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Effect of selected microbial isolates from maize rhizosphere on disease incidence and severity of maize common smut disease caused by *Ustilago maydis*

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ABSTRACT

The study was conducted to evaluate the effect of selected microbial isolates from the maize rhizosphere on the incidence and severity of maize common smut disease, which causes a decline in maize production. The rhizosphere contains microorganisms with potential antagonistic activities against microbial diseases. However, there is limited information regarding their use as biocontrol agents. The maize varieties Dk 8033 and Duma 43 were used because they have a short maturity period, are recently released, and are highly preferred by farmers. In-vitro screening of microbial isolates against *Ustilago maydis* was conducted using the dual culture method in a completely randomized design, with three replicates. Greenhouse experiments followed a completely randomized design with ten treatments, also in triplicate. Data on growth inhibition, disease incidence, and severity were collected. Significant differences ($P \leq 0.05$) were observed among isolates. The highest in-vitro inhibition was recorded by fungal isolate MF14 (22.0 mm, unidentified), followed by *Serratia* sp. (19.0 mm), *Bacillus* sp. (16.0 mm), and *Aspergillus* sp. (15.0 mm). In greenhouse trials, MF14 showed the lowest disease incidence (49.3%), while *Serratia* sp. had the highest (84.3%). These findings highlight the potential of selected microbial isolates, especially MF14, as promising biocontrol agents for managing maize common smut.

Contribution/Originality: There is limited information regarding maize rhizospheric microflora, which may be important in identifying biocontrol potential against Ustilago maydis, a fungal pathogen of maize. Therefore, this study aims to evaluate disease incidence and severity using microbial isolates from the maize rhizosphere as biocontrol agents to identify potential biocontrol agents against Ustilago maydis, with the goal of increasing maize production.

1. INTRODUCTION

The rhizosphere refers to the soil zone immediately surrounding plant roots, characterized by increased microbial activity [1]. Studies have identified this area as a hotspot of microbial diversity, with plant roots supporting a wide range of microbial communities [2, 3]. The release of nutrients through root exudates contributes to the development of a dynamic, nutrient-enriched environment in the rhizosphere. In maize, seeds are known to release various compounds, including amino acids, sugars, and weak organic acids that influence the chemical properties of the adjacent soil [4].

The rhizosphere plays a vital role in protecting plant roots from pathogenic attacks by serving as a frontline defense zone [5]. Microorganisms that inhabit this region have demonstrated potential as effective biocontrol agents. According to Hong et al. [6] soil microbes can influence plant health by either stimulating, inhibiting, or completely suppressing the growth of soil-borne pathogens. This antagonistic activity has been well documented in bacterial genera such as *Pseudomonas*, *Burkholderia*, and *Bacillus*, as well as fungal genera like *Trichoderma*. Pathogens are subject to microbial antagonism during both the initial and later stages of infection [7]. However, there is limited research on the antagonistic capabilities of maize rhizosphere microorganisms, particularly in Kenya, against plant pathogens such as *Ustilago maydis*.

Biological control offers a promising approach to managing plant pathogens, but its effectiveness depends on selecting microbial agents that are adapted to the local environments where maize is cultivated [8]. The large-scale introduction of non-native microbes can disturb native microbial communities and potentially alter the ecological balance within the rhizosphere [8]. In some cases, these introduced organisms may unintentionally affect non-target species, including beneficial microbes, alongside the intended pathogens. This highlights the importance of exploring native microbial populations associated with maize roots to identify environmentally compatible and effective biocontrol candidates.

Plant disease incidence (percentage of infected plants), often based on visual symptoms of disease, and disease severity (percentage of leaf covered or affected) are important in the development of disease control methods. Therefore, in this study, there was a need to quantify disease incidence and severity while using microbial antagonists from the rhizosphere of maize varieties DK 8033 and Duma 43 as biocontrol agents against *Ustilago maydis*.

