Efficacy of different fungicides by in vitro against Colletotrichum gloeosporioides, the causal agent of mango anthracnose

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Abstract
The cultivation of mango is found to be increased due to its high income and nutritive value. The crop is widely grown throughout the world. Productivity of mango is decreased due to various biotic and abiotic stresses. Among biotic, anthracnose is the fungal disease infecting in both field and storage with an economical loss to the tune of 15-20 per cent so that management through fungicides is the necessary practice to reduce the loss. In laboratory condition different fungicides were tested against Colletotrichum gloeosporioides by using poison food technique. Among systemic fungicides viz., difencinoazole 25% EC, propiconazole 25% EC and tebuconazole 25% EC completely inhibited the growth of fungus while in non-systemic fungicides propieb 70% WP significantly inhibited the fungus and among combi-fungicides tryfloxystrobin 25% + tebuconazole 50% was successful by completely inhibiting the growth of C. gloeosporioides at all the three tested concentrations.

Keywords: Mango, anthracnose, fungicides, per cent inhibition, Colletotrichum gloeosporioides

Introduction
Mango (Mangifera indica L.) is grown throughout the tropics and subtropics of the world it belongs to the family Anacardiaceae. It is considered as the king of fruits due to its wide ecological range, delicious taste, excellent flavor, high nutritive and medicinal value with great religious historical significance (Lakshmi et al., 2011) [6]. Mango is ranked second only to banana both in quantity and value and fifth in total production among the major fruit crops worldwide with estimated production of 26 million tons per annum (Anon, 2016) [1], India ranks first in production by contributing 52 per cent of the world production.

In India, the crop is grown in an area of 2.2 million hectares with a production of 21.02 million tonnes and productivity per hectare is 8.17 tonnes. The major growing states in India are Uttar Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu. The crop in Karnataka occupied an area of 0.192 million hectares with a production of 1.829 million tonnes and productivity of 9.5 tonnes per hectare (Anon, 2018) [2].

Although mango is considered to be an hardy plant and India is the largest producer of mango. The productivity is low mainly due to a wide range of biotic and abiotic stresses. Among the biotic stresses, fungal diseases are the major once responsible for field and transit losses. Diseases like powdery mildew (Oidium mangiferae Barathet), anthracnose [Colletotrichum gloeosporioides (Penz.) Penz and Sacc], stem end rot (Botryodiplodia theobromae Pat) and black mould (Meliola mangifera Earle) are economically important among the fungal diseases. Butler (1918) [3] reported Colletotrichum gloeosporioides for the first time in India as a causal organism of coffee leaf spot and in 1924 McRae [7] reported it as causal organism of mango anthracnose.

Colletotrichum gloeosporioides is an asexual or anamorph (imperfect) state of the pathogen belongs to the family Phyllachoraceae of the division Ascomycota. It is a facultative parasite. The sexual or teleomorph (perfect) state is Glomerella cingulata. The fungus occurs on a broad range of host species producing acervuli within the host tissue during asexual (mitotic) phase of its life cycle.
Anthracnose is a fungal disease infecting mango in both field and storage. It reduces marketable yield from 10 to 80 per cent in developing countries (Poonpolgul and Kumphai, 2007 and Kumar et al., 2010) (11) under field condition and also being post-harvest disease, causing an economical loss to the tune of 15-20 per cent (Ploetz and Prakash, 1997) (10). Keeping the point in view, laboratory studies were under leayed to evaluate different group of fungicides by in vitro against the growth of the pathogen.

Materials and Methods
Collection of disease samples and isolation of the pathogen
The leaves, twigs and floral parts of mango which were infected by anthracnose were collected from the farmer fields and were used for isolation of the pathogen. The process of isolation of pathogen is explained below.

Isolation of the pathogen
The pathogen was isolated from mango leaves showing typical anthracnose symptoms by tissue segment method (Rangaswami and Mahadevan, 1999) (12) on potato dextrose agar medium (PDA). Small bits measuring about 3 mm size were cut off from the leaves showing lesions in such a way that, it contained both infected and healthy parts. Then these bits were surface sterilized in 0.1 per cent mercuric chloride (HgCl₂) solution for 30 seconds followed by three washings in sterilized distilled water. The bits were dried by transferring on to sterilized discs of blotting paper, and then subsequently transferred on to potato dextrose agar (PDA) medium in Petriplates under aseptic conditions. The inoculated plates were incubated at 27 ± 1 °C for seven days for the growth of the pathogen.

