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Biological control of the shoot disease of *Amaranthus hybridus* caused by *Choanephora cucurbitarum* by the phylloplane microorganisms

Ngwoke C. A.[1]*, Igiebor F. A.[1]**

[1]*Department of Microbiology, Faculty of Science, Benson Idahosa University, Benin City, Nigeria*
[2]*Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria*

**Corresponding author, Email address:*cngwoke@biu.edu.ng
***Corresponding author, Email address:* francis.igiebor@lifesci.uniben.edu

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Abstract: The pathogen, *Choanephora cucurbitarum* is the causal agent of *Amaranthus hybridus* shoot disease, which is associated with the young part of the shoot. Control measures that have been adopted include inspection and quarantine procedures, cultural methods and fungicide applications. These have been effective but have a lot of side effects and disadvantages. Due to the damage caused by this pathogen, there is obvious need for biocontrol measures with no side effects, to eradicate this pathogen. The growth rate of *Amaranthus hybridus* plants was determined under field conditions. Microflora associated with *A. hybridus* shoot was screened under field conditions for potential use as biocontrol agents of *Amaranthus* shoot disease. The test for antagonism between the selected prospective biocontrol agents and *C.cucurbitarum* was carried out *invivo* on PDA plate. Field trials were also carried out.*A. hybridus* plants grew to a mean height of 54.8cm at the sixth week, from the original mean height of 8.6cm in the fourth week, within three weeks, showing a cumulative increase in height of 42.2cm and a growth rate of 2.2+ 1.1 cm per day. Microorganisms associated with *A. hybridus*shoot under field conditions were *Bacillus*sp, *Erwinia* sp., *Serratia* sp., and *Aspergillus flavus*. Zone of inhibition developed between the pathogen, *C. cucurbitarum* and the isolates *Bacillus* sp., *Erwinia* sp., and *Aspergillus flavus* on agar plates. The highest zone of inhibition (12.4mm) was obtained when *Bacillus*sp was paired with the pathogen *C. cucurbitarum* while the lowest zone of inhibition (8.0mm) was obtained when *Erwinia* sp was paired with the pathogen on potato dextrose agar plates. No zone of inhibition developed between the pathogen and *Serratia* sp, on agar plates. Field trials with *Bacillus*sp as a candidate bioagent supported the result obtained *invivo*, showing its importance in the resistance of the shoot disease of *A. hybridus* caused by *C. cucurbitarum*a as well as confirming the fact that biological control operates in nature.

1. Introduction

Amaranthus hybridus, a member of the family Amaranthaceae, belongs to the order Caryophyllales and is closely related to the family Chenopodiaceae (Chakravarthi *et al*., 2019). It is widely grown in the tropical regions of West Africa, including Nigeria, where it serves as an essential green vegetable in local diets. Beyond West Africa, species of *Amaranthus* such as *A. quitensis*, *A. powellii*, *A. edulis*, *A. caudatus*, *A. cruentus*, and *A. hypochondriacus* are cultivated in America and Asia, primarily for their nutrient-rich seeds, often used as a substitute for wheat (Singh *et al*., 2021).