Ustilago maydis is a fungal pathogen specific to maize and the causal agent of the maize disease, common smut [9, 10]. The continued cultivation of maize on the same land without rotation encourages the persistence and spread of this disease, as the pathogen's teliospores can survive in soil and crop residues for several years. This study highlights the potential of microbial isolates as biocontrol agents against *U. maydis*, offering promising prospects for improving maize production and supporting the country's agricultural economy. Employing these microbial antagonists presents an eco-friendly and sustainable alternative to existing disease management strategies.

2. MATERIALS AND METHODS

2.1. Study Site

The experiment was conducted between April 2023 and July 2024 at Maseno University, utilizing the Botany Department's microbiology laboratory, greenhouse, and the university research farm. The greenhouse conditions included daytime temperatures of 25°C–40°C, nighttime temperatures of 20°C–30°C, a 14/10-hour photoperiod, and relative humidity ranging from 70% to 90%. Maseno was selected to ensure uniform environmental conditions and to safeguard crop growth. Geographically, the site is situated at 0°10′0″ S, 34°36′0″ E along the Kisumu–Busia Road, at an elevation of 1,503 meters (4,934 feet) above sea level. The region experiences both long and short rainy seasons, with an annual average rainfall of 1,750 mm and a mean temperature of 28.7°C [11].

2.2. Pathogen Isolation

A collection of maize smut gall samples was randomly conducted from naturally infected maize plants at the end of the growing season at Maseno University farm in 2023. Potato dextrose agar and 20% carrot solution were used to obtain pure cultures of *Ustilago maydis* and for propagation of sporidia (basidiospores), respectively. The galls were chopped, and teliospores were separated from the gall tissues by sieving through a tea strainer. The teliospores were surface-sterilized by immersion in 1% copper sulfate solution for 20-60 seconds and filtered through two layers of sterile cheesecloth, which prevented the teliospores from passing through. Subsequently, the

teliospores on the cheesecloth were washed in three changes of sterile distilled water and dried on sterile filter paper, then transferred onto PDA supplemented with antibiotics (streptomycin sulfate) in petri dishes. The dishes were incubated at 25°C for 4-5 days until sporidia of *U. maydis* emerged. When the sporidia reached about a pinhead size, they were taken from cultures and transferred into 500 ml Erlenmeyer flasks containing 20% sterile carrot solution, then incubated at 25°C for 7 days to facilitate sporidia multiplication. The flasks were shaken once or twice a week. For inoculum preparation, basidiospore suspensions in the Erlenmeyer flasks were stirred to achieve a homogeneous solution, and basidiospores were counted using a hemocytometer. The suspensions were diluted to appropriate concentrations with sterile carrot solutions and adjusted to 4×10^6 sporidia per ml. Similarly, teliospore suspensions were prepared at 1×10^6 teliospores per ml and added to the basidiospore suspensions [12].

2.3. Pathogenicity Test of Ustilago Maydis Isolate

Pathogenicity assays were conducted under greenhouse conditions at Maseno University using a completely randomized design with three replicates, following a modified protocol adapted from Aydoğdu and Boyraz [12]. Two seeds of each maize variety, DK 8033 and Duma 43, were manually planted in 15 cm diameter pots filled with sterilized topsoil collected from the university's research farm. Di-ammonium phosphate (DAP) fertilizer was applied at planting at a rate of 1.5 g per pot. Top dressing was carried out at the second leaf stage using calcium ammonium nitrate (CAN) at 2.5 g per pot. When plants reached a height of 40–60 cm, 2 ml of prepared inoculum comprising 4×10^4 sporidia/ml and 1×10^6 teliospores/ml was injected into the apical node using a sterile hypodermic syringe. Disease development was assessed by monitoring the appearance of characteristic U. maydis symptoms 15 days post-inoculation.

