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Proceeding Paper

Formulation and Evaluation of Sugarcane-Bagasse-Based Biocontrol Agents for Sustainable Phytopathogen Management †

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Abstract: Biocontrol agents are microbiological-based alternatives to agrochemicals due to their effective and sustainable attributes in controlling phytopathogens. This research highlights the formulation of biocontrol agents using sugarcane-bagasse as a carrier matrix and the evaluation of the formulants in phytopathogen management. The isolated rhizospheric bacteria were screened for the antibiosis trait responsible for biocontrol activity using the agar streak method. Bacterial isolates with antibiosis potential were further identified phenotypically. The carrier was prepared by oven drying the sugarcane-bagasse at 90 °C for three days while grinding and sieving using a mesh sieve of 1.16 mm was done afterwards. For the biocontrol formulation, 200 mL of biocontrol inoculum was added to 20 g of sugarcane-bagasse for each organism to form the final products. Water and adhesion capacities were conducted on the three formulations and, the antagonistic potential of the formulants were evaluated using the maize growth profile after 21 days. A total of nine isolates were obtained; only three (3) showed antibiosis antagonistic activity and were further utilized for the formulations branded ZEEMYC (*Mycobacterium* spp.), ZEEPAS (*Pseudomonas* spp.), and ZEEBAC (*Bacillus* spp.), respectively. The water capacities of the three formulations were between 6.9 g and 9.9 g, respectively, while adhesion capacity was also observed. On day five (5), maize seeds planted in all pots sprouted, except diseased seeds without a biocontrol agent (DSs). On day 11, plant height, shoot length, and root length ranged between 36.5 cm and 39 cm, 31 cm and 34 cm, and 5 cm and 7 cm for plants with a biocontrol agent. Those of the control (healthy seeds without biocontrol) were 42 cm, 34.5 cm, and 7.5 cm, while barely visible growth was observed in the DSs. This study displays the potential of natural-based biocontrol agents in controlling the phytopathogen *Aspergillus niger and contributes significantly to SDG 2*.

Keywords: biocontrol; plant growth promoting rhizobacteria; bioformulation; microbial inoculants; sugarcane bagasse; antibiosis

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1. Introduction

Phytopathogens pose a significant threat to global agricultural productivity, leading to substantial crop losses and economic damage. In response to growing concerns about the environmental and health risks associated with chemical pesticides, researchers have been exploring sustainable and eco-friendly alternatives for phytopathogen management [1]. One of such environmentally friendly pathogen management approach is the use of biological control (biocontrol) agents. Biocontrol agents are natural or modified organisms or microbes that effectively control phytopathogens to enhance the yield of plants [2]. They can serve as alternatives to agrochemicals due to their sustainable attributes in controlling phytopathogens, and significant impact in sustainable farming

practices. Plant-growth-promoting rhizobacteria (PGPR), which are a broad range of microorganisms that use multiple mechanisms or sometimes a combination of processes to stimulate plant development and control a range of phytopathogens, have been used effectively in the biocontrol of phytopathogens. Inoculants from *Bacillus* spp., *Rhizobia* spp., *Azosprilium lipoferum*, *A. brasilense*, *Azotobacter* spp., *Pseudomonas* spp., and *Bradyrhizobium* spp. are among the most commonly used PGPR-based biofertilizers and biocontrol agents commercially available in Africa [3].

In the biocontrol of plant-based diseases, rhizospheric microbes possess several mechanisms that allow for antagonistic potential against different diseases. These mechanisms include, but are not limited to, the following: siderophore and hydrogen cyanide production, antibiosis activity, induced systemic response, hyperparasitism, and others. Antibiosis is a process in biocontrol mechanisms which is achieved by the production of secondary metabolites, like volatile compounds and antibiotics, by beneficial microbes which help to antagonize pathogens in the host plants [4].

Presently, beneficial bacteria are currently used in the formulation of inoculants in two forms: solid and liquid carriers. These inoculant formulations are used in a variety of commercially important agricultural crops [5]. Carrier-based bioinoculants demonstrate efficiency through their capacity to influence the shelf life of the inoculant. Thus, the judicious choice of a suitable carrier is paramount not only in ensuring the preservation of the inoculant's shelf life during storage but also in enhancing its efficacy in agricultural fields [6]. Sugarcane (*Saccharum officinarum*) is renowned for its extensive cultivation and the significant byproduct it generates in the form of bagasse, which is the dry, fibrous residue that remains after the extraction of sugarcane juice [7]. This agro-waste has been used and reported as a suitable and inert carrier matrix for the bioformulation of products involving microbial inoculations. Therefore, the objective of this research is to assess the effectiveness of biocontrol agents formulated using sugarcane-bagasse as a carrier matrix in combating phytopathogens and promoting plant growth.

