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FUNGI ASSOCIATED WITH GUAVA ANTHRACNOSE AND MANAGEMENT OF *COLLETOTRICHUM GLOEOSPORIOIDES* THROUGH BIOLOGICAL AND CHEMICAL MEANS

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A B S T R A C T

Guava (*Psidium guajava* L.) belongs to family *Myrtaceae*, is the fourth most important fruit crop of Pakistan. In spite of its importance in the livelihood and upliftment of the economy of farmers, the production of guava has been reduced due to anthracnose problem thorought the Pakistan. Results of investigation revealed that *C. gloeosporioides* was established as major causal organism. In vitro biological control of *Colletotrichum gloeosporioides* by *Aspergillus flavus* gave good results and appeared to be the most effective against the test pathogen followed by *Aspergillus niger* and *Trichoderma harzianum*, while *Aspergillus fumigatus* gave poor results. Out of six fungicides tested against *Colletotrichum gloeosporioides*, systemic fungicides gave more good results than non systemic fungicides. Least colony growth was observed in case of Derosal which gave effective control against *C. gloeosporioides* followed by Bayletan, Daconil, Ridomil Gold, Mancozeb and Alliete. By the application of these strategies the anthracnose problem can be managed properly with better economic benefits and small risk of health hazard effects. These studies whould be useful for high quality guava fruit production and to control this disease.

Keywords: Guava, Anthracnose, Antagonistic, Biocontrol.

INTRODUCTION

Guava is widely distributed throughout the tropics, while India and Mexico are major producers. Its annual production in Pakistan was 495 thousand tones and ranked 4th among guava producing countries (Pakistan economic survey, 2015-16. Fruit contains 82 percent water, 0.7 percent protein, 11 percent carbohydrates and good amounts of vitamins A, B and C including some minerals, alkaloids, tannins, (Joseph and Priya, 2011). Guava is mixed with cornmeal and other ingredients to make breakfast food flakes (Amusa *et al*., 2005).. It can also be used in sugar syrup, while its juice may be used in the preparation of ice cream. The fresh cuts of guava are used as salad. A total 10 diseases have been reported on guava of which anthracnose is recognized as the second most important disease (Rahman *et al*., 2003).

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Among diseases anthracnose of guava is one that impairs the quality of fruits (Amusa *et al.,* 2005). This disease attack on all above ground parts of plant causes the death of branches spots on unripe fruits develop especially during the rainy season. The most characteristic symptom includes appearance of small pin heads sized spots. In moist weather acervuli are produced in abundance on dead twigs. Disease is favoured by comparatively temperature ranges 25 -30°C and high relatively humidity (Soares *et al.,* 2014). The fruits are usually infected at early maturity, but the fungus remains quiescent until symptoms develop during ripening. The primary symptoms on mature fruits are circular, sunken lesions with dark brown centres and these lesions often merge to produce large rotten areas on ripening. Mechanical and physiological injuries created during and after harvest are the usual sites of invasion by 'wound pathogens' which as a group cause the most devastating post harvest disease (Pandey

et al., 1997). This disease has become a serious obstacle for the cultivation of guava. Its food value and market price is falling and has threat to germplasm preservation (Rahman *et al*., 2003). Various approaches including chemical sprays and cultural practices have been used to control anthracnose with partial success (Ansari, 2000). In the absence of proper management and control options, there is danger that the fruit growers may shift to some other crop. Keeping in view the significance of the problem, research was conducted to record the disease incidence and severity in different districts of Punjab and its management through the use of antagonists and chemicals.

MATERIALS AND METHODS

Isolation, purification and identification of associated fungi: Guava twigs and fruits showing symptoms of infection were collected from orchards of five districts of Punjab. Parts showing the symptoms of anthracnose were sliced into 3-5mm long pieces. These pieces were surface sterilized for 3 minutes in 1% NaOCl and then were rinsed in 4 successive changes of sterile distilled water. These pieces were plated on PDA in petri dishes and were incubated for six days under alternating 12 hr light and dark periods at 25°C. The fungal isolates were examined under a microscope. The identity of these fungi were determined by using morphological characters and descriptions in existing publications of Webster (1980) and Barnett (1972).The frequency of each isolated fungus from each part in the cultured specimens was calculated using the following formula.

$$\text{Colonization \%} = \frac{\text{No. of pieces colonized by fungus}}{\text{Total No. of pieces}} \times 100$$

