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IMPROVEMENT OF BIOCONTROL EFFICACY OF *Stenotrophomonas rhizophila* STRAIN pstu-hort-14 WITH SODIUM BICARBONATE AND LEMONGRASS EXTRACT TO CONTROL ANTHRACNOSE OF MANGO

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Abstract

The study was conducted at the Postharvest and Plant Biotechnology Laboratory, Department of Horticulture, Patuakhali Science and Technology University, Patuakhali, Bangladesh during the period from July to December 2017 to test the potential of sodium bicarbonate and lemongrass extract to increase the biocontrol efficacy of *Stenotrophomonas rhizophilla* strain PSTU-Hort-14. All treatments were arranged in a completely randomized design (CRD) with replications and repeated twice. This bacterium was found to be highly compatible with 20% lemongrass extract and 2% sodium bicarbonate (SBC) or mixture of both. Both of these have suppressive activity against *Colletotrichum gloeosporioides* of mango and could be used as enhancer for biocontrol efficacy of PSTU-Hort-14 during mango storage. The survival and proliferation of PSTU-Hort-14 in mango wounds and on fruit surfaces was not affected by lemongrass extract and SBC throughout the storage period. In addition, PSTU-Hort-14 strain was able to colonize and multiply on the surface of mango fruits. The combination of PSTU-Hort-14 with lemongrass extract and SBC was more effective in controlling the anthracnose disease than PSTU-Hort-14 alone or other treatments including fungicide Dipheniconazol® both in inoculated or naturally infected fruits stored at 12±1ºC and 90±5% RH for 18 and 14 days, respectively. However, this combination offered a greater control by reducing 98.2% of the disease over control in naturally infected fruits at the end of 14 days storage at 12±1ºC and 90±5% RH and six days post ripening at 28 ± 2ºC, which was superior to that found with Dipheniconazol® or other treatments tested.

Keywords: Anthracnose, Biocontrol efficacy, Lemongrass extract, Mango, Sodium bicarbonate.

Introduction

Postharvest pathogens caused significant losses in fruits and they are normally controlled by using synthetic fungicides. Recently, biological control has been developed

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as an alternative to synthetic fungicide treatment and considerable success has been achieved upon utilizing antagonistic microorganisms to control both pre harvest and postharvest diseases (Janisiewicz and Korsten, 2002). Alternatives to chemical control, biological controls often less effective than chemical fungicides especially for postharvest disease management. So, the efficacy of antagonists in controlling postharvest disease must be enhanced (Janisiewicz and Korsten, 2002). In order to enhance biocontrol ability of antagonists against postharvest fungal pathogens, certain strategies, such as supplemented with calcium salts, carbohydrates, amino acids and other nitrogen compounds with antagonists, have been proposed (Tian *et al*., 2001; EI-Ghaouth *et al*., 2000). Organic and inorganic salts are antimicrobial agents against a range of phytopathogenic fungi. Among them, sodium bicarbonate (SBC), commonly known as baking soda (Hadzic *et al*., 2019), which is generally regarded as safe (GRAS) by the United States Food and Drug Administration.

The potential of SBC for the enhancement of biocontrol ability of antagonists has been investigated in controlling postharvest decay. Liu *et al*. (2011) claimed that the performance of SBC combined with *Bacillus subtilis* 11 exhibited better control of pear ring rot disease caused by *Botryosphaeria berengeriana* than that of individual treatments. Smilanick *et al*. (2006) found that the effectiveness of sodium bicarbonate (SBC) and carbonate was improved significantly in the control of citrus green mold when the treatment was followed by the application of the biological control agent *Pseudomonas syringae* Esc 10. Similarly, SBC also increased the bio efficacy of different biocontrol agent to control different postharvest diseases, such as *Candida oleophila* (Lanza *et al*., 2004), and *Bacillus subtilis* (Obagwu and Korsten, 2003).

A novel approach to extend postharvest shelf life is the use of edible coatings of natural antimicrobial compounds is preferred since they pose minimal risk to health (Baldwin *et al*., 1995). Presently, wax coating is practiced for postharvest commodities. Wax coatings are mostly made of synthetic waxes and fatty acids, oils, shellac, emulsifier, plasticizers, antifoam agents, and surfactants (Baldwin, 1994). These coatings are somewhat effective in delaying ripening, but in general, do not prevent decay. Thus, combining lemongrass extract and sodium bicarbonate with *Stenotrophomonas rhizophila* strain PSTU-Hort-14 will make it possible to exploit the antifungal and eliciting properties of these chemicals and biological activity of this bacterium. Therefore, the present study was undertaken to select suitable enhancer (s) that increases the biocontrol efficacy of antagonistic bacteria to suppress the disease.

Materials and Methods

Culture and preparation of conidial suspension of *C. gloeosporioides*

Colletotrichum gloeosporioides, the causal organism of anthracnose of mango was collected and isolated from naturally infected mango fruits. The purified isolate of *C. gloeosporioides* was culture on potato dextrose agar (PDA) at 28 ± 2°C for seven days. The spores were harvested from the cultured and the concentration of conidia in the filtered suspension were adjusted to 5×10^5 conidia ml^{-1} with sterile distilled water using a haemacytometer (Obagwu and Korsten, 2003).

