Recent Advances on the Use of Natural and Safe Alternative Method to Control Grey Mould of Grapes

Mahendra Dahiwale^{1,*}, N S Suryawanshi²

¹Department of Botany, K. M. Agrawal College of Arts, Commerce and Science, Kalyan 421301, Dist.-Thane, Maharashtra, India

²Department of Botany, K V Pendharkar College of Arts, Science and Commerce, Dombivali, India

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Abstract Grey mould of grapes (*Vitis vinifera* L. var. *red globe*) caused by *Botrytis cinerea* Pers. ex. Fr. is recorded as an economically serious pathogen found in stored and imported grapes in India and which may cause heavy loss in all grape producing countries. Therefore the management of grey mould is equally important. In conventional agriculture the application of fungicides to grapes after harvest to reduce decay has been increasingly, curtailed by the development of pathogen resistance, the lack of knowledge of fungicide, negative public approach regarding the safety of pesticides and consequent restrictions on fungicide use. The study of alternative methods to control of grey mould decay has developed over several decades. Twenty five isolates of *B.cinerea* were isolated from infected samples of grape collected from various localities of Maharashtra. Out of which isolate *Bc-19* was highly sensitive (290.8 µg/ml) while isolate *Bc-11* was resistant (1095.1 µg/ml). Sensitivity of *B. cinerea* was tested using thiophanate methyl by food poisoning methods. Experiments were performed in Research Laboratory, KVP to find out the natural and safe alternative method to control of resistant *Bc-11*. Six fungicides viz. dithane M-45, thiram, zineb, benomyl, carbendazim and captafol were used individually and in mixture with thiophanate methyl. Benomyl gave a significant PCE individual at 50 µg/ml (70.01) and at 100 µg/ml PCE value (83.12) when compared with thiophanate methyl at 100 µg/ml (58.12), Captafol 50 µg/ml and 100 µg/ml (64.18), zineb 50 µg/ml (62.13) and 100 µg/ml (75.15) PCE values, thiram at 50 µg/ml (54.12) and 100

µg/ml (PCE- 65.10) and dithane M-45 at 50 µg/ml PCE (38.02) and 100 µg/ml (48.15) PCE values. Dithane and thiram was less effective. *Trichoderma viride* was found to be the most effective in reducing the colony diameter of *B. cinerea* (69.89 - 68.12). Thus *T. viride* proves better and safe for the management of *B. cinerea* of grape as a biocontrol agent.

Keywords Grey Mould, Grapes, *Botrytis cinerea*, Thiophanate Methyl, *Trichoderma*, Antagonism

1. Introduction

The most postharvest decay of table grapes (*Vitis vinifera* L. var. *red globe*) caused by *Botrytis cinerea* Pers. ex. Fr. is recorded as an economically serious pathogen found in stored and imported grapes in India and which may cause heavy loss in all grape producing countries. It is an opportunistic pathogen on a wide variety of crops, causing a disease known as grey mould through infections via wound [11]. The world wide post harvest loss is great and immense due to *B. cinerea* ranged between 10 to 50%. The use of chemical becomes essential when use of resistant varieties, fungal cultural practices or alternation of the environment is inadequate to manage this pathogen. Nowadays, chemicals play role in crop protection [9,10]. In cold stored grapes (-1 °C), the main decay pathogens are *B.cinerea* Pers., *Cladosporium herbarum* Link., *Alternaria alternata* Keissler, *Rhizopus stolonifer* Ehrenb,

Aspergillus niger Bainer and *Penicillium expansum* Link. Further losses occur during grading, packing, transport and finally marketing as fresh produce. Among these pathogens *B.cinerea* Pers. is most trouble because of its vigorous growth rate and ability to spread among grapes [7,8]. However, the use of systemic fungicides are not allowed on grapes and there are increasing regulatory restrictions on the use of chemical fungicides.

The use of alternative method to control postharvest decay of table grape extensively reviewed. Therefore minimize the economic losses and improve the quality of grapes. To develop management strategies to reduce fungicide used and adopt natural and safe alternative method to control the grey mould of grape. Wherein biological control would be significant [3]. *Trichoderma* is known to play an important role in natural and safe alternative because of it is easy to isolate and culture, grow rapidly on many medium and affect of a wide range of plant pathogen [1,2,4].

