Efficacy of Selected Bacterial Antagonists (*Burkholderia* sp.) in Managing *Colletotrichum truncatum* Infection of Chilli Fruits

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Abstract
Anthracnose disease causes high yield losses in chilli mainly due to postharvest fruit decay. The disease affects fruit yield and quality; therefore, control measures have to be applied at the correct time. In a new paradigm shift from synthetic fungicides to a safer and environmentally friendly alternative for managing post-harvest decays in fruits and vegetables, efficacy of some selected antagonistic *Burkholderia* species against anthracnose fruit decay was assessed in this study. Chilli fruits at different fruit growth stages, green (21 days after fruit setting), colour breaking (28 days after fruit setting) and red (35 days after fruit setting) stages were used for the assay in order to find out the most suitable stage to apply these antagonists to prevent anthracnose disease development. Detached chilli fruits of the three growth stages were first treated with overnight broth cultures of the antagonists and incubated for 24 hours in a humid chamber. Inoculation with pathogen was done 24 hours after incubation by using both wounding and non-wounding methods. Recovery from anthracnose was effective at both green and colour breaking stages of chilli fruits. Fruits at colour breaking stage showed a remarkable disease recovery in response to applied bacterial antagonists both in wounded (32.52% - 80.97%) and non- wounded (100%) inoculations. Green stage chilli fruits also showed a significant disease recovery (at $p<0.05$ level) ranging from 19.58% - 100% in non-wounded inoculation. Red chilli fruits showed a rotting tendency when treated with antagonists, implying that, this fruit growth stage is not appropriate in applying antagonists as a control measure to prevent anthracnose. Therefore, harvesting period of chilli is the most appropriate time to treat fruits with these antagonists in order to prevent postharvest anthracnose incidence. Results of this study prove the suitability of application of these antagonists at the colour breaking stage of chilli to effectively manage postharvest anthracnose fruit decay.

Keywords: Antagonists, Anthracnose, *Burkholderia* sp., Chilli fruits, Post-harvest
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Introduction

Chilli anthracnose is a destructive postharvest disease which causes heavy yield losses in tropical and subtropical regions. It mainly diminishes the fruit quality and thereby its marketability. Therefore, overcoming this disease is a worthwhile proposition in view of economic losses incurred by this.

Developing fruits in the field (preharvest) and mature fruits during storage (postharvest), can both be infected by the causal agent, *Colletotrichum truncatum* (Ranathunge *et al.*, 2012). The ability of this pathogen to cause latent or quiescent infections has led this disease mainly to be a postharvest disease. Control measures can be effectively applied at pre harvest stages to manage the initial inoculum. However, proper postharvest practices can slow down the disease development and symptom appearance, which leads to rapid fruit decay. Because of the easiness of operation, provision of effective, but safe postharvest treatments are applicable during or just before storing.

The widely known control measure in post-harvest fruit decays is the use of synthetic fungicides such as iprodione. In place of hazardous agrochemicals, biological control is gaining increasing popularity as a reliable alternative. Many studies have tested the efficacy of biocontrol active microorganisms on postharvest fungal pathogens, with useful results to reduce losses in fruits and vegetables during postharvest and storage periods (Janisiewicz and Korsten, 2002). Recently, postharvest application of antagonistic microorganisms is becoming popular and appears to be better for controlling postharvest diseases of fruits and vegetables.
In this study the effectiveness of eight selected bacterial antagonists of *Burkholderia* sp. On

C.truncatum infection in different chilli fruit growth stages was assessed.

Materials and methods

Bacterial antagonists

Eight bacterial isolates, isolated from rhizosphere soil from a forest floor and compost and screened for their antagonistic properties against *Colletotrichum truncatum* (Sandani *et al.*, 2014, 2015) were used in this study.

Pathogen

C. truncatum was isolated from infected chilli fruits collected from Akuressa area in Sri Lanka. Collected chilli fruits were surface sterilized and the pathogen was aseptically isolated. Resulted *C. truncatum* was verified through microscopic observations according to literature. The isolate was maintained on PDA slants at 4 ºC.

Effect of bacterial antagonists at different growth stages of chilli fruits

Treatment with antagonists

Thirty six detached chilli fruits (var MI-2) from each green (21 days after fruit setting (DAFS)), colour breaking (28 DAFS) and ripened stages (35 DAFS) were surface sterilized with 5% sodium hypochlorite and sterilized distilled water. Four sets of chilli fruits at each growing stage were dip inoculated in each of the overnight broth cultures of the antagonists (10^8cells/ml). Treated fruits were air dried within the lamina air flow and all the fruits were then incubated in a humid chamber (at 28 ºC, 95% - 100% RH, under dark conditions).

Inoculation of fruits with the pathogen

Twenty four hours after incubation, 16 fruits were spot inoculated with 10^6 spores/ml spore suspension of *C. truncatum* by wounding while another set of 16 treated fruits were spot inoculated with 10^6spores/ml without wounding. Four chilli fruits which were not treated with antagonists also were spot inoculated with *C. truncatum* spore suspension by wounding and without wounding as controls. These chilli fruits were incubated in a humid chamber (at 28 ºC, 95%-100% RH, dark conditions) for 10 days and observed for development of symptoms. Lesion diameter of each was measured and lesion area and percent inhibition of lesion development (PILD) were calculated.