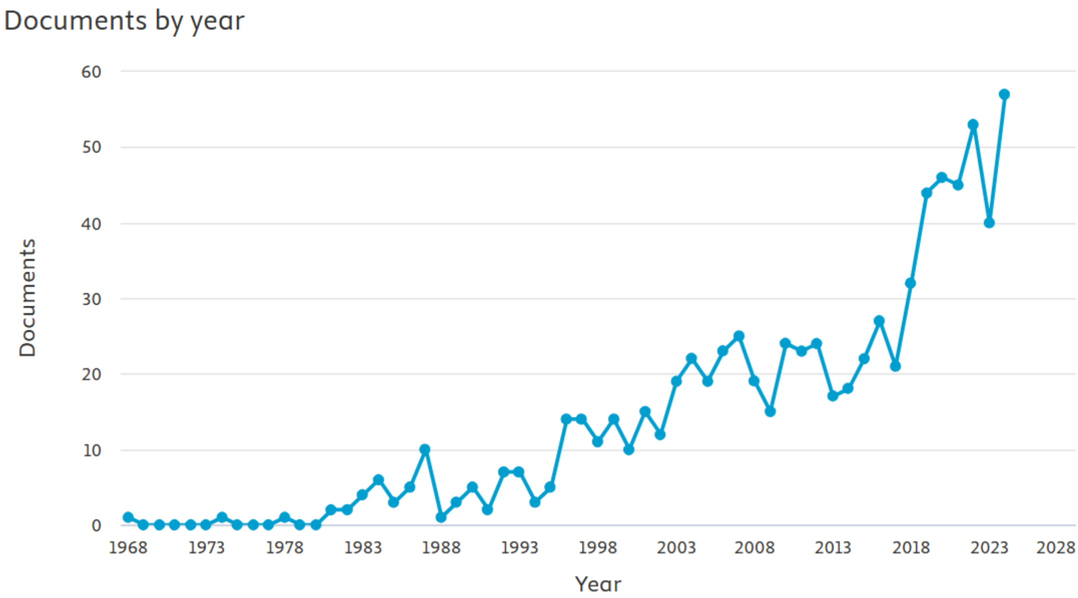
In Nigeria and other parts of West Africa, both the leaves and young succulent stems of *Amaranthus hybridus* are consumed as vegetables. They are valued for their rich protein and vitamin content, making them irreplaceable in the diet of many Nigerians (Adeoluwa *et al*., 2020). Other vegetables commonly cultivated in Nigeria include *Solanum gilo*, *Solanum aethiopicum*, *Capsicum annuum*, *Celosia argentea*, and *Hibiscus sabdariffa*. Among these, *Amaranthus* species, popularly referred to as "African spinach," are the most widely grown and consumed. The leaves are particularly rich in vitamin A, calcium, potassium, and proteins, while the seeds contain lysine and a protein content of approximately 15%, with 6.2% of the total protein comprising methionine, an essential amino acid (Achigan-Dako *et al*., 2014). Many tropical vegetables, including *Amaranthus*, grow wild and can be cultivated year-round. Planting typically occurs during the rainy season to maximize growth, and harvest begins 4–6 weeks post-transplantation. Rather than harvesting the entire plant, growers often cut the young shoots with clean, sharp tools, allowing new branches to develop from axillary buds for subsequent harvesting (Usman *et al*., 2023).

Photo 1. *Amaranthus hybridus*

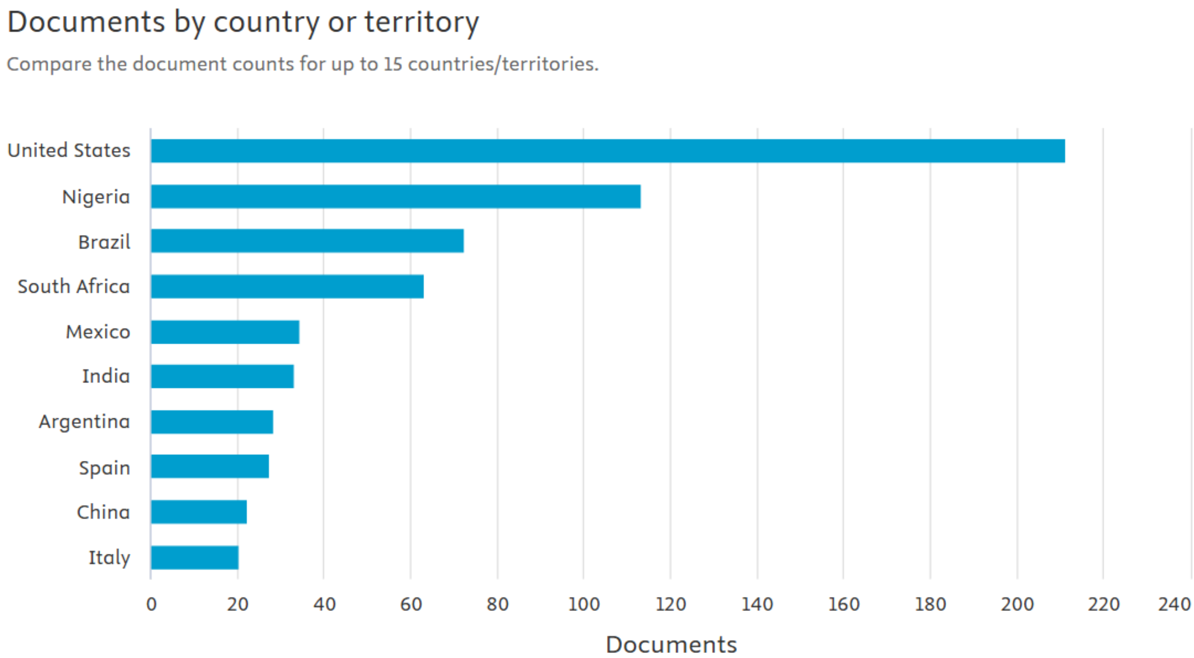
The versatility of *Amaranthus* allows its use in salads, sauces, porridges, stews, and soups. Medical professionals often recommend it for convalescent patients due to its rich nutritive profile. However, the crop is susceptible to various diseases; most notably one caused by the fungal pathogen *Choanephora cucurbitarum*. This pathogen belongs to the division Zygomycota, class Zygomycetes, order Mucorales, and family Choanephoraceae (Liu *et al*., 2019). *C. cucurbitarum* also affects other crops such as rice (Isibhakhomen *et al*., 2021), cotton (Asad *et al*., 2020), and millet (Olayemi *et al*., 2022). The earliest association of this fungus with *Amaranthus* diseases was reported by Palm and Jochems (1924). *Choanephora cucurbitarum* thrives in warmer soils and produces conidia at an optimum temperature of 25°C, while larger sporangia develop at approximately 28°C (Webster and Weber, 2021). The sporangia contain one or a few spores, serving as the primary means of fungal spread through wind, water, and insect vectors. Given the significant damage caused by this pathogen to *Amaranthus* and other vegetables, there is an urgent need for effective control measures.