In vitro evaluation of fungicides
Poisoned food technique was followed to test the efficacy of different fungicides against the growth of Colletotrichum gloeosporioides. List of fungicides along with their concentration evaluated is furnished in Table 1. Suspension of each of the fungicide was prepared in PDA by adding required quantity of respective fungicide to obtain the desired concentrations based on the active ingredient present in each chemical. Fifteen ml of poisoned medium of each fungicide was poured separately into sterilized Petri plates and then allowed to solidify.

Mycelial disc of the fungus measuring 0.5 cm was taken from the periphery of seven days old culture and was placed in the center of the plates. Inoculated plates were incubated at 27 ± 1 °C. Control was maintained without addition of any fungicide. Three replications were maintained for each treatment. Observations were drawn for the growth of the pathogen in all the treated plates, whenever the growth of the fungus touched the periphery in control plate, the colony growth of the pathogen in each treated plate was measured in four directions and average was worked out. The per cent inhibition of growth by the fungicide was calculated by using below mentioned formula given by Vincent (1927) (10). And data were analyzed statistically using Completely Randomized Block Design with Factorial concept.

\[ I = \frac{C - T}{C} \times 100 \]

Where
\( I = \) Per cent inhibition
\( C = \) Radial growth of the fungus in control plate
\( T = \) Radial growth of fungus in treated plate

Details of fungicides used for in vitro evaluation against C. gloeosporioides

| Sl. No. | Common name | Systemic fungicides | Concentration |
|--------|-------------|---------------------|--------------|
| 1      | Difenconazole 25% | EC                  | 1            |
| 2      | Hexaconazole 5%  | EC                  | 2            |
| 3      | Propiconazole 25% | EC                  | 3            |
| 4      | Tebuconazole 25% | EC                  | 4            |
| 5      | Azoxystrobin 23% | SC                  | 5            |
| 6      | Carbendazim 50% | WP                  | 6            |
| 7      | Thiophenate methyl 70% | WP              | 7            |

| Sl. No. | Non systemic fungicides | Concentration |
|--------|--------------------------|--------------|
| 1      | Chlorothalonol 75% WP    | 1            |
| 2      | Copper oxy chloride 50% WP | 2            |
| 3      | Copper hydroxide 50% WP  | 3            |
| 4      | Propineb 70% WP          | 4            |
| 5      | Mancozeb 75% WP          | 5            |
| 6      | Zineb 75% WP             | 6            |

| Sl. No. | Combi – fungicides | Concentration |
|--------|-------------------|--------------|
| 1      | Carbendazim 12% + mancozeb 63% | 1            |
| 2      | Pyraclostrobin 5% + metiram 55.5% | 2            |
| 3      | Tryfloxystrobin 25% + tebuconazole 50% | 3            |
| 4      | Azoxystrobin 23% SC + tebuconazole 25% | 4            |
| 5      | Tricyclazole 18% + mancozeb 62% | 5            |
| 6      | Captan 70% + hexaconazole 5% | 6            |
| 7      | Iproidione 25% + carbendazim 25% | 7            |
| 8      | Hexaconazole 5% + zineb 75% | 8            |

Results and Discussion
Efficacy of systemic fungicides against C. gloeosporioides
The efficacy of seven systemic fungicides was tested under in vitro against the growth of C. gloeosporioides. Among the seven systemic chemicals evaluated (Table -1 and Plate -1) significantly complete mycelial inhibition (100%) was noticed in the plates treated with viz.,, difenconazole, tebuconazole and propiconazole at all the three concentrations followed by hexaconazole with 85.41 per cent inhibition. Thiophenate methyl was found to be significantly less effective (28.49%) whereas carbendazim recorded an inhibition zone of 44.26 per cent.

| Sl. No. | Fungicides         | Per cent inhibition |
|--------|--------------------|---------------------|
|        | Concentrations (%) |                     |
|        | 0.05               | 0.10                | 0.15                | Mean               |
| 1      | Difenconazole 25%  | 100.00 (90.00)      | 100.00 (90.00)      | 100.00 (90.00)     | 100.00 (90.00)     |
| 2      | Hexaconazole 5%    | 83.60 (66.11)       | 86.04 (68.06)       | 86.59 (68.52)      | 85.41 (67.55)      |
| 3      | Propiconazole 25%  | 100.00 (90.00)      | 100.00 (90.00)      | 100.00 (90.00)     | 100.00 (90.00)     |
| 4      | Tebuconazole 25%   | 100.00 (90.00)      | 100.00 (90.00)      | 100.00 (90.00)     | 100.00 (90.00)     |
| 5      | Carbendazim 50%    | 41.00 (39.82)       | 45.08 (42.17)       | 46.70 (43.11)      | 44.26 (41.70)      |
| 6      | Azoxystrobin 23%   | 32.76 (34.92)       | 36.83 (37.37)       | 37.67 (37.86)      | 35.75 (36.72)      |
| 7      | Thiophenate methyl 70% | 16.55 (24.01)   | 29.89 (33.14)       | 39.04 (38.67)      | 28.49 (32.26)      |
propiconazole, difenconazole and hexaconazole were effective against *C. gloeosporioides* by recording an inhibition of 100, 85.50 and 85.77 per cent respectively, while thiophenate methyl recorded the least inhibition of 55.62 per cent. The effectiveness of triazole fungicides like propiconazole, tebuconazole and difenconazole was attributed to their interference with biosynthesis of fungal sterol and inhibition of ergosterol biosynthesis. In many fungi ergosterol is the structural component of the cell wall and its absence cause irreparable damage to cell wall leading to the death of fungal cell. Inhibition of sterol biosynthesis pathway in fungi by triazole fungicides was studied by Nene and Thapliyal (2002) [9]. Most of the fungicides at higher concentrations usually exhibit superior efficacy as reported by Sudhakar (2000) [15] and Shivakumar (2015) [14].