Percent inhibition of lesion development was determined using the following formula.

$$PILD = \frac{L1 - L2}{L2} * 100$$

Where,

L1= Average lesion diameter on control fruits

L2= Average lesion diameter on antagonists treated fruits

The experiment was done in completely randomized design with four replicates. The experiment was repeated twice for accuracy.

Statistical analysis

Collected data were subjected to ANOVA procedure and the means were compared by Duncan Multiple Range Test in SAS 9.1.3 software.

Results and discussion

Effect of bacterial antagonists at different growth stages of chilli fruits

Out of the three different growth stages of chilli fruits studied, anthracnose recovery from these bacterial antagonists was 100% effective at the colour breaking stage (Table 1). In green stage also a significant disease recovery (at $p<0.05$) was observed (Table 2). Ripened chilli fruits tended to be rotten when treated with the antagonists.

Table 1: Average area of lesions resulted on chilli fruits at colour breaking stage and Percent Inhibition of Lesion Development (PILD) after 10 days of incubation.

Treatment	Wounded		Non-wounded	
	Average Lesion Area (mm^2)	PILD (%)	Average Lesion Area (mm^2)	PILD (%)
F2	225.00	32.52^{e}	0.00	100^{a}
F35	30.10	80.97^{a}	0.00	100^{a}
F65	54.70	60.17^{c}	0.00	100^{a}
F79	93.15	43.36^{d}	0.00	100^{a}
F80	94.34	55.75^{c}	0.00	100^{a}
C31	95.95	69.02^{b}	0.00	100^{a}
C39	206.14	39.15^{d}	0.00	100^{a}
C54	44.58	44.69^{d}	0.00	100^{a}
Control	426.00	0^{f}	75.55	0b

Values in a column followed by same letter are not significantly different at $p<0.05$ level

Non wounded inoculation gave promising results compared to wounded inoculation. Even though a complete disease recovery couldn't be realized, the control of anthracnose disease was significantly successful in all the treatments in wounded inoculation (Table 1). As shown in Table 1, all the antagonists could control the anthracnose disease in spite of the non-wounded inoculation of *C. truncatum*, indicating the possibility of usage of these bacterial strains

as coatings in chilli fruits at their colour breaking stage to prevent the postharvest decay due to anthracnose.

Table 2: Average lesion area and Percent Inhibition of Lesion Development (PILD) on green chilli fruits after 10 days of incubation

Antagonist	Average Lesion Area (mm^2)	PILD (%)
F2	0	100[a]
F35	0	100[a]
F65	29.47	49.48[c]
F79	0	100[a]
F80	21.65	56.70[b]
C31	92.92	19.58[d]
C39	0	100[a]
C54	0	100[a]
Control	115.51	0[e]

Values in a column followed by same letter are not significantly different at $p<0.05$ level

This study revealed the high efficacy of the eight selected *Burkholderia* strains in managing *Colletotrichum truncatum* infection on chilli fruits. Previous studies have shown that the same antagonistic strains were highly effective opponents of the fungus in *in vitro* growth inhibition (Sandani *et al.*, 2014, 2015), spore germination inhibition (Sandani *et al.*, 2015), minimizing seed colonization (Sandani *et al.*, 2016) and avoiding foliar infection (Sandani *et al.*, 2016). Apart from that the applicability of these antagonists in managing fruit infection is a key factor of importance as the most severe economic damage is caused by anthracnose fruit decay in chilies. Furthermore, application of biocontrol measures could be more effective at postharvest stages.

According to the results of this study, a remarkable disease recovery from all the eight tested antagonists could be observed at the colour breaking stage of chilli fruits (Table 1). This implies that the chilli fruits can successfully be treated with the antagonists just after harvesting to achieve the best anthracnose management. Treatment of antagonists at mature green stage will also assist in reducing the postharvest decay as antagonists can colonize the fruit surface and prevent the disease incidence caused by this quiescent pathogen.

Control of postharvest fruit decays with the help of microbial antagonists is an effort taken by many scientists over several decades. Adikaram *et al.* (2002) has used *Aureobasidium pullullans* in several ways to control grey mold of strawberry fruits. In a review by Abano and Sam-Amoah (2012) the control of fruit decay by microbial antagonists is due to complex interactive mechanisms which include direct parasitism, antibiotic production, production of lytic enzymes, competition for nutrients, induced resistance *etc.* The mechanism exerted by these antagonists may be one or several of these. The exact mechanism underlying a particular host pathogen system is needed to be investigated thoroughly in further studies.

The findings of this preliminary study is required to be followed up by further studies considering the standard guidelines in developing this application to a commercial proposition. Application of these antagonists in recovering from anthracnose would be a noteworthy green approach in striving to eradicate hazardous chemical usage.

Conclusion

The selected eight *Burkholderia* antagonistic bacterial strains are effective in managing chilli anthracnose disease and the most appropriate stage of chilli fruits to apply these antagonists is their colour breaking stage for a complete recovery from the disease.

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