Experimental treatments

Five different concentration (1%, 1.5%, 2%, 2.5% & 3%) of SBC and four concentration (10%, 20%, 30% & 40%) of lemongrass extract were separately evaluated for their efficacy in controlling *C. gloeosporioides*. Both of the concentrations were also used to observe the compatibility with bacterial strain PSTU-Hort-14 (10^8 CFU mL^{-1}). In *in vivo* test four different combinations of bacterial strain PSTU-Hort-14, SBC and lemongrass extract were used..

Preparation of aqueous suspension of bacterial strain PSTU-Hort-14

Fourteen isolates were selected based on their antagonistic activity tested against *C. gloeosporioides* in *In-vitro*. In preparing aqueous antagonist suspension, isolates were grown on nutrient agar media (NA) at 28 ± 2°C for 24 hours. A loop of each culture were then transferred to 250 mL conical flask containing 50 mL of nutrient broth and incubated on a rotary shaker at 150 rpm for 72 hours at 31°C. To enumerate the colony forming units (CFU), cultures were serially diluted in sterile distilled water and plated on nutrient agar. The number of CFU were counted after 48h of incubation at 28 ± 2°C. At the time of use, the suspensions of PSTU-Hort-14 were adjusted to approximately 10^8 CFU ml^{-1} by spectrophotometer standard growth curve.

Preparation of sodium bicarbonate solutions

Solutions of SBC at concentrations of 0 (control, water only), 1, 1.5, 2, 2.5 and 3% (w/v) were used. Different concentrations of SBC were filtered through a 0.45µm pore filter before adding them to the autoclaved PDA. Desired concentration of lemon grass extract solutions were prepared by following Mehedi *et al.* (2020). Biocontrol activity of bacterial strain PSTU-Hort-14 enhanced with sodium bicarbonate and lemongrass extract on mango fruits pre inoculated with *C. gloeosporioides.*

Fruit inoculation and lesion measurement

A total of 108 mango fruits at color stage two (according to MAFC) were surface sterilized with 75% ethanol followed by rinsing with distilled water. Each of the fruit were wounded (3 mm deep and 5 mm diameter) with a sterilized cork borer. Two wounds were made at the mid area of fruit with six centimeters apart. Each of the wound were then inoculated with 50 µL conidial suspension of *C. gloeosporioides* (5×10^5 spores ml^{-1}) and held at 28 ± 2ºC for two hours (Gamagae *et al*., 2003). Each of the 12 inoculated fruits were then dipped for 15 min in i) aqueous suspension of bacterial strain PSTU-Hort-14 (10^8 CFU ml^{-1}); ii) 2% SBC solution and iii) 20% lemongrass extract solution.

In case of combination treatments (i) 12 inoculated fruits were initially dipped in 2% SBC solution for 15 minute and allowed to air dry for five minutes. Then the fruits were immersed in aqueous solution of bacterial strain PSTU-Hort-14 for 15 min. (ii) 12 inoculated fruits were initiallysoaked in bacterial suspension for 15 minute and allowed to air dry for five minutes. then immersed in 20% lemongrass extract solution for 15 min. (iii) 12 inoculated fruits were initially immersed in bacterial suspension for 15 minute and allowed to air dry for five minutes and then immersed in 20% lemongrass extract solution amended with 2% SBC solution for another 15 min. The control treatments consisted of a set of 12 inoculated fruits that were immersed either in sterilized

distilled water or commercial fungicide Difenoconazol® @ 0.50 ml l^{-1} acted as negative and positive controls, respectively. Fruits were allowed to air dry for five min after treatment. Each fruit were wrapped using 70 g offset paper and held at 12ºC and 90±5%RH for 18 days (Rahman *et al*., 2007). Data on lesion diameter were recorded on each alternate day starting from ten days after inoculation. The experiments were arranged with 12 replications and repeated twice.

Biocontrol activity on naturally infected fruits

Fruits selection and sterilization were done as in artificially inoculated fruits. Fruits were allowed to treat as in previously described (fruit inoculation and lesion measurement) without wounding the fruits. Treated fruits were packed and stored for 14 days. At completion of storage time, fruits were removed from storage and ripened with ethylene at room temperature (28 ± 2ºC). Ten mL/L 2% ethylene were placed in sealed polyethylene bag of fruits for 24 hours. Ethylene was then removed by opening the sealed polythene bag and the fruits were allowed to ripen at room temperature for another six days. Data on anthracnose incidence and severity were recorded everyday started on third day of post-storage, when disease symptoms began to appear in ripened fruits. Disease incidence and severity were calculated using formulae and scales (Uddin *et al.*, 2023).

$$\text{Disease incidence (DI) (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

$$\text{Disease Severity (DS)(\%)} = \frac{\Sigma\,(\text{Severity rating} \times \text{number of fruits in that rating})}{\text{Total number of fruits assessed} \times \text{highest scale} \times 100}$$

Experimental design and statistical analysis

All treatments in this study were arranged in a completely randomized design with five replications and experiments were repeated twice. The recorded data on different parameters of the experiment were tabulated and analyzed with following design of experiment (Gomez and Gomez, 1984) adopting a statistical programme MSTAT-C. All the treatment means were calculated and the analyses of variances (ANOVA) of different parameters considered were done by 'F' variance test. The means were separated by Least Significant Difference (LSD) test at 5% level of significance.

