Pak. J. Agri. Sci., Vol. 50(4), 707-710; 2013
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

OCCURRENCE OF GUAVA ANTHRACNOSE IN PUNJAB (PAKISTAN) AND ITS INTEGRATED MANAGEMENT

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Survey of guava (*Psidium guajava* L.) orchards was conducted to assess the incidence and severity of fruit anthracnose of guava in Sheikhupura, Sargodha, Faisalabad, Hafizabad, Jhang and Chiniot districts of Punjab province. Maximum disease incidence was recorded in Shiekhupura (24%) followed by Sargodha (18.37%) while minimum in Chiniot (9%). Disease severity in Sheikhupura, Sargodha, Faisalabad, Hafizabad, Jhang and Chiniot districts was recorded as 55%, 42%, 35%, 46%, 31% and 22% respectively. Efficacy of different chemicals (mancozeb, daconil, ridomil gold, derosal, bayleton, aliette), biological agents (*Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Trichoderma harzianum*) and Plant growth promoting rhizobacteria (PGPR) (*Bacillus subtilis* and *Pseudomonas fluorescens*) was tested *in vitro* against *Colletotrichum gloeosporioides*, the causal agent of anthraconose of Guava. Aliette was the most effective at all concentrations followed by mancozeb which was effective at 20 and 40 ppm concentrations but less at 60 ppm. Derosal was least effective at its all concentrations. *Aspergillus flavus* and *Pseudomonas florescence* were found to be the most effective treatments among fungi and PGPR evaluated respectively in inhibiting the colony growth of *C. gloeosporioides.* These findings may provide information regarding chemical and biological control against *C. gloeosporioides* under *in vitro* conditions and serve as guide for future field trials.
Keywords: Antagonistic fungi, anthracnose, guava, disease management, PGPR

INTRODUCTION

Guava (*Psidium guajava* L.*)* an important member of family *Myrtaceae* L. is assumed to be originated from Southern part of Mexico. It contains vitamins A, B, C plus some minerals and good for health (Baradi, 1975). It is the fourth important fruit of the Pakistan. In Pakistan it is grown on an area of 665 thousand ha with a total annual production of 5495 thousand tons with yield of 28,842 kg/ha (FAO, 2011). Guava plant growth is related to nutrient acquisition (Swain and Padhi, 2012) and guava branch architecture influences foliage fauna (Ghaffar *et al*., 2011).
Among fungal diseases of guava, anthracnose of guava, caused by *Colletotrichum gloeosporioides* (B. Weir & P.R. Johnst), gets more attention and is the most commonly observed disease that can affect young developing flowers, fruit and has been reported in all guava-growing areas around the world (Kumar *et al*., 2007).
Different management strategies for the recovery of guava trees were studied to evaluate their effects on the productivity of trees affected by various pathogens (Padilla *et al.*, 2003). Organic sulfur (dithiocarbamates) fungicides like zineb, maneb and heterocyclic nitrogen compounds gave adequate control against *Colletotrichum gloeosporioides* (Ruehl and Ledin, 1960). Different species of antagonistic fungi have been used widely against *C. gloeosporioides* and can cause maximum growth inhibition (Pandey *et al*., 1993). Plant growth promoting rhizobacteria (PGPR), especially *Pseudomonas fluorescens*, *Bacillus subtilis* and some yeast antagonists are also promising candidates as bioprotectants. *Pseudomonas fluorescens* had the greatest inhibitory effect on *C. gloeosporioides* under in vitro conditions (Vivekananthan, 2006).
Keeping in view the importance of guava, present investigation was aimed to investigate the disease incidence and severity of anthracnose of guava and to determine the efficacy of fungicides, antagonistic fungi and PGPR on the growth of *Colletotrichum gloeosporioides* under in vitro conditions.