Various control strategies have been employed, including inspection, quarantine, cultural practices, and fungicide application. However, these approaches have limitations. Fungicides are often costly, may contribute to pathogen resistance, and are subject to strict regulatory controls in many countries (Pimentel and Burgess, 2021). Cultural methods, though effective, are labor-intensive and may damage crops, making them less attractive to commercial farmers (Sharma *et al*., 2018). Biological control offers a more sustainable alternative to conventional methods. This approach reduces the inoculum density or disease-causing activity of pathogens by using other organisms, either naturally or by manipulating the environment, host, or antagonists (Garrett, 1970; Baker and Cook, 2021).

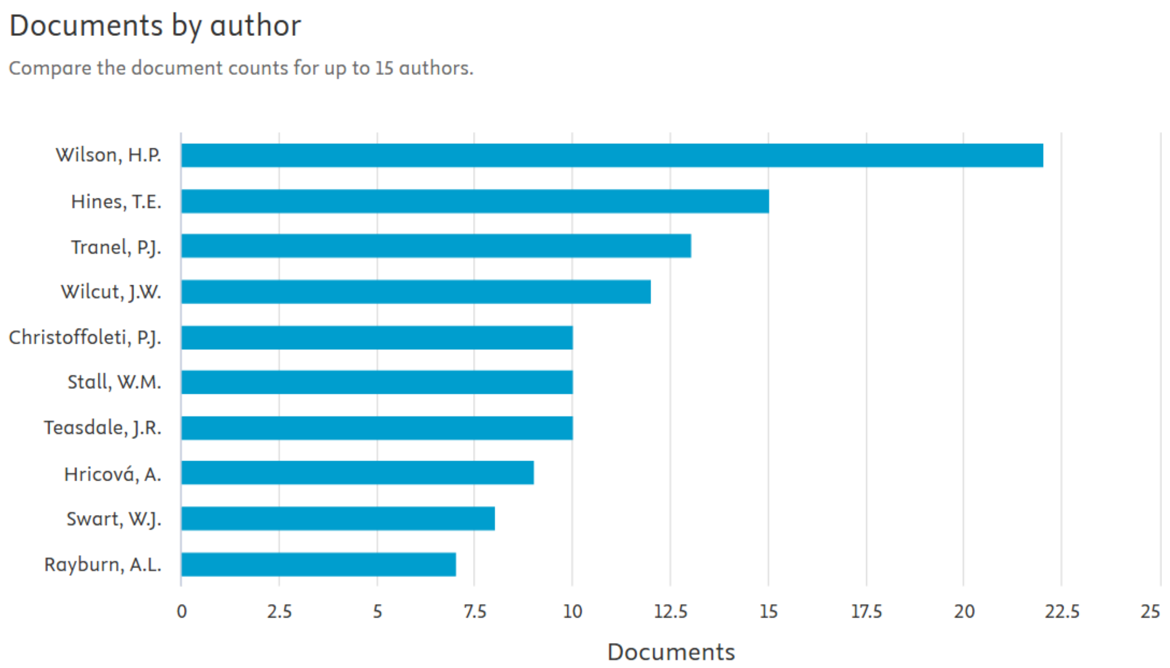
There are more than 800 documents on "*Amaranthus hybridus*" according to Scopus, analysis shows that this kind of natural plant draws the attention of researchers, leading to an increase in research in these later years, reaching an average of 50 papers per year (Scheme 1). Scheme 2 indicates that Nigeria takes the second place after the united State (US), Brazil, South Africa and Mexico occupying the 3th, 4thand 5th ranks. Wilson H.P., from US, is the most published author (22 documents), and the most cited paper belongs to Hirschberg and McIntosh, (1983).



