Application of antagonistic fungi to control anthracnose disease of grape

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Anthracnose of the grape varieties Bigblack, Nanpha, Blackopal, Loose perlette and White malaca are caused by *Colletotrichum gloeosporioides*. All isolates obtained from grape anthracnose were shown to be pathogenic; isolate WMF01 was the most virulent on all tested varieties of grape. Assays using crude extracts from *Chaetomium cupreum* CC, *C. globosum* CG, *Trichoderma harzianum* PC01, *T. hamatum* PC02, *Penicillium chrysogenum* KMITL44 and antibiotic substances Rotiorinol, Chaetoglobosin-C and Trichotoxin A50 were carried out to test bioactivity. All extracts and compounds inhibited the growth of *C. gloeosporioides* strain WMF01, with average ED_{50} values between 1 to 50 ppm. Applications of bioproducts of *Chaetomium*, *Penicillium* and *Trichoderma*, and a mixture of those bioproducts in a powder formulation and a chemical control were conducted in the field to control anthracnose disease of 5-varieties of grape. All bioproducts significantly reduced the disease incidence on leaves, twigs and fruits of grape in all varieties as compared to the chemical control.

Key words: antagonistic fungi, Chaetoglobosin-C, *Chaetomium*, *Penicillium*, Rotiorinol, *Trichoderma*, Trichotoxin A50

Introduction

The grape (*Vitis vinifera*) is one of the most economically important fruit crops in the world and has many uses. Fruit is eaten fresh or made into juice, fermented to wines and brandy and dried into raisins and sultanas (Roger and Goheen, 1998). Anthracnose is one of the most damaging diseases of grape and is caused by *Colletotrichum gloeosporioides* and *C. acutatum* and are

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responsible for yield losses in commercial grape production. In wet humid regions the disease incidence and severity on various cultivars of grape can be very serious (Kummuang *et al.*, 1996). Fungicides have been extensively used to control anthracnose of grape, but cause environmental pollution and leave residues in the agricultural soil and on products. Chemical usage has been effective, although resistance to these fungicides is developing. Biological control of plant pathogens has been shown to have potential to control many diseases in plantations. *Chaetomium*, *Penicillium* and *Trichoderma* species are biological control agents that have the potential to control plant diseases. Field trails have shown that *Chaetomium* formulated bioproducts have promise as broad spectrum mycofungicides to control many diseases (Soytong and Soytong, 1997). Pot experiments have shown that *Bacillus subtilis* S9 mixed with fungal antagonists, such as *C. cupreum* and *C. globosum* and *Trichoderma viride* could potentially act synergistically to control plant diseases caused by the pathogenic fungi, *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum* f.sp. *niveum* (Lin and Li, 2002). Fang and Tsao (1995) reported that *Penicillium funiculosum* could inhibit the growth of *Phytophthora cinnamoni*, *P. parasitica* and *P. citrophthora* (root rot of *Azalea* and orange). Biological control agents have become important in integrated disease management for improved plant production. In this study we therefore investigate the effect of antagonistic fungi to control anthracnose disease of grape.

Materials and methods

Isolation of pathogen from anthracnose disease of grape

Isolates of *C. gloeosporioides* were obtained from leaves, twigs and fruits of grape, (varieties Bigblack, Nanpha, Blackopal, Loose perlette and White malaca) showing anthracnose symptoms in vineyards at Pechphimai District, Nakornratchasima Province, Thailand. All isolates were tested for pathogenicity to grape using Koch's Postulate. The most virulent isolate was chosen for further experimentation.

The bioactivity test

The bioactivity assay used crude extracts from *Chaetomium cupreum* CC (extracted by MeOH), *C. globosum* CG (extracted by EtOAc), *Trichoderma harzianum* PC01 (extracted by EtOAc), *T. hamatum* PC02 (extracted by EtOAc), *Penicillium chrysogenum* KMITL44 (extracted by EtOAc) and pure compounds, Rotiorinol from *C. cupreum* CC, Chaetoglobosin-C from *C.*

globosum CG and Trichotoxin A50 from *T. harzianum* PC01 (fig.1) to establish ability to inhibit spore production of the most virulent isolate (*C. gloeosporioides*). The bioassays were carried out on Potato dextrose agar (PDA) medium amended with each crude extract or pure compound at concentrations of 0, 10, 50, 100 and 500 ppm. The test pathogen was cultured on PDA and incubated at room temperature (28-30°C) for 8 days. A 0.3 cm diameter plug with mycelium was placed in the middle of a PDA plate and incubated at room temperature for 7-10 days. A randomised block design with 4 replications was used. Data collection were statistical analysis of variance (ANOVA) and compared the treatment means with Duncan 's multiple range test (DMRT) at P = 0.01 and analysis value of effective dose (ED_{50}).

