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In-Vitro Inhibitory Effect of *Bacillus Amyloliquefaciens* on Isolated Foliar Diseases of Sorghum

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Abstract *Bacillus amyloliquefaciens* is a bio control agent that is reported to promote plant performance alongside having antifungal properties. The bacterium is found often naturally around the rhizosphere of and normally colonizes sorghum roots. To counter the adverse effects of sorghum foliar diseases, the inhibitory potential of *Bacillus amyloliquefaciens* bacterium was tested against isolated foliar disease causing pathogens. This experiment was done under *in vitro* conditions in a completely randomized design where four isolates; *Colletotrichum sublineola*, *Sporisorium sorghi*, *Exserohilum turcicum* and *Gloeocerspora sorgi* where treated with *Bacillus amyloliquefaciens* in three replications. Mycelial growth inhibition was assessed by taking measurements on isolate growth towards the control agent as a subject to the distance travelled away from the control agent and expressed as a percentage. To further this assessment, a greenhouse experiment was set where, different rates of *Bacillus amyloliquefaciens* bacterium was assessed against covered kernel disease. This was done in a completely randomized design with three replications. From the study, the bacterium significantly reduced the mycelial growth of the test fungi where *Colletotrichum sublineola* was the most inhibited by 58 % while *Exserohilum turcicum* was least inhibited by the bacterium (35%). On the other hand, increasing bacterium treatment rates consequently reduced covered kernel disease severity compared to control where no bacterium was applied. *Bacillus amyloliquefaciens* should be included in an integrated disease management system as seed dressers to reduce prevalence of sorghum foliar diseases in Western Kenya.

Keywords: *Bacillus amyloliquefaciens, foliar diseases, sorghum, management*

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1. Introduction

Sorghum is a high-yield potential crop that is broadly adapted to many environments that limit the performance of other cereals. This is due to the fact that it is characterized by the C4 carbon cycle making it a photosynthesis-efficient crop with high water use efficiency and hence can withstand drought conditions. Furthermore, sorghum is known to tolerate salinity and grows well in both sandy and clay soils with pH ranges 5.0 - 8.5 and also can do well in soils with less permeability. The root system of this crop is well developed and can penetrate to a depth of 1.5-2.5 meters into the soil and therefore can get water from a lower soil profile. Sorghum crop stems can regrow from rhizomes after harvesting, making it possible to re-harvest the crop in subsequent seasons [1,2].

A large proportion of sorghum production worldwide is concentrated in the United States, followed by India, China and Africa. The world's total sorghum production is estimated to be 61.69 million metric tons in about 98 million hectares and the annual economic losses due to production constraints are estimated to be around $ 130 million. In Africa, the leading producer of sorghum is Nigeria followed by Sudan, Ethiopia, Uganda, Rwanda, Tanzania and Kenya [3]. The major sorghum-producing areas in Kenya are western, eastern and coastal parts of the country and this is majorly done by smallholder farmers, this means that the yield gap is larger than the potential that Kenya could produce if sorghum was well adopted like other major cereals such as maize, rice and wheat. To meet her demand Kenya imports sorghum from neighbouring countries such as Uganda, Tanzania and Sudan [4]. This implies that there is a need for more efforts toward crop performance improvement through addressing both biotic and a biotic constraints as well as socio cultural issues. Several pathogens cause diseases in sorghum seedlings, roots, stalks, leaves, panicles and grains under favourable environmental conditions [5].

Rust, grey leaf spot, leaf blight, Smuts and anthracnose

are some of the most significant sorghum diseases which limit crop productivity in most of the sorghum-growing regions of Kenya. Smuts affect the panicle while rusts, leaf blight, leaf spots are foliar diseases [6]. Anthracnose is soil-borne and is most severe during panicle formation stages under favourable conditions affecting the quality and quantity of sorghum grains and is transmitted by conidia. These diseases are reported to cause losses of up to 90 % in susceptible varieties [7]. Most of these diseases are seed-borne and thus the best management strategy will be to ensure clean disease free seeds. It is difficult to control most of these fungal pathogens because of their ability to persist in the environment as their Inoculum can be found on seeds, soil stubble or even air. With an aim of improving yield of sorghum in western Kenya, *in vitro* and *in vivo* studies were done to determine the potential effects of *Bacillus amyloliquefaciens* in inhibiting the development of foliar diseases of sorghum.