Results

Effect of SBC on mycelia growth and spore germination of *C. gloeosporioides*

At 3% SBC treatment the complete inhibition of mycelial growth of *C. gloeosporioides* was found which is similar to 2% and 2.5% SBC treatment but significantly different from 1% and 1.5% SBC treatments (Plate 1). After seven days of incubation increase in concentrations of SBC significantly ($p \leq 0.05$) inhibited the radial growth of *C. gloeosporioides*. These results showed that SBC inhibited the growth of *C. gloeosporioides* . In control no inhibition was observed (Plate 1).

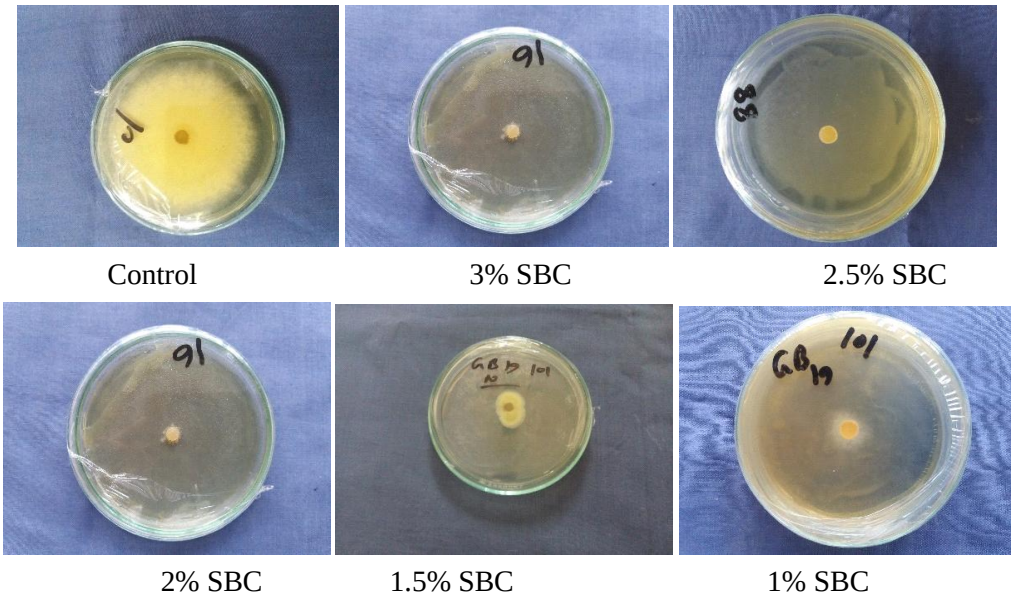


Fig. 1. Effect of different concentrations of SBC on the radial growth of C. gloeosporioides after seven days of incubation at 28 ± 2ºC

The spore germination of *C. gloeosporioides* was significantly lower on PDA plates amended with higher concentrations of SBC compared to control by microscopic observation. On PDA plates treated with concentrations of more than 2% SBC after seven hours of incubation no spore germination was observed. On the contrary, few spores were germinated in 1-1.5% SBC amended plates but numerous were found on the control plates. The results of 2 and 2.5% SBC similar with the results of 3% SBC treatment are shown in the Plate 1.

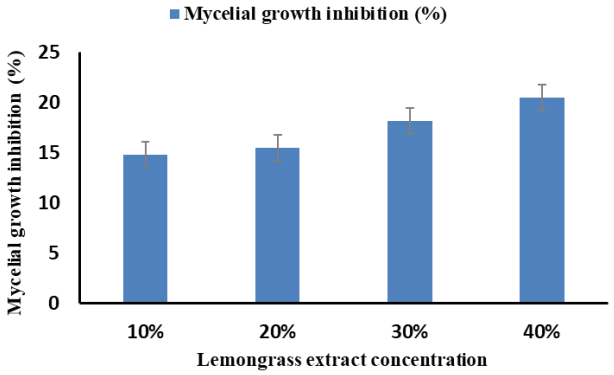


Fig. 2. Effect of different concentrations of lemongrass extract on the percentage inhibition of the radial growth of *C. gloeosporioides* after six days of incubation at 28 ± 2ºC. Each value is the mean of five replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error

Effect of lemongrass extract on mycelia growth and spore germination of *C. gloeosporioides*

At 40% lemon grass extract concentration treatment the inhibition of mycelial growth of *C. gloeosporioides* was recorded in 20.08% whereas in 30% lemon grass solution inhibited the 18.28% mycelial growth and 20% lemon grass extract solution controlled 15.8% mycelial growth which is statistically similar to 10% solution treatment (Fig. 1). With increasing concentrations of lemongrass extract the inhibition of spore germination of *C. gloeosporioides* are decreased. When PDA was amended with 10% of lemongrass extract, the highest inhibition of spore germination (20%) was recorded which was statistically similar with 20% lemongrass extract treatment (Table 1). However, after seven hours of incubation spore germination in control plates was found 100%. After 11 hours, 40% lemongrass extract amended plate showed 2.2% inhibition. After 13 hours of incubation, in all treatment did not show any inhibition on spore germination.. Germination of spores was not totally inhibited by lemongrass extract, only the rate of germination was reduced (Table 1).