In vitro biological control of pathogen: *In vitro* screening experiment was conducted to find out the antagonistic effect of the different isolated fungi against *C.gloeosporioides* on PDA by dual culture technique (Dhingra and Sinclair, 1985). Discs of mycelium (10 mm diameter) of each of the selected fungi were cut from the edge of an actively growing fungal colony with a cork borer. Test plates were prepared by pouring 20 ml of PDA per plate. After solidification, one mycelial disc of different isolated fungi and one disc of test fungal pathogen were placed separately on the edge of the each PDA petri plate at opposite direction. Three replicated plates were used for each isolated fungus and pathogen. The plates were arranged on the laboratory desks following completely randomized design. The plates received only mycelial discs of the test pathogens served as control. The plates were incubated in the laboratory having ambient temperature of 25°C until mycelium of the test pathogens *C. gloeosporioides* cover the whole control plate. Then mycelial growth of each antagonistic fungi and test pathogen was calculated. Inhibition percentages of *C. gloeosporioide* were calculated based on the growth of the pathogen on PDA plates following the formula as suggested by Sundar *et al.* (1995).

$$\text{\% Inhibition} = \frac{X - Y}{X} \times 100$$

Where, X= Growth of control plate Y= Growth of test plate.

Experiment was conducted following Completely Randomized Design (CRD) with three replications. The significant difference, if any, among the means were compared by (DMR).

In vitro chemical control of *Colletotrichum gloeosporioides*: Six fungicides were tested *in vitro* to evaluate their effect on colony growth of *C.gloeosporioides* by poisoned food technique (Dhingra and Sinclair, 1985). Fungicidal suspensions of different concentrations were prepared by dissolving requisite quantities of each fungicide in warm PDA. The fungicides were thoroughly mixed with the medium by shaking with hands after autoclaving. About 15 ml of sterilized medium was poured in each 9 cm sterilized petridish. After solidification, the plates were inoculated by placing 5mm discs of 7 days old PDA cultures of *C.gloeosporioides*. Three replicated plates were used for each concentration of every fungicide. Three replicated PDA plates received no fungicides and served as control. The inoculated plates were incubated at 28°C and data on the radial colony diameter was recorded after 4-5 days of incubation when the growth of the control plates completely covered the plate. Diameter of the colonies on PDA with and without fungicide was measured from the bottom side of the petri dishes. Inhibition of radial growth was computed based on colony diameter on control plate using the following formula as stated by Sundar *et al.* (1995).

$$\text{\% Inhibition} = \frac{X - Y}{X} \times 100$$

Where, X= Growth of control plate Y= Growth of test plate.

Experiment was conducted following (CRD) with three replications. The significant difference, if any, among the means were compared by (DMR).

RESULTS

Isolation, purification and identification of associated fungi: Total 11 fungi namely *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Alternaria* sp., *Rhizopus* sp., *C.gloeosporioides*, *B.theobromae, Helmenthosporium* sp., *Curvularia* sp., *Pythium* sp. and *Fusarium solani*, were isolated from twigs and fruits of guava plants. Of these *Colletotrichum gloeosporioides* was the most abundant fungus that was isolated from all the districts as well as from twigs and fruits (Table 1).

Effect of antagonists against *C. gloeosporioides*: Among the selected antagonists *Aspergillus flavus* was appeared to be the most effective against the test pathogen showing the least (14.1 mm) colony growth of pathogen and highest percentage of inhibition (83.1%) of colony growth of pathogen. *Aspergillus niger* showed (20.8 mm) colony growth of pathogen and percentage of inhibition was (75.2%) of colony growth of pathogen. Unknown *Aspergillus sp*. inhibited 63.3 % of colony growth of pathogen showing average colony growth (30.8 mm) in dual cultue plate technique. While *Trichoderma harzianum* exhibited (42.5 mm) colony growth of pathogen and 49.4 % was the percentage of inhibition. The lowest percentage of inhibition was 37.5 % in case of *Aspergillus fumigatus* with 52.5 mm of colony growth of pathogen. The average growth of control plate was 84.1 mm as shown in Figure 1.1, 1.2, 1.3.

Table 1. Fungi isolated from twigs and fruits of anthracnose infected guava plants in different districts of Punjab.