Efficacy of non-systemic fungicides against *C. gloeosporioides* under *in vitro*
Among the six non-systemic fungicides tested (Table -2 and Plate -2) propineb 70% WP was found to be significantly superior with the record of 100 per cent growth inhibition of pathogen at lowest concentration of 0.1 per cent followed by the next best effective fungicide as mancozeb 75% WP (84.09%). The other fungicides viz., Chlorothalonil 75% WP and copper oxychloride 50% WP were found to be moderate effective. Copper hydroxide 50% WP recorded as significantly least effective (40.06%) fungicide. The results obtained were in accordance with the report of Ranjitha et al. (2019) [13], who reported that, mancozeb is highly effective against *C. gloeosporioides* with 76.83 per cent mycelial inhibition. The effective inhibition of the pathogen by propineb was mainly due to its mode of action, that is alteration in the lipid metabolism of pathogen (Golakiya et al., 2020) [5] and similar results were obtained by Moreira et al. (2017) [8] and Ekbote et al. (1994) [4].

![Plate 1: *In vitro* efficacy of systemic fungicides against *C. gloeosporioides* of mango](image)

Similar results were obtained by Ranjitha *et al.* (2019) [13] that

| Sl. No. | Fungicides               | Per cent inhibition (Mean) |
|--------|-------------------------|---------------------------|
|        |                         | 0.10 | 0.20 | 0.30 |               |
| 1      | Chlorothalonil 75% WP   | 54.85 (47.79) *  | 86.67 (68.58) | 86.67 (68.58) | 76.06 (60.71) |
| 2      | Copper oxychloride 50% WP | 65.22 (53.86) | 72.52 (58.38) | 76.56 (61.04) | 71.43 (57.69) |
| 3      | Copper hydroxide 50% WP | 30.67 (33.63) | 33.96 (35.64) | 55.56 (48.19) | 40.06 (39.27) |
| 4      | Propieb 70% WP          | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| 5      | Mancozeb 75% WP         | 62.74 (52.38) | 89.52 (71.11) | 100.00 (90.00) | 84.09 (66.49) |
| 6      | Zineb 75% WP            | 32.96 (35.04) | 39.15 (38.73) | 58.30 (49.78) | 43.47 (41.25) |

*Figures in parentheses indicate angular transformed values*
Effectivity of combi – fungicides against *C. gloeosporioides* under *in vitro*

Among the eight combi fungicides tested against the pathogen under *in vitro*, the tryfloxystrobin 25% + tebuconazole 50% (Table 3 and Plate -3) showed 100 per cent inhibition of mycelial growth of the pathogen even at the lowest concentration of 0.1 per cent followed by pyraclostrobin 5% + metiram 55.5% with 95.86 per cent inhibition and captan 70% + hexaconazole 5% with 94.95 per cent mycelial inhibition and significantly least mycelial inhibition was recorded by carbendazim 12% + mancozeb 63% (45.18%) of mycelial inhibition. There is a significance difference in respect of efficacy between each of the fungicide. Ranjitha *et al.* (2019) [13] reported that, tryfloxystrobin 25% + tebuconazole 50% was an effective fungicide against *C. gloeosporioides* with 94.86 per cent inhibition. Further 89.19 per cent inhibition of *C. gloeosporioides* with the same fungicide was obtained by Golakiya *et al.* (2020) [5]. From the results, it is confirmed that, triazole group of fungicides either alone or in combination with other fungicides were more effective. There was positive correlation between the concentration and inhibition in growth of the pathogen. It is also observed that with the increase in concentration of any fungicide, there will be a corresponding inhibition in growth of the pathogen. Except some fungicides *viz.*, difenconazole, propiconazole, tebuconazole, propineb and tryfloxystrobin 25% + tebuconazole 50% which resulted hundred per cent inhibition even at lowest concentration.