Table 1. Effect of different concentrations of lemongrass extract on percentage inhibition of spore germination of *C. gloeosporioides* after 7, 9, and 11 hours of inoculation at 28 ± 2ºC

Time after incubation (hr)	% inhibition of spore germination				
	Control	10%	20%	30%	40%
7	0.00	19.00 a	18.00 a	15.20 a	12.60 a
9	0.00	10.00 b	8.60 b	7.60 b	5.40 b
11	0.00	4.80 c	4.00 c	3.00 c	2.20 c
13	0.00	0.00	0.00	0.00	0.00
LSD(0.05)	ND	1.57	1.68	2.01	1.54
Level of Significance	ND	*	*	*	*
CV%	ND	13.63	16.41	23.26	22.79

**Significant at 5% level of probability, ND= Analysis not done*

Effect of SBC and lemongrass extract on mango fruit colonization by *Stenotrophomonas rhizophila* (PSTU-Hort-14)

On the wounds and surfaces of mango fruit in the presence of lemongrass extract or lemongrass extract amended with SBC the population of the total *Stenotrophomonas rhizophila* (PSTU-Hort-14) was monitored. The concentration of *S. rhizophila* in wounds and on fruit surfaces were 5.44 $\log_{10}$ CFU per wound and 5.55 $\log_{10}$ CFU cm^{-2}, respectively immediately after dipping the fruits in the cell suspension of *S. rhizophila* (PSTU-Hort-14), which were statistically similar with other treatments (Table 2). The population of *S. rhizophila* in wounds markedly increased in all treatments after 18 days of storage at 12ºC and 90±5% RH. However, the population reached the maximum level

of 6.23, 6.67 and 6.77 $\log_{10}$ CFU per wound in wounds treated with *S. rhizophila* - lemongrass extract, the combination of *S. rhizophila* + lemongrass extract + SBC and *S. rhizophila* + SBC, respectively, which were not significantly different from each other (Table 2).

Table 2. Effect of lemongrass extract, SBC and their combination on the populations of *Stenotrophomonas rhizophila* (PSTU-Hort-14) in mango wounds and on surface during storage at 12±1°C and 90±5% RH for 18 days

Treatments	Wound population Log CFU cm^2		Surface population Log CFU cm^2	
	Immediate after treatment	At the end of storage	Immediate after treatment	At the end of storage
Bacteria alone	5.17 d	6.48 c	5.50 c	5.76 a
Bacteria + Lemongrass extract	5.67 a	6.23 d	5.74 a	6.55 c
Bacteria + SBC	5.35 c	6.77 a	5.46 d	6.37 d
Bacteria + Lemongrass extract + SBC	5.44 b	6.67 b	5.55 b	6.70 b
LSD $_{(0.05)}$	0.074	0.060	0.070	0.063
Level of Significance	*	*	*	*
CV%	1.02	0.69	0.94	0.74

**Significant at 5% level of probability*

Similarly, significantly higher population *S. rhizophila* was recorded on fruit surface (6.70 $\log_{10}$ CFU cm^{-2}) in combined treatment of *S. rhizophila* + Lemongrass extract + SBC at the end of the storage period (Table 2). This was statistically similar with *S. rhizophila* + lemongrass extract and *S. rhizophila* +SBC. Therefore, the survival and proliferation of *S. rhizophila* in mango wounds and fruit surface were not affected by lemongrass extract and SBC or its mixture. Moreover, it seemed that coating with lemongrass extract enhanced the multiplication of *S. rhizophila*.

Biocontrol activity of *S. rhizophila* (PSTU-Hort-14) enhanced with SBC and lemongrass extract on mango fruits pre-inoculated with *C. gloeosporioides*

The values of area under disease progress curves (AUDPC) derived that the ability of the treatments to delay the onset of disease symptoms as well as to reduce the disease severity was expressed as the percentage disease reduction. By AUDPC disease development on inoculated fruits was also evaluated. The highest AUDPC was recorded for the water-treated control fruit which was significantly different from other treatments at the end of storage period (Table 3). Whereas, the lowest AUDPC was recorded in pre-inoculated fruits treated with the combination of *S. rhizophila* +lemongrass extract +SBC

