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# Management of Post Harvest Disease of Mango Anthracnose

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**Management of anthracnose, a post harvest disease of mango (*Mangifera indica* L.) incited by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was produced by using native antagonistic microflora. Under *in vitro* study, the *Trichoderma* isolates *Trichoderma fasciculatum* and *Trichoderma koningii* showed the highest antagonistic activity against *Colletotrichum gloeosporioides* in dual culture isolated from fructoplane and phylloplane respectively. *T.fasciculatum* proved to be the best compatible antagonist with different fungicides evaluated. *In vivo* screening of potential antagonist *T.fasciculatum* on mango fruits revealed that post-inoculation (pre-treatment) method is superior over the pre-inoculation method in management of anthracnose disease. The possibility of exploitation of fungicidal compatible bioagent in the integrated management of anthracnose with low fungicidal residue will delay in development of resistance in the pathogen will be discussed.**

**Key words:** Mango, anthracnose, *Colletotrichum gloeosporioides* and *Trichoderma.*

Mango (*Mangifera indica* L.) is native to India and South East Asia. India is the largest producers of mangoes in the world when compared to half of the global production and the largest exporter. Andhra Pradesh ranks the first in production and productivity in India. Devastating disease like anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. reduce the fruit quality and responsible for 30 to 60% of harvest losses[1]. The incidence of this reach almost 100% in fruits produced under wet or very humid conditions[2]. The post harvest phase is the most economically significant throughout the world. Post harvest thermal and chemical treatments reduces anthracnose severity of the fruits[3] but the adverse effect of synthetic chemical residues on human health, environment and the development of resistance in the pathogen to chemicals used for controlling the disease have lead to intensified efforts to develop alternative methods. Biological control using microbial antagonists has emerged as one of the most promising alternatives, used either alone or as integrated control strategy to reduce the use of fungicides. The information on biological control of post harvest disease of mango anthracnose is scanty. Considering the severity of the disease and the losses associated with it, an investigation was made using native potential antagonists either single or in combination for successful management of the anthracnose disease.

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## MATERIAL AND METHODS

### Isolation and pathogenicity of pathogen

The pathogen was isolated from infected Baneshan mango fruits collected from mango orchards at Agricultural Research Station, Anantharajupeta, Kadapa (Dt), Andhra Pradesh, (India) by using tissue segment method[4]. The pathogen was purified by single spore isolation method[4], identified using standard mycological keys[5] and was maintained on potato dextrose agar (PDA) for further studies. Wound inoculation method was used to test the pathogenicity on Baneshan mango fruits[6].

### Screening of native potential bioagents

Serial dilution plate technique was used for the isolation of native antagonistic microflora from phylloplane and fructoplane of mango[7]. The antagonistic activity of microflora isolates against *C.gloeosporioides* was determined by dual culture technique under *in vitro*[6].

### Efficacy and compatibility of native potential antagonists with different fungicides under *in vitro*

The commonly used systemic and non-systemic fungicides *viz.,* carbendazim, hexaconazole, propioconazole, thiophanate-methyl, prochloraz, thiram, captan, mancozeb and copper oxychloride were tested respectively at 50, 25, 25, 50, 50, 750, 750, 1000 and 1000 ppm concentrations against *C.gloeosporioides* isolates by poisoned food technique[8]. The compatibility studies were performed by poisoned food technique for fungal antagonists[8] and spectrophotometric method for bacterial antagonists[9].

### *In vivo* screening of potential antagonist *Trichoderma fasciculatum* on mango fruits

Native potential fungicide compatible antagonist was used for *in vivo* screening by pre-inoculation and post-inoculation methods[10]. The details of the treatments imposed in integrated disease management of *C.gloeosporioides* is given in Table 3. In pre-inoculation method, treatments were given after inoculation of the pathogen, whereas in post-inoculation method, treatments were given before the inoculation of the pathogen. Mango fruits were washed thoroughly in tap water, surface sterilized by dipping in 0.1% mercuric chloride for 30 seconds, then three washes with distilled water and air dried on sterilized blotting paper. A circular inoculation site with 1 cm diameter was marked on the surface of the fruits and wounds were made by puncturing the rind to a depth of 2 mm on the marked area using sterile needle. A drop of conidial suspension ($2x10^4$ conidia/ml) of the pathogen prepared from 10 days old culture was kept on the marked area and left for air drying. Then the mangoes were packed in sterile polythene covers with air holes and loosely tied and incubated at 28±2ºC for seven days for the development of symptoms. The diameter of the lesions was measured on the 7th day after inoculation of the pathogen. Both pre-inoculation and post-inoculation method includes five different treatments as listed below:

| S. No. | Treatment designation | Treatment |
|---|---|---|
| 1. | A | Treating fruits with potential antagonist for ten minutes |
| 2. | B | Treating fruits with fungicide solution for ten minutes |
| 3. | C | Fungicide treatment for 10 minutes, twenty four hours after treating with antagonist |
| 4 | D | Antagonist treatment for 10 minutes, twenty four hours after treating with fungicide |
| 5. | E | No treatment |

### Statistical analysis

Completely Randomized Design (CRD) was used for radial growth, per cent disease incidence, poisoned food technique, dual cultural technique and spectrophotometric method and Factorial Completely Randomized Design (RBD) for *in vivo* screening of antagonists[11].