2. Materials and Methods

2.1. Collection and Isolation of PGPR Strains

A rhizospheric soil sample was collected from the rhizosphere of selected plants using standard methods according to Verma and Yadav. [8]. The PGPR strain was isolated from the sample using serial dilution and spread plate methods according to Sivasakthi et al. [9] to obtain pure isolates.

2.2. Evaluation of Antagonistic Activity Using Antibiosis Method

The agar streak method was employed to evaluate the antagonistic activity of isolated rhizospheric bacteria against phytopathogens to determine their antibiosis potential. The isolated rhizospheric bacteria and a fungal pathogen (collected from a culture collection center) identified as *Aspergillus niger* were used for the assay according to Sellem et al. [10]. Isolates with inhibition zones (haloes without mycelial development or deformed hyphae) larger than 2 mm were selected and identified phenotypically according to Ehis-Eriakha et al. [11].

2.3. Inoculum Preparation and Formulation of Sugarcane-Bagasse-Based Biocontrol Agents

Rhizobacterial strains with antibiosis properties were used for the preparation of the inoculum according to Riaz et al. [12]. For the formulation, 60 g of the prepared and sterilized sugarcane bagasse was introduced into bacterial pellets in approximately 1:10 ratio (weight/volume). The mixture of sugarcane-bagasse and liquid culture was vortexed for 45 min in support of homogenous mixing of the bacterial cell within the bagasse matrix and dried at room temperature (28 $\pm$ 10 °C) (Figure 1). The experiment was performed in triplicate. The containers were sealed in airtight sterilized packs to prevent any potential contamination according to Ansari and Jaikishun [13], with slight modifications.

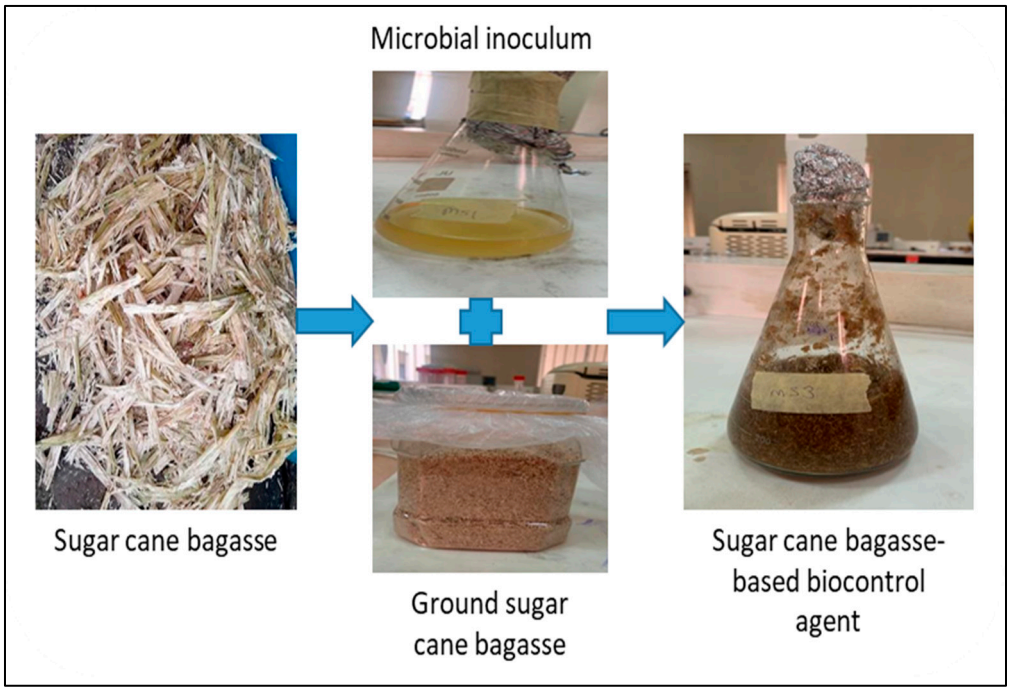

Figure 1. Bioformulation of sugarcane-bagasse-based biocontrol agent.