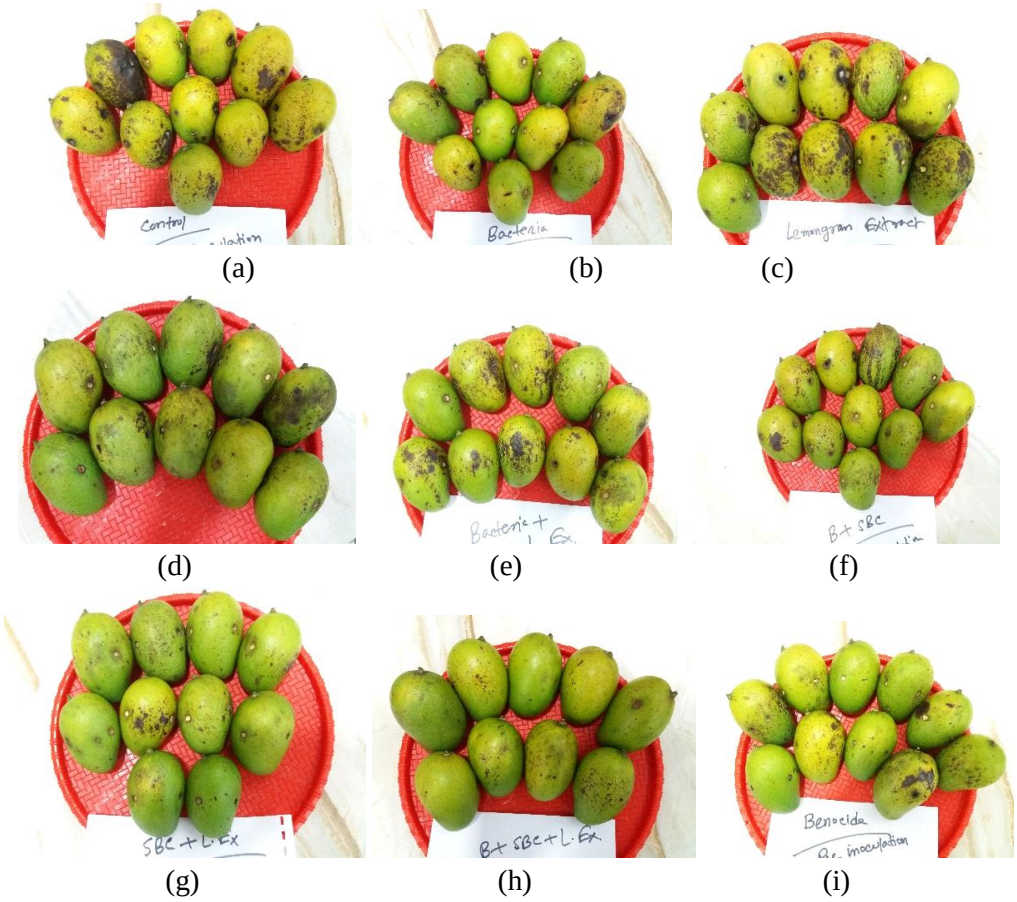
followed by the fungicide Dipheniconazol® treatment, which is statistically different from *S. rhizophila*+ SBC. In respect of AUDPC there was no statistical difference between the treatments of *S. rhizophila* suspension and *S. rhizophila*+ lemongrass extract.

In this study, during the whole storage period the highest lesion expansion rate was recorded for water-treated control fruits, which was statistically different than all treated fruits. The disease reduction over control is a good indicator that reflected the progress of disease over time. Significantly lower rate of lesion expansion was recorded in fruit treated with Dipheniconazol® followed by *S. rhizophila* +SBC treatment. The combination of *S. rhizophila* + lemongrass extractwas statistically similar with *S. rhizophila* treated fruits. However, anthracnose symptoms did not develop on inoculated fruits subjected to the combination of *Stenotrophomonas rhizophila* +lemongrass extract +SBC during the whole storage period. Thus, there was no lesion expansion for this treatment (Table 3).

Table 3. Effect of *Stenotrophomonas rhizophila*, lemongrass extract, SBC and their combinations on the reduction of anthracnose disease in pre-inoculated mango fruits stored at 12ºC for 18 days

Treatments	AUPDC (cm^2)	Lesion expansion rate (mm day^{-1})	Disease reduction over control (%)
Control	30.75 a	5.30	--
Lemongrass extract	25.36 b	3.01	20.16 h
SBC	15.78 c	3.45	40.21 g
SBC + Lemongrass extract	12.43 d	3.00	50.43 f
Bacteria	7.49 e	2.61	70.11 e
Bacteria + Lemongrass extract	4.42 f	2.50	71.60 d
Bacteria + SBC	3.70 g	1.71	89.15 c
Dipheniconazol® (0.5 mlL^{-1})	0.09	0.21	98.00 b
Bacteria + Lemongrass extract + SBC	0.00	0.00	100 a
$LSD_{(0.05)}$	0.089	--	0.720
Level of Significance	*	NS	*
CV%	0.38	133.23	0.57

*Significant at 5% level of probability, NS= Non significant



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Fig. 2. Effect of Stenotrophomonas rhizophila, sodium bicarbonate (SBC 2%) and Aloe vera (lemongrass extract 20%) and their combinations on the lesion diameter of anthracnose caused by C. gloeosporioides in pre-inoculated mango fruits stored at 12±1°C and 90±5% RH for 18 days. Two hours after inoculation, fruits were dipped in (a) sterilized distilled water (control), (b) cell suspension of Stenotrophomonas rhizophilan (1×108 CFU mL-1), (c) lemongrass extract (20%), (d) Sodium bicarbonate (SBC) solution (2%), (e) lemongrass extract (20%) + Stenotrophomonas rhizophila, (f) SBC (2%) + Stenotrophomonas rhizophila, (g) SBC (2%) + lemongrass extract (20%) (h) Stenotrophomonas rhizophila + 2% SBC amended with 20% lemongrass extract and (i) Dipheniconazol® (0.50 mlL-1). Dipping time in each solution/suspension was 15 min. Photographs on lesion development were taken after 14 days of inoculation.