Fungi isolated	Colonization percentage at different districts									
	Sargodha		Faisalabad		Lahore		Khanewal		Multan	
	Twig	Fruit	Twig	Fruit	Twig	Fruit	Twig	Fruit	Twig	Fruit
Aspergillus flavus	6.67	26.40	13.30	6.67	13.30	13.30	6.67	20.00	13.30	20.0
Aspergillus niger	6.67	6.67	6.67	6.67	6.67	6.67	13.30	6.67	6.67	6.67
Aspergillus fumigatus	6.67	6.67	-	6.67	-	-	6.67	6.67	-	6.67
Alternaria sp.	6.67	-	6.67	-	-	13.30	-	-	6.67	-
Rhizopus sp.	-	-	6.67	20.00	6.67	6.67	6.67	6.67	-	-
C. gloeosporioides	40.00	40.00	40.00	33.30	40.00	40.00	60.00	60.00	46.6	33.3
B. theobromae	20.00	20.00	-	13.30	13.30	20.00	6.67	-	20.00	20.0
Helmenthosporium sp.	-	-	6.67	6.67	6.67	-	-	-	6.67	13.3
Curvularia sp.	-	-	-	6.67	13.30	-	-	-	-	-
Pythium sp.	-	-	13.30	-	-	-	-	-	-	-
Fusarium solani	13.30	-	6.67	-	-	-	-	-	-	-

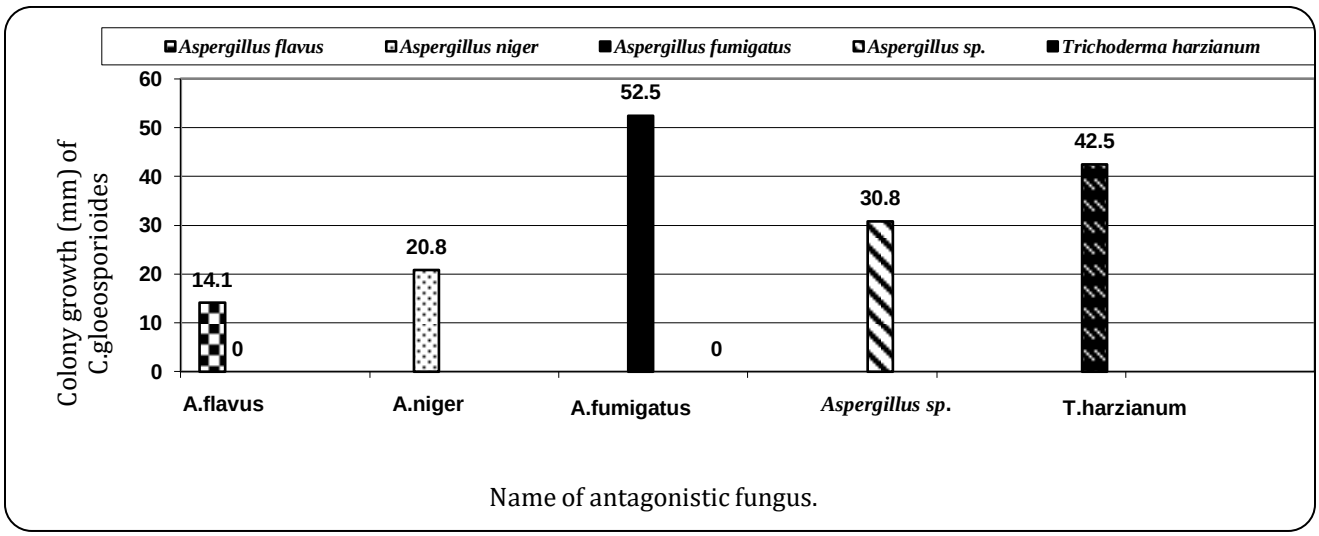

Figure 1.1. Effect of antagonistic interaction on average colony growth (mm) of *C. gloeosporioides* in dual culture plate technique.