Scheme 1. The increase of publication on *Amaranthus* with time



Scheme 2. The increase of publication on *Amaranthus* with time



Scheme 3. The increase of publication on *Amaranthus* with time

Given the widespread cultivation of *Amaranthus* by peasant farmers in Nigeria, the objective of this study is to explore the use of selected microorganisms as biocontrol agents against shoot diseases of *Amaranthus* under field conditions, presenting a cost-effective and durable solution for crop protection (Azandémè-Hounmalon *et al.*, 2023).

2.0 Methodology

2.1 Plant material and cropping Conditions

The experiments were carried out at the Faculty of Agriculture farm, University of Benin, Benin City, Edo state, located on Latitude 6°20′1.32N and Longitude 5°36′0.53E and 239.16 meters (784.65 feet) above sea level. Benin City has a tropical or savannah climate with a yearly temperature of 28.78°C (83.8°F), which is -0.68% lower than Nigeria's average. Relative humidity is 81.51%.

Seeds of *Amaranthus hybridus* employed in this work were bought from New Benin Market, Benin City. The seeds were seeded in a bed made of wooden crate, containing two parts of soil to one part chicken manure. The seedlings were transplanted when they were 4weeks old into 600cm x 180cm beds and planting spacing was maintained at 18cm x 18cm. Twenty *A. hybridus* plants were randomly selected, marked and their growth rates determined every day with a ruler at the subsequent second, third, fourth, fifth and sixth weeks, following the seedling transplant.

2.2 Antibiotic Mixture

Streptomycin powder and commercially available penicillin V tablets were used to prepare this mixture. 5g of streptomycin was dissolved in 100ml of sterile water and 5 tablets of penicillin V was dissolved in 10ml of sterile water (Chudobova *et al.*, 2024; Haddou *et al.*, 2024; Taibi *et al.*, 2024). The antibiotic mixture was made by adding 20ml of pipetted streptomycin solution to 10ml of the penicillin solution. The resulting antibiotic solution was incorporated into agar plates at the rate of 0-6ml of the mixture per plate.

2.3 Screening of microflora of A hybridus shoot under field conditions for potential use as a biocontrol agent

Two *A hybridus* plants were randomly selected. One centimeter of the apices was cut with a sterile razor blade, defoliated and their diameter determined. The two apices were shaken together in 20ml of sterile water and serial dilutions were prepared and plated out by pour plate technique using 20ml of molten potato dextrose agar (Terrones-Fernandez *et al*., 2023). After incubation at room temperature for 24hrs, the predominant bacterial colonies were isolated. For fungal isolates, fungi colonies were determined after 72 hours of incubation.

2.4 Isolation of Choanephora cucurbitarum on A. hybridus plants

The pathogen, *C. cucurbitarum* was isolated from a diseased *A hybridus* plant, showing the characteristic blight of the young shoot. The affected plant was incubated overnight in a humidified polyethylene bag to allow the production of fresh spores by the pathogen. The spores were then picked with a sterile inoculating needle and inoculated into potato dextrose agar plates containing the antibiotics mixture. The plates were incubated at room temperature for 48 hours on a table near a fluorescent light to allow for diffuse light condition necessary for maximum sporulation (Webster, 1970). The outgrowing fungus was then isolated and identified.

2.5 Testing for antagonism between the selected prospective biocontrol agents and C. cucurbitarum.

Spores of *C. cucurbitarum* from a 2-day old culture were inoculated 4cm away from the edge of the petri dish on potato dextrose agar. A prospective bacteria agent was streaked perpendicularly 2cm from the pathogen on the opposite end of the agar plate. The plates were incubated at room temperature for two days at the end of which any zone of inhibition formed was measured (Ikediugwu *et al*., 1994; Griffith *et al*., 2007).

2.5.1 Preparation of spore suspension of C. cucurbitarium

A 2-day old culture of *C. cucurbitarium was* used in preparing the spore suspension. Spores were harvested into 20ml of sterile potato dextrose broth in a sterile MacCartney bottle. This was then filtered through a sterilized muslin cloth to remove the mycelium and the resulting suspension was adjusted to 15,000 spores/ml.

