

Original Research Article

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In vitro* Evaluation of Systemic and Combi Fungicides against Anthracnose of Guava (*Psidium guajava* L.) caused by *Colletotrichum psidii

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Guava (*Psidium guajava* L.) is an important fruit crop of India and is considered to be poor men's apple. Its production is low in India because of several important diseases by which it suffers. Among diseases, Anthracnose is one that impairs the quality and has become a serious obstacle for the cultivation of guava fruits. Keeping in view the significance of the problem, research was conducted to screen the different chemicals under *in vitro* for the management of the disease. Among combi fungicides evaluated, SAAF, MATCO and SECTIN completely inhibited mycelial growth of the pathogen at all the three different concentrations (100 %). Similarly under systemic fungicides, Hexaconazole and Difenconazole showed 100 per cent inhibition under 500, 1000 and 2000 ppm followed by Thiophanate methyl (55.33 %).

Introduction

Guava (*Psidium guajava* L.) is an important tropical fruit crop of India and is considered as poor men's apple. The crop is commonly cultivated in many tropical and subtropical regions and it belongs to Myrtaceae family. Guava is native to Mexico, Central America, the Caribbean and northern South America. Due to its high level of pectin content, guavas are extensively used to make candies, preserves, jellies, jams, and marmalades. Fruit contains 82 per cent water, 0.7 per cent

protein, 11 per cent carbohydrates and good amounts of vitamins A, B and C including some minerals, alkaloids, tannins, (Joseph and Priya, 2011). In 2016, world production of guavas was 46.5 million tonnes, led by India with 41% of the total. Other major producers were China (10 %) and Thailand (7 %). In recent days its production is low in India because of many important diseases by which it suffers. There are a number of pre and post-harvest diseases of guava. Pre harvest diseases like Wilt, Canker, Leaf spot, Decline and Sooty mould etc. affect the plant growth

and production, while post-harvest diseases such as Anthracnose, Fruit rot, Dry rot and several others spoil the fruits in field, storage and in transit. Due to the perishable nature of the fruit, several post-harvest diseases are very important since they cause considerable yield loss to the crop. A total of ten important diseases have been reported on guava of which anthracnose is recognized as the second most important disease (Rahman *et al.*, 2003) that impairs the quality of fruits (Amusa *et al.*, 2005). It is a serious problem causing heavy loss in guava production in India.

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 relative 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 of this problem an experiment was conducted at College of Sericulture, Chintamani during 2019-2020 to know the efficacy of different contact, systemic and combi fungicides against mycelial growth of the pathogen under *in vitro*.

Materials and Methods

Isolation of the pathogen from Anthracnose infected plant sample

The pathogen (*Colletotrichum psidii*) from the Anthracnose infected fruit samples collected from premises of College of Sericulture, Chintamani and were isolated separately by following standard tissue isolation technique. The infected tissues along with healthy portions were cut into small bits and were surface sterilized with 0.1 % Sodium hypochlorite solution for 30 seconds followed by dipping in Ethyl alcohol and washed three times in sterile distilled water and transferred them to potato dextrose agar. The plates were incubated at room temperature (28±1⁰C) and observed periodically for fungal growth. The colonies which developed from the tissue bits were transferred to PDA slants, subcultured and used for further studies.

In vitro* evaluation of systemic and combi fungicides against *Colletotrichum psidii

The bio-efficacy of five systemic and combi fungicides were evaluated under *in vitro* conditions against *Colletotrichum psidii* for inhibition of radial growth on the PDA using Poisoned Food Technique (Sharvelle, 1961). The combi fungicides were evaluated at 1000, 2000 and 3000 ppm concentrations, whereas systemic fungicides were tested at 500, 1000 and 2000 ppm concentrations.