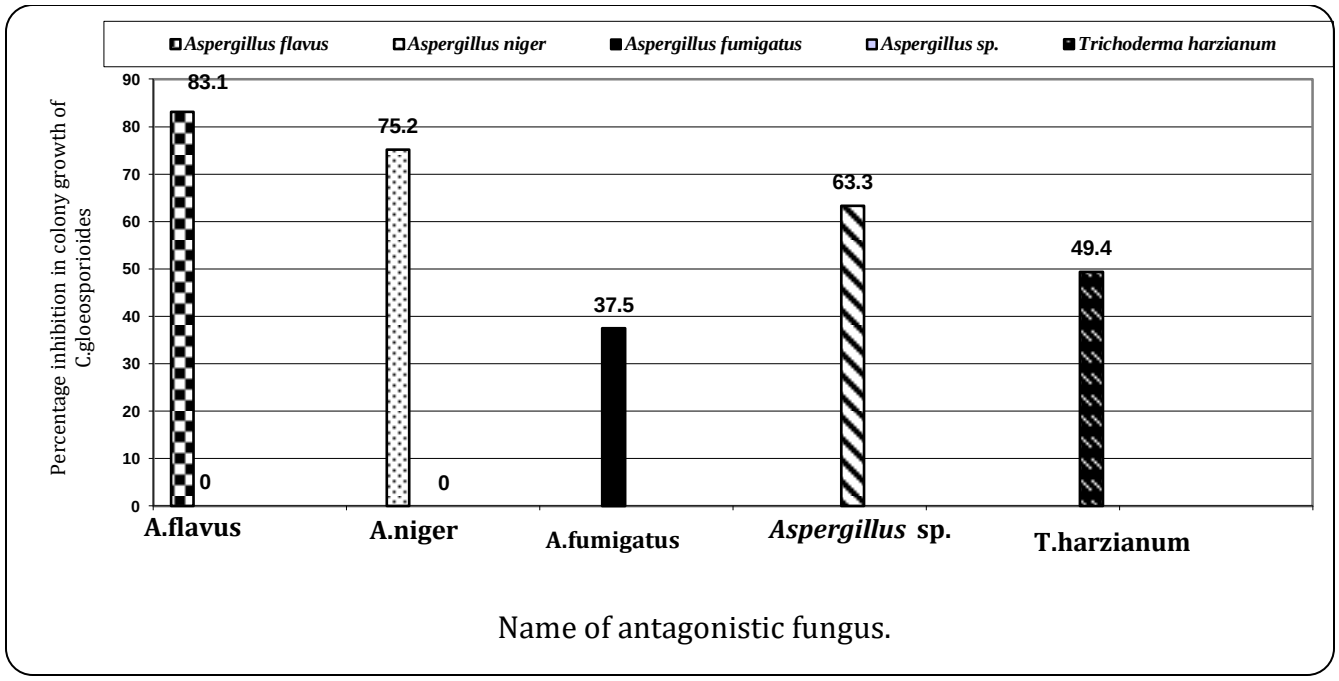

Figure 1.2. Effect of antagonistic interaction on percentage inhibition in average colony growth (mm) of *C. gloeosporioides* in dual culture plate technique.

Figure 1.3. Overall view of antagonistic interaction.

Effect of different chemicals on mycelial growth of *C. gloeosporioides*: Systemic fungicides gave good results than non-systemic fungicides. Least colony growth was observed in case of Derosal at 60 ppm, 20 ppm and 40 ppm with 7 mm, 10 mm and 11.6 mm growth respectively.

Bayletan at 20 ppm, Daconil at 20 ppm, Ridomil Gold at 60 ppm showed 29 mm, 37.3 mm and 42.6mm growth of pathogen respectively. 46.5 mm, 47.6mm and 54.5 mm average growth of *C.gloeosporioides* were observed in case of Daconil at 60 ppm, Bayletan at 40 ppm and 60 ppm respectively. While Daconil showed 55.8 mm at 40 ppm, and Mancozeb at 60 ppm showed 62.1mm of growth. Mancozeb at 20 ppm, Alliete at 40 ppm, Mancozeb at 40 ppm showed 66mm, 67.10mm and

69.6mm of growth respectively. 72.1mm growth of pathogen was observed in Ridomil Gold at 40 ppm and in Alliete at 20 ppm. While Alliete showed 74.5 mm at 60 ppm and Ridomil Gold showed maximum colony growth of 76mm respectively. From fungicides means, it appeared that response of each fungicide is different. Best response was in Derosal followed by Bayletan, Daconil, Ridomil Gold, Mancozeb and Alliete. Means of dose rates showed similar statistically response of 20 and 60 ppm which was different from 40 ppm (Table 2).

Table 2. Effect of different fungicides on mycelial growth of *C.gloeosporioides* by poisoned food technique after 6 days.

