Archives Of Phytopathology And Plant Protection

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gapp20

In vitro screening of biological and chemical agents together on the growth of Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. causing inflorescence die back in arecanut

K. Nidhina[a] & Vinayaka Hegde[b]

[a] Sir Syed Institute For Technical Studies, Kannur University, Kannur, India

[b] Plant Pathology Section, Central Plantation Crops Research Institute, Kasaragod, India

Published online: 09 Dec 2013.

To cite this article: K. Nidhina & Vinayaka Hegde (2014) In vitro screening of biological and chemical agents together on the growth of Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. causing inflorescence die back in arecanut, Archives Of Phytopathology And Plant Protection, 47:15, 1905-1911, DOI: 10.1080/03235408.2013.861648

To link to this article: http://dx.doi.org/10.1080/03235408.2013.861648

Archives of Phytopathology and Plant Protection, 2014
Vol. 47, No. 15, 1905–1911, http://dx.doi.org/10.1080/03235408.2013.861648

In vitro screening of biological and chemical agents together on the growth of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causing inflorescence die back in arecanut

K. Nidhina[a]* and Vinayaka Hegde[b]

[a]*Sir Syed Institute For Technical Studies, Kannur University, Kannur, India;* [b]*Plant Pathology Section, Central Plantation Crops Research Institute, Kasaragod, India*

(*Received 19 October 2013; accepted 30 October 2013*)

Colletotrichum gloeosporioides is a destructive pathogen of many crop species causing diseases in many annual, biennial and perennial plants. A study was undertaken to find out the effect of biological and chemical agents together on the growth of *C. gloeosporioides* causing inflorescence die back in arecanut at the Department of Plant Pathology, CPCRI, Kasaragod. To reduce the release of chemical pesticides to the environment, integrated control strategies have been adopted extensively by combining both bioagents and chemical agents. So in the present study *in vitro* experiments were conducted with two compatible *Trichoderma* sp. viz., *Trichoderma virens* and *Trichoderma viride* and fungicides viz. Blitox 50 W and Mixol 72. The results indicated that all the treatments had a significant inhibitory effect on the growth of *C. gloeosporioides* and reduced its colony diameter. High percent inhibition was found when 0.05% of Mixol 72 was used with *T. virens* (87.61%). The least inhibition was shown by *T. virens*+0.05% Blitox 50 W (80.95%). It is concluded that the combination of bioagents with fungicides provided higher disease suppression than achieved with fungicides and bioagents when used alone.

Keywords: *Colletotrichum gloeosporioides*; *Trichoderma* sp.; fungicides; IDM

1. Introduction

The arecanut palm, *Areca catechu* L. (Family: Arecaceae), is an important cash crop of India. Arecanut or betelnut palm is affected by a number of diseases during different stages of its growth and development. Dieback of inflorescence and button shedding caused by *C. gloeosporioides* (Penz.) Penz. and Sacc. was recorded as one of the reasons for low fruit set in arecanut. *C. gloeosporioides* is a polyphagous species with a wide host range. The genus *Colletotrichum* is known for its variability in cultural and morphological characters (Van Arx 1970).

Though there is recommendation of spraying fungicides namely mancozeb, there is no much studies on the effect of new fungicides or bioagents on the pathogen. The recommendation is also based on the studies carried out during 1970s and 1980s. The use of fungicides sometimes becomes unavoidable. However, their dose and frequency of application can be minimised by integration with other methods for effective control of plant pathogens. The use of antagonistic micro-organisms in

*Corresponding author. Email: nidhinak960@gmail.com

controlling plant diseases continues receiving increasing attention (Adebanjo & Bankole 2004). Successful use of the genus *Trichoderma* in the biological control of several plant pathogens has also been well documented (Paavanen-Huhtala et al. 2000). Biological control helps to overcome some of the problems created today in agriculture due to excessive use of chemicals for disease control. However, it is very difficult to achieve desirable level of plant disease control with the application of biocontrol agents alone. Hence, an integrated disease management (IDM) has been suggested for the management of many plant diseases.

Development of an ecofriendly integrated management of the disease needs information on compatibility of common bioagents with fungicides. Integration of compatible bioagent with pesticide may enhance the effectiveness of disease control (Pappavizas & Lewis 1981). The combination of biocontrol agents with fungicides would provide similar disease suppression as achieved with higher fungicide use (Monte 2001). This study was aimed at finding out the effect of *Trichoderma* sp. alone and in combination with fungicides on the growth of *C. gloeosporioides*.