2.4. In-Vitro Antagonism Assays to Evaluate the Ability of Microbial Isolates from the Rhizosphere to Inhibit Growth of Ustilago Maydis

These experiments were conducted in the Botany Department laboratory at Maseno University. Fungal isolates were tested using a dual culture assay on PDA medium, following the methods outlined in Alwathnani and Perveen [13] and Dhanya et al. [14]. Plugs (5 mm in diameter) cut from five-day-old fungal cultures of both the pathogen (*U. maydis*) and the test fungi (potential antagonists) were excised using a sterile cork borer. Antagonist plugs were positioned approximately 2 cm inward from the edge of the Petri dish, while a second 5 mm plug of *U. maydis* was inoculated on the same plate. Plates were incubated at 25 ± 2 °C, and pathogen-only plates served as controls. The experiment was arranged in a completely randomized design, replicated three times. Inhibition zones were measured after seven days of incubation.

For bacterial isolates, the antagonists were line-streaked 5 cm away from the fungal pathogen plug on the same PDA plate, using a sterile loop, following the method of Dhanya et al. [14]. This experiment also followed a completely randomized design, replicated three times. After incubation at 28 ± 2 °C for seven days, inhibition was recorded as the distance between bacterial growth and the pathogen. Based on inhibition results, two fungal and two bacterial isolates with the strongest antagonistic effects were selected for further greenhouse experiments.

2.5. In- Vivo Greenhouse Experiment to Evaluate the Effect of Selected Microbial Isolates on Disease Incidence and Severity of Maize Common Smut Disease

2.5.1. Experimental Design and Set-Up

The experiment was conducted following the procedures described in Frommer et al. [15] and Juma et al. [16]. The microbial isolates selected for evaluation included two bacterial strains, MB2 (*Serratia* sp.) and NB16 (*Bacillus* sp.) and two fungal isolates, MF13 (*Aspergillus* sp.) and MF14 (unidentified). These isolates were chosen based on their demonstrated antagonistic efficacy against *Ustilago maydis* in preliminary in vitro assays. Plastic pots (30 × 30 cm, 20 L capacity) with drainage holes were sterilized using a sodium hypochlorite solution and filled with

steam-sterilized topsoil collected from the Maseno University farm. Seeds of the maize varieties DK 8033 and Duma 43 were sown manually at a depth of 5 cm. Di-ammonium phosphate (DAP) fertilizer was applied at planting at a rate of 1.5 g per pot. Each pot received two seeds, which were later thinned to one seedling. The pots were spaced 1 m apart and arranged in a completely randomized design, with treatments replicated three times for both maize varieties. Top dressing was performed at the second leaf stage using calcium ammonium nitrate (CAN) at a rate of 2.5 g per pot. Manual weeding was conducted twice during the crop growth period.

2.5.2. Inoculation and Pathogenicity Tests

The experiment was conducted based on the procedures described in Juma et al. [16] and Frommer et al. [15] One month after planting, maize plants were inoculated with *Ustilago maydis* by injecting 2 ml of spore suspension near the leaf whorls. Antagonistic cultures *Serratia* sp., *Bacillus* sp., *Aspergillus* sp., and MF14 were cultured on potato dextrose agar (PDA) at 25 °C for 14 days (for fungi) and on nutrient agar (NA) at the same temperature for 48 hours (for bacteria). The biomass from these cultures was aseptically scraped using a sterilized spatula, suspended in sterile distilled water, and filtered through nylon mesh. Spore and bacterial concentrations were adjusted to 1.0×10^6 spores/ml and 1.0×10^4 colony-forming units (cfu)/ml, respectively, using a hemocytometer. Suspensions were pipetted into hemocytometer chambers using sterile tips, and spore or cell counts were made under a microscope using standard grid volumes (0.1 µl) to estimate concentration per milliliter.

Infected maize plants were then treated by injecting the respective microbial suspensions, while control plants received sterile distilled water. All treatments were replicated three times and arranged in a completely randomized design. Plants were observed daily for disease progression, with particular attention to gall formation, which typically became visible three weeks post-inoculation. Soil was watered daily to maintain adequate moisture throughout the experimental period.