Treatments	Average colony growth (mm) at different concentrations (ppm)		
	20 ppm	40 ppm	60 ppm
Mancozeb	66.00	69.60	62.10
Daconil	37.30	55.80	46.50
Ridomil gold	76.00	72.10	42.60
Derosal	10.00	11.60	7.00
Bayletan	29.00	47.60	54.50
Alliete	72.10	67.10	74.50

Effect of different chemicals on percentage of inhibition of *C. gloeosporioides*: Maximum inhibition was observed in Derosal at 60, 20 and 40 ppm. Bayletan at 20 ppm, Daconil at 20 ppm, Ridomil Gold at 60 ppm showed 66.66%, 57.1% and 51 percent inhibition respectively. 46.5%, 45.2% and 37.3 percent of inhibitions were observed in case of Daconil at 60 ppm, Bayletan at 40 and 60 ppm respectively. While Daconil showed 35.86 percent of inhibition at 40 ppm, and Mancozeb at 60 ppm showed 28.6 percent of inhibition. Mancozeb at 20 ppm, Alliete at 40 ppm, Mancozeb at 40 ppm showed 24.1, 22.8 and 20 percent of inhibitions respectively.17.1 percent of inhibition was observed in Ridomil Gold at 40 ppm and in Alliete at 20 ppm. While Alliete showed 14.3 percent at 60 ppm and Ridomil Gold showed least percent of inhibition about 12.6 respectively (Figure 2.1).

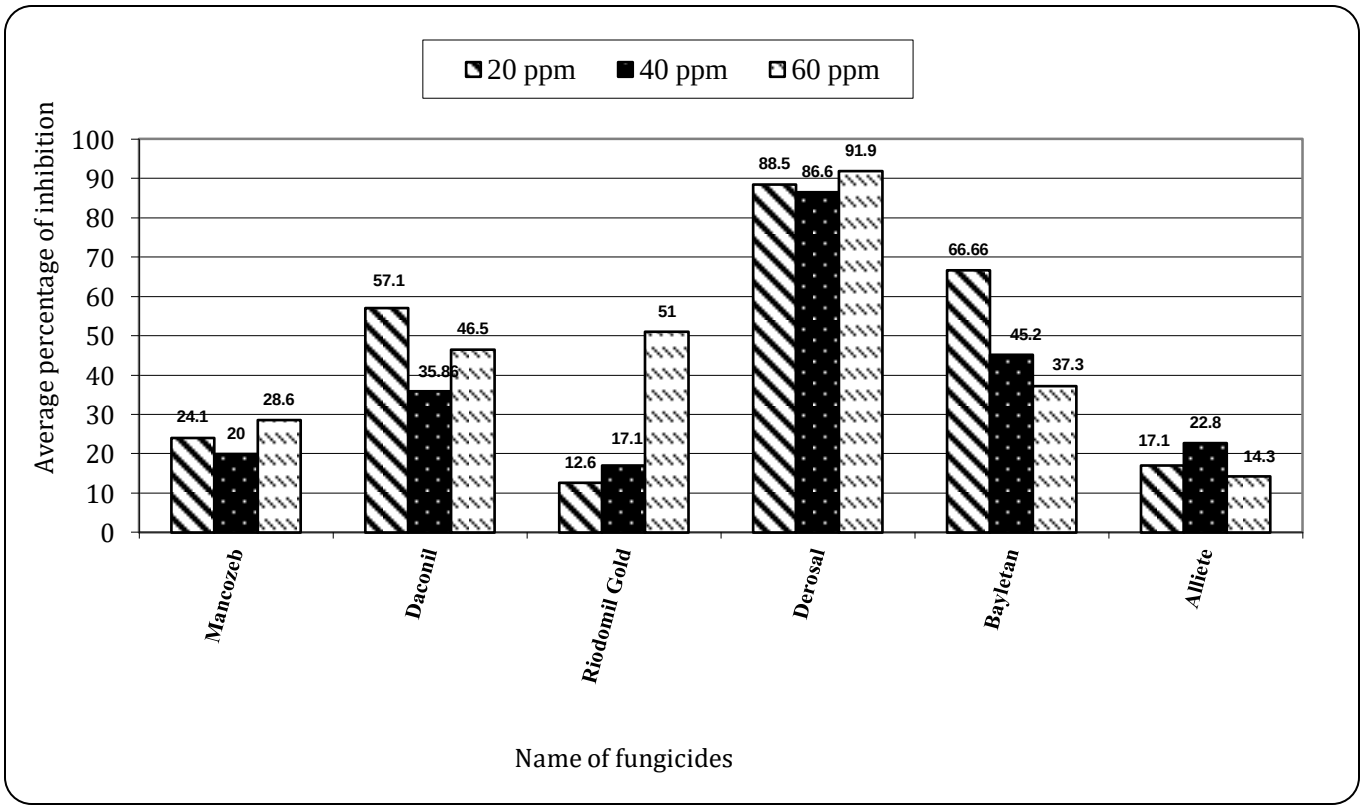

Figure 2.1. Effect of fungicides on percentage inhibition in average colony growth of *C. gloeosporioides*.