The application of bioproducts to control anthracnose disease in field

The biological products in powder formulations were tested to control anthracnose disease of the same varieties of grape. Four replications and 5 treatments were as follows:

1. *Chaetomium*
2. *Penicillium*,
3. *Trichoderma*,
4. Mixture of *Chaetomium*, *Penicillium* and *Trichoderma*
5. Chemical control (Benomyl).

The tested bio-products were developed and produced by the standard formulation using the method of K. Soytong (2004). The bio-products were sprayed into the soil at the rate of 5 g/plant for each treatment every 4 months, and spraying plants above-ground at a rate of 20 g/20 litres of water every 30 days and interval spraying with a fungal elicitor (crude extracts from *C. cupreum* CC, *C. globosum* CG, *T. harzianum* PC01, *T. hamatum* PC02 and *P. chrysogenum* KMIT44. Each experimental unit (100 m^2) was prepared by applying biological fertilizer developed by K. Soytong (2004) at the rate of 2 kg/plant and adjusting the soil pH with lime (1 kg/plant). Data collected and computed statistical analysis of variance (ANOVA) using a randomized complete block design, Duncan 's multiple range test (DMRT) at P = 0.01 was calculated to compare treatment means.

Results

Isolation of pathogen from anthracnose disease of grape

Colletotrichum gloeosporioides was isolated from anthracnose symptoms on grape and shown to be pathogenic isolates using Koch's postulate. The most virulent isolate (strain WMF01) was used in the bioassay.

Bioactivity assay

Results showed that all crude extracts and pure compounds (Rotiorinol, Chaetoglobosin-C and Trichotoxin A50) significantly inhibited the spore production of the pathogenic strains at the highest concentration (500 ppm.) when compared with the non-treated control. Rotiorinol and the crude extracts of *C. cupreum* and *T. hamatum* inhibited spore production the greatest at 88.67%, 87.75% and 81.87% per cent, respectively. The ED_{50} values of the bioassays are shown in Tables 1 and 2.

The application of bio-products to control anthracnose disease in field

A one-year field trail of bio-product application was evaluated to establish whether control of anthracnose in grape varieties could be achieved using formulations of *Chaetomium*, *Penicillium* and *Trichoderma*, and a mixture of these formulations. The application of these bio-products significantly reduced disease levels and incidence on leaves, twigs and fruits of in all tested varieties of grape when compared to those in the chemical treatment (Tables 3, 4).

Discussion

The use of biological control methods to reduce disease incidence caused by plant pathogens is continually being developed and is being used in a variety of crops (Soytong *et al.*,2001). In our study, we tested fungi with antagonistic properties to reduce anthracnose disease incidence in grape. The ability of the antagonistic fungi to reduce sporulation in culture was shown and disease control was confirmed in field trails. Amemiya *et al*. (1994), Saowapak and Soytong (2002) and Rajathilagam and Kannabiran (2001) reported that anti-fungal substances extracted from *C. globosum*, *T. harzianum* PC01, *T. hamatum* PC02 and *T. viride* (with chloroform) could inhibit the growth of several plant pathogens including *Verticillium dahliae*, *Fusarium oxysporum*

Table 1. Number of spores produced following bioactivity test.

Biocompounds	Number of *C. gloeosporioides* WMF01 spores (× 10^6 spore/ml) produced at each concentration (ppm)				
	0	**10**	**50**	**100**	**500**
Ch. cupreum CC (MeOH)	35.00 a[1]	12.87 b	10.25 bc	6.50 bc	4.25 c
Ch. globosum CG (EtOAc)	31.25 a	13.87 b	11.37 b	10.37 b	9.62 b
T. harzianum PC01 (EtOAc)	32.50 a	16.25 b	12.25 b	9.37 b	8.75 b
T. hamatum PC02 (EtOAc)	33.75 a	11.50 b	10.25 b	9.50 b	5.75 b
P. chrysogenum KMITL44 (EtOAc)	32.50 a	18.75 b	12.87 c	10.87c	9.62 c
Rotiorinol	35.00 a	11.12 b	9.62 bc	7.50 bc	3.75 c
Chaetoglobosin-c	30.00 a	13.25 b	12.12 b	9.62 b	8.87 b
Trichotoxin A50	33.12 a	14.87 b	10.75 b	9.37 b	8.12 b

[1]Average of four replications. If letters are the same in each row this means they are not significantly different using Duncan's multiple range test.

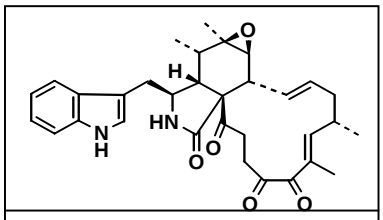
f.sp. *lycopersici*, *Colletotrichum gloeosporioides* and *C. capsici*. Chaetoglosin-c extracted from *Trichoderma* can reduce root rot of citrus caused by *P. parasitica* after treatment for 30 days, and induced resistance to the pathogen in plant. Its integration with other treatments (Integrated Pest Management, IPM systems) could effectively control root disease of *Citrus* (Usuwan *et al.*, 1999).