Table 1 presents the layout of the research, which consists of ten treatments (i.e., DK 8033 seedling inoculated with Serratia sp. (MB2) + Ustilago maydis, DK 8033 seedling inoculated with Bacillus sp. (NB16) + Ustilago maydis, Duma 43 seedling inoculated with Serratia sp. (MB2) + Ustilago maydis, Duma 43 seedling inoculated with Bacillus sp. (NB16) + Ustilago maydis, DK 8033 seedling inoculated with Aspergillus sp. (MF13) + Ustilago maydis, DK 8033 seedling inoculated with MF14 (fungal isolate) + Ustilago maydis, Duma 43 seedling inoculated with Aspergillus sp. (MF13) + Ustilago maydis, Duma 43 seedling inoculated with MF14 (fungal isolate) + Ustilago maydis, DK 8033 seedling inoculated with sterile distilled water + Ustilago maydis (control), and Duma 43 seedling inoculated with sterile distilled water + Ustilago maydis (control). Each treatment was replicated three times.

Table 1. Treatments in the greenhouse experiment.

Treatment	Description
N + MB2	DK 8033 inoculated with *Serratia* sp. (MB2) + *Ustilago maydis*
N + NB16	DK 8033 inoculated with *Bacillus* sp. (NB16) + *Ustilago maydis*
M + MB2	Duma 43 inoculated with *Serratia* sp. (MB2) + *Ustilago maydis*
M + NB16	Duma 43 inoculated with *Bacillus* sp. (NB16) + *Ustilago maydis*
N + MF13	DK 8033 inoculated with *Aspergillus* sp (MF13) + *Ustilago maydis*
N + MF14	DK 8033 inoculated with MF14 (fungal isolate) + *Ustilago maydis*
M + MF13	Duma 43 inoculated with *Aspergillus* sp. (MF13) + *Ustilago maydis*
M + MF14	Duma 43 inoculated with MF14 (Fungal isolate) + *Ustilago maydis*
N + Control	DK 8033 inoculated with sterile distilled water + *Ustilago maydis* (Control)
M + Control	Duma 43 inoculated with sterile distilled water + *Ustilago maydis* (Control)

2.5.3. Disease Incidence

Fifteen days post-inoculation, the incidence of smut disease (%) was assessed. Data collection involved evaluating all treatments and the control by recording the number of symptomatic plants. Disease symptoms

included leaf chlorosis, shoot stunting, dwarfism, morphological deformities such as plant slanting, the formation of small galls on leaves, stems, and basal regions, tassel galls, and plant mortality [17, 18].

$$DI = \frac{\text{Total no.of diseased plants}}{\text{Total no.of observed plants}} \times 100$$

Where DI is disease incidence.

2.5.4. Disease Severity

Severity of common smut symptoms was evaluated using a rating scale below, based on the percentage of plants exhibiting galls or typical symptoms of *Ustilago maydis*, as described by Frommer et al. [15].

1: No symptom.

2:1-3% of the plants are infected (One leaf has symptoms).

3: 4-10% of the plants are infected (Two leaves have symptoms).

4: 11-25% of the plants are infected (Three leaves have symptoms).

5: 25-50% of the plants are infected (Four leaves have symptoms).

6: 51-75% of the plants are infected (Four leaves and tassel have symptoms).

7: 76-100% of the plants are infected (Five leaves and tassel have symptoms).

2.6. Data Analysis

The collected data were analyzed statistically using SAS version 9.1 software to evaluate the effects of fungal and bacterial antagonist treatments on the inhibition zone of *Ustilago maydis*, as well as smut disease incidence and severity under greenhouse conditions. Treatment means were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

3. RESULTS

3.1. In-Vitro Antagonism Assays to Evaluate the Ability of Microbial Isolates from the Rhizosphere to Inhibit Growth of Ustilago Maydis

3.1.1. Bacteria

The bacterial antagonists tested exhibited varying degrees of inhibition against *Ustilago maydis*, as indicated by the different sizes of growth inhibition zones (Table 2). Isolate MB2 (*Serratia* sp.) produced the largest inhibition zone (19.0 mm), followed by NB16 (*Bacillus* sp.) with 16.0 mm. MB2 demonstrated a statistically significant difference from all other bacterial isolates ($P \leq 0.05$). Additionally, isolates MB2, NB16, and NB17 showed significantly greater inhibition compared to NB22, NB25, MB12, MB3, MB6, NB18, NB23, MB9, MB4, and MB1. However, no significant differences were observed among NB16, NB17, NB19, NB24, and MB8. The smallest inhibition zones were recorded for isolates NB23, MB9, and MB4, with no significant differences among them.

