Journal of Biological Control, 24 (4): 360–362, 2010

Research Note

Effect of time of application of biocontrol agents for the management of crown rot disease in banana (*Musa acuminata* L.)

G. SANGEETHA* and S. USHARANI

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar 608 002, Chidambaram, Tamil Nadu, India.

**Corresponding author E-mail: sangeethaau@hotmail.com*

ABSTRACT: Crown rot disease caused by *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. and *Colletotrichum musae* (Berk and Curtis) Arx. is the major problem in banana cv. Robusta. Efficacy of application time of biocontrol agents in post-harvest environment in relation to pathogen infection is an important factor in controlling the crown rot disease of banana. *Trichoderma viride* isolates TV3 and TV4 which showed effectiveness against both pathogens *in vitro* were applied at different time intervals in relation to the time of pathogen inoculation in order to know the effective application time of biocontrol agents. Biocontrol agents were applied 2 and 4 h before pathogen inoculation; simultaneous challenge inoculation;and 2, 4 h after pathogen inoculation. The results indicated that the highest reduction of crown rot disease of banana was achieved when the biocontrol agents were applied 4 and 2 h before pathogen inoculation (preventive method of application) compared with the other treatments.

KEY WORDS: Banana crown rot, *Trichoderma viride*, time of application

(Article chronicle: Received: 22.07.2010; Sent for revision: 23.08.2010; Accepted: 03.09.2010)

INTRODUCTION

Banana (*Musa acuminata* L.) fruit is highly perishable and its storage life is often affected by many post-harvest diseases. Crown rot caused by a complex of fungal pathogens (*Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. and *Colletotrichum musae* (Berk and Curtis) Arx**.,** is a major problem in all banana growing regions (Sepiah *et al.,* 1990). This disease affects the quality, producing aesthetic damage and drop of fingers. A high incidence of crown rot can cause premature ripening during transit (Jones, 2000). There are many reports of successful use of biocontrol agents to control post-harvest diseases of vegetables and fruits (Tronsmo, 1991; Tronsmo and Dennis, 1997; Okigbo and Ikediugwa, 2000; Sivakumar *et al.,* 2001). Species of *Trichoderma* to control post-harvest diseases of banana have been evaluated and their efficacy is already well proved by many researchers (Golam *et al*., 1998; Thangavelu *et al*., 2007; Sangeetha *et al*., 2009). However, there is no information on the application time of biocontrol agents against crown rot disease of banana caused by a complex of pathogens. The aim of this research was to evaluate the effect of application time of biocontrol agents, in order to control crown rot disease of banana in respect to pathogen infection.

L. theobromae and *C. musae* were isolated from crown rot infected banana fruits and further purified by the hyphal tip method. Pathogenicity test was conducted for both *L. theobromae* and *C. musae* isolates and Koch's postulates were successfully proved in cv. Robusta. *T. viride* isolates TV3 and TV4 were isolated from soil samples collected from organic banana orchards in Tiruchirapalli district of Tamil Nadu, India. The two *T. viride* isolates were evaluated *in vitro* to test the antagonism against both *L. theobromae* and *C. musae* by dual culture technique (data not shown).

The experiment was conducted in Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu during 2006-2007 with eight treatments. For the experiment, banana cv. Robusta (AAA group) was harvested at 75–80% maturity from a commercial plantation in Tiruchirapalli district, Tamil Nadu. Banana hands free of visual defects with uniform shape and weight were selected. The hands were thoroughly washed in running tap water to remove dust, surface sterilized with 70% ethanol and allowed to dry for 6 h at room temperature. Biocontrol agents were applied at different time intervals in relation to the time of pathogen inoculation. Combination of two

Trichoderma isolates *viz.,* TV3 and TV4 were used in this study which already showed effectiveness against both test pathogens in *in vitro* assays (data not shown). A cocktail of conidial suspensions of both isolates of *T. viride viz.*, TV3 and TV4 (10^9 conidia mL^{-1}) was used for the study. The *Trichoderma* isolates were applied at 4 and 2 h before pathogen inoculation; simultaneous challenge inoculation; and 2, 4 h after pathogen inoculation. For pathogen inoculation, 200 µl spore suspension containing cocktail of *L. theobromae* (100 µL containing 10^5 spores mL^{-1}) and *C. musae* (100 µL containing 10^5 spores mL^{-1}) were utilized. Inoculated fruits were placed inside perforated polythene bags to maintain high humidity and incubated at 28 ± 2°C. One 'set' of treatments were incubated at room temperature (28 ± 2°C) and disease assessment was made after 10 days based on crown rot severity scale (0-5 scale; where 0-no disease. 1, 2, 3, 4-rot progression of 25, 50, 75 and 100% respectively and 5-rot extended up to the pedicel) (Finlay and Brown, 1993). Another 'set' of identical treatments was incubated under cold storage (14°C, 90% Relative Humidity) and disease assessment was made after 25 days as described above. In each case, four hands were inoculated to maintain the required number of replications. The experiment was conducted based on Completely Randomized Block Design (CRD).

The results indicated that the highest disease reduction was achieved when the biocontrol agents (isolates TV3 and TV4) were applied 4 and 2h before to inoculation of pathogens, in both storage regimes. At room storage, when the biocontrol agents applied 4 h and 2h and after pathogen inoculation, however, the disease incidence was reduced to the rot score 3.7 and 3.0 respectively compared to pathogen alone inoculate hands (rot score 4.3). In cold storage also the disease incidence was reduced to 2.0 and 2.7 when the biocontrol agents applied in 2h and 4h after pathogens inoculation compared to pathogen alone inoculate hands having the rot score 4.0. Simultaneous challenge inoculation of biocontrol agents with pathogen was found to be moderately effective in both storage regimes. Disease reduction was less if there was a delay in application of biocontrol agents. The results revealed that *T. viride* isolates were not effective against already established infections in the fruits under postharvest conditions. Combined application of bio control agents *viz.*, isolates TV3 and TV4 on fruits either 2 or 4 h before pathogen inoculation / infection was effective as that of the fungicide carbendazim in controlling the crown rot disease of banana. In control fruits (without pathogen inoculation) also the slight disease incidence was noticed, it might be due to the latent infection of crown rot pathogens.