Table 3: *In vitro* efficacy of combi – fungicides against *C. gloeosporioides* of mango

| Sl. No. | Fungicides                        | Per cent inhibition |
|---------|-----------------------------------|---------------------|
|         |                                   | 0.10    | 0.20    | 0.30    | Mean   |
| 1       | Pyraclostrobin 5% + metiram 55.5%| 87.59   | 100.00 | 100.00  | 95.86  |
| 2       | Tryfloxystrobin 25% + tebuconazole 50% | 100.00 | 100.00 | 100.00  | 90.00  |
| 3       | Tricyclazole 18% + mancozeb 62%  | 41.00   | 54.63  | 72.67   | 56.10  |
| 4       | Carbendazim 12% + Mancozeb 63%   | 39.52   | 41.59  | 54.44   | 45.18  |
| 5       | Azoxystrobin 23% + tebuconazole 50% | 80.67 | 91.89  | 100.00  | 90.85  |
| 6       | Captan 70% + hexaconazole 5%     | 84.85   | 100.00 | 100.00  | 94.95  |
| 7       | Iprodione 25%+ carbendazim 25%  | 82.67   | 86.66  | 88.68   | 86.00  |
| 8       | Hexaconazole 5% + zineb 75% WP  | 81.67   | 83.59  | 83.85   | 83.04  |

Figures in parentheses indicate angular transformed values
Plate 3: *In vitro* efficacy of combi – fungicides against *C. gloeosporioides* of mango

References

1. Anonymous. Horticultural Statistics at a Glance by Horticulture Statistics Division Department of Agriculture, Cooperation & Farmers Welfare Ministry of Agriculture & Farmers Welfare Government of India 2018a, 500.

2. Anonymous, www.Hortiglance.com 2018.

3. Butler EJ. Fungi and Disease in Plants. Thacker Spink and Co. Calcutta 1918, 547.

4. Ekbote SD. Studies on anthracnose of mango (*Mangifera indica* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. M.Sc. (Agri.) Thesis, Univ. Agric. Sci. Dharwad, (India) 1994.

5. Golakiya BB, Akbari LF, Marakna NM. *In vitro* evaluation of different fungicides against pomegranate anthracnose caused by *Colletotrichum gloeosporioides*. Int. J Chem. Stud 2020;8(4):3669-3674.

6. Lakshmi BKM, Reddy PN, Prasad RD. Cross infection potential of *Colletotrichum gloeosporioides* Penz. Isolates causing anthracnose in subtropical fruit crops. Trop. Agric. Res 2011;22:183-193.

7. McRae W. Economic botany part-III. Mycology. Annual Report (1922-23), Board of Science. Advice India 1924, 31-35.

8. Moreira RR, Castellar C, Hamada NA, May D, Mio LL. Sensitivity of *Colletotrichum* species, associated to Glomerella leaf spot in apple, to mancozeb and thiophanate methyl. Modern fungicides and antifungal compounds 2017;7:159-160.

9. Nene YL, Thapliyal PN. Fungicides in plant disease control (3rd edition). Oxford and IBH Publishing Company Private Limited 1993, 691.

10. Ploetz CRL, Prakash O. Foliar, floral and soil borne diseases. In: The Mango. R.E. Litz (Eds.), CAB International, Wallingford, UK. Fruit Dis 1997, 281-325.

11. Poonpolgul S, Kumpha S. Chilli pepper anthracnose in Thailand. Country report. In: First Int Symp. Chilli Anthracnose. Ed. Oh, D. G. and Kim, K.T., Republic of Korea: National Horticultural Research Institute, Rural Development of Administration 1999, 23(abs).

12. Rangaswami G, Mahadevan A. Diseases of crop plants in India. 4th Ed., Prentice Hall of India Pvt. Ltd., New Delhi 1999, 65-66.

13. Ranjitha N, Devapp V, Sangeetha CG. Evaluation of fungicides against *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc. The incitant of mango anthracnose. J Pharmaco. Phytochem 2019;8(3):812-814.

14. Shivakumar KV. Studies on variability and management of anthracnose of mango caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. M. Sc. (Agri) Thesis, Univ, Agric. Sci. Raichur, (India) 2015.

15. Sudhakar. Biology and management of *Stylosanthes* anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. M.Sc. (Agri.) Thesis, Uni. Agric. Sci. Dharwad, (India) 2000.

16. Vincent JM. Distribution of fungal hyphae in the presence of certain inhibitors. Nature 1927;159:850.