Table 2. Zones of growth inhibition (mm) of bacterial isolates from the rhizosphere of maize against *Ustilago maydis*.

Bacterial isolate + Pathogen	Zone of inhibition (mm)
MB2	19.00^{a}
NB16	16.00^{b}
NB17	16.00^{b}
NB19	15.67^{bc}
NB24	15.00^{bc}
MB8	14.33^{bcd}
NB22	14.00^{cd}
NB25	14.00^{cd}
MB12	14.00^{cd}
MB3	14.00^{cd}

Bacterial isolate + Pathogen	Zone of inhibition (mm)
MB6	14.00^{cd}
NB18	13.00^{de}
NB23	12.00^{e}
MB9	12.00^{e}
MB4	11.67^{e}
MB1	5.00^{f}
Mean	9.15
LSD	1.93
P. value	< 0.0001
% C.V.	12.81

Note: a,b,c,d,e,f, means that in a column having the same superscript letter(s) do not differ significantly at $p \leq 0.05$. Means followed by different superscript letters along the column are significantly different at $p \leq .05$. Means with more than one letter down the columns are intermediates. Values are means of three replications. **C.V**: Coefficient of variation, **LSD:** Least significant difference, **P. value**: Probability value, **M:** Duma 43 Maize variety, **N:** DK 8033 Maize variety**, B:** Bacteria.

3.1.2. Fungi

The fungal isolates demonstrated varying antagonistic activity against *Ustilago maydis*. Among these, MF14 (unidentified) and MF13 (*Aspergillus* sp.) were the most effective, producing inhibition zones of 22.0 mm and 15.0 mm, respectively. Both isolates were significantly more inhibitory than the others ($P \leq 0.05$) (Table 3). Isolates MF6, MF3, and NF21 did not differ significantly in their suppressive effects on the pathogen. Similarly, MF3, NF21, MF17, MF1, and MF12 also showed no statistically significant differences among themselves.

Table 3. Zone of growth inhibition (mm) of fungal isolates from the rhizosphere of maize against *Ustilago maydis*

Fungal isolate and pathogen	Zone of inhibition (mm)
MF14	22.000^{a}
MF13	15.000^{b}
MF5	8.667^{c}
MF3	7.000^{cd}
NF21	6.000^{cde}
MF17	4.000^{de}
MF1	2.667^{e}
MF11	2.333^{e}
Mean	8.46
LSD	4.11
P value	< 0.0001
%C. V	28.04

Note: a,b,c,d,e, means in a column having same superscript letter(s) do not differ significantly at $p \leq 0.05$. Means followed by different superscript letters down the column indicate significant differences at $p \leq .05$. Means with more than one letter down the columns are intermediates. Values are means of three replications. **C.V:** Coefficient of variation, **LSD**: Least significant difference, **P. value**: Probability value, **M:** Duma 43 Maize variety, **N:** DK 8033 Maize variety, **F-** Fungi.

3.2. In- Vivo Greenhouse Experiment to Evaluate the Effect of Selected Microbial Isolates on Disease Incidence and Severity of Maize Common Smut Disease

3.2.1. Disease Incidence

A greenhouse experiment was conducted to assess the effect of selected microbial isolates on the incidence of common smut disease (*Ustilago maydis*) in maize. Four microbial antagonists, *Bacillus* sp., *Serratia* sp., *Aspergillus* sp., and MF14 (an unidentified fungal isolate) were evaluated for their biocontrol potential on two maize varieties: Duma 43 (coded as "M") and DK 8033 (coded as "N") (Table 4). Among the treatments, the fungal isolate MF14 applied to the DK 8033 variety (N-MF14) resulted in the lowest disease incidence (49.3%), indicating the highest level of disease suppression. This was followed by M-MF14 (53.03%), demonstrating that MF14 was effective in both maize varieties, with slightly better performance in DK 8033. The bacterial isolate, Bacillus sp., showed moderate biocontrol efficacy, with disease incidences of 81.1% (N-NB16) and 84.3% (M-NB16). These values, although significantly lower than the controls, indicate a less potent suppression effect compared to MF14.