MATERIALS AND METHODS

***Assessment of percent disease incidence and disease severity*:** Survey was conducted in guava orchards of Chiniot, Faisalabad, Hafizabad, Jhang, Sheikhupura, and Sargodha Districts of Punjab province, Pakistan. Samples showing typical disease symptoms were collected. Randomly 5 orchards were selected from each District. Data regarding disease incidence on trees was recorded on visual basis and five plants were selected in each orchard to record disease incidence on trees by given formula.

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected trees}}{\text{Total no. of trees}} \times 100$$

Severity index was calculated by using the following formula.

$$\text{Disease severity index (\%)} = \frac{\text{(Sum of all disease ratings)} \times 100}{\text{(No. of trees observed x Maximum disease grade)}}$$

***Efficacy of various fungicides for the management of Colletotrichum gloeosporioides*:** The tested material culture (*Colletotrichum gloeosporioides*) was multiplied on PDA for experimentation. Six fungicides, mancozeb, daconil, ridomil gold, derosal, bayleton, aliette were tested at the concentrations of 20, 40 and 60 ppm to evaluate their effect on colony growth of *C. gloeosporioides* by poisoned food technique (Dhingra and Sinclair, 1985). Three replicated plates were used for each concentration of every fungicide with Completely Randomized Design (CRD). 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 8 days of incubation when the growth of the control plates completely covered the plate. Inhibition of radial growth was computed based on colony diameter on control plate using the formula (Sundar *et al.*, 1995):

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

(X= growth of control plate; Y= growth of test plate).

***Biological control of Colletotrichum gloeosporioides through antagonistic fungi*:** Antagonistic effect of *Aspergillus flavus*, *A. niger*, *A. fumigatus*, and *Trichoderma harzianum* was studied against *Colletotrichum gloeosporioides* on PDA by dual culture technique (Dhingra and Sinclair, 1985). Each treatment was replicated three times in completely randomized design. The plates received only mycelia discs of the test pathogens served as control. The plates were incubated at ambient temperature of 25°C until mycelium of the test pathogens *C. gloeosporioides* cover the whole control plate. Mycelial growth of each antagonistic fungi and test pathogen was calculated. Inhibition percentage of *C. gloeosporioides* was calculated based on the growth of the pathogen on PDA plates (Sundar *et al.*, 1995).

***Biological control of Colletotrichum gloeosporioides through Plant growth promoting rhizobacteria (PGPR)*:** Tests for antibiosis between PGPR strains (*Bacillus subtilis* and *Pseudomonas fluorescens*) and *Colletotrichum gloeosporioides* were conducted on PDA plates. 6-mm-diameter plug of *C. gloeosporioides* mycelia taken from the edge of a colony on agar was placed at the center of each PDA plate. PGPR bacterial cells were streaked in a straight line 3 cm from the *C. gloeosporioides* inoculation plug. The plates were incubated at 25ºC for 7 days, and then checked for inhibition zones and any changes in morphology of the *C. gloeosporioides* mycelia. Two PGPR strains were tested on each plate, and the experiment was conducted thrice and mean of colony growth was recorded (Zhang *et al*., 2010).

RESULTS

Survey results indicated that disease attacks on all above ground parts of plant. The most characteristic symptom includes appearance of small pin heads sized spots. In moist weather acervuli are produced in abundance on dead twigs full sudden decline of branch or even the whole tree. The mean disease incidence for all the districts was 15.58%. Maximum disease incidence was recorded in Shiekhupura (24%) followed by Hafizabad (18%) and minimum in Chiniot (9%). Maximum disease severity occurred in Sheikhupura (55%) followed by Hafizabad (46%) and Sargodha (42%). Maximum disease incidence as well as maximum disease severity was found in Sheikhupura district (Fig. 1).