Present investigation was carried out to evaluate a number of *Trichoderma* species and isolate, alone and in combination with different fungicides viz. benomyl, dithane, zeneb, thiram and captafol for the controlling *B.cinerea*.

2. Materials and Methods

2.1. Isolation of *Botrytis cinerea*

Figure 1a. Healthy Fruit of Grape

Figure 1b. Infected Fruit of Grapes

Infected samples were collected from different localities of Maharashtra in 2008 to 2011. The infected samples were surface sterilized with 0.1% HgCl_2 solutions for one minutes with gentle agitation. Samples were rinsed with sterile distilled water (SDW) up to 5 to 6 times then cut into smaller pieces and place aseptically at equidistant onto sterile potato dextrose agar (PDA) augmented with streptomycin (20 $\mu\text{g/ml}$) in petriplates. Plates were incubated at $22 \pm 2^\circ\text{C}$ in dark for seven days. The pure culture of the pathogen was obtained. The pathogen was purified using PDA medium. Seven days old culture of *B. cinerea* were used for the experiment. Sensitivity of *B. cinerea* was tested using thiophanate methyl by food poisoning methods [6]. Isolate *Bc-11* was resistant while isolate *Bc-19* are sensitive were obtained. Resistant *Bc-11* was used for the experiment (Table 2).

2.2. Isolation of *Trichoderma* spp.

Three isolates of *Trichoderma* were isolated from mangrove soil of Thane creek. Approximately 20 gms of soil were added to 100 ml of SDW and shaking well for 15 min. The resultant suspension used to inoculate in PDA. All plates were incubated at room temperature $22 \pm 2^\circ\text{C}$. The fungal colonies appearing after 7 days were picked and restreaked until pure culture were obtained. The fungal colonies were identified and used against *B.cinerea*. PDA broth pour in 250 ml conical flask and inoculate the *T.viride*. and *T.harzianum* and inoculate at $22 \pm 2^\circ\text{C}$. After seven days *T.viride*. and *T.harzianum* were separated from the broth mycelial mats were used. Dried mycelial mat were grinded in powder form. Powder of *Trichoderma* and fungicides were used against *B.cinerea* in vitro.

3. Results and Discussions

Table 1 and fig 2 revealed that twenty-five isolates of *B. cinerea* were isolated from infected samples of grape using PDA. The table 2 showed that sensitivity of *B.cinerea* isolates to thiophanate methyl were tested. The thiophanate methyl sensitivity was determined by measuring the linear growth of *B.cinerea* colony on agar plate after eight days of inoculation. Plates without thiophanate methyl serve as control. Each treatment was replicated three times. There are quite variations in MIC of thiophanate methyl against grey mould pathogen. MIC ranged from 530 to 1280 $\mu\text{g/ml}$. The isolate *Bc-19* was sensitive and *Bc-11* was resistant. It is revealed that powder of *Trichoderma* and fungicides were used against *B.cinerea* in vitro. *T. viride* was found overgrew of *B.cinerea* therefore in proved that *T. viride* shows strong antagonistic properties (Fig 3).

Table 1. *In vitro* sensitivity of thiophanate methyl against *Botrytis cinerea*

Isolates	ED ₅₀ (µg/ml)	MIC(µg/ml)
Bc-1	380.2	980.2
Bc-2.	140.1	349.3
Bc-3	250.4	590.1
Bc-4	390.3	600.5
Bc-5	193.0	585.9
Bc-6	280.4	591.7
Bc-7	265.8	615.4
Bc-8	400.4	475.6
Bc-9	280.9	585.7
Bc-10	470.2	995.6
Bc-11	510.7	1095.1
Bc-12	360.5	594.2
Bc-13	310.6	695.3
Bc-14	195.7	312.5
Bc-15	210.4	600.5
Bc-16	300.8	635.3
Bc-17	270.3	615.1
Bc-18	295.7	780.1
Bc-19	130.2	290.8
Bc-20	330.4	627.1
Bc-21	400.3	885.9
Bc-22	310.7	625.4
Bc-23	290.9	631.3
Bc-24	420.3	905.2
Bc-25	345.1	680.3