Aspergillus and Serratia sp. also showed moderate disease suppression, with incidences ranging from 63.93% to 67.80%, and no significant differences between the two maize varieties. Notably, no significant difference was observed among treatments N-MF13, N-MB2, M-MF13, and M-MB2, suggesting comparable biocontrol potential for these isolates (Table 4).

Table 4. Disease incidence in maize plants following treatment with selected microbial isolates from the rhizosphere of DK 8033 and Duma 43 maize varieties.

Treatment	Disease incidence (%)
N- DD (Control)	100.00^{a}
N- NB16	81.10^{c}
N- MF13	65.53^{de}
N- MF14	49.30^{g}
N- MB2	66.13^{de}
M- DD (Control)	100.00^{a}
M- NB16	84.30^{b}
M- MF13	63.93^{e}
M- MF14	53.03^{f}
M- MB2	67.80^{d}
Mean	73.113
LSD	2.733
P .value	<0.0001
% C. V	2.2

Note: a,b,c,d,e,f,g means in a column having same superscript letter(s) do not differ significantly at $p \leq 0.05$. Means with more than one letter down the columns are intermediates. Values are means of three replications. **C.V**: Coefficient of variation, **LSD:** Least significant difference, **P. value:** Probability value, **DD:** Distilled water, **M**: Duma 43 maize variety, **N**: DK 8033 maize variety, **NB16:** *Bacillus* sp., **MF13:** *Aspergillus* sp., **MF14:** unidentified isolate, **MB2:** *Serratia* sp.

3.2.2. Disease Severity

The control treatments (M-DD and N-DD), which involved no microbial intervention, recorded the highest disease severity (100%), confirming the full susceptibility of both maize varieties in the absence of biological control. All microbial treatments significantly reduced disease severity ($p < 0.05$) compared to the controls. Among the microbial antagonists, the fungal isolate MF14 demonstrated the highest efficacy, particularly in the DK 8033 variety (N-MF14), where it reduced disease severity to 49.30%, followed closely by M-MF14 at 53.03%. These values represent a substantial suppression of disease symptoms and highlight MF14 as the most promising biocontrol agent in this study. The bacterial isolate NB16 (*Bacillus* sp.) showed moderate disease suppression, with disease severity of 81.10% (N-NB16) and 84.30% (M-NB16). While these treatments significantly outperformed the controls, they were less effective compared to the MF14 isolate. The bacterial isolate MB2 (*Serratia* sp.) and the fungal isolate MF13 (*Aspergillus* sp.) produced intermediate levels of disease suppression. Their severity values ranged between 63.93% and 67.80%, with no significant difference among N-MB2, M-MB2, N-MF13, and M-MF13 treatments. This suggests comparable biocontrol potential for these two antagonists across both maize varieties (Table 5).

Table 5. Disease severity in maize plants after treatment with selected microbial isolates from the rhizosphere of Duma 43 and DK 8033 maize varieties.