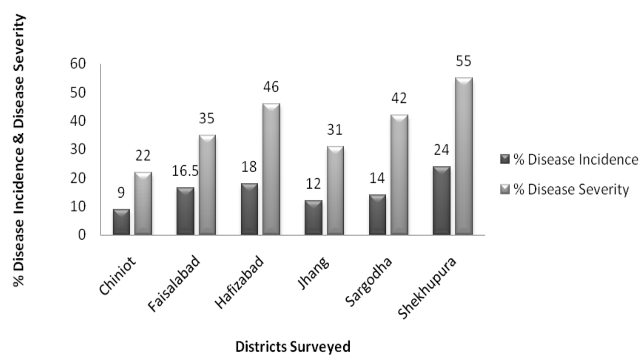

Figure 1. Disease incidence and disease severity of guava anthracnose in various districts of Punjab

The effect of six fungicides was tested by food poisoning technique on the mycelial growth of *Colletotrichum gloeosporioides* after 8 days. Ridomil gold was the most effective followed by mancozeb and daconil at 20 and 40 ppm concentrations. Bayleton was effective at 40 ppm concentration. Derosal was the least effective at all concentrations (Fig. 2).

In the *in vitro* studies, all the organisms tested for their antagonistic action inhibited the growth of the pathogen. *Aspergillus flavus* was found to be the most effective against *Colletotrichum gloeosporioides* and showed the least (13 mm) colony growth of pathogen. *Aspergillus fumigatus* showed (21 mm) colony growth of pathogen. *Aspergillus niger* inhibited the colony growth of pathogen showing average colony growth (29 mm) in dual culture plate technique. The lowest growth inhibition was recorded in case of *T. harzianum* with (37 mm) of colony growth of pathogen. The average growth of the fungus in control plate was (87 mm). The results showed that *A. flavus* was the most effective in inhibiting the colony growth of *C. gloeosporioides* while *T. harzianum* was the least effective (Fig. 3).

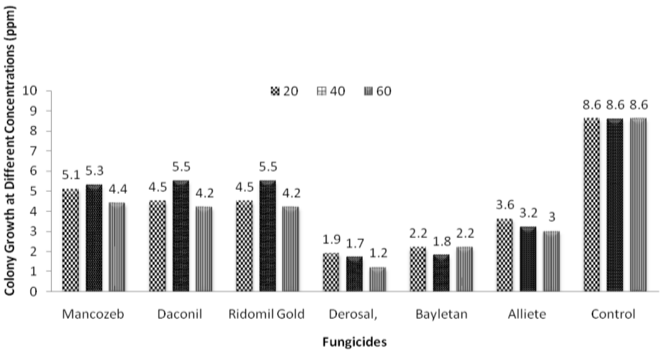

Figure 2. Effect of different fungicides on mycelial growth of *C. gloeosporioides*

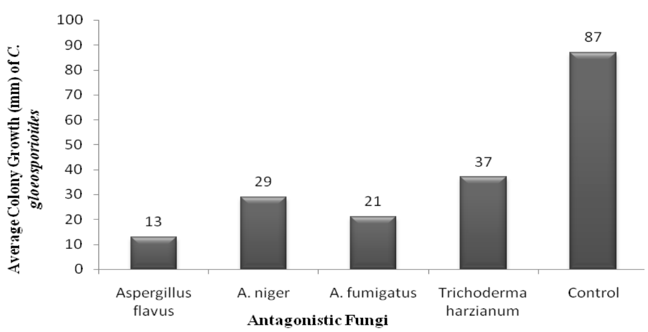

Figure 3. Effect of antagonistic interaction on average colony growth (mm) of *C. gloeosporioides*

Among PGPR, *Pseudomonas fluorescens* was found to be the most effective in inhibiting the fungus growth. *Bacillus subtilis* was less effective as compare to *P. fluorescens* in inhibiting the colony growth of *C. gloeosporioides* but significant difference was notices as compared to control (Fig. 4).