Table 2. Effect of biocompounds on *Colletotrichum gloeosporioides* WMF01 spore production.

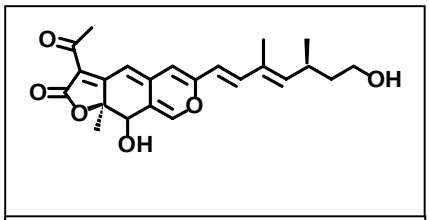
Biocompounds	Growth Inhibition[1] (%) at each concentration (ppm)				
	10	**50**	**100**	**500**	**ED_{50} (ppm)**
Ch. cupreum CC (MeOH)	61.20 c [2]	69.21 bc	81.29 ab	87.75 a	1
Ch. globosum CG (EtOAc)	53.84 b	61.53 ab	65.25 a	67.73 a	2
T. harzianum PC01 (EtOAc)	44.37 b	58.68 a	69.06 a	70.81 a	7
T. hamatum PC02 (EtOAc)	64.79 b	68.81 b	70.68 b	81.87 a	1
P. chrysogenum KMITL44 (EtOAc)	42.15 b	60.34 a	66.98 a	71.54 a	16
Rotiorinol	67.92 c	72.27 c	78.66 b	88.67 a	1
Chaetoglobosin-c	54.18 b	57.70 ab	66.18 a	68.81 a	2
Trichotoxin A50	54.53 b	66.96 ab	70.91 a	74.32 a	2

[1]Growth Inhibition (GI) = R1-R2/R1 × 100: R1=Number spores of tested pathogen produced in the control (0 ppm) plate and R2: Number spore of tested pathogen produced at each concentration.

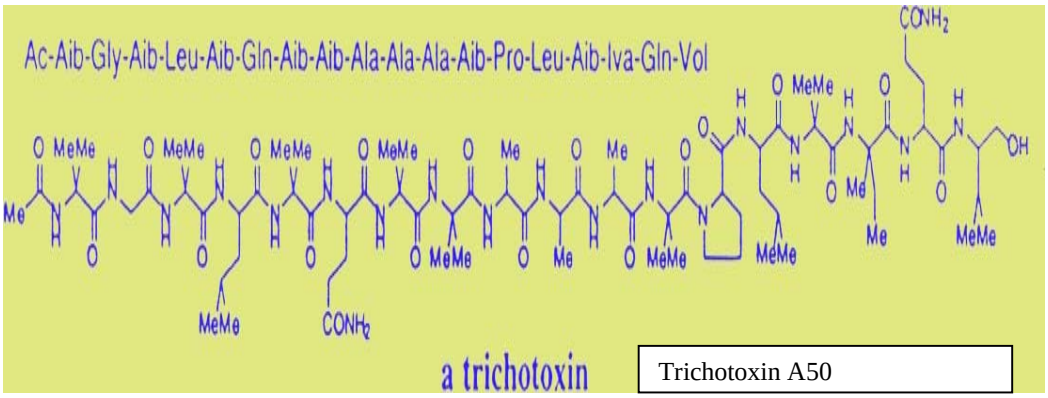
[2]If letters are the same in each row this means they are not significantly different using Duncan's multiple range test.



Chaetoglobosin - C



Rotiorinol



Trichotoxin A50

Fig. 1. Antibiotic substances produced from antagonistic fungi.

In field trials, we were able to check the efficacy under defined abiotic and biotic conditions despite significant differences in ecological climate. In this study we showed the effectiveness of using bio-products to control anthracnose disease on 5-varieties of grape caused by *C. gloeosporioides*. It was observed that all bioproducts treatments had significantly reduced the incidence of anthracnose disease when compared chemical treatment. This was similar to previous research applying Chaetomium products which gave a good control of anthracnose disease of palms and application of bioproducts:- *Chaetomium*, *Penicillium* and *Trichoderma* into soil amended with organic compost and cultural practices showed effective control to several plant pathogenic fungi such as *Phytophthora* spp. causing root rot of durian, tangerine, black pepper, strawberry and lime (Soytong *et al*., 2001). In this case, the presence of antagonistic fungi and soil microflora may also influence

biocontrol activity by inhibiting the growth and development of plant pathogenic fungi or by metabolising antibiotic substances. The rhizosphere component of bioproducts from antagonist fungi have potential efficacy as far as introduced fungi are concerned; a root colonizer is a fungi that becomes distributed along the root in soil, propagates, and survives for long time, increasing colonization and reduction of pathogens when compared with chemical control (data not shown). Our research studies applying bioproducts become more integrated into management strategies in protection and curative of plant diseases. Similarly, the application of bioproducts from *Chaetomium* can protect and cure Thielaviopsis bud rot of *Hyophorbe lagenicaulis* in Thailand (Soytong *et al*., 2000). We can then develop effective applications and utilizations of bioproducts for the best control of plant pathogenic fungi.

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