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Host Specificity of *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* Isolates from Mango, Papaya and Rambutan and their Response to *Trichoderma harzianum*

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Abstract

Anthraxnose (*Colletotrichum gloeosporioides*) and stem end rot (*Botryodiplodia theobromae*) are the two most prevalent diseases that contribute significantly to post harvest loss of papaya, mango and rambutan in Sri Lanka. The problem is compounded by the home garden system of horticulture practised in the country. The objective of this study was to test the ability of these pathogens to cause disease by cross infection between crops and to provide information that would facilitate an integrated non chemical means of controlling post harvest loss due to disease. Thus *C. gloeosporioides* and *B. theobromae* were isolated from respective disease carrying mango, papaya and rambutan fruits. Pure cultures of each isolate were maintained on Potato Dextrose Agar at 28°C. The antagonistic effect of a local isolate of *Trichoderma harzianum* was tested via in vitro bioassays against the above isolates. Host specificity trials were conducted on mango (var. Karthakolomban) and papaya (var. Red Lady) at the 10% — 25% stage of maturity and rambutan (cv. Malwana special selection), at full ripe stage. Cross inoculation potential of isolates was confirmed by testing the ability of the respective organisms to produce characteristic disease symptoms when inoculated onto each of the above host tissue. Lesion diameter was recorded over 5 days with fruits incubated at 28°C ± 2°C. While the three *C. gloeosporioides* isolates produced disease lesions on all hosts, respective isolates were observed to produce larger lesions (diameter 2.9cm-5.8 cm) on their original host compared with the alternate hosts (diameters 1.1 cm — 2.6 cm). However, the three *B. theobromae* isolates were equally effective in causing stem-end rot on the three hosts examined. *T. harzianum* was observed in vitro to be antagonistic to all isolates of the respective anthracnose and stem end rot causing pathogens.

Introduction:

B. theobromae and *C. gloeosporioides* cause heavy postharvest loss in Sri Lanka. *B. theobromae* has an exceptionally large number of host plants around the world as it is able to attack non specific enzymes, which affect substances and processes, found commonly in plants (Ragab et al., 1971). In previous studies on cross inoculation potential of *C. gloeosporioides*, isolates from avocado and mango showed larger lesions on their original hosts while all isolates tested produced lesions on all other hosts except citrus (Sanders G. M. and Korsten L., 2002). Thus, host specificity of pathogens is an important factor to be considered, when developing measures for reducing loss due to disease, particularly in countries like Sri Lanka where fruit crops are produced in small holdings under mixed cropping systems.

The soil organism, *T. harzianum* has been used as a control measure against plant pathogens. Mortuza *et al* (1997), report direct parasitism and coiling around hypahe of *Lasiodiplodia theobromae*. Thus this study was initiated to investigate the possibility of using Sri Lankan isolates of *T. harzianum* to reduce postharvest loss of papaya, mango and rambutan due to stem-end rot and anthracnose diseases.

Methodology:

Isolation of the pathogens -The pathogens *C. gloeosporioides* and *B. theobromae* were isolated from infected mango, rambutan and papaya fruits. Diseased tissue was obtained from the outer margin of lesions, washed, dipped for 1 minute in 70% ethanol, re-washed with sterile distilled water and plated on Potato Dextrose Agar (PDA) medium. Growing edges of the mycelia emerging from the tissue were sub-cultured to obtain pure cultures of the pathogens.

Isolates were tested for pathogenicity via Koch's postulates, using healthy fruits washed with water, before surface sterilization with 70% ethanol. The pathogens (on 0.7cm agar discs) were placed on wounds made on the surface of the healthy fruits. Inoculated fruits were incubated in a moist chamber at room temperature (28 ± 2 ° C).