2. Materials and methods

The experiment was conducted in the Plant Pathology Laboratory of CPCRI, Kasaragod to evaluate the compatibility of *Trichoderma* sp. with fungicides and the effect of *Trichoderma* sp. alone and in combination with fungicides on the growth of *C. gloeosporioides,* causal agent of inflorescence die back of arecanut.

2.1. Sources of fungicides and bioagents

Four fungicides viz. Blitox 50 W, Sectin, Mixol 72 and Dithane M 45 (Table 1) and bioagents such as *T. harzianum, T. viride* and *T. virens* were obtained from the Plant Pathology Laboratory of CPCRI, Kasaragod.

2.2. Collection of samples

Inflorescence showing symptoms of *Colletotrichum* infection was collected from Kanhangad in Kasaragod district of Kerala. During the collection of samples, disease symptoms were recorded in detail.

Table 1. Details of fungicides used in the *in vitro* studies against *C. gloeosporioides*.

Sl. no.	Trade name	Active ingredients	Formulation	Manufacturers/distribution
1	Blitox 50W	Copper oxychloride 50% WP	Wettable powder	M/s Rallis India Ltd
2	Dithane M 45	Mancozeb 75% WP	Wettable powder	Dow Agro Science India Pvt. Ltd
3	Mixol 72	Metalaxil M 8% + Mancozeb 64% WP	Wettable powder	Jai Bharat Crop India Pvt. Ltd., Jammu
4	Sectin	Fenamidone 10% + Mancozeb 50% WG	Wettable powder	Bayer Crop Science Ltd., Gujarat

2.3. Isolation and identification of the fungus

The fungus was isolated from the infected inflorescence following tissue segment method (Agotini & Timmer 1992). The infected tissues were cut into small pieces and were surface sterilised by dipping in 0.1% $HgCl_2$ solution for about 30 s. The cut pieces were then placed onto sterilised potato dextrose agar plates and incubated at room temperature. The mycelial growth emerged from the isolated tissue after 48 h of incubation was subcultured. Based on the morphology of the mycelial colony as well as the characteristics of the conidia, the fungus was identified as *C. gloeosporioides*. The pure culture of the pathogen was established by single spore isolation (Choi et al. 1999) and maintained on PDA slants.

3. Antifungal assay

Compatibility tests were conducted under *in vitro* conditions to find out safer fungicides against *Trichoderma* sp. Four fungicides viz., Blitox 50 W, Sectin (0.05, 0.1, and 0.2%), Mixol 72 and Dithane M45 (0.025, 0.05, 0.1%) were evaluated at different concentrations by poisoned food technique (Nene & Thapliyal 1982).

Appropriate quantities of each fungicides were mixed with sterile distilled water to make 10 ml fungicide stock solutions of required concentration. One millilitre of this stock solution was incorporated into 49 ml PDA medium to get the final concentration. The medium was gently shaken to ensure proper and uniform distribution of fungicide. Fifteen millilitres of poisoned medium were then poured into each of the sterile Petri plate. After solidification of medium, each plate was inoculated centrally with mycelial disc of 6 mm diameter cut from periphery of four-day-old culture of *Trichoderma* sp. growing on PDA medium. The plates were incubated at room temperature for five days. Three replications were maintained for each treatment (concentration). In control, 1 ml sterile distilled water alone was added. Radial growth of the fungus in each plate was recorded after five days of incubation by measuring the diameter of colony in two directions at right angles to each other. The average of two measurements was taken as the colony diameter. The percentage inhibition of growth was calculated using the formula given by Mukherjee et al. (2011).

$$I = (C - T)/C \times 100$$

where I – Percent inhibition, T – mycelial growth in treatment and C – mycelial growth in control.

Experiments were also carried out to test the relative efficacy of fungicides, *Trichoderma* sp. which is compatible with the fungicides against *C. gloeosporioides* alone and its combination with the fungicides. Ten milliltres fungicide stock solutions of required concentration were prepared. One millilitre of this stock solution was incorporated into 49 ml PDA medium to get the final concentration. The medium was gently shaken to ensure proper and uniform distribution of fungicide. Fifteen millilitres of poisoned medium were then poured into each of the sterile Petri plate. Petri plates were inoculated in the centre with 6 mm diameter of 5- day-old culture of *C. gloeosporioides*. The other half of the plate was inoculated with 6 mm diameter mycelia disc from the 4-day-old culture of *Trichoderma* sp. In control, 1 ml sterile distilled water alone was added. The plates were incubated at room temperature for five days. Radial growth of the fungus in each plate was recorded after

five days of incubation by measuring the diameter of colony in two directions at right angles to each other. The average of two measurements was taken as the colony diameter. The percentage inhibition of growth was calculated using the same formula given above.