2.6 Effect of Bacillus sp on the development of C. cucurbitarium shoots disease in the field

Twenty (20) *A. hybridus* plants were selected randomly and sprayed with cell suspensions of Bacillus sp, using a Harry brand sprayer. The cell suspension was prepared by inoculating three loopfuls of bioagent from a 2-day old culture in 1 litre of potato dextrose broth, which was left overnight before use. At the end of two weeks, ten plants were sprayed with the spore suspension of *C. cucurbitarum.* The spraying of plants was done in the evening to minimize the drying effect of the sun and to enable the bioagent establish during the cool night. Ten *A. hybridus* plants not previously protected with the bioagent were sprayed with the spore suspension of the pathogen *C. cucurbitarium* at each week. This served as the control.

2.7 Morphological and Biochemical tests for bacteria isolates.

Appropriate morphological and biochemical tests were carried out to identify the bacteria isolates. These were gram stain, motility test, Citrate utilization test, oxidase and catalase tests, indole tests, sugar fermentation test, methyl red, vogas Proskauer (VP) tests, glucose, lactose and spore stain (Igiebor *et al*., 2024; Osarumwense *et al*., 2019; Osarumwense and Igiebor, 2018).

3. Results and Discussion

A paragraph explaining the results obtained and citing (Figure 1; Figure 2) and Table citing (Table 1; Table 2).