2.4. Determination of the Water Absorption and Adhesion Capacity of the Formulant

Water absorption capacity was determined using the method described by Norhasnan et al. [14] and calculated using the following equation:

$$\%M = \frac{W_t - W_0}{W_0} \times 100$$

where W_t is the sample's weight at a recorded immersion time, and W_0 is the weight of the dried sample. Adhesion capacity was also determined according to the method of Baliyan et al. [15].

2.5. Physicochemical Analysis of Soil Sample Prior to Cultivation

The pH of the soil sample was measured using a pH meter. Soil electrical conductivity and soil organic matter tests were also conducted according to the method of Salihu and Iyya [16]. Temperature, total organic carbon, and heavy metal constituents of the soil were also determined based on standard methods.

2.6. Evaluation of Formulated Biocontrol Agents on Maize Cultivation under Greenhouse Conditions

The formulated biocontrol inoculants were evaluated for their biocontrol potential against phytopathogens in controlled laboratory conditions. The ability of the biocontrol bacteria to induce plant defense responses and suppress disease development was investigated. The performance of seeds and soil treated with the sugarcane-bagasse-based formulant was compared to that of control groups to assess their effectiveness. Five (5) different groups were prepared: ZEEMYC (*Mycobacterium* spp. + phytopathogen + healthy maize seeds), ZEEPAS (*Pseudomonas* spp. + phytopathogen + healthy maize seeds), ZEEBAC (*Bacillus* spp. + phytopathogen + healthy maize seeds), Control A (healthy maize seeds), and Control B (diseased maize seeds). The planting of seeds was performed following the method of Ju et al. [17] using a block randomized design in triplicates, and the experimental design is presented in Table 1.

Table 1. Experimental design.

Samples	Experiment
POT 1	2 kg soil + 30 g ZEEMYC + phytopathogen + healthy seed
POT 2	2 kg soil + 30 g ZEEPAS + phytopathogen + healthy seed
POT 3	2 kg soil + 30 g ZEEBAC + phytopathogen + healthy seed
POT 4 (Control A)	2 kg soil + healthy seed
POT 5 (Control B)	2 kg soil + diseased seed (healthy seed impregnated with phytopathogen)

Key: ZEEMYC—formulated *Mycobacterium* spp. with bagasse, ZEEPAS—formulated *Pseudomonas* spp. with bagasse, ZEEBAC—formulated *Bacillus* spp. with bagasse, phytopathogen: *Aspergillus fumigatus*.

2.7. Data Analysis

Data generated from the monitoring indices were subjected to different statistical tools and models, such as one way analysis of variance (ANOVA) SPSS version 22 and standard deviation.

3. Results

Out of the 20 bacterial isolates obtained, 3 isolates showed the highest antibiosis activity against *Aspergillus niger*, as shown in Table 2. The zones of inhibition were higher than 2 mm and, hence, scored positive for antibiosis activity. The three isolates were Gram-positive and Gram-negative, while other phenotypic properties displayed by the individual isolates revealed a close relatedness to *Mycobacterium* spp. (MS1), *Pseudomonas* spp. (MS3), and *Bacillus* spp. (CS2), respectively, and assigned tentative identities (Table 3).

Table 2. Antibiosis antagonistic activity of the isolates.

Isolates	Zone of Inhibition	Antibiosis Activity
MS1	3.5 mm	+
MS3	5 mm	+
CS2	3 mm	+

Table 3. Morphological and biochemical characteristics of biocontrol organisms.

Isolate	Cultural Properties	Gram Stain	Shape	Oxidase	Catalase	H_2S	Citrate	Urease	Indole	Glucose	Sucrose	Lactose	Maltose	Fructose	Tentative Identity of Isolates
MS 1	Round, Cream, Raised, Smooth. Entire, Opaque Dry, Small.	-	Rod	+	+	-	+	+	-	+	-	-	+	-	*Mycobacterium* spp.
MS 3	Round, Yellowish-green, Flat, Smooth, Entire, opaque, Dry, Small	-	Rod	+	+	+	-	-	+	+	-	-	+	+	*Pseudomonas* spp.
CS 2	Round, Cream, Raised. Smooth, Entire, Opaque Dry, Large.	+	Rod	-	+	+	-	+	-	+	+	-	+	+	*Bacillus* spp.

Key: +: positive; -: negative; r: rod; c: cocci.