This study showed that the combination of *S. rhizophila* +-lemongrass extract +SBC treatment was the most effective treatment in suppressing the anthracnose disease on pre-inoculated fruits followed by Dipheniconazol® in terms of disease reduction over the control. Pre-inoculated fruits treated by the combination of *S. rhizophila* +lemongrass extract +SBC resulted in complete control (100%) of the disease. The lowest disease reduction (20.16%) was recorded in lemongrass extract treatment compared to water-treated control (Plate 2).

Biocontrol activity on naturally infected fruits

Disease incidence

With ripening period the incidence of the disease gradually increased. The naturally infected fruits subjected to the combination of *S. rhizophila* +lemongrass extract +SBC showed significantly lowest anthracnose incidence than in fruits dipped in *S. rhizophila* suspension, lemongrass extract +SBC and Dipheniconazol®. At the third and fourth day of shelf period, water-treated control fruits showed anthracnose spots and the disease incidence increased to 65 and 100% after 14 days and 18 days of storage respectively (Figure 2). At the end of the ripening period the lowest disease incidence (12%) was recorded in fruits subjected to the combination of *S. rhizophila* +lemongrass extract +SBC treatment, followed by 2% SBC + *S. rhizophila* (50%). At the end of the post storage period disease incidence recorded in fruits treated with *S. rhizophila* suspension, Dipheniconazol® and lemongrass extract + SBC were 35, 20 and 35% after three days of ripening, and increased gradually to 70, 45 and 75%, respectively (Fig. 2).

The data of present study showed that the combination of *S. rhizophila* with lemongrass extract + SBC was not only effective in reducing the disease incidence but also delayed the onset of anthracnose infection. This treatment delayed the anthracnose appearance on fruits by four days compared to water-treated control fruits. Anthracnose incidence was only noticed after five days of ripening on a few fruits (10%), which did not increase until the end of the ripening period.

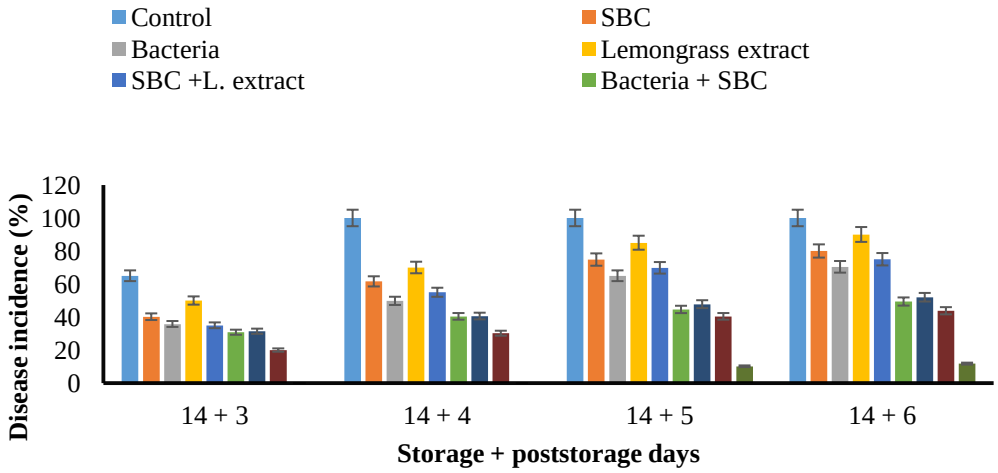


Fig. 2. Effect of *Stenotrophomonas rhizophila*, sodium bicarbonate (2% SBC) and lemongrass extract (20% lemongrass extract) and their combinations on the incidence of anthracnose in naturally infected mango fruits stored at 12ºC for 14 days and six days post storage under ambient temperature (28 ± 2ºC). Each value is the mean of twelve replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error.

Disease severity

In all fruit treatments, disease severity was significantly ($P \leq 0.05$) lower compared to water treated control fruits during 14 days of storage and six days post ripening at 28 ± 2°C (Figure 3).. The combination of *S. rhizophila* +lemongrass extract +SBC was the most effective treatment, which showed significantly lower anthracnose severity on naturally infected fruits than in fruits dipped in *S. rhizophila* suspension, lemongrass extract + SBC and Dipheniconazol®.

The combined treatment *S. rhizophila* + lemongrass extract + SBC reduced the disease severity with complete absence of symptoms until 14 days of storage at 12ºC and four days of post storage period. At the end of post storage period, disease severity was recorded as 2.15% for the fruits subjected to the combination of *S. rhizophila* + lemongrass extract + SBC. In contrast, at the end of post storage period anthracnose symptoms appeared in water-treated control fruits at the end of the 14 days storage and disease severity increased gradually reaching 84.88%.