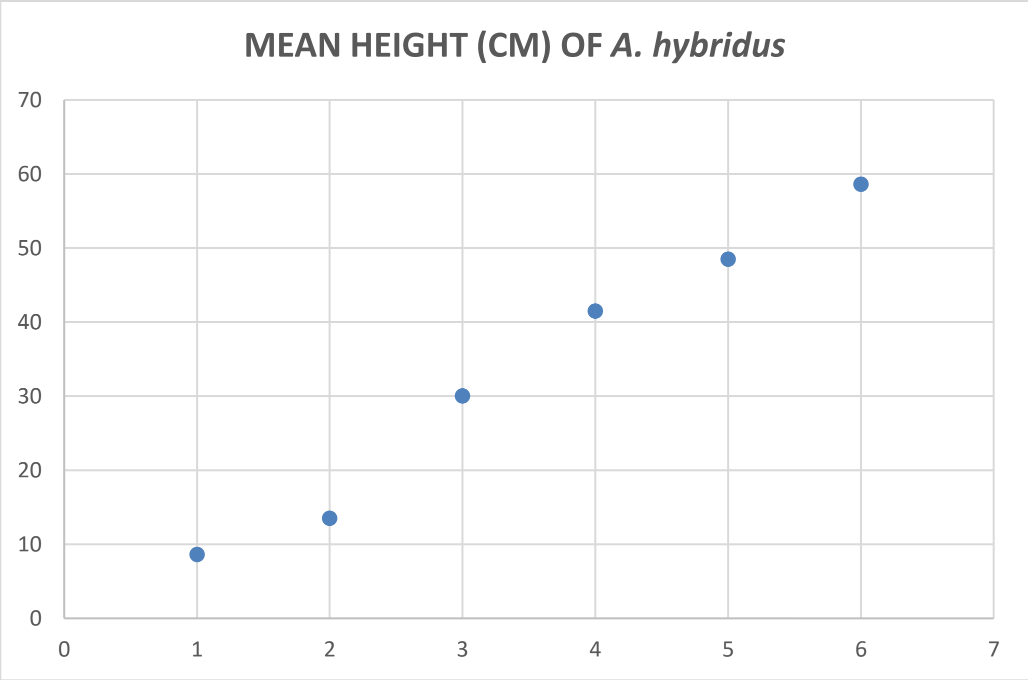


Figure 1. Growth rate (cm) of *A. hybridus* under field conditions

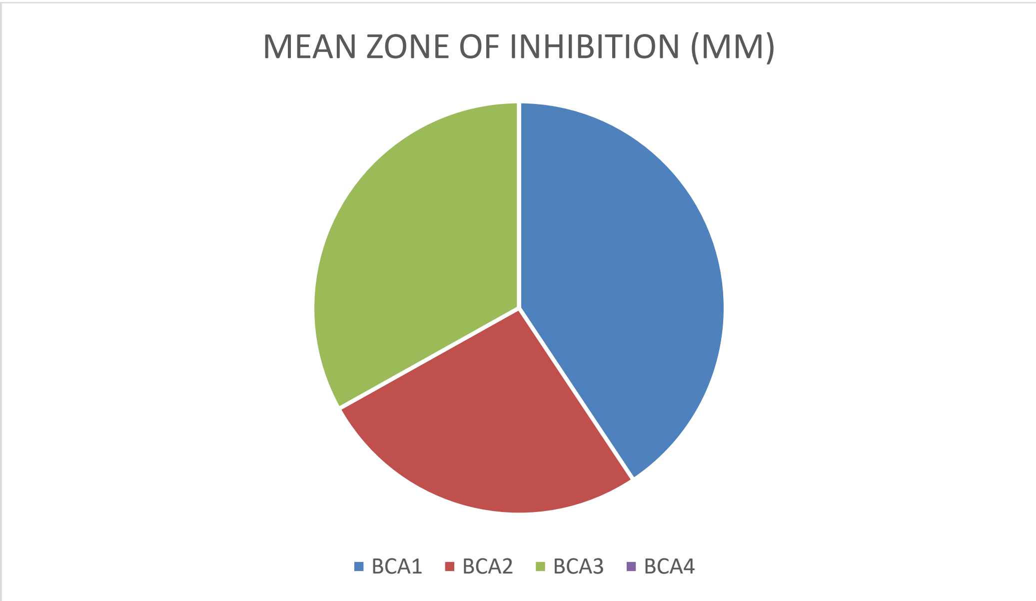


Figure 2. Zone of inhibition (MM) between the biocontrol agents and the pathogen *C. cucurbitarium* on PDA plates

Field grown *A. hybridus* plants attained a mean height of 54.8cm from an original mean height of 8.6cm within three weeks. This gave a cumulative growth of 45.2 cm and a growth rate of 2.2±1.1cm per day. This is in accordance with the result obtained by Emoghene (1989) that greenhouse grown *A. hybridus* plants attained a mean height of 49.4cm from the original mean height of 7.5cm in 25days, giving a fast growth rate of 1.7cm per day. The shoot disease of *Amaranthus hybridus* is caused by *Choanephora cucurbitarium* which is associated with the young apical region of the shoot. *C. cucurbitarium* is aggressively pathogenic on the young apical zone of *A. hybridus* plants (Ikediugwu, 1981).

Table 1. Development of *C. cucurbitarium* shoot disease in the field following inoculation with *Bacillus* sp.

Time(days)	*Treatments*	
	Bacillus sp + C. cucurbitarium	*C. cucurbitarium* alone
1	-	-
2	-	+
3	-	+

Key: - = No disease development; + = disease development

Table 2a. Morphological identification of biocontrol agents

Sample code	Ends	Form	Motility	Edge	Elevation	Colour	Surface	Growth on nutrient agar
BCA1	round	rod	+	entire	Convex	cream	Smooth	Good
BCA2	round	rod	+	entire	Convex	White	Smooth	Good
BCA3	round	rod	+	entire	Convex	pink	Smooth	Good

Table 2b. Biochemical identification of biocontrol agents

Sample	Gram stain	Citrate	Catalase	Oxidase	Indole	VP	MR	lactose	glucose	Identified organism
BCA1	+	+	+	+	-	+	-	-	+	*Bacillus*sp.
BCA2	-	+	+	-	+	+	-	-	+	*Erwinia*sp
BCA3	-	+	+	-	-	+	-	-	+	*Serratia*sp

Key: - = Negative result; + = Positive result

Table 3. Cultural morphology and microscopic characteristics of fungi isolate

Sample code	Colony morphology	Microscopic characteristics	Presumptive isolate
BCA4	Olive green in colour butgolden on the reverse sideof the plate	Thick walled conidiosphorewith hyaline and long aseptate shape	*Aspergillus flavus*

Plate 1. (a) *Amarantus hybridus* plants grown under filed conditions. (b) *Amarantus hybridus* plants grown under field conditions, showing the characteristic blight of the young shoot caused by the pathogen *Choanephora cucurbitarium*.

The isolated phylloplane microorganisms associated with the shoot apex of *A. hybridus* plants grown under field conditions was identified as *Bacillus* sp, *Ervinia* sp, *Serratia* sp and *Aspergillus flavus*. The results suggest that these are the microorganisms on the shoot of *A. hybridus* plants. *Erwinia* sp was reported by Schwartz *et al*. (1991), as a phylloplane microorganism of pear and apple trees while *Serratia* sp was reported to be a normal flora of some plants and vegetables (Pelczar *et al*., 1986). *Bacillus* sp was also isolated from the leaves of Geranuim cultivars by Rytter *et al*. (1989). Awomolo (1985) reported that *A. flavus* and *A. niger* was the causal agents associated with the softening of *Amaranthus viridis* leaves.