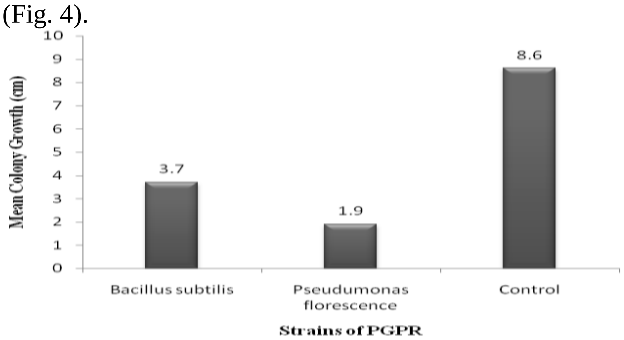

Figure 4. Effect of PGPR on average colony growth of *C. gloeosporioides*

DISCUSSION

Guava decline is becoming more common and severe in the guava orchards of the Punjab, Pakistan and is inflicting substantial losses to the gardens. The disease is found in all guava growing districts of the Punjab surveyed for this purpose with varying degrees. However, it is more severe in the Sheikhupura district followed by Hafizabad and Sargodha, whereas its severity was minimal in Chiniot. It seems that environmental conditions, particularly the soil texture and the water table, for the development of the disease were more favorable in Sheikhupura compared to other districts and Moist weather favors the production of acervuli on dead plant parts (Hossain and Meah, 1992).

The results showed that out of six fungicides belonging to different chemical groups, three (aliette, mancozeb and ridomil gold) were more effective while from the biological control agents *A. flavus* and *P. florescence* were the most effective. These findings are in conformity with Mahoney and Tattar (1980). The effectiveness of six fungicides applied with and without the surfactant in controlling *Colletotrichum gloeosporioides* was also tested (Cole *et al*., 2005). The fungicides tested were mancozeb, copper hydroxide, trifloxystrobin, chlorothalonil, myclobutanil, and azoxystrobin. These fungicides were also incorporated into potato dextrose agar (PDA) to determine the effective concentration to obtain 50% inhibition of mycelial growth of *C. gloeosporioides*. It was assessed from the present investigation that the biological control provides the effective control over *C. gloeosporioide.* Culture of *Aspergillus niger* caused more than 50% growth inhibition of *C. gloeosporioides* (Pandey *et al*., 1993). Volatiles are produced that inhibit the growth but their effect decreased with the increase of incubation time. Different isolates of bacteria and T-CB-Pin-01 (a commercial strain of *Trichoderma harzianum*) could reduce disease severity caused by *C. gloeosporioides* (Yenjit *et al*., 2004). *Aspergillus* spp. lysed the cytoplasm of *C. gloeosporioides* on PDA and inhibited about 99.09% colony growth. *T. harzianum* did not show any clear pattern of hyphal interaction and inhibited the growth about 90.90% (Evueh and Ogbebor, 2008).

The fungicides have different mode of actions through which these inhibit the growth of pathogens. Aliette (Aluminium tris O-ethyl phosphonate) is a systemic fungicide and no resistance is likely to develop against it. Mancozeb is broad spectrum, non-systemic and contact with protective action which acts by disrupting the lipid metabolism. Ridomil gold (Metalaxl-M) has residual and systemic action and inhibits germination of the spores. These fungicides disrupted the mechanism of *C. gloeosporioides* and inhibit its development. Our results are in conformity with the other scientists (Gullino *et al*., 1985; Evueh and Ogbebor, 2008).

Inhibitory effect on the growth of *C. gloeosporioides* may be due to different enzymatic activities such as pectin lyase by *A. niger*. *Trichoderma harzianum* have mycoparasitism and antibiotic (toxin) production (Howell, 2003). Among the PGPR tested *P. fluorescens* showed the greatest inhibitory effect on *C. gloeosporioides* under *in vitro* conditions and same results were found (Vivekananthan, 2006).

The results showed the incidence of anthracnose in all districts surveyed indicating the problem of anthracnose spreading through guava orchards and certain measures must be adopted to check this problem. *In vitro* evaluations of different fungicides and biological agents against *Colletotrichum gloeosporioides* may provide useful preliminary information regarding efficacy of different antagonistic fungi against another fungi within a short period of time and serve as guide for further field experiments in future and as an environment friendly tool, may be the best alternative to chemicals.

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