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DEVELOPING A BIOCONTROL METHOD AGAINST, *BOTRYODIPLODIA*
THEOBROMAE & *COLLETOTRICUM MUSAE* CAUSING CROWN ROT OF
'EMBUL' BANANA

A THESIS PRESENTED BY

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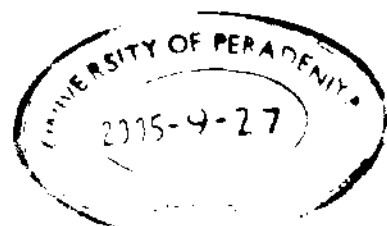
DEVELOPING A BIOCONTROL METHOD AGAINST, *BOTRYODIPLODIA THEOBROMAE* & *COLLETOTRICUM MUSAE* CAUSING CROWN ROT OF 'EMBUL' BANANA

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Two common pathogens associated with crown rot in the Asian region, *Colletotrichum musae* and *Botryodiplodia theobromae* (*Lasiodiplodia theobromae*), on 'Embul' (*Musa*, AAB), bananas were assessed for their relative ability to cause disease. Experiments were conducted by inoculating banana hands or fingers with either of these pathogens or in combination. All crowns or stems developed disease at varying degrees of severity and inoculation of pathogens onto healthy crowns or stems always increased severity. However, the combined effect of both pathogens was always less than their individual effects. *B. theobromae* inoculated hands showed significantly ($P=0.05$) higher crown rot lesion development rates. The highest values for lesion development were on fingers inoculated with *B. theobromae*. Generally, when both pathogens were inoculated together, the frequencies of recovery of each pathogen from hands or fingers, were significantly lower (Dunn's test, $P=25\%$) than when inoculated individually. The results suggest that *B. theobromae* is relatively more robust, and there does not appear to be a synergistic effect between *Colletotrichum musae* and *Botryodiplodia theobromae*.

Two bacteria (*Flavobacterium* sp. [W5481 (a)] and *Pantoea agglomerans* (W5482) on the above mentioned pathogens were tested on 'Embul' (*Musa*, AAB) bananas, to determine their effectiveness as an alternative, to use of fungicides and to determine their mode of antagonism. Both live cells and culture filtrates were tested by germination assays, agar well diffusion, and TLC bioassays. Effects on banana tissues were tested either using banana peel disks (bioassay) or banana hands. Conidial germination of pathogens was significantly ($P=0.05$) reduced in formulations with live cells and cell free culture media

significantly ($P=0.05$) reduced in formulations with live cells and cell free culture media (CFCM), although CFCM of *P. agglomerans* were not effective on *C. musae*. On agar well diffusion assays too, CFCM of *Flavobacterium sp.* was effective, and that of *P. agglomerans* was not. TLC-bioassays showed that extent of inhibition, and Rf values of active areas of methanolic extracts of spent media, varied according to the growth medium used for preparation of CFCM and according to the test fungus. Autoclaving the CFCM partially reduced its activity on mycelial growth, but not on conidial germination.

The rot development on peel disks was significantly ($P=0.05$) suppressed by the treatments with live cells but not with CFCM. None of the biocontrol applications were as effective as the fungicide, thiabendazole. On banana hands, viable cell preparations of each antagonist reduced crown rot significantly ($p= 0.05$). Antibiotics of *Flavobacterium sp.* appeared to be more potent directly on the pathogens. In spite of the differences in antibiotic production by the antagonists, live cells of each antagonist separately suppressed crown rot to the same extent.

Experiments were conducted to determine nutrient competition between antagonist and pathogens, at the site of inoculation by adding the extra nutrient (10 % sucrose in Davis and Mingioli minimal medium or 10% sucrose). There was no indication that these treatments enhanced the efficacy of the antagonists. However, although there was no significant difference in disease incidence, the additional hydration of the infection site by adding nutrient solution or sterile distilled water appeared to favor the pathogen establishment on banana tissue.

Of the two pathogens, *B. theobromae* appeared to be more liable to control by both antagonists. Finally the results obtained from crown rot suppression data showed that there is a chance to develop an environmental friendly, effective control method for crown rot development using these antagonistic bacteria: *Pantoea agglomerans* and *Flavobacterium sp.*