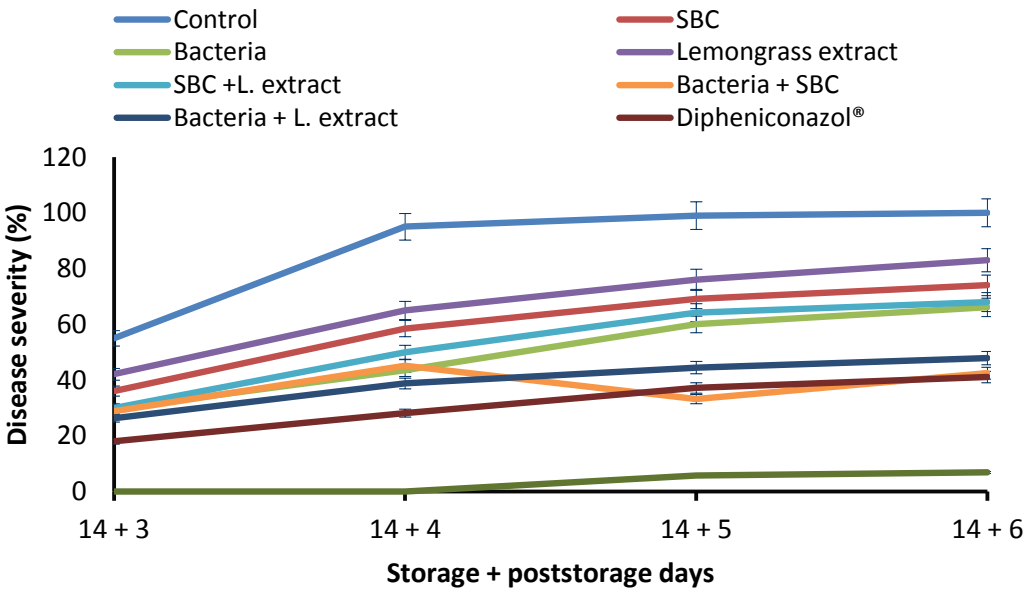


Fig. 3. Effect of *Stenotrophomonas rhizophila*, sodium bicarbonate (2% SBC) and lemongrass extract (20%) and their combinations on the severity of anthracnose in naturally infected mango fruits stored at 12ºC for 14 days and six days post storage under ambient temperature (28 ± 2ºC). Each value is the mean of eight replications. Means were separated by Least Significant Difference (LSD) test at 5% level of significance. Vertical bars represent standard error

From the AUDPC values derived that the efficacy of treatments to delay the onset of disease symptoms as well as to reduce the lesion expansion rate was expressed as percentage disease reduction. The AUDPC was also calculated to evaluate the treatment efficacy against disease progress over time. At the end of storage and post

storage period, the highest AUDPC was recorded in water-treated control fruits and the lowest value was found in fruits subjected to combination of *Stenotrophomonas rhizophila* +lemongrass extract + SBC. AUDPC of Dipheniconazol® was was statistically similar with the treatment combination of lemongrass extract-*S.s rhizophila* and SBC-*S. rhizophila*.

Table 4. Effect of *Stenotrophomonas rhizophila*, 2% sodium bicarbonate (SBC), lemongrass extract or their combinations on the reduction of anthracnose disease in naturally infected mango fruits stored at 12±1°C for 14 days and six days ripening under ambient temperature (28 ± 2°C)

Treatments	AUPDC (cm^2)	Lesion expansion rate (mm day^{-1})	Disease reduction over control (%)
Control	72.50 a	19.90 a	--
Lemongrass extract	51.30 b	13.73 b	30.19 h
SBC	44.54 c	11.50 c	40.30 g
SBC + Lemongrass extract	36.40 d	9.52 d	53.05 f
Bacteria	13.48 e	3.80 e	84.73 e
Dipheniconazol® (0.5 mL^{-1})	9.54 f	3.14 g	88.04 b
Bacteria + Lemongrass extract	9.14 g	2.85 h	85.52 d
Bacteria + SBC	10.27 h	3.26 f	85.71 c
Bacteria + Lemongrass extract + SBC	2.21 i	0.54 i	98.70 a
LSD(0.05)	0.730	0.509	1.879
Level of Significance	*	*	*
CV%	1.26	3.22	1.43

**Significant at 5% level of probability*

The rate of lesion expansion was significantly higher in water-treated control than other treatments and the lowest rate was recorded in fruits subjected to the combination of *S. rhizophila* + lemongrass extract + SBC (Table 3) almost similar trend was observed among the treatments on the lesion expansion rate in water-treated control fruits. The lesion expansion rate of Dipheniconazol®, lemongrass extract + *S. rhizophila*, SBC + *S. rhizophila* treatments were statistically similar.

In the present study results showed that the combination of *S. rhizophila* + lemongrass extract + SBC was the most effective treatment in controlling anthracnose disease in naturally infected mango fruits. In treated fruits disease severity was reduced by 98.70% at the end of 14 days storage and six days post storage period. The level of disease control obtained was superior to that obtained with Dipheniconazol® treatment. The lowest disease reduction was recorded in fruits treated with 30.19% lemongrass extract solution followed by SBC and SBC+lemongrass extract (Table 4).

Plate 3. Effect of *Stenotrophomonas rhizophila*, sodium bicarbonate (SBC 2%) lemongrass extract (20%) and their combinations on the incidence and severity of anthracnose in naturally infected mango fruits stored at 12±1°C and 90±5% RH for 14 days and six days under ambient temperature (28 ± 2ºC). (A) Sterilized distilled water (control), (B) cell suspension of *Stenotrophomonas rhizophila* (1×10^8 CFU mL^{-1}), (C) lemongrass extract (20%), (D) Sodium bicarbonate (SBC) solution (2%), (E) lemongrass extract (20%) + *Stenotrophomonas rhizophila*, (F) SBC (2%) + *Stenotrophomonas rhizophila*, (G) SBC (2%) + lemongrass extract (20%) (H) *Stenotrophomonas rhizophila* + 2% SBC amended with 20% lemongrass extract and (I) Dipheniconazol® (0.50 mlL^{-1}). Dipping time in each solution/suspension was 15 min. Photographs on anthracnose incidence and severity were taken after 14 days + 3 days under ambient temperature, when symptoms were developed on fruit surfaces