2. Materials and Methods

2.1. Isolation and Identification of Sorghum Foliar Diseases

Fungal isolation was done through the agar plate method where potato dextrose agar media was used. Thirty nine (39) grams of the PDA were weighed and added to one litre of sterile distilled water. After thorough mixing using a magnetic stirrer on a hot plate maintained at 60°C, this media was sterilized in an autoclave at 15 PSI and 121°C for 15 minutes, After which sterile media was dispensed onto sterile Petri dishes in laminar flow hood and left to solidify. Plant parts sampled showing symptoms of foliar diseases that were identified in the field were analyzed at the crop protection laboratory at University of Eldoret department of seed crop and horticultural sciences. Small infected sections were carefully excised within the boundary of the lesions to about 2mm pieces and surface sterilized with 10 % sodium hypochlorite for 20 seconds to remove any external organisms that may have accompanied the samples. These samples were further rinsed three times with sterile distilled water to remove any remains of the sterilizing agent and then dried using sterile filter papers. Clean and dry plant parts were plated onto Petri dishes and incubated for seven days. After seven days, growing fungi was identified by describing the cultural and microscopic characteristics. Plant pathological manuals, reference journals and microscope were used to differentiate and identify the isolates [8,9,10].

2.2. Evaluation of Inhibitory Effects of *Bacillus amyloliquefaciens* on Identified Fungal Pathogens

Dual culture technique was used to evaluate the inhibitory potential of *Bacillus amyloliquefaciens* where an experiment was set in a completely randomized design (CRD) where the bacterium was tested against identified fungal isolates such as *Colletotrichum sublineola*, *Sporisorium sorghi*, *Exserohilum turcicum* and *Gloeocerspora sorgi* each replicated thrice. Assessment was done on the inhibitory potential of treatments applied on mycelial growth characteristics of the test fungi.

Freshly grown fungal culture that was seven days old was cultured at the centre of the plate containing PDA along the diameter line. At the periphery of the plate, along the diameter line a spot of the bacterium was smeared and incubated at room temperatures. A control was set in which a fungal isolate was cultured and sterile distilled water used in place of *Bacillus amyloliquefaciens*. In terms of inhibitory potential of *Bacillus amyloliquefaciens*, evaluation was done my measuring the area covered by the fungus treated with the bacterium in comparison to the area covered by the control culture. While percentage inhibition was done by subtracting area covered by the test fungi under treatment (r) from area covered under control as a subject to control (R) expressed as a percentage [11]. Growth patterns of the fungus following the treatment with the bacterium were assessed at the interval of 24 hours for seven days after which growth inhibition was calculated. These data were subjected to analysis using GenStat Release 16.1, VSN International Ltd.

2.3. Assessment of *Bacillus amyloliquefaciens* As a Bio Control of Sorghum Covered Kernel Disease Under Greenhouse Conditions

One sorghum genotype that was reported to be susceptible to major fungal diseases of sorghum was used for this study. Seeds were dressed with the Inoculum of *Bacillus amyloliquefaciens* at different rates (0, 200, 300 and 400 ml in 1 gram of seeds) before planting in pots and this experiment was set in a completely randomized design in three replicates. Regular agronomic management practices such as watering, weeding and close monitoring were employed to ensure optimal growth throughout the study. After bacterium inoculation, regular scouting for the covered kernel disease under different rates of bacterium was done and scored on a severity scale of 1-5.

3. Results and Discussion

3.1. Results

3.1.1. Isolation and Identification of Causal Agents of Sorghum Foliar Diseases

Colletotrichum sublineola, *Sporisorium sorgi*, *Exserohilum turcicum* and *Gloeocercospora sorgi* were isolated from samples with symptoms of Anthracnose, leaf blight and leaf spot and covered kernel diseases respectively. *Colletotrichum sublineola* was characterized by raised cottony mycelium, whitish to yellowish pigmentation, hyaline and smooth conidia with no septations [12]. On the other hand, *Exserohilum turcicum* was found to form whitish grey colonies on potato dextrose agar medium and the cultures turned darkish grey during sporulation. Conidiophores were in small groups with septations, their margins were smooth and straight and dark brownish in colour [13]. While *Gloeocercospora*

sorgi formed yellowish to brownish mycelial growth and formed long conidia, curved and hyaline with numerous septations. These cultures produced numerous mass of spores with sclerotia being hyaline, curved and septate [14]. And finally, *Sporisorium sorgi* was characterized by brownish mycelium. The spores were found to be thick walled, globose to cylindrical in shape [15].