The bioformulation was successfully performed using the three biocontrol agents absorbed in the sugarcane-bagasse carrier matrix and the final products were branded as ZEEMYC, ZEEPAS, and ZEEBAC for *Mycobacterium* spp., *Pseudomonas* spp., and *Bacillus* spp., respectively. The water capacities of the formulants ranged between 6.9 g and 9.9 g (Table 4), while adhesion activity was also evidenced, establishing the successful formulation of the biocontrol agent.

Table 4. Water capacity of sugarcane-bagasse-based inoculant.

Inoculant	Water Capacity
ZEEMYC	9.9 g
ZEEPAS	6.9 g
ZEEBAC	8.9 g

Prior to maize cultivation, a comprehensive analysis of the soil physicochemical properties indicated that the soil sample was well suited for agricultural purposes. The levels of phosphate, nitrate, electrical conductivity (E. conductivity), and other critical parameters were found to be well within the typical ranges when compared to undisturbed arable soil conditions. Furthermore, an assessment of heavy metal concentrations and organic content revealed that the sample was uncontaminated (as shown in Table 5.. Upon concluding the cultivation phase on day 28, it became evident that the ZEEPAS treatment had produced the highest plant height (43.7 cm), closely trailed by the ZEEBAC treatment (39.97 cm). In contrast, the ZEEMYC treatment and control group A, which received healthy seeds, resulted in slightly shorter plant heights, with ZEEMYC exhibiting the lowest height at 36.77 cm. Notably, no growth was observed in control group B throughout the entire sampling period (as detailed in Table 6) and pictorially presented in Figure 2.

Table 5. Mean values of the soil physicochemical properties prior to cultivation.

S/N	Parameter	Mean Value
1	pH	7.785
2	Temperature (°C)	25.80
3	Conductivity (uscm)	85.15
4	Moisture Content (%)	12.23
5	Color	Brownish/Ditto
6	Phosphorus (mg kg^{-1})	29.23
7	Nitrate (mg kg^{-1})	16.52
8	Organic Carbon (%)	0.32
9	Organic Matter (%)	0.56
10	Nickel (mg kg^{-1})	0.18
11	Zinc (mg kg^{-1})	0.44
12	Lead (mg kg^{-1})	0.098

Table 6. Plant parameters of maize plant at different growing stages.

Days	Growth Parameters	ZEEPAS (cm)	ZEEBAC (cm)	ZEEMYC (cm)	Control A (cm)	Control B
Day 7	Plant height	11.65 ± 0.31^{b}	11.97 ± 3.41^{c}	10.47 ± 0.81^{b}	9.03 ± 1.68^{b}	No growth
	Shoot length	9.80 ± 0.35^{b}	9.89 ± 0.19^{b}	8.37 ± 2.82^{c}	7.93 ± 3.59^{b}	
	Root length	$2.40 \pm \pm 0.69^{b}$	1.93 ± 0.12^{b}	2.56 ± 0.97^{b}	1.54 ± 0.94^{b}	
Day 14	Plant height	16.71 ± 5.70^{b}	18.57 ± 2.48^{b}	16.77 ± 5.87^{b}	14.77 ± 5.02^{b}	No growth
	Shoot length	13.61 ± 5.55^{b}	15.40 ± 5.05^{b}	15.21 ± 5.01^{b}	13.73 ± 5.46^{b}	
	Root length	3.77 ± 2.13^{ab}	3.60 ± 2.43^{b}	2.67 ± 1.15^{b}	2.07 ± 0.12^{b}	
Day 28	Plant height	43.70 ± 1.60^{a}	39.97 ± 0.74^{a}	36.77 ± 0.31^{a}	38.13 ± 0.35^{a}	No growth
	Shoot length	34.37 ± 1.80^{a}	35.40 ± 0.95^{a}	31.80 ± 0.30^{a}	34.80 ± 1.87^{a}	
	Root length	6.47 ± 0.60^{a}	7.73 ± 0.32^{a}	5.27 ± 0.40^{a}	5.00 ± 0.10^{a}	

Data presented as mean ± SD; superscripts for means for groups in homogeneous subsets indicate diverse significant differences at $p \leq 0.05$.

Figure 2. Plant growth and development of maize (*Zea mays*) plants at days 7, 14, and 28. Key: ZEEPAS: *Pseudomonas* spp. inoculant, ZEEMYC: *Mycobacterium* spp. inoculant, ZEEBAC: *Bacillus* spp. inoculant, Control A: healthy seeds without biocontrol agent; Control B: diseased seeds without biocontrol agent.