Discussion

Findings of this study showed that, postharvest application of *S. rhizophila*, SBC and lemongrass extract alone in artificially inoculated and naturally infected mangos resulted in a significant reduction of anthracnose severity compared to water-treated

fruits. Moreover, it was shown that *S. rhizophila* was effective as a postharvest dip treatment on wound invaders and in latent infection of mango caused by *C. gloeosporioides*. These findings are in agreement with Obagwu and Korsten (2003), who reported that combinations of SBC and *S. rhizophila* was very effective in controlling green and blue molds of citrus and superior to either treatment alone. Similarly, in postharvest treatment, *S. rhizophila* combined with 2% SBC significantly reduced the ring rot of pear (Liu *et al.*, 2011). However, the addition of 2% SBC amended with 20% lemongrass extract to *S. rhizophila* created friendly environment. This is supported, in part, by its higher performance on artificially inoculated and naturally infected mango. This combined treatment effectively reduced the postharvest anthracnose during storage by delaying the onset of infection and slowed down the infection process.

The combination of biocontrol activity of *S. rhizophila*, antifungal property of SBC, and improved micro environment by lemongrass extract resulted in improved consistency and efficacy of disease control. The observed preventive and curative activities of the combination of *S. rhizophila* + SBC + lemongrass extract showed the synergy between the antagonist and SBC-lemongrass extract. In addition, in artificially inoculated fruits, treatment with the combination showed to confer a level of disease control equivalent to the fungicide Dipheniconazol®. However, it was superior to Dipheniconazol® when applied on naturally infected mango. These findings are in agreement with Obagwu and Korsten (2003), who found that the level of disease control provided by *S. rhizophila*, when applied alone, was inferior to control provided by fungicide 'fungazil', but it was equivalent to fungicide 'fungazil' when *S. rhizophila* applied with 2% SBC. Similar trend of anthracnose control in mango was also achieved with the combined application of 2% sodium bicarbonate in wax formulation and *Candida oleophila* (Gamagae *et al*., 2004).

Performance of Dipheniconazol®, when applied on naturally infected mango, was lower compared to the combined treatment *S. rhizophila* + SBC + lemongrass extract. The lower performance of fungicides might be due to the quiescent infection of mango caused by *C. gloeosporioides*. Being a quiescent infection, anthracnose is difficult to control by postharvest treatment (Janisiewicz and Korsten, 2002; Yakoby *et al.*, 2001), even along with synthetic fungicides (Ippolito *et al.*, 2004). This is because the infecting hyphae are protected once the pathogen has penetrated the plant cuticle (Yakoby *et al.*, 2001).

The mechanism by which SBC, lemongrass extract and *S. rhizophila* (PSTU-Hort-14) integrated to control *C. gloeosporioides* is not fully understood. This might be happened by multi-layer protection of fruits created by lemongrass extract, SBC and *S. rhizophila* (PSTU-Hort-14). Firstly, lemongrass extract produces a bio-edible-coating, which reduce the transpiration and respiration and maintain the firmness of fruits (Mart´ınez-Romero *et al*., 2006). As a result, fruit remain fresher, which indicate a certain level of self-defense from pathogen. Secondly, SBC is fungistasis in nature (Davide *et al*., 2004), so it can give short period protection from fungal pathogen. Since, *S. rhizophila* (PSTU-Hort-14) isolated from mango surface so, it can be attached and multiplied on the surface of mango. So, whenever *C. gloeosporioides* germinate after the action of SBC, *S. rhizophila* (PSTU-Hort-14) will imposed further action on *C. gloeosporioides*.

Conclusion

Based on the experimental data, the biocontrol efficacy of *Stenotrophomonas rhizophila* strain PSTU-Hort-14 against mango anthracnose was significantly enhanced by the incorporation of 20% lemongrass extract supplemented with 2% sodium bicarbonate (SBC). This combined treatment demonstrated superior disease suppression compared to PSTU-Hort-14 alone or its combinations with either lemongrass extract or SBC individually. Notably, it outperformed even the chemical fungicide Difenoconazole® in controlling anthracnose. Given its enhanced efficacy and eco-friendly nature, the integrated use of *S. rhizophila* PSTU-Hort-14 with lemongrass extract and SBC presents a promising alternative to chemical fungicides. It is recommended that this biocontrol formulation be further evaluated under field conditions and developed as a sustainable management strategy for postharvest anthracnose in mango.

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Author's Contribution

M.J.U.: Conceptualization, methodology, investigation, data curation, formal analysis, resources, software, validation, visualization and writing – original draft. M.R & M.F.H.: Concept, planning, supervision, validation, resources, funding acquisition, project administration, and reviewed. M.N.H.M: Methodology, data curation, formal analysis, software, reviewed, revised and edited the content. U.R.T.: Methodology, investigation, data curation, statistical analysis. S.M.A.I: Methodology, validation, data curation, statistical analysis and writing – review & editing. All authors equally contributed to the preparation of the manuscript and have approved the final version for submission.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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