3.1.2. Inhibitory Effects of *Bacillus amyloliquefaciens*

Mycelial growth in *Colletotrichum sublineola* was the most inhibited by *Bacillus amyloliquefaciens* by 58 % while *Exserohilum turcicum* was least inhibited by the bacterium ((35%) which had a lower significance difference in terms of mycelial growth inhibition from *Sporisorium sorgi* (42%) and *Gloeocercospora sorgi* (45%) (Figure1).

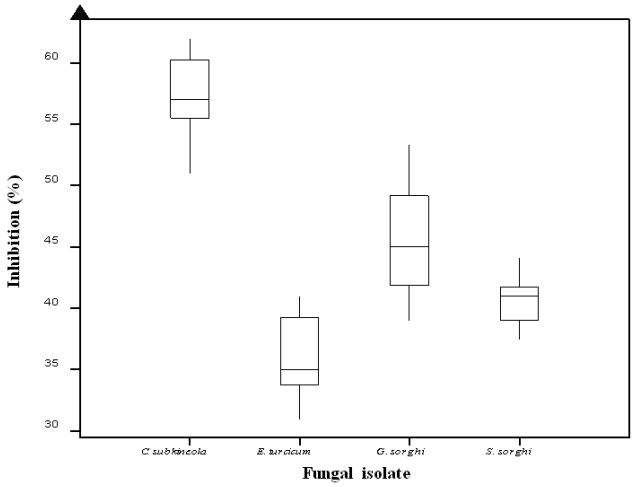

Figure 1. Percentage inhibition of selected fungal pathogens of sorghum by *Bacillus amyloliquefaciens*

Mycelial inhibition was least in *Sporisorium sorgi* while *Colletotrichum sublineola* had the highest inhibition. *Gloeocercospora sorgi* and *Exserohilum turcicum* had moderate inhibition. This was following a dual culture technique where isolated foliar fungal diseases of sorghum were cultured together with *Bacillus amyloliquefaciens* bacterium at equidistant positions and the rate of inhibition examined at intervals for seven days by measuring the distance away and to the fungus (Figure 2).

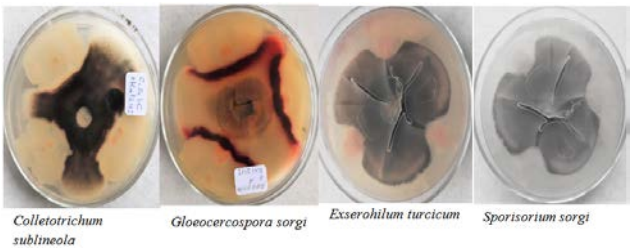

Figure 2. Effects of *Bacillus amyloliquefaciens* bacterium on mycelial growth of isolated fungal pathogens

3.2. Effects of Different Rates of *Bacillus amyloliquefaciens* on Covered Kernel Disease Severity

On a severity scale of 1-5, sorghum genotype seeds treated with 400 ml of bacterium per a kilogram of seeds had the lowest disease severity. Increasing bacterium treatment rates consequently reduced disease severity compared to control where no bacterium was applied. When compared with a positive check, fungicide; bacterium at a higher rate responded almost similarly in suppressing disease severity (Figure 2). Disease severity was inversely proportional to the rates of Bacillus amyloliquefaciens bacterium this is evident in Figure 3 where the highest severity was recorded in the control treatment and the lowest rate (200 ml per kilogram of seeds)

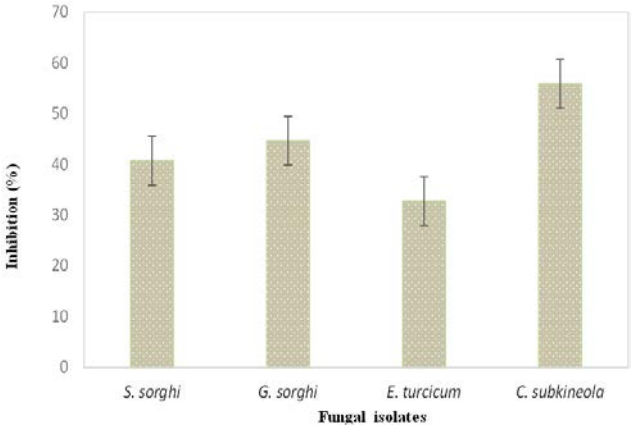

Figure 3. *In vitro* inhibition of sorghum foliar disease pathogens by *Bacillus amyloliquefaciens* bacterium. Error bars represents standard errors