ED₅₀ . Fungicide concentration causing 50 % reduction in radial growth

MIC - Minimal inhibitory concentration

Table 2. Sensitive and resistant isolate of *Botrytis cinerea*

Isolate	ED ₅₀ (µg/ml)	MIC(µg/ml)
Sensitive (Bc-19)	130.2	290.8
Resistant (Bc-11)	510.7	1095.1

Six fungicides viz. dithane M-45, thiram, zineb, benomyl, carbendazim and captafol were used individually and in mixture with thiophanate methyl. The concentration was used at 50 µg/ml and 100 µg/ml in potato dextrose agar medium (PDA). After 6 days growth of *B.cinerea* was observed and percentage control efficacy (PCE) was measured (Table 3). The results in table 3 showed that benomyl gave a significant PCE individual at 50 µg/ml (70.01) and at 100 µg/ml PCE value (83.12) when compared with thiophanate methyl at 100 µg/ml (58.12). Carbendazim at 50 µg/ml (65.03) and 100 µg/ml PCE values increased upto 70.05 as compared with thiophanate methyl. Captafal 50 µg/ml and 100 µg/ml (64.18) and (66.33) respectively and fallowed by zineb (62.13), (75.15) PCE values. Thiram at 50 µg/ml (54.12) and 100 µg/ml (PCE- 65.10) and dithane M-45 at 50 µg/ml PCE (38.02) and 100 µg/ml (48.15) when

compared with thiophanate methyl PCE values. Dithane and thiram was less effective individually (Table 3).

Fungicides were mix with thiophanate methyl in PDA. Test fungus inoculated at the centre of petriplate, after 6-7 days colony diameter was measured and PCE value determined. PCE was highly increased with benomyl, carbendazim, captafol, zineb, thiram and dithane M-45. It was going upto 42.08 -75.88 at 50 µg/ml concentration while PCE values ranged from 60.10 - 86.12 at 100 µg/ml concentration. Benomyl use as systemic fungicide PCE 75.88 as 50 µg/ml and 86.12 at 100 µg/ml as compared with individually and in mixture with thiophanate methyl PCE value appeared more fruitful and fallowed by other fungicides.

Figure 2. Pure culture of *B.cinerea* Pers.ex Fr. on PDA **Figure 3.** *Trichoderma viridae* overgrew on *B.cinerea*

Table 3. PCE of thiophanate methyl individually and in mixture with other fungicide against resistant mutant (*Bc*-EMS-4) of *B.cinerea* Pers. ex. Fr on PDA plate.

Sr. No.	Fungicide (µg/ml)	PCE individual	PCE mixture with Thiophanate methyl		
1	Benomyl				
	50	70.01	75.88		
	100	83.12	86.12		
2	Dithane M-45				
	50	38.02	42.08		
	100	48.15	60.10		
3	Zineb				
	50	62.13	64.12		
	100	75.15	78.17		
4	Thiram				
	50	54.12	62.50		
	100	65.10	78.08		
5	Carbendazim				
	50	65.03	70.10		
	100	70.05	75.12		
6	Captafol				
	50	64.18	70.10		
	100	66.33	72.13		
7	Thiophanate Methyl (290.8 µg/ml)	58.12	-		
S. E.		50% - 4.680	4.289	100% - 10.033	11.114
C. D at 0.05		10.352	9.487	22.192	24.584
0.01		12.738	11.674	27.309	30.252

Values are the mean of three replicates.

Table 4 revealed that the effects of the three species of *Trichoderma* that had the greatest potential of antifungal activity. *T. viride* and *T. harzianum* was found to be the most effective in reducing the colony diameter of *B. cinerea* (69.89 - 68.12). It also reported that *T. viride* and *T. harzianum* significantly suppressed *Fusarium udum* in soil and roots of pigeon pea [9]. Reference [8] observed that the isolate of *T. virens* and *T. viride* were the most effective in which maximum growth inhibition of mycelium of *Fusarium oxysporium* was recorded i.e., 77.7% and 77.55%. Reference [5] reveals that *Trichoderma* emerged as most potential bioprotectants for management of wide variety of plant diseases. Table 5 revealed that all treatment significantly reduced the inhibition zone of *B.cinerea*. The treatment with powder

of *Trichoderma* and fungicides (dithane M-45, carbendazim, thiophanate methyl, antracol and benomyl) gave overall steady results.