## RESULTS AND DISCUSSION

The target pathogen *C.gloeosporioides* isolated from infected mango fruits was tested on Baneshan mango fruits for its pathogenicity and proved Koch's postulates. A total of twenty four putative antagonistic microflora was isolated and

evaluated for its antagonistic activity against test pathogen (Table 1). Of all the twenty four biocontrol agents evaluated, nine ($T_1$ to $T_9$) out of fifteen mycoflora were identified as *Trichoderma* spp. based on their colony and morphological characteristics as reported by different workers[12,13]. In dual culture studies, the test microbes inhibited the growth of *C.gloeosporioides* at varying degrees (Table 1). The native *Trichoderma* isolate $T_1$ from phylloplane showed highest per cent of inhibition of 79.93% followed by fructoplane isolate $T_7$ which inhibited 71.38 per cent growth of the pathogen. Statistical analysis revealed that there is significant difference between per cent inhibition

**Table 1.** *In vitro* evaluation of the efficacy of antagonistic microflora against growth of *C.gloeosporioides* by dual culture technique

| S. No. | *Antagonistic isolates | Habitat | **Mycelial growth (mm) | Per cent inhibition over control |
|---|---|---|---|---|
| 1. | $T_1$ | phylloplane | 18.05 | 79.93 |
| 2. | $T_2$ | phylloplane | 32.98 | 63.36 |
| 3. | $T_3$ | phylloplane | 31.80 | 64.60 |
| 4. | $T_4$ | phylloplane | 30.40 | 66.20 |
| 5. | $T_5$ | phylloplane | 40.60 | 54.88 |
| 6. | $T_6$ | fructoplane | 34.40 | 61.70 |
| 7. | $T_7$ | fructoplane | 25.76 | 71.38 |
| 8. | $T_8$ | fructoplane | 34.78 | 61.35 |
| 9. | $T_9$ | fructoplane | 36.97 | 59.00 |
| 10. | $F_{10}$ | phylloplane | 42.15 | 53.00 |
| 11. | $F_{11}$ | phylloplane | 26.10 | 71.00 |
| 12. | $F_{12}$ | phylloplane | 39.97 | 55.59 |
| 13. | $F_{13}$ | fructoplane | 44.29 | 50.79 |
| 14. | $F_{14}$ | fructoplane | 62.10 | 31.00 |
| 15. | $F_{15}$ | fructoplane | 66.97 | 25.59 |
| 16. | $B_1$ | phylloplane | 33.22 | 63.09 |
| 17. | $B_2$ | phylloplane | 42.90 | 52.33 |
| 18. | $B_3$ | phylloplane | 67.33 | 25.09 |
| 19. | $B_4$ | phylloplane | 72.77 | 19.14 |
| 20. | $B_5$ | phylloplane | 77.78 | 13.58 |
| 21. | $B_6$ | fructoplane | 64.78 | 28.20 |
| 22. | $B_7$ | fructoplane | 74.20 | 17.56 |
| 23. | $B_8$ | fructoplane | 43.89 | 51.23 |
| 24. | $B_9$ | fructoplane | 82.55 | 8.28 |
| | Control | | 90.00 | - |
| | SEm | | 1.3151 | 0.4369 |
| | CD (0.05) | | 3.7716 | 0.8921 |

*$T_1$-$T_9$: Trichoderma isolates; $F_1$ to $F_{15}$: Fungal isolates other than Trichoderma; $B_1$ to $B_9$: Bacterial isolates.

** Mean of three replications

of $T_1$ and $T_7$. The efficacy of different fungicides revealed that the complete inhibition of the pathogen was observed with all fungicides except mancozeb which inhibited only 61.19 per cent[14]. Benzimidazoles like carbendazim, thiophanate-methyl and benomyl are most effective in controlling *C.gloeosporioides* from mango and other crops than non-systemic fungicides like mancozeb and copper oxychloride[15-18]. The present results are in accordance with the earlier findings.