The requisite quantities of fungicides were incorporated aseptically to PDA medium cooled to 45°C, so as to, give the required

concentrations. Twenty milli litre of the poisoned medium was poured into flat-bottomed sterile Petri dishes. The plates were then inoculated by cutting half cm of seven days old mycelial discs of *Colletotrichum psidii* with a sterile Cork borer and incubated at $28\pm 1^{\circ}\text{C}$. Three replications were maintained for each treatment. The fungus growth on the PDA without any fungicide served as control. The radial growth (mm) of the colony was recorded when maximum growth (7 days) in control plates was noticed. The per cent inhibition of the mycelial growth of the fungus was determined by using the Vincent's formula (Vincent, 1947). The percent values were converted into angular transformations, the data were analysed statistically.

$$I = \frac{(C-T)}{C} \times 100$$

Where, I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment (fungicide)

Results and Discussion

Isolation of the pathogen from Anthracnose infected plant sample

The collected diseased sample of Guava fruit was observed under microscope to know the presence of pathogen in the infected plant part. After confirming, the presence of pathogen under microscope, diseased plant samples showing the typical Anthracnose symptoms were subjected to isolation. *C. psidii* was isolated on PDA media by tissue isolation method. The isolated fungus was further purified by single spore isolation method and the purified culture was maintained on PDA slants for further studies. Similar type of isolation method was carried out by Haider *et al.*, (2016) for Guava

Anthracnose with the use of infected fruits and twigs.

In vitro evaluation of systemic and combi fungicides against *Colletotrichum psidii*

Evaluation of fungicides *in vitro* is a handy tool to screen large number of fungicides at different concentrations. In the present study, the laboratory evaluation of fungicides by poison food technique revealed that among systemic fungicides evaluated, complete inhibition of mycelial growth was observed in Hexaconazole and Difenconazole (100 %) and they were also exhibited significant difference over other chemicals screened. The next chemical found was Thiophanate methyl which inhibited 55.33 per cent of mycelial growth (Table 1 and Plate 1). Next best is with Carbendazim (44.03 %) and inferior result obtained by Azoxystrobin (39.50 %). Similar results obtained by Haider *et al.*, (2016) for Guava Anthracnose with the use of infected fruits and twigs.

Out of three different concentrations, concentration at 2000 ppm showed maximum inhibition of mycelial growth (66.77 %) and was found significantly superior over other two concentrations like 1000 ppm (56.97 %) and at 500 ppm (45.68 %).

In the interactions of both fungicides and concentrations, Hexaconazole and Difenconazole at all the three concentrations completely inhibited mycelial growth of the pathogen (100 %) and minimum inhibition was found in Carbendazim at 500 ppm (15.25 %). Similar results obtained by Haq *et al.*, (2013) stated that Ridomil gold was the most effective followed by Mancozeb and Derosal (Carbendazim) was the least effective at all concentrations to control Guava Anthracnose disease. Contradictorily, Haider (2016) reported that Derosal (Carbendazim) at 60 ppm concentration, inhibited maximum

mycelia growth of *C. psidii*. Among combi fungicides screened, SAAF, MATCO and SECTIN completely inhibited mycelial growth of the pathogen at all the three different concentrations (100 %) (Table 2) and were found significantly superior to other

two chemicals like CURZATE (92.56 %) and MELODY (88.89 %). Both the concentrations of 2000 ppm and 3000 ppm completely inhibited pathogen's growth followed by 1000 ppm (74.05 %).

Table.1 *In vitro* evaluation of combi fungicides against *Colletotrichum psidii* causing anthracnose of Guava by using poisoned food technique

| Sl. No. | Fungicides | Concentration / Per cent Inhibition | | | Mean |
|--|------------|-------------------------------------|----------|----------|---------|
| | | 1000 ppm | 2000 ppm | 4000 ppm | |
| 1 | SAAF | 100.00 | 100.00 | 100.00 | 100.00 |
| 2 | MATCO | 100.00 | 100.00 | 100.00 | 100.00 |
| 3 | SECTIN | 100.00 | 100.00 | 100.00 | 100.00 |
| 4 | MELODY | 66.67 | 100.00 | 100.00 | 88.89 |
| 5 | CURZATE | 77.67 | 100.00 | 100.00 | 92.56 |
| 6 | Control | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | S. Em± | C.D @1% |
| Fungicide (A) | | | | 0.19 | 0.73 |
| Concentration (B) | | | | 0.13 | 0.52 |
| Fungicide x Concentration (A x B) | | | | 0.33 | 1.27 |