Treatment	Disease severity (%)
N- DD (Control)	100.00^{a}
M- DD (Control)	100.00^{a}
M- NB16	84.30^{b}
N- NB16	81.10^{c}
M- MB2	67.80^{d}
N- MB2	66.13^{de}
N- MF13	65.53^{de}
M- MF13	63.93^{e}

Treatment	Disease severity (%)
M- MF14	53.03^{f}
N- MF14	49.30^{g}
Mean	73.113
LSD	2.733
P. Value	< 0.0001
% C. V	2.2

Note: a,b,c,d,e,f,g, means followed by different superscript letters along the column are significantly different at P < 0.05. Values represent the mean of three replicates. Means with more than one letter down the columns are intermediates. **C.V:** Coefficient of variation, **LSD:** Least significant difference, **P. value:** Probability value, **DD:** Distilled water, **M**: Duma 43 Maize variety, **N**: DK 8033 Maize variety, **NB16**: *Bacillus* sp., **MF13**: *Aspergillus* sp., **MF14**: unidentified isolate, **MB2**: *Serratia* sp.

4. DISCUSSION

4.1. In-Vitro Antagonism Assays to Evaluate the Ability of Microbial Isolates from the Rhizosphere to Inhibit Growth of Ustilago Maydis

4.1.1. Bacteria

Bacterial isolates obtained from the rhizosphere of maize exhibited statistically significant antagonistic activity against *Ustilago maydis*, as evidenced by distinct zones of growth inhibition (Table 2). The inhibition zones ranged from 5.00 mm to 19.00 mm ($p < .0001$). Among the isolates, MB2 (*Serratia* sp.) demonstrated the highest antifungal activity (19.00 mm), followed by NB16 and NB17, both with zones of 16.00 mm.

These findings suggest that the ability to suppress *U. maydis* varies among isolates, likely due to differences in the type and quantity of bioactive metabolites they produce. This observation aligns with earlier studies highlighting the biocontrol potential of rhizosphere-associated bacteria. For example, *Serratia* spp. are known producers of chitinases and other antifungal enzymes capable of degrading fungal cell walls [2]. The strong inhibition observed for *Serratia* sp. in this study supports previous reports and highlights its potential as an effective biocontrol agent. Additionally, several *Bacillus* species are recognized for producing antibiotics and bioactive compounds that inhibit fungal growth.

The current findings are consistent with prior research indicating that *Bacillus amyloliquefaciens* can reduce fungal contamination in post-harvest grains and enhance plant resistance when applied as a seed coating [19-21]. Other isolates, including NB19, NB24, and MB8, showed moderate antifungal activity, with inhibition zones ranging from 14.33 mm to 15.67 mm. In contrast, MB1 (*Enterobacter* sp.) exhibited minimal activity (5.00 mm), significantly lower than the group mean of 9.15 mm. This variation further supports the hypothesis that antagonistic efficiency is linked to the metabolic diversity of bacterial isolates. Comparable conclusions have been drawn in studies involving *Pseudomonas fluorescens* and *Bacillus* spp., where antifungal efficacy differed according to each strain's capacity to produce secondary metabolites such as hydrogen cyanide, siderophores, and lipopeptides [22, 23]. These results are also consistent with observations by Ab Rahman et al. [24] who reported that bacterial biocontrol agents act either directly via antimicrobial secretion or indirectly by inducing systemic resistance in plants.

4.1.2. Fungi

Fungal isolates from the maize rhizosphere exhibited antagonistic activity against *Ustilago maydis*, with inhibition zones ranging from 2.33 mm to 22.00 mm (Table 3). Isolate MF14 demonstrated the strongest inhibition (22.00 mm, $p < .0001$), followed by MF13 (15.00 mm). Moderate activity was observed in MF5 (8.67 mm) and MF3 (7.00 mm), while MF1 (2.67 mm) and MF11 (2.33 mm) were the least effective, both below the group mean (8.46 mm). The high LSD (4.11 mm) and coefficient of variation (28.04%) reflect significant differences among isolates.

The strong activity of MF14 aligns with prior reports on *Trichoderma* and *Aspergillus* spp., which produce antifungal enzymes such as chitinases and proteases [25, 26]. These findings are consistent with studies showing that soil fungi can inhibit plant pathogens [27, 28]. Similar inhibitory effects by *Aspergillus* and *Penicillium* species

against other fungal pathogens further support the potential of maize rhizosphere fungi as biocontrol agents [13, 29].

