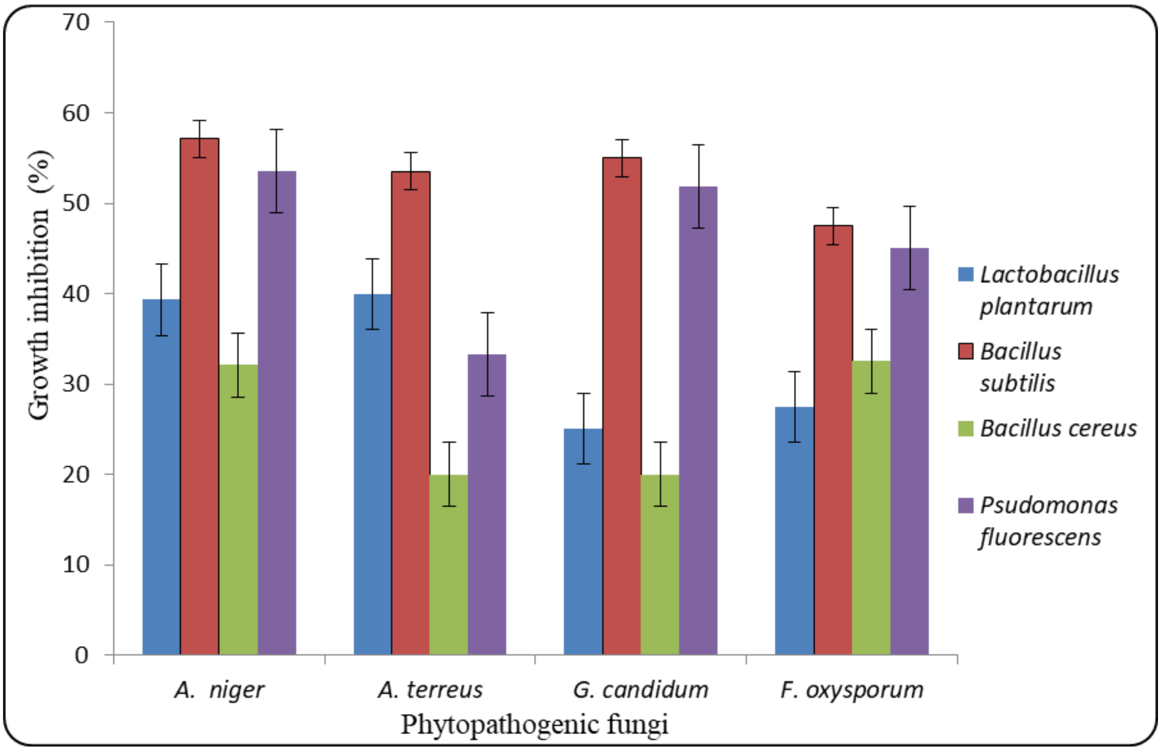


**Figure 2: *In-vitro* antagonistic activity of bacterial strains against phytopathogenic fungi associated with diseased chilli pepper fruits**

## DISCUSSION

The findings of this study showed that *Aspergillus niger*, *Aspergillus terreus*, *Geotrichum candidum and Fusarium species* caused postharvest rot of chilli pepper fruits. These fungi have been previously implicated as fruit rot pathogens by Balogun *et al.* (2015); Onuorah and Orji (2015); Vrushali and Vanmare (2015); Ezikanyi (2016) and Wenyue *et al.* (2018). The fungal strains were able to grow on the fruits and induced some levels of disease symptoms like fruit rots, black rots, sunken spots and brown discoloration with varying degrees of pathogenicity. Among the four isolates, *Aspergillus niger* exhibited the highest level of virulence (i.e. mycelia and/or rots covering most fruit surfaces). Balogun *et al.* (2015) also reported similar result with *Aspergillus niger having* the highest level of virulence on pepper fruits obtained from various markets in Ilorin, North central Nigeria. Though, *Aspergillus terreus* has been used as an antagonist to control various pathogenic fungi, it has also been reported to cause diseases in various fruits resulting in infections and allergies (Louis *et al.*, 2013). In addition, fungal isolates such as *Aspergillus niger* and *Fusarium species* have been reported to produce several secondary metabolites or mycotoxins like ochratoxin, oxalic acid, malformin A and malformin C which are potentially harmful to humans and animals (Onuorah and Orji, 2015; Baiyewu *et al.*, 2017).

The antagonistic activity of some bacterial strains has been widely employed in the management of many plant diseases. The results of the present study revealed that *Bacillus subtilis, Lactobacillus plantarum, Bacillus cereus* and *Pseudomonas fluorescens significantly inhibited the mycelial growth of phytopathogenic fungal isolates (Aspergillus niger*, *Aspergillus terreus*, *Geotrichum candidum and Fusarium species).* The results corroborate the previous studies that demonstrated the *in vitro* growth inhibitory activities of several bacterial strains on fungal pathogens (Aydi-Ben-Abdallah *et al*., 2020; Oloyede *et al*., 2022). Wang *et al.* (2011) also reported that metabolites of *Lactobacillus plantarum* IMAU10014 exhibited high inhibitory activity against some phytopathogenic fungi. The bacterial strains could produce secondary antifungal metabolites and other toxic volatile compounds such as nicin (produced by *Lactobacillus plantarum*), organic acids, hydrogen peroxide and phenolic compounds which diffused into the culture medium and served as bioactive agents that inhibited the growth of fungal pathogens as previously reported by Oloyede *et al.* (2019) and Oloyede *et al.* (2022). *The strains could also* antagonize fungal pathogens by competing aggressively for niches and essential nutrients, or by inducing systemic acquired resistance (Cawoy *et al.,*2011).

## CONCLUSION

This study revealed that *Bacillus subtilis and Pseudomonas fluorescens showed strong antagonistic activity* against *Aspergillus niger*, *Aspergillus terreus*, *Geotrichum candidum and Fusarium species that* cause postharvest rots of chilli pepper fruits, indicating that these strains could be used as biocontrol agents to reduce postharvest diseases of chilli pepper fruits. However, there is a need to conduct further studies on the *in vivo* applications of these bacterial strains as effective biological control agents.

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*(Manuscript received:8th April, 2024; accepted: 12th February, 2026).*