Figure 2.2. Overall view of colony growth of *C. gloeosporioides* in all tested fungicides.

DISCUSSION

Different fungi have been isolated and identified but *C. gloeosporioides* was isolated from most of the diseased guava fruit sample collected from different districts. The pathogen has a wide host range and successfully invades mango, pear and apple fruits supported by Wahid (2001) and Peres *et al*. (2002). The *C. gloeosporioides* isolates were more aggressive on guava fruits than the other tested fruits, like apple, pear and mango. Antagonistic effects of different saprophytic fungi indicated the importance of many such fungi as a possible biocontrol agent. *Aspergillus* species overgrew *C. gloeosporioides* in culture and eventually displaced it. *Aspergillus flavus* inhibited the growth of pathogen about 83.1 % more than *Trichoderma harzianum*. These results were similar to those achieved by Evueh and Ogbebor (2008) in which *Aspergillus* sp. lysed the cytoplasm of *C.gloeosporioides* on PDA and inhibited 99.09% of fungal growth. While *Trichoderma* sp. did not show any clear pattern of hyphal interaction and inhibited the growth about 90.90%. This could possibly be as a result of competition between saprophytic and pathogenic microorganisms for nutrients. *C. gloeosporioides* was inhibited and this is as a result of the production of metabolites. As *Aspergillus* species except *A. fumigatus* caused more than 50% of inhibition similar results were obtained by Pandey *et al.* (1993). While *Trichoderma harzianum* showed 49.4% inhibition similar results were obtained by Osando and Waudo (1994) who observed that different isolates of *Trichoderma* sp. exhibited different level of antagonism against *Armillaria* root rot fungus which may be due to severe vacuolation, shrinkage and coagulation that is produced by *T. harzianum* against the cytoplasm of the pathogen hyphae. Shovan *et al*. (2008) also showed the similar results in which the screened isolates of *Trichoderma* showed significantly variable antagonism ranging from 50.93 to 89.44% reduction of the radial growth of *C. dematium.*

Derosal gave good results against *C. gloeosporioides* which are principally alike to the results obtained by Vir and Guar (1973), Leroux and Gred (1974), Pandey (1988), Oh and Kang (2002) and Everett *et al*. (2005) who found that Carbendazim (Derosal) gave better control of anthracnose disease of guava. It is due to the sensitivity of different isolates of *C. gloeosporioides* to benzimidazole group that was according to Zauberman *et al*. (1976) who reported that *C. gloeosprioides* is extremely sensitive to compounds which possess the methyl benzimidazol-2-yl carbamate (MBC) toxic moiety.

It could be used as the substitute for the other fungicides as described by Bernstein *et al*. (1995) that isolates of *C.acutatum*, from a variety of hosts that were resistant to benomyl. If the variation is made between benzimidazoles and triazoles in greenhouse and field experiments, thiabendazole and derosal were more effective than hexaconazole and propiconazole.After the

Derosal, Bayletan was found affective Jiskani *et al*. (2000) revealed that bayleton 25 WP was found to be the best for control of disease followed by calixin, baytan and topass as compared to control (no fungicide).

Among the non-systemic fungicide Daconil was effective described by Smith and Black (1993). It gave good results *in vitro* against *C. gloeosporioides* by Gullino *et al*. (1985) and in field by Cole *et al*. (2005). Mancozeb did not give satisfactory results as Shovan *et al*. (2008) described Dithane M-45 (Mancozeb) and Cupravit were of low quality to control the *C.dematium.* Similarly for Alliete, Brown (1992) showed that fosetyl-Al did not give control for brown rot when fruits were inoculated 3 days before. If all these fungicides are available the first preference should be given to Carbendazim followed by Bayletan and Riodomi Gold with alternating programme. In other case of non availibilty of systemic fungicides Daconil can be used.

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