Table 4. Management of *Botrytis cinerea* using biocontrol agent

Sr. No.	Fungicides	PCE	PCE mixture with thiophanate methyl
1	<i>Trichoderma viride</i>	64.06	69.89
2	<i>Trichoderma harzianum</i>	51.85	68.12
3	<i>Trichoderma koeningi</i>	42.54	59.12
4	Thiophanate methyl (290.8 $\mu\text{g/ml}$)	64.74	-

Means of Three replicates

Table 5. Integrated management of *Botrytis cinerea* on CDA medium

Sr.No.	Antagonist	Dose (µg/ml)	PCE
1	<i>Trichoderma viride</i> + Benomyl	100	51.21 (4.2)
2	<i>Trichoderma viride</i> + Dithane M-45	100	53.65 (4.4)
3	<i>Trichoderma viride</i> + Zeneb	100	58.53 (4.8)
4	<i>Trichoderma viride</i> + Thiram	100	50.00 (4.1)
5	<i>Trichoderma viride</i> + Carbendazim	100	47.56 (3.9)
6	<i>Trichoderma viride</i> + Captafol	100	40.12 (3.1)
7	<i>Trichoderma harzianum</i> + Dithane M-45	100	52.43 (4.3)
8	<i>Trichoderma harzianum</i> + Carbendazim	100	54.87 (4.5)
9	<i>Trichoderma harzianum</i> + Thiophanate methyl	100	63.41 (5.2)
10	<i>Trichoderma harzianum</i> + Antracol	100	51.21 (4.2)
11	<i>Trichoderma harzianum</i> + Benomyl	100	48.78 (4.0)
12	<i>Trichoderma harzianum</i> + Captafol	100	40.20 (3.0)
13	<i>Trichoderma koningi</i> + Dithane M-45	100	56.09 (4.6)
14	<i>Trichoderma koningi</i> + Carbendazim	100	54.87 (4.5)
15	<i>Trichoderma koningi</i> + Thiophanate methyl	100	65.85 (5.4)
16	<i>Trichoderma koningi</i> + Antracol	100	41.46 (3.4)
17	<i>Trichoderma koningi</i> + Benomyl	100	43.90 (3.6)
18	<i>Trichoderma koningi</i> + Captafol		38.12 (3.1)
19	Control		-- (8.2)

Means of three replicates

4. Conclusion

1. Grape (*Vitis vinifera* L.) is an important fruit of India and its losses are much higher because operations to protect grapes from mechanical damage during storage. In storage condition various fungal pathogens destroyed the fruits. The grey mould is a very destroying pathogen on grapes therefore management is equally important.
2. A total of 25 isolates of *B. cinerea* were tested for their thiophanate methyl sensitivity by food poisoning method. There was quite a large variation in the sensitivity among the isolates collected from various places. In *vitro* isolate Bc-19 was sensitive while isolate Bc-11 was resistant. Bc-11 resistant isolate was used further studies for the management.
3. Use of thiophanate methyl was found highly effective in controlling of grey mould caused by *B. cinerea*. Individually thiophanate methyl was more effective.
4. *Trichoderma viride* was highly effective PCE followed by *T. harzianum*, *T. koningi*.
5. Currently biological control is considered very promising alternatives to fungicide in control of post-harvest decay of grapes. Fungal antagonist *T. viride* and *T. harzianum* was found greatest inhibitory effect (69.89 % and 68.12%).
6. Biological agents viz. *T. viride*, *T. harzianum* and *T. koningi* are ecofriendly and considered best for

management of grey mould disease caused by *B. cinerea*, reducing mycelia growth upto 83.52 percentage.

7. Integration approaches of biocontrol agent i.e., *T. viride*, *T. harzianum* with thiophanate methyl was beneficial in controlling of this disease but not more effective.

Research during the previous five decades indicates another potential option for fruit disease management. That is the biological control of fruit diseases. Bio-control assumes special significance in being an eco-friendly and cost-effective strategy that should be used in integration with other strategies for a greater level of protection with sustained fruit production.

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