4. Results and discussion

The development of a suitable disease management strategy is essential because of the economic loss caused by the inflorescence dieback of arecanut. Integrating fungicide resistant antagonists with suitable fungicidal treatment has importance in the framework of IDM. In the present study, laboratory experiment was conducted to test the possibility of combining *Trichoderma* sp. viz., *T. harzianum, T. viride* and *T. virens* with fungicides. Though all the fungicides inhibit *Trichoderma*, the fungicides showing ≤ 50% inhibition were considered as compatible. The sensitivity of *Trichoderma* species was rated as follows by considering the percent inhibition on the growth of *C. gloeosporioides*.

70–100%	High sensitivity
51–69%	Less sensitivity
≤50%	Poor sensitivity

Out of the three *Trichoderma* sp. *T. harzianum* was more sensitive to all the fungicides tested and *T. virens* was least sensitive (Table 2). *T. viride* and *T. virens* were compatible with Blitox 50 W (0.05, 0.1%) and Mixol 72 (0.025, 0.05%). All the three *Trichoderma* sp. were highly sensitive to Dithane M 45 even at the lowest concentration (0.025%). Similar results were reported by Tapwal et al. (2012) that mancozeb is not compatible with *T. viride*. In a similar study, *T. harzianum* was found highly sensitive to mancozeb, Tebuconazole and Thiram, less sensitive to benomyl, triadimenol and dichlofluanid (Mclean et al. 2001). Thus, the present investigations provide evidence for the compatibility of *Trichoderma* with synthetic chemicals and thus Blitox 50 W (0.05 and 0.1%) and Mixol 72 (0.025 and 0.05%) were used with *T. virens* and *T. viride* for further studies.

Results from dual culture assay showed that maximum inhibition was shown by *T. virens* (61.90%). These results are similar to the findings of Malathi et al. (2002) and Verma et al. (2006). Absence of direct parasitism and less competition may also be one of the reasons due to which *T. viride* was not successful in controlling *C. gloeosporioides.* On the other hand, Blitox 50 W and Mixol 72 also reduced the colony diameter of the pathogen (Table 3). Mixol 72 showed 78.09% inhibition followed by Blitox 50 W (61.90%) at 0.05%. The high percent inhibition was found when 0.05% of Mixol 72 was used with *T. virens* (87.61%). The least inhibition was shown by *T. virens*+0.05% Blitox (80.95%). Blitox 50 W exhibits greater inhibition (86.66%) when used with *T. viride* at 0.1%. *T. virens* shows least inhibition when used in combination with Blitox 50 W since it is less compatible than *T. viride*. In the same way, *T. viride* shows least inhibition when used with Mixol 72 since it is less compatible than *T. virens.* Thus, the percent inhibition was found to be higher when fungicides are used with bioagents than using alone.

Table 2. *In vitro* compatibility of fungicides with *Trichoderma* species.

Fungicide	Concentration (%)	% Inhibition			Mean	Grand mean
		T. virens	*T. viride*	*T. harzianum*		
Dithane M-45	0.025	50.00 (45.02)	100.0 (90.04)	100.0 (90.04)	83.33 (75.03)	91.15 (81.37)[a]
	0.05	70.37 (57.05)	100.0 (90.04)	100.0 (90.04)	90.12 (79.04)	
	0.1	100.0 (90.04)	100.0 (90.04)	100.0 (90.04)	100.0 (90.04)	
Mixol 72	0.025	6.67 (14.97)	21.11 (27.36)	58.52 (49.93)	28.76 (30.75)	51.97 (47.08)[b]
	0.05	18.89 (25.76)	50.00 (52.32)	87.41 (69.26)	52.10 (49.11)	
	0.1	49.26 (44.59)	86.67 (68.61)	89.26 (70.91)	75.06 (61.37)	
Mean		49.19 (46.24)[c]	76.29 (69.73)[b]	89.19 (76.70)[a]		
CD ($p \leq 0.05$) for *Trichoderma*-0.29; fungicide-0.24; concentration-0.29; interaction-0.71						
Sectin	0.05	50.74 (45.44)	54.81 (47.78)	71.48 (57.75)	59.01 (50.32)	78.76 (66.03)[a]
	0.1	75.56 (60.40)	100.0 (90.04)	84.81 (67.10)	86.79 (72.51)	
	0.2	84.81 (67.10)	100.0 (90.04)	86.67 (68.61)	90.49 (75.25)	
Blitox 50 W	0.05	24.81 (29.89)	29.26 (32.75)	38.52 (38.38)	30.86 (33.67)	48.31 (44.18)[b]
	0.1	50.00 (45.02)	35.93 (36.84)	72.59 (58.46)	52.84 (46.77)	
	0.2	56.30 (48.64)	43.70 (41.40)	83.70 (66.22)	61.23 (52.09)	
Mean		57.38 (49.41)[c]	60.61 (56.48)[b]	72.96 (59.42)[a]		
CD ($p \leq 0.05$) for *Trichoderma*-0.32; fungicide-0.26; concentration-0.32; interaction-0.79						

Means with the same letter are not significantly different.