This finding is partially in agreement with the report of Chaultz and Wilson (1990) who reported that in the control of citrus blue mould, the efficacy of *Debaryomyces hansenii* was more when applied simultaneously or before *Penicillium digitatum*. Similar results were also observed by Moreno and Paningbatan (1995) who had noticed less disease incidence in mangoes when *T. viride* was applied before inoculation with *Diplodia natalensis*. For better control with the application of biocontrol agents in post harvest environment, the bioagents should be applied immediately after dehanding operation, before the pathogen gains entry through cut

Table 1. Effect of time of application of biocontrol agents on severity of crown rot incidence in cv. *Robusta*

Treatments	Time intervals between treatment (h)	Rot severity (0–5 scale)	
		Room storage (28 ± 2°C)	Cold storage (14°C)
Biocontrol agents applied prior to pathogen inoculation	4	1.0^{ab}	0.3^{a}
Biocontrol agents applied prior to pathogen inoculation	2	1.0^{ab}	0.7^{a}
Challenge inoculation of the biocontrol agents along with pathogen	0	1.7^{b}	1.7^{b}
Biocontrol agents applied after pathogen inoculation	2	3.0^{c}	2.0^{bc}
Biocontrol agents applied after pathogen inoculation	4	3.7^{cd}	2.7^{c}
Control (no pathogen)	–	1.0^{a}	0.3^{a}
L. theobromae + *C. musae*	–	4.3^{d}	4.0^{d}
Carbendazim (0.1%)	–	1.0^{ab}	0.3^{a}
CD (P = 05)		0.71	0.87

*Mean of three replications . In a column means followed by a common letter are not significantly different at 5 per cent level by DMRT

wounds. If biocontrol agents are applied prior to pathogen infection, the bioagents occupy the cut wounds effectively and compete with the pathogens when they attempt to enter.

Hence, the time of application of biocontrol agents is yet another factor to be considered for the efficient performance of the biocontrol agents mainly under postharvest conditions.

REFERENCES

Chaultz, E. and Wilson, C. L. 1990. Post-harvest biocontrol of green and blue mould and sour rot of citrus fruit by *Dabaryomyces hansennii*. *Plant Disease,* **74**: 134–137.

Finlay, A. R. and Brown, A. E. 1993. The relative importance of *Colletotrichum musae* as a crown-rot pathogen on Windward Island bananas. *Plant Pathology*, **42**: 67–74.

Golam, M., Mortuza, M. G. and Ilag, L. L. 1998. Biological control for *Colletotrichum* rot in banana fruits by *Trichoderma* species. *Bangladesh Journal of Plant Pathology,* **14**: 21–24.

Goos, R. D., Elsie A., Cox, G. and Stotsky, I. 1961. *Botryodiplodia theobromae and its association with Musa species. Mycologia,* **53**: 262–277.

Jones, D. R. 2000. *Diseases of Banana*. Abaca and Enset. CABI Publishers. UK. pp. 173–206.

Moreno, L. S. and Paningbatan, R. A. 1995. Biological control of mango stem end rot caused by *Diplodia natalensis* with *Trichoderma viride*. *Philippine Phytopathology*, **31**: 103–116.

Okigbo, R. N. and Ikediugwa, F. E. O. 2000. Studies on biological control of post-harvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride*. *Journal of Phytopathology,* **148**: 351-355.

Punithalingam, E. 1976. *Botryodiplodia theobromae*, CM1 descriptions of pathogenic fungi and Bacteria. Common Wealth Agricultural Bureaux. p. 519.

Sangeetha, G., Usharani, S. and Muthukumar, A. 2009. Biocontrol with *Trichoderma* species for the management of postharvest crown rot of banana. *Phytopathologia Mediterranea,* **48**: 214–225.

Sepiah, M., Acedo, A. L., Sabari, S. D., Ilag, L. L. and Kuthubutheen, A. J. 1990. Post-harvest physiology of banana, pp. 104–111. In: Hassan, A. and Pantatico, Er. B. (Eds.), *Banana Fruit Development – Post Harvest Physiology, Handling and Marketing in ASEAN*. ASEAN Food, Handling Bureau, Malaysia.

Sivakumar, D., Wijeratnam, R. S. W., Marikar, F. M. M. T., Abeyesekere, M. and Wijesundera, R. L. C. 2001. Biocontrol agentsic effect of *Trichoderma harzianum* on post-harvest pathogens of rambutans. *Acta Horticulturae,* **553**: 389–392.

Sutton, B. C. and Waterson, J. M. 1970. *Colletotrichum musae*. CMI Descriptions of Pathogenic Fungi and Bacteria. **221**: 1–5.

Thangavelu, R., Sangeetha, G. and Mustaffa, M. M. 2007. Cross-infection potential of crown rot pathogen (*Lasiodiplodia theobromae*) isolates and their management using potential native bioagents in banana. *Australasian Plant Pathology,* **36**: 595–605.

Tronsmo, A. 1991. Biological and integrated controls of *Botrytis cinerea* on apple with *Trichoderma harzianum*. *Biological Control,* **1**: 59–62.

Tronsmo, A. and Dennis, C. 1997. The use of *Trichoderma* species to control strawberry fruit rots. *The Netherlands Journal of Plant Pathology*, **83**: 449–455.