Considerable zone of inhibition developed between the pathogen *C. cucurbitarum* and colonies of *Bacillus* sp, *Erwinia* sp and *A. flavus* on potato dextrose agar plates. However, there was no zone of inhibition between *C. cucurbitarum* and the colonies of *Serratia* sp on potato dextrose agar (PDA) plates. The result suggests that an inhibitory substance was produced by *Bacillus* sp, *Erwinia*sp and *A. flavus* which resulted in the antagonism of the pathogen *C. cucurbitarum* on agar plates. The result is in accordance with the findings of Kemf and Wolf (1989), in the use of *Erwinia herbicola*as a biocontrol agent of *Fusarium culmorum* and *Puccinia recondite* on wheat and Rytter*et al*. (1989) in the biological control of Geraniuim rust by *Bacillus subtilis*. The result of the field experiment showed that *Bacillus* sp, *Erwinia* sp and *A. flavus* were the predominant microflora associated with *A. hybridus* plants. *Bacillus* sp, *Erwinia* sp and *A. flavus* antagonized *C. cucurbitarum* on agar plates and these bacteria species also showed promise in the biocontrol of *A. hybridus* shoot disease caused by *C. cucurbitarum*. This indicates their importance in resistance to the shoot disease of *A. hybridus* caused by *C. cucurbitarum*as well as confirming the fact that biological control operates in nature.

Conclusion

The long lasting and highly effective activity of a simple application of these biocontrol agents to control the plant disease of *A. hybridus* is highly relevant to developing countries where peasant farmers lack the finance to support spraying schedules with sophisticated equipment. This technique clearly has the potential to be applied by small scale growers of *A. hybridus* in West Africa and elsewhere.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

References

Achigan-Dako E. G., Sogbohossou E. O., Maundu, P. (2014). Current knowledge on *Amaranthus* spp.: Research avenues for improved nutritional value and yield in leafy amaranths in sub-Saharan Africa. *African Journal of Plant Science*, 8(10), 451–463. https://doi.org/10.5897/AJPS2014.1212

Adeoluwa O. O., Ogbeide, O., Adebooye, O. (2020). Nutritional and health-promoting properties of African indigenous leafy vegetables. *Food Research International*, 100(1), 190–207. https://doi.org/10.1016/j.foodres.2019.01.023

Asad S., Gohar, M., Zaman, M. (2020). Mycological profile and plant pathogenicity of *Choanephora cucurbitarum*. *International Journal of Mycology*, 12(2), 101–110.

Awomolo Y.A. (1985) Microbial Spoilage of leaf vegetables from Uselu Market in Benin City Nigeria. B.ScThesis, University of Benin. 34pp.

Azandémè-Hounmalon G. Y., Jhonn Logbo, Anicet Gbèblonoudo Dassou, Landry Lokossi, Evrard Akpla, Komi K. Mokpokpo Fiaboe, Manuele Tamò M. (2023), Investigation of amaranth production constraints and pest infestation reduction by basil intercropping, Journal of Agriculture and Food Research, Volume 12, 100627, ISSN 2666-1543, https://doi.org/10.1016/j.jafr.2023.100627

Baker K.F., Cook, R.J. (1983). The Nature, Practice of Biological Control of Plant Pathogens. St Paul, MN:APS Press. 539pp

Chakravarthi M., Singh, A., Rao, K. J. (2019). Phylogenetic insights into the Caryophyllales: Morphological and molecular perspectives. *Botany Letters*, 166(3), 283–294. https://doi.org/10.1080/23818107.2019.1651202

Chudobova D, Dostalova S, Blazkova I, Michalek P, Ruttkay-Nedecky B, Sklenar M, Nejdl L, Kudr J, Gumulec J, Tmejova K, et al. (2014). Effect of Ampicillin, Streptomycin, Penicillin and Tetracycline on Metal Resistant and Non-Resistant Staphylococcus aureus. *International Journal of Environmental Research and Public Health.* 11(3), 3233-3255. https://doi.org/10.3390/ijerph11030323

Emoghene A.O. (1989). Biological Control of Shoot Disease of *Amaranthus viridis* caused by *Choanephora cucurbitarium* with *Bacillus subtilis*. M.ScThesis University of Benin, Benin City. 115pp

Griffith G. W., Easton, G. L., Detheridge, A., Roderick, K., Edwards, A., Worgan, H. J., Nicholson, J., Perkins, W. T. (2007). Copper deficiency in potato dextrose agar causes reduced pigmentation in cultures of various fungi. *FEMS Microbiol Lett*., 276(2), 165-171.