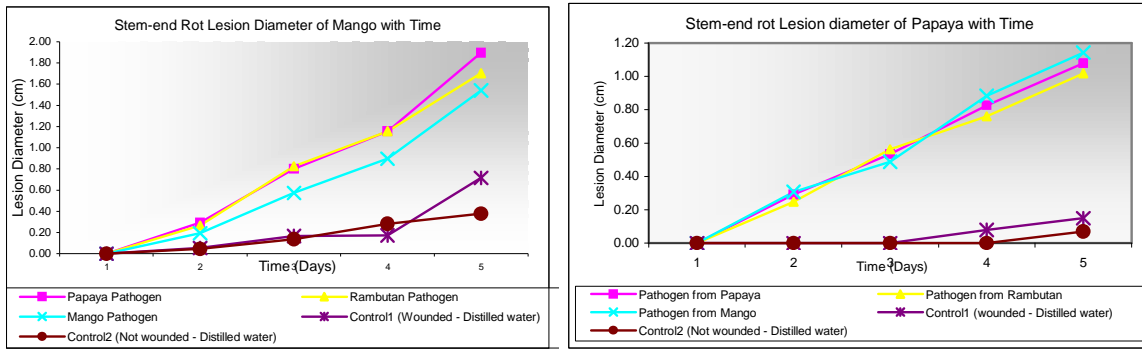
Cross inoculation study-Papaya var. Red Lady and mango var. Karthakolamban at 10% to 25% ripe stage and rambutan cv. Malwana at full ripe stage were selected for the experiments. Healthy mature fruits were washed in water and with 0.03% NaOCl and allowed to dry before inoculation. Inoculation of the fruits with the pathogens for the cross inoculation trials was carried out with seven day old pure cultures of *B. theobromae* and *C. gloeosporioides* isolated from diseased mango, papaya and rambutan respectively. Mycelial discs of diameter 0.9cm, were obtained from the periphery of the above cultures and used for inoculating healthy fruits including the original host type. A wound of diameter 0.9cm was made on the surface of 10 replicates from each fruit type immediately before inoculation with the respective *C. gloeosporioides* isolates. A sterile no. 04 cork borer was used in each case. Inoculations were repeated with *B. theobromae* isolates from papaya, rambutan and mango, on a further set of 10 replicates of each of the three fruits tested. Sterile distilled water was used to inoculate one set of 10 wounded controls and one set of 10 unwounded control fruits respectively. Each set of fruits was incubated separately in moist chambers lined with perforated polythene and newspaper, at room temperature (28 ± 2 ° C). Lesion diameter was measured daily in duplicate, at right angles to each other for each fruit, for five days .

In-vitro Bioassays with *T. harzianum*- Dual culture method (Dennis and Webster,1971) and slide culture technique (Sivakumar *et al*, 2000) were used for bioassays. A mycelial disc obtained from the periphery of a 7day old culture in each case- *C. gloeosporioides* and *B. theobromae*- was placed 1.5cm from the centre of a PDA plate while a mycelial disc obtained from *T. harzianum* was placed 3cm away from the mycelial disc of the pathogen. A sterile agar disc instead of the biocontrol agent disc was used in the control experiment. All plates were incubated at 28° C.

Results and Discussion:

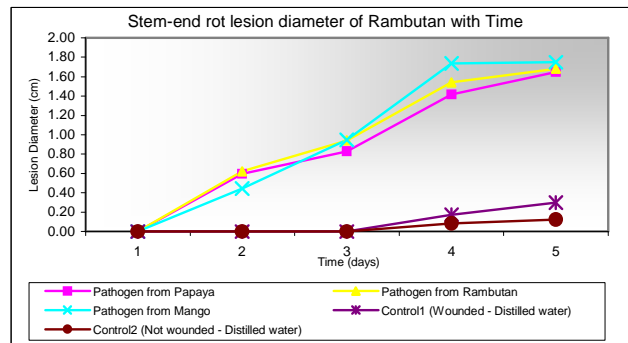
C. gloeosporioides was established as the causative organism of anthracnose disease in mango, papaya and rambutan and *B. theobromae* as the causative organism of stem-end rot disease of mango, rambutan and papaya via the pathogenicity tests.

Cross inoculation study- All *B. theobromae* isolates caused similar levels of infection with very little variation in lesion size on all fruit types, but differed significantly from the controls (Figure 1a, b, c).



(a)

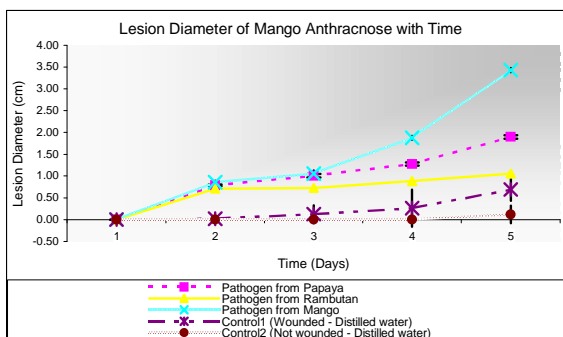
(b)