4. Discussion

Multiple rhizobacteria with the ability to inhibit the growth of plant pathogens using diverse mechanisms are usually present in the rhizosphere of plants [18]. These mechanisms often entail the production of chemical compounds referred to as antibiotics, which are expressed by various microorganisms contingent upon their genetic makeup and serve as agents that antagonize phytopathogens. The chemicals are diverse, some of which have broad spectrum potentials targeting a wide range of phytopathogens. In this study, three selected rhizobacterial isolates with antibiosis potential were successfully utilized for the production of a sugarcane-bagasse-based biocontrol agent. Antibiosis is one of the most studied biocontrol mechanisms in plant disease control, and the synthesis of different antibiotics by microorganisms associated with plants participating in the biological control of plant pathogens has been widely acknowledged as a significant mechanism contributing to the mitigation of disease symptoms, especially within the context of soil con-

ditions [4]. In this study, the rhizospheric bacteria displayed biocontrol of phytopathogen potential through antibiosis mechanisms in mitigating *Aspergillus niger*, which is a known phytopathogen associated with different plants. The biocontrol formulation with sugarcane-bagasse and rhizobacteria with biocontrol attributes successfully promoted the growth of diseased *Zea mays* with no evidence of stunted growth or diseased parts. The formulant effectively enhanced the growth of the plant as evidenced in the plant parameters in comparison with the growth of a healthy plant under the same conditions.

The rhizobacteria utilized in this research were carefully selected from a pool of isolated rhizobacteria based on their ability to demonstrate antibiosis and *in vitro* antagonistic traits. The antibiosis activity of the selected bacteria was assessed and scored based on the formation of zones of inhibition measuring up to 2 mm, as seen in Table 1, and, notably, the result corresponds with the findings of Liu et al. [19]. The antibiosis attribute underscores the ability of microbes to produce secondary metabolites, which improves the bacterium's ability to either compete with pathogens by inhibiting the activity of the pathogens or by triggering host defenses. Plant responses to bioinoculants are influenced by soil physicochemical parameters and edaphic variables [20]. As a result, several soil physiochemical parameters were determined prior to the cultivation experiment on the soil. The soil parameters revealed an optimum quantity of nitrates, phosphorus, and organic matter, which are rate-limiting factors for plant growth, while the heavy metals present are essential for optimum plant growth and the concentrations were within permissible limits [21].

Understanding how different treatments impact the growth of plants is essential for optimizing cultivation practices and achieving desired plant outcomes. This study assessed the growth parameters of maize using different sugarcane bagasse inoculant treatments. In this study, the different biocontrol agents harboring the three selected bacterial strains showed varied plant growth patterns. However, based on statistical analysis, no significant differences ($p \leq 0.05$) were observed among treatments and between treatments and Control A, except at day 7 for plant height between ZEEBAC and other treatments and for shoot length between ZEEMYC and other treatments, including Control A. This demonstrates the effectiveness of the formulated biocontrol agents in suppressing the phytopathogen *Aspergillus niger* and promoting plant growth comparatively measurable with plants grown with healthy seeds. Again, Control B showed no visible growth, just a sprout within the soil layer, which is more remarkable evidence displaying the biocontrol potential of ZEEMYC, ZEEPAS, and ZEEBAC. Plant pathogens have deleterious effects on plants, such as reduced yield, poor growth, or no growth, which consequently promote food insecurity [22]. The rapid and healthy plant growth observed in the three treated pots could also be attributed to the nutritional constituents of the sugarcane-bagasse carrier matrix, which has served as a biofertilizer in previous studies. Hassan et al. [23] accessed the effects of carrier-based biofertilizer (using maize straw and sugarcane husk as carriers) containing *Bacillus* and *Pseudomonas* species on wheat growth; the biofertilizer increased plant growth and also decreased heavy metal concentrations in soils. Detraska [24] also conducted a similar experiment to evaluate the effects of *Streptomyces* spp. immobilized with sugarcane-bagasse on plant growth promotion.

5. Conclusions

The assessment of maize growth parameters under the various bioformulations demonstrated that these bioinoculants had a positive impact on plant growth and suppressed the phytopathogen *Aspergillus niger*. This research conclusively demonstrates the biocontrol potential of sugarcane bagasse-based bioformulants in effectively managing and controlling the phytopathogen *Aspergillus niger,* contributing to sustainable agricultural practices. This study also reveals that the bioformulant could serve as an effective, sustainable, and eco-friendly alternative to agrochemicals in combating plant diseases, enhancing plant growth, and securing food production for the growing global population. More significantly, this research aligns with the second sustainable development goal (SDG 2).

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