4.2. In- Vivo Greenhouse Experiment to Evaluate the Effect of Selected Microbial Isolates on Disease Incidence and Severity of Maize Common Smut Disease

4.2.1. Disease Incidence

In the greenhouse experiment, selected rhizospheric microbial isolates NB16 (*Bacillus* sp.), MF13 (*Aspergillus* sp.), MF14 (unidentified fungal isolate), and MB2 (*Serratia* sp.) demonstrated varying levels of effectiveness in reducing the incidence of common smut in two maize varieties: DK 8033 and Duma 43 (Table 4). Control plants treated with distilled water (N-DD and M-DD) showed 100.00% disease incidence, while all microbial treatments significantly reduced disease incidence ($p < .0001$). Isolate MF14 provided the greatest suppression, reducing incidence to 49.30% in DK 8033 and 53.03% in Duma 43 well below the group mean of 73.11%. MF13 followed, with reductions to 65.53% and 63.93%, respectively. NB16 and MB2 showed moderate efficacy, with incidence levels ranging from 66.13% to 84.30%, depending on the maize variety.

These results align with previous findings, such as those by Cheng et al. [27] showing fungal isolates effectively suppressing smut diseases in cereals. The differential efficacy among isolates likely reflects variations in their production of antifungal compounds, including enzymes, siderophores, and secondary metabolites. Prior studies by Dutta et al. [30] have reported that rhizospheric microbes employ diverse antagonistic strategies, such as rapid root colonization, acidification, and antimicrobial secretion. Additionally, *Serratia* spp. are known to inhibit fungal pathogens through antifungal compound production and biofilm formation, further supporting their role in disease management [30].

4.2.2. Disease Severity

The selected microbial isolates NB16 (*Bacillus* sp.), MF13 (*Aspergillus* sp.), MF14 (unclassified fungal isolate), and MB2 (*Serratia* sp.) significantly reduced disease severity caused by *Ustilago maydis* under greenhouse conditions (Table 5). All treatments demonstrated a significant reduction in severity compared to untreated controls, confirming their biocontrol potential. Variations in suppression levels likely reflect differences in the types and quantities of bioactive secondary metabolites produced by each isolate.

These results align with previous studies showing that *Bacillus* and *Aspergillus* spp. mitigate fungal disease severity through the secretion of hydrolytic enzymes such as chitinases, glucanases, and proteases, which degrade fungal cell walls [26, 31]. For instance, *Bacillus subtilis* strains have been shown to suppress maize pathogens through the enzymatic breakdown of pathogenic structures [31] while *Aspergillus* spp. reduce soilborne diseases through both enzymatic and metabolite-mediated mechanisms [26]. Furthermore, the results are consistent with findings in other crops where rhizospheric *Serratia* spp. and fungal isolates reduced disease severity through antimicrobial production and induction of systemic resistance [32].

Collectively, these findings support the role of microbial antagonists as effective and sustainable biocontrol agents in maize disease management.

5. CONCLUSION AND RECOMMENDATION

Maize plant diseases such as common smut remain a significant threat to maize cultivation, affecting both smallholder farmers' food security and their income. This study aimed to evaluate the effect of selected microbial antagonists on the incidence and severity of maize common smut disease. The fungal and bacterial isolates exhibited potential antagonistic activities against the pathogen, *Ustilago maydis*, from the *in vitro* experiment, displaying them as potential microbial biocontrol agents. In vivo studies to control common smut in the greenhouse revealed that the four selected potential antagonists, MF13 (*Aspergillus fumigatus*), MF14 (unidentified), NB16 (*Bacillus sp*), and

MB3 (*Serratia sp*), had varying levels of disease control regarding incidence and severity data recorded. MF13, MF14, NB16, and MB3 may therefore be recommended for use as potential biocontrol agents against *Ustilago maydis* after further exploring their performance on the best inoculation method, formulation, and ecological fitness. Further studies should focus on identifying the most effective antagonistic strain concerning the timing of application, inoculation method, and ecological fitness in an effort to control maize smut disease.

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