Table 3. *In vitro* evaluation of fungicides and *Trichoderma* sp. together against *C. gloeosporioides*.

Sl. no.	Treatments	% Inhibition
1	0.05% blitox	61.90[g]
2	0.1% bitox	67.61[f]
3	0.025% mixol	50.47[i]
4	0.05% mixol	78.09[e]
5	*T. virens*	61.90[g]
6	*T. viride*	58.09[h]
7	0.05% blitox + *T. virens*	80.95[de]
8	0.05% blitox + *T. viride*	83.81[bcd]
9	0.1% bitox + *T. virens*	82.85[cd]
10	0.1% bitox + *T. viride*	86.66[ab]
11	0.025%mixol + *T. virens*	85.71[abc]
12	0.025%mixol + *T. viride*	82.85[cd]
13	0.05% mixol + *T. virens*	87.61[a]
14	0.05% mixol + *T. viride*	84.76[abc]

CD ($p \leq 0.05$) 2.50; Means of three replications.
Means with the same letter are not significantly different.

4. Conclusion

IDM is a disease control approach that uses all available management strategies to maintain disease pressures below an economic injury threshold. In the present study, the combination of biological and chemical agents has provided higher percent inhibition than when they used alone. It may enhance the effectiveness of disease control, and help in reducing the usage of higher doses of fungicides and better management of *C. gloeosporioides*. Thus, the present investigation has opened up new areas and given rise to new ideas on the control of inflorescence dieback of arecanut caused by *C. gloeosporioides.* However, further studies are needed to evaluate their potential under field conditions.

Acknowledgement

The authors express their gratitude to Central Plantation Crops Research Institute, Kasaragod for providing necessary facilities during the present investigations.

References

Adebanjo A, Bankole SA. 2004. Evaluation of some fungi and bacteria for biocontrol of anthracnose diseases of cowpea. J Basic Microbiol. 44:3–9.

Agotini JP, Timmer LW. 1992. Selective isolation procedure for differentiation of two strains of *Colletotrichum gloeosporioides* of citrus. Plant Dis. 76:1176–1178.

Choi YW, Hyde KD, Ho WH. 1999. Single spore isolation of fungi. Fung Diver. 3:29–38.

Malathi P, Viswanathan R, Padmanaban P, Mohanraj D, Sundar AR. 2002. Microbial detoxification of *Colletotrichum falcatum* toxi. Curr Sci. 83:745–749.

Mclean KL, Hunt J, Stewart A. 2001. Compatibility of the biocontrol agent *T. harzianum* c52 with selected fungicides. New Zeal Plant Prot. 54:84–88.

Monte E. 2001. Editorial paper: Understanding *Trichoderma*: between agricultural biotechnology and microbial ecology. Int. Microbiol. 4:1–4.

Mukherjee A, Khandker S, Islam MR, Shahid SB. 2011. Efficacy of some plant extracts on the mycelial growth of *Colletotrichum gloeosporioides*. J Bangladesh Agril Univ. 9:43–47.

Nene YL, Thapliyal PN. 1982. Fungicides in plant diseases control. New Delhi: Oxford and IBH. p. 163.

Paavanen-Huhtala S, Avikainen H, Yli-Mattila T. 2000. Development of strain specific primers for a strain of *Gliocladium catenulatum* used in biological control. Eur J Plant Pathol. 106: 187–198.

Pappavizas GC, Lewis JA. 1981. Introduction and augmentation of microbial antagonists for the control of soil borne plant pathogens. In: Papaviazas GC, editor. Biological control in crop production. Totowa, NJ: Totowa; p. 305–322.

Tapwal A, Kumar R, Gautham N, Pandey S. 2012. Compatibility of *Trichoderma viride* for selected fungicides and botanicals. Int J Plant Pathol. 3:89–94.

Verma N, MacDonald L, Punja ZK. 2006. Inoculum prevalence, host infection and biological control of C*olletotrichum acutatum*: causal agent of blueberry anthracnose in British Columbia. Plant Pathol. 55:442–450.

Van Arx JA. 1970. A revision of the fungi classified as *Gloeosporium*. 2nd ed. Vaduz: J. Cramer. p. 203.