Haddou S., Elrherabi A., Loukili E.H., Abdnim R., al. (2024). Chemical Analysis of the Antihyperglycemic, and Pancreatic α-Amylase, Lipase, and Intestinal α-Glucosidase Inhibitory Activities of Cannabis sativa L. Seed Extracts. *Molecules.* 29(1), 93. https://doi.org/10.3390/molecules29010093

Hirschberg J., McIntosh, L. (1983) Molecular basis of herbicide resistance in Amaranthus hybridus, *Science*, 222(4630), 1346-1349

Igiebor F. A., Michael, F. C., Haruna, O., Ikhajiagbe, B. (2024). Impact of plant-based nanoparticles synthesized from *Carica papaya* and *Bryophyllum pinnatum* against selected microorganisms. *Studia Universitatis Babeş-Bolyai Biologia*, 69(1), 87-106.

Ikediugwu F.E.O. (1981). A shoot disease of *Amaranthus* spp in Nigeria associated with *Choanephora cucurbitarum*.*J. Hort. Sci.* 56(4), 289-293

Ikediugwu F.E.O., Emoghene A.O., Ajiodo P.O. (1994). Biological Control of the shoot disease of *Amaranthus hybridus* caused by *Choanephora cucurbitarum* with *Bacillus subtilis*. *J. Hort. Sci*., 69, 1-6

Isibhakhomen S. E., Wirek-manu, F.D., Page, D., Renard, C. (2021). Traditional leafy vegetables as underutilized sources of Micronutrients in a rural farming community in South West Nigeria: Estimation of vitamin C, Carotenoids and Mineral contents. *J. Clinical Nut*., 32(2), 40-45

Kemp H.J., Wolf, G. (1989). *Erwinia herbicola* as a biocontrol agent of *Fusarium culmorum* and *Puccina recondite* on wheat. *Psycopathology*, 78, 194-198

Liu Y., Wang H., Sun J. (2019). Morphological and physiological adaptations of *Choanephora cucurbitarum* in vegetable crops. *Fungal Biology*, 123(8), 633–641. https://doi.org/10.1016/j.funbio.2019.03.005

Osarumwense J.O., Igiebor F.A. (2018). Assessment of Indigenous Bacteria from Biodiesel Effluents Contaminated Site. *Journal of Applied Sciences and Environmental Management*, 22 (2), 157-160.

Osarumwense J.O., Igiebor, F.A., Idahosa, D.E. (2019). Isolation, Characterization and Identification of Bacterial isolates from Auto-mechanic Workshop contaminated with Hydrocarbon. *The Pacific Journal of Science and Technology*, 20 (1), 349-355.

Palm B.T., Jochems, S.C. (1924). A disease of *Amaranthus* caused by *Choanephora cucurbitarum*. *Phytopathology*, 14, 490-494

Pelczar M.J., Chan, C.S., Krieg, M. R.(1986). Microbiology (5th ed) McGraw Hill Inc, London, 908pp

Pimentel D., Burgess, M. (2021). Impact of pesticides on health and the environment. *Agriculture and Human Values*, 38(3), 537–549. https://doi.org/10.1007/s10460-021-10232-6

Rytter J.L. Lukezic, F.L., Craig, R. and Moorman, G.W. (1989). Biological Control of Geranium rust by *Bacillus subtilis*. *Phytopathology*, 79, 367-370

Schwartz T.F., Bernard T., Geider. K. (1991), Diversity of the fire blight pathogen in production of dihydrophenyl alanine, a virulence factor of some *Erwinia amylovora* strains

Taibi M, Elbouzidi A, Haddou M, *et al.* (2024), Chemical Profiling, Antibacterial Efficacy, and Synergistic Actions of Ptychotis verticillata Duby Essential Oil in Combination with Conventional Antibiotics. *Natural Product Communications.* 19(1). https://doi.org/10.1177/1934578X231222785

Terrones-Fernandez I., Casino P., López A., Peiró S., Ríos S., A. Nardi-Ricart, E. García-Montoya, D. Asensio, A. M. Marqués, R. Castilla, P. J. Gamez-Montero, Piqué N. (2023) Improvement of the Pour Plate Method by Separate Sterilization of Agar and Other Medium Components and Reduction of the Agar Concentration, *Microbiology Spectrum*, 11(1), 1-12,

Usman A., Ahmed, M., Musa, I. (2023). Enhancing sustainable cultivation of leafy vegetables in West Africa: Challenges and solutions. *Agronomy*, 13(4), 678–691. https://doi.org/10.3390/agronomy13040678

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