Control of Post-Harvest Disease (Stem End Rot) of Rambutan and Annona Species by Using a Bio-Control Agent (*Trichoderma* spp.)

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Introduction

Recent studies have shown the importance of products of underutilised fruits, such as jams, juices and candied fruits to nutrition, income generation and poverty reduction of small-scale entrepreneurs in developing countries (AZAM-ALI, 2004). Underutilised tropical fruits such as rambutan *(Nephelium lappaceum*) and annona (*Annona* sp.) provide important contributions to small-holder livelihoods. However, heavy post-harvest losses significantly reduce the full potential for income generation.

The Industrial Technology Institute (ITI) , Sri Lanka, is involved in developing biological non chemical methods for control of post-harvest diseases of tropical fruits as well as working together with the International Centre of Underutilised Crops on promising underutilised species, such as rambutan, annona and woodapple. The project presented here was part of this research programme and was intended to contribute toward the larger national effort.

Botryodiplodia sp. is known to cause stem end rot disease on many fruits and is listed as a major pre- and postharvest disease for rambutan (SIVAKUMAR et al., 1997) and *Annona* species (DE Q. PINTO, 2005). The effectivness of *Trichoderma* sp. as biological control agents (BC-agents) against certain diseases has been proved in a number of studies (SIVAKUMAR et al., 2000; BARBOSA et al., 2001; WANTOCH-REKOWSKI, 2004).

This study established that BC-agents were readily available in Sri Lanka. The bio-assays conducted proved the effectiveness of the BC-agents against *Botryodiplodia* sp.. Cross inoculation studies also indicated that pathogens isolated from fruits of different species were capable of causing disease in fruits other than the original host from which they were isolated.

Material and method

Bio-assay:

Petri-dishes containing 15ml sterile PDA-Medium (Potato-Dextrose-Agar) were used for the Bio-assay experiment. Four wells were cut in the outer area of the plate by useing a sterile cork-borer (No. 5). The wells were filled with 0.8ml spore suspension of the BC-agent and a mycelial disk (taken from a seven-day-old pathogen culture using a cork-borer (No. 5)) was placed in the middle of the plate. All plates were held in an incubator at 28°C. Five replicates were observed for each pathogen and the control, where in the latter case wells were filled with sterile destilled water.

Observations were recorded daily for seven days by measuring the diameter of the pathogens mycelial growingbetween the wells.

Cross-inocculation:

After a surface sterilization of the fruits by dipping them for 5min in a 5% sodium hypochlorite solution, wells were cut into the fruit near the stem and a seven day old mycelial disk was inserted. The fruits were held in humidity chambers for five days. Ten fruits were used per pahogen and the two control treatments. In control 1 the wells were filled with 1ml sterile destilled water. In control 2 fruits were only subjected to surface sterilization without any futher treatment.

The percentage serverity of infection was recorded daily and the experiment was replicated twice.

Results

Isolates:

Twelve isolates of *Trichoderma* were obtained from six different soil-sources and eight isolates of the pathogen from diseased fruits.
One pathogen from each fruit was taken for futher experiments.

Bio-assay:

The bio-assay experiment showed that three of the Trichoderma isolates have a high, four have moderate and three have low biocontrol activity (Table 1, Figure 1) while three isolates observed to be non effective. Two of the *Trichoderma* strains with a high BC-activity were isolated from rambutan orchards in Warakapola and the third was found in a soil-sample, taken from the sourrounding area of an *Annona muricata* tree in Wijayapura.

None of the isolates taken from *Annona squamosa* sources showed high BC-activity, while one was moderately effective against the pathogens.

Code	Source	BC-activity
Tra I	Urapola	++
Tra II	Warakapola	+++
Tra III	Warakapola	+++
Tsq I	Matale	0
Tsq II	Matale	+
Tsq III	Wijayapura	++
Tsq IV	Medawachchiya	0
Tmu I	Galagedera	++
Tmu II	Galagedera	++
Tmu III	Galagedera	0
Tmu IV	Matale	+
Tmu V	Wijayapura	+++

Table 1: Overview of the Trichoderma strains and their bio-control (BC) activity (+++=high BC activity, ++=moderate BC activity, +=low BC activity, 0=non BC activity)