Table.2 *In vitro* evaluation of Systemic fungicides against *Colletotrichum psidii* causing anthracnose of Guava by using poisoned food technique

| Sl. | Fungicides | Concentration / Per cent Inhibition | | | Mean |
|--|--------------------|-------------------------------------|----------|----------|---------|
| | | 500 ppm | 1000 ppm | 2000 ppm | |
| 1 | Azoxystrobin | 27.33 | 32.33 | 58.83 | 39.50 |
| 2 | Carbendazim | 15.25 | 46.00 | 70.83 | 44.03 |
| 3 | Hexaconazole | 100.00 | 100.00 | 100.00 | 100.00 |
| 4 | Thiophanate methyl | 31.50 | 63.50 | 71.00 | 55.33 |
| 5 | Difenconazole | 100.00 | 100.00 | 100.00 | 100.00 |
| 6 | Control | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | S. Em+ | C.D @1% |
| Fungicide (A) | | | | 0.53 | 2.04 |
| Concentration (B) | | | | 0.38 | 1.45 |
| Fungicide x Concentration (A x B) | | | | 0.92 | 3.54 |

Plate.1 *In vitro* evaluation of systemic fungicides for the management of *Colletotrichum psidii*

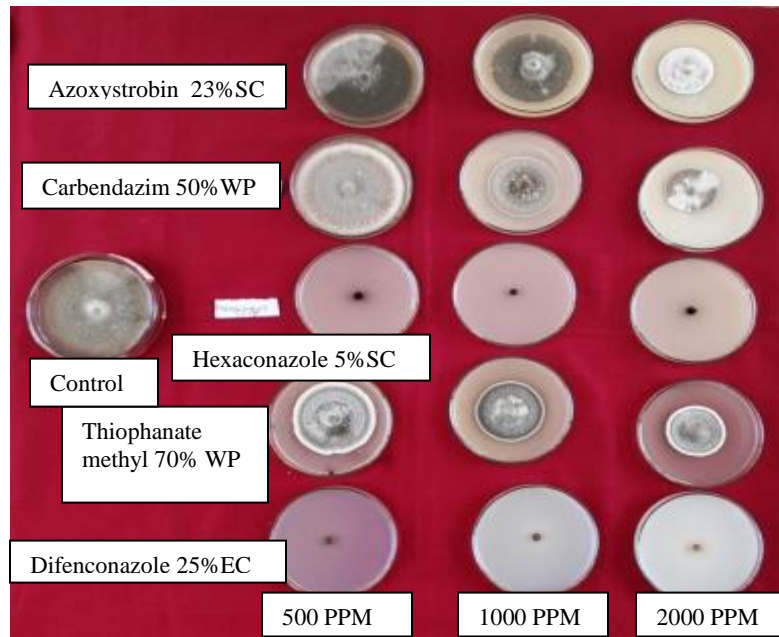
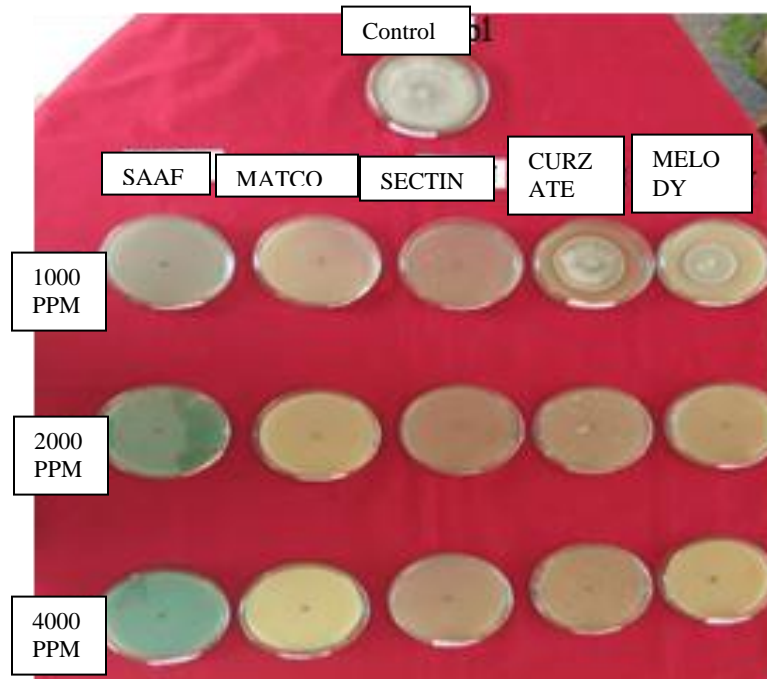


Plate.2 *In vitro* evaluation of combi fungicides for the management of *Colletotrichum psidii*



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