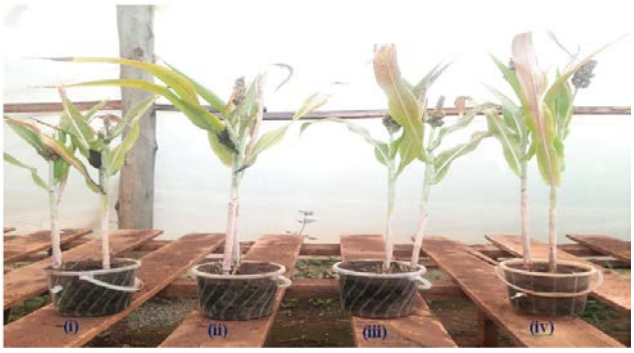

Figure 4. Severity of covered kernel disease at different rates of bacterium: (i) - 0ml/kg (ii) - 200ml/kg, (iii)-300ml/kg & (iv)-400 ml/kg of seeds

3.2. Discussion

Bacillus amyloliquefaciens was found to inhibit mycelial growth of sorghum foliar diseases *invitro* and this may be attributed to production of enzymes, antibiotics and sideophores that may have triggered antagonistic reactions between the fungus and the bacterium resulting to observed changes [16]. In some instances, the bacterium may act through hyper parasitism or direct competitions. The degrees of inhibition were less in other fungal pathogens; for instance *Exserohilum turcicum* was less inhibited by the bacterium since it may have caused a zone of inhibition making the fungus resist the actions of the bacterium [17]. These results are in harmony with those of earlier scholars who reported that some bacterial organisms produce enzymes that degrades fungal structures inhibiting their growth and development [18,19]. They further concluded

that some strains attach, penetrate and lyses the fungal hyphae. Furthermore, some strains of plant growth promoting bacterium produces several antibacterial and antifungal substances that induce cytolysis, damage membrane structural integrity, impede mycelial development, and prevent spore germination. Increasing the rates of the bacterium means increasing the chances of antagonism of the test fungi thus the observed results.

4. Conclusion

Sorghum production in western Kenya is affected by foliar diseases such as anthracnose, leaf blight and leaf spot diseases. These diseases can be significantly managed by the use of resistant cultivars as well as by incorporating bio control agents such as *Bacillus amyloliquefaciens* as seed dressants. This bacterium not only reduces disease severity but also improves plant growth and development. Further studies should be done to ascertain the correct rates that are cost effective of *Bacillus amyloliquefaciens* bacteria that can be used to improve plant growth and also to reduce disease infections. This will help in incorporation of the bacterium in an integrated disease management system to ensure continuous production of sorghum in the tested regions.

References

[1] Borrell A, van Oosterom E, George-Jaeggli B, Rodriguez D, Eyre J, Jordan DJ, et al. Chapter 5 - Sorghum. In: Sadras VO, Calderini DF, editors. Crop Physiology Case Histories for Major Crops [Internet]. Academic Press; 2021 [cited 2024 Jul 28]. p. 196–221. Available from: https:// www.sciencedirect.com/ science/ article/pii/B9780128191941000050.

[2] Khoddami A, Messina V, Vadabalija Venkata K, Farahnaky A, Blanchard CL, Roberts TH. Sorghum in foods: Functionality and potential in innovative products. Crit Rev Food Sci Nutr. 2023 Apr 3; 63(9): 1170–86.

[3] Afify AEMMR, El-BELTAGI HS, El-Salam SMA, Omran AA. Effect of Soaking, Cooking, Germination and Fermentation Processing on Proximate Analysis and Mineral Content of Three White Sorghum Varieties (Sorghum bicolor L. Moench). Not Bot Horti Agrobot Cluj-Napoca. 2012 Sep 24; 40(2): 92–8.

[4] Cox S, Nabukalu P, Paterson AH, Kong W, Nakasagga S. Development of Perennial Grain Sorghum. Sustainability. 2018 Jan; 10(1): 172.