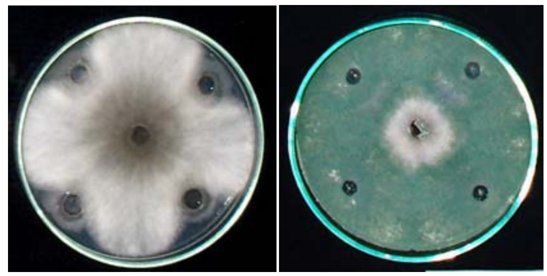

Figure 1: Trichoderma strain without (left) and with high (right) bio-control activity

Cross-inoculation experiment:

The pathogens which were cross-inoculated to the rambutan fruits showed an equal serverity after the third day. On the first two days the severity that was caused by Bmu I pathogens was a little higher (Figure 2).

The cross-inocculaton experiment with Annona muricata fruits did not lead to satisfacory results. The reason being that the serverity of infection in the two controls were the same or higher to the serverity caused by the pathogens. A statistically significant higher severity between Bmu I and Bsq III compared to the others can be seen at the fith day (Figure 3).

The variability in the cross-inoculation experiment with *Annona squamosa* fruits was high; nevertheless we can conclude that the pathogens of the various fruits do infect the others (Figure 4).

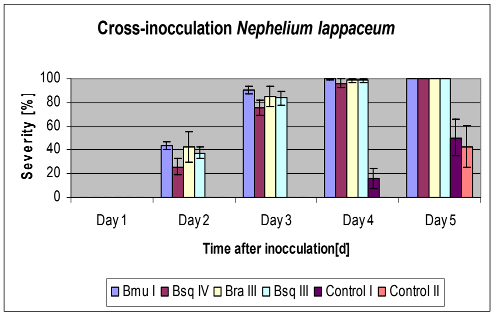

Figure 2: Results of cross-inoculation experiment with *Nephelium lappaceum*

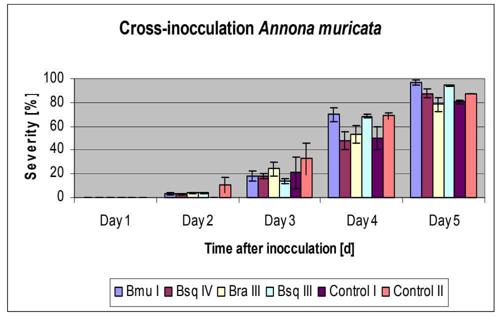

Figure 3: Results of cross-inoculation experiment with *Annona muricata*

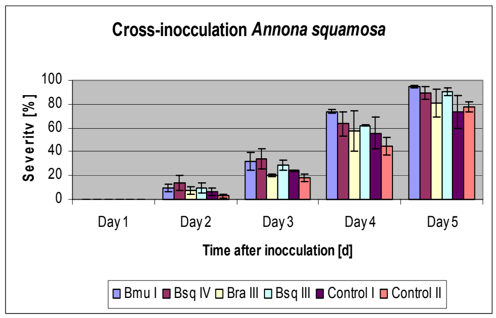

Figure 4: Results of cross-inoculation experiment with *Annona squamosa*

Conclusion

The study shows that there is a ready availability of *Thrichoderma* strains in Sri Lanka and that some of these strains have biocontrol effectivities. SIVAKUMAR et al. (2000) isolated *Trichoderma harzianum* from rambutan orchards in Sri Lanka and proved its antogonistic effect against *Botryodiplodia theobromae.*

The *Trichoderma* strains isolated from soil-samples from rambutan plantations in this study had good BC-activity compared to other strains of this BC-agent. However only Tmu V a *Trichoderma* strain which was isolated from soil surrounding a *Annona muricata* tree could completely control the pathogen Bsq IV isolated from a red *Annona squamosa* fruit.

All pathogens which were isolated from various fruits are able to infect all fruits. This result shows that cross infection could occur between rambutan and annona in the field and during transportation and storage.

Future research in this area should include in vivo studies on the effectiveness of the *Trichoderma* strains as BC-agents. This could be done by introducing the BC-agents into the soil of rambutan or annona plantations or by dipping the fruits into a solution of the BC-agents after havest. The effect of *Trichoderma* against other microorganisms especially against those that are beneficial tocrops should also be investigated.

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