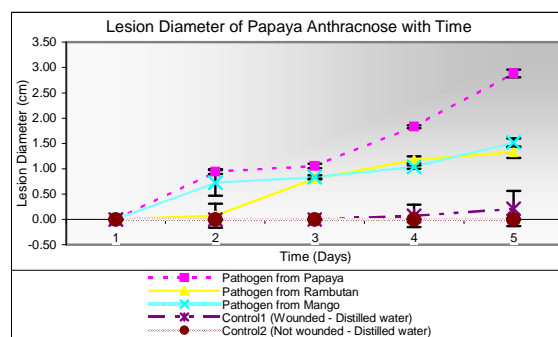
(c)

Figure 1: Increase in Stem-end rot Lesion Diameter over time in Mango (a) Papaya (b) and Rambutan (c)

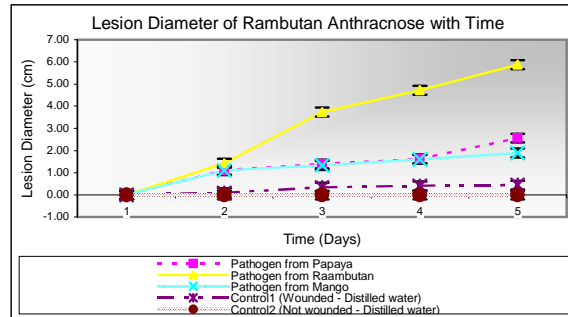
C. gloeosporioides isolates produced lesions on all hosts. However lesion diameters of the anthracnose isolates from mango, papaya and rambutan were less when cross inoculated compared with lesion diameters recorded on their original host (Figure 2a, b, c).



(a)



(b)

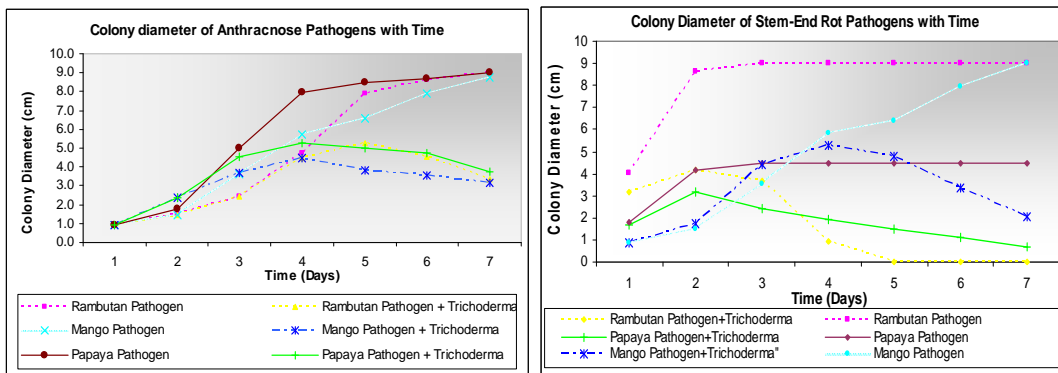


(c)

Figure 2: Increase in Anthracnose Lesion Diameter over time in Mango (a) Papaya (b) and Rambutan (c)

The host specificity of the rambutan isolate was more apparent on day 3 compared with the host specificity observed on day 4 in the mango and papaya anthracnose isolates.

In-vitro Bioassays with *T. harzianum*- The bioassays clearly indicated the antifungal effect of *T. harzianum* on *B. theobromae* and *C. gloeosporioides* isolates from mango, papaya and rambutan as indicated in Figure 3. *T. harzianum* was observed to form coiling structures around both pathogens in the respective slide culture assays.



(a)

(b)

Figure 3: Antifungal effects of biocontrol agent *T. harzianum* against (a) *C. gloeosporioides* and (b) *B. theobromae* from Papaya, Rambutan and Mango

Conclusions and Outlook

The study established the possibility of cross infection between host organisms in the case of both pathogens with respect to examined varieties of hosts. Future studies must include biochemical and genetic variations in respective pathogen isolates so as to understand further the cross infection process and the mechanisms that the anthracnose and stem end rot pathogens adopt to invade host tissue leading to the heavy losses of valuable sources of human nutrition. The fungicidal effect of *T. harzianum* observed in the bioassays suggests the possibility of using this organism as a non-chemical means of reducing pathogen inoculum in the soil. This could result in the development of an integrated, eco friendly, safe and convenient means of reducing post harvest loss in not only home garden fruit production systems but also in commercial plantations.

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