[5] Prazak R. Prospects for Sorghum cultivation in Poland. Acta Agrobot [Internet]. 2016 [cited 2024 Jul 28]; 69(2). Available from: http://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-3b57914d-f997-4692-b681-aea591a42681.

[6] Okong'o CA. Screening of sorghum (sorghum bicolor) genotypes for resistance to covered kernel smut disease (sporosorium sorghi) for western kenya [Internet] [Thesis]. 2021 [cited 2024 Jul 28]. Available from: http:// repository.rongovarsity.ac.ke/ handle/ 123456789/2366.

[7] Ngugi HK, King SB, Abayo GO, Reddy YVR. Prevalence, Incidence, and Severity of Sorghum Diseases in Western Kenya. Plant Dis. 2002 Jan; 86(1): 65–70.

[8] Dorley O, Were J, Ochuodho J, Auma E. Evaluation of Antifungal Properties of Botanical Extracts in the Management of Common Spoilage Fungi of Rice (Oryza sativa L.). Am J Microbiol Res. 2023 Feb 3; 11: 1–13.

[9] Mwatabu ME, Omondi WJ, Chemulanga CJ, Ouma OE. Efficacy Assessment of Inhibitory Fungal Concoctions Against Mycotoxin Fungi Affecting Maize Grain Quality in Western Kenya. 2023 Mar 31 [cited 2024 Jul 28]; Available from: http:// repository.rongovarsity.ac.ke/ handle/123456789/2477.

[10] Ruto CE, Were JO, Kinyua MG. Symptomatology and Identification of Major Fungal Pathogens of Pyrethrum (Chrysanthemum cinareriifolium) in Kenya. J Biol Agric Healthc. 2024; 14(1): 92.

[11] Chemitei K, Makumba B, Mwamburi L, Ochuodho J. Bio-Control of Net-Blotch and Scald Pathogens of Barley Using Paenibacillus Polymyxa KAI245 Isolated from Sorghum Rhizosphere in Western Kenya. 2019 May 1: 28–36.

[12] Koima IN, Kilalo DC, Orek CO, Wagacha JM, Nyaboga EN. Identification and Characterization of Colletotrichum Species Causing Sorghum Anthracnose in Kenya and Screening of Sorghum Germplasm for Resistance to Anthracnose. J Fungi. 2023 Jan; 9(1): 100.

[13] Patil L, Jagtap GP, Hingole DG, Pawar S, Gaikawad G, Ghante P, et al. The Pharma Innovation Journal 2022; 11(12): 702-708 Studies on morphological, cultural, physiological and pathological variability in isolate of Exserohilum turcicum, incitant of northern leaf blight of maize. 2022.

[14] Aslam HMU, Peters NT, Killiny N, Riaz H, Hameed A, Aslam S, et al. An Insight into Infectious and Noninfectious Diseases of Sorghum Species. In: Sustainable Summer Fodder. CRC Press; 2023.

[15] Ahn E, Fall C, Botkin J, Curtin S, Prom LK, Magill C. Inoculation and Screening Methods for Major Sorghum Diseases Caused by Fungal Pathogens: Claviceps africana, Colletotrichum sublineola, Sporisorium reilianum, Peronosclerospora sorghi and Macrophomina phaseolina. Plants. 2023 Jan; 12(9): 1906.

[16] Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, et al. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem. 2013 May 1; 60: 182–94.

[17] Köhl J, Kolnaar R, Ravensberg WJ. Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy. Front Plant Sci [Internet]. 2019 Jul 19 [cited 2024 Jul 28]; 10. Available from: https:// www. frontiersin.org/journals/plant-science/ articles/ 10.3389/ fpls.2019.00845/full.

[18] Al-Rajhi AMH, Alharbi AA, Yahya R, Abdel Ghany TM. Induction of Hydrolytic Enzyme Production and Antibiosis via a Culture of Dual Fungal Species Isolated from Soil Rich with the Residues of Woody Plants in Saudi Arabia. | BioResources | EBSCOhost [Internet]. Vol. 17. 2022 [cited 2024 Jul 28]. p. 2358.

[19] Xu SJ, Jing ZQ, Guo ZJ, Li QQ, Zhang XR. Growth-promoting and disease-suppressing effects of Paenibacillus polymyxa strain YCP16-23 on pepper (Capsicum annuum) plants. Trop Plant Pathol. 2020 Aug 1; 45(4): 415–24.