It is now well established that the development of fungicide resistance in pathogen can lead to poor disease control, if not timely managed. However, it is difficult to predict the

actual risk of fungicide resistance because of many interacting factors between the pathogen and fungicide. The integration of chemicals with antagonistic fungi such *Trichoderma* spp. which are resistant to a good number of chemicals is one of the most attractive ways to reduce the amount of fungicides used[19]. Considering high inhibition activity of the antagonists $T_1$ and $T_7$, their compatibility with different fungicides which already tested against *C.gloeosporioides* was assessed. These results revealed that the both antagonists $T_1$ and $T_7$ are 100 per cent compatible with mancozeb (Table 2). Moreover, the isolate $T_7$ was also found to be compatible with thiram to the extent of 76.44 per cent. The results are in agreement that *Trichoderma* spp. can tolerate many fungicides as reported by several workers[19]. Both the antagonists $T_1$ and $T_7$ were identified as *Trichoderma koningii* ($T_1$) (accession no. 6623) and *Trichoderma fasciculatum* ($T_7$) (accession no. 6624) respectively at Indian Agricultural Research Institute (IARI), New Delhi, India. The antagonist, *T.fasciculatum* isolated from fructoplane having compatibility with thiram to the extent of 76.44% has been selected for further studies. Moreover, the thiram has also given 100% inhibition of the test pathogen.

Any biocontrol agent having ability to suppress the disease needs to be applied through an established method for its consistent performance. Biocontrol, using antagonistic organisms offers reliable approach either alone (or) integration with other disease management practices[20]. In such approach, fungicides need to be used with biocontrol agents without toxic effect[21]. It may even better if the biocontrol is effective as well as compatible so that it can be used in integrated disease management system. In such approach, this study was carried out with the objective of selecting a suitable method of application for managing mango anthracnose. These results revealed that post-inoculation (pre-treatment) method proved to be superior over the pre-inoculation method. The pre-inoculation method (Fig 1A) gave higher lesion diameter compared to post-inoculation (Table 3 & Fig 2B). Treatment A (*T.fasciculatum* ($10^7$spores/ml)) gave the least lesion diameter in case of pre-inoculation method (12.832 mm). Whereas in post-inoculation method, treatment B (thiram @ 750ppm application only) gave the lesion diameter of 11.840 mm when compared to control. Statistical analysis showed that there was no significant difference between treatment A and treatment C, where antagonist treated initially followed by the fungicide treatment. Applying the yeast antagonist, *Pichia guilliermondii* to citrus fruit in combination with fungicide substantially reduced the concentration

**Table 2.** *In vitro* evaluation of compatibility of potential *Trichoderma* antagonists with fungicides by poisoned food technique

| Fungicides | Concentration (ppm) | *Mycelial growth (mm) | | *Per cent compatibility over control | |
|---|---|---|---|---|---|
| | | *Trichoderma* koningii | *Trichoderma* fasciculatum | *Trichoderma* koningii | *Trichoderma* fasciculatum |
| Carbendazim | 50 | 0.00 | 0.00 | 0.00 | 0.00 |
| Hexaconazole | 25 | 13.10 | 10.93 | 14.83 | 12.00 |
| Propioconazole | 25 | 0.00 | 0.00 | 14.41 | 0.00 |
| Thiophanate-methyl | 50 | 39.00 | 13.33 | 15.79 | 14.78 |
| Prochloraz | 50 | 0.00 | 0.00 | 17.78 | 0.00 |
| Thiram | 750 | 16.30 | 68.80 | 14.28 | 76.44 |
| Captan | 750 | 24.00 | 43.77 | 13.93 | 48.87 |
| Mancozeb | 1000 | 90.00 | 90.00 | 100.00 | 100.00 |
| Copper oxychloride | 1000 | 68.47 | 23.57 | 0.00 | 26.32 |
| Control | - | 90.00 | 90.00 | - | - |
| SEm | - | 0.3771 | 0.3730 | 0.42228 | 0.4424 |
| CD (0.05) | - | 1.1124 | 1.1004 | 0.8883 | 0.9294 |

* Mean of three replications

**Table 3.** Effect of pre-inoculation and post-inoculation treatments of native potential antagonist *T.fasciculatum* in integrated disease management of *C.gloeosporioides*

| S. No. | Treatment designation | Treatments | Pre-inoculation method lesion size (mm) | Post-inoculation method lesion size (mm) | Mean |
|---|---|---|---|---|---|
| 1. | 1A &11a | Application of *T.fasciculatum* @ $10^7$ spores/ml | 13.33 | 12.33 | 12.832 |
| 2. | 1B &11b | Application of thiram @750 ppm | 13.68 | 10.00 | 11.840 |
| 3. | 1C & 11c | Application of *T.fasciculatum* followed by thiram | 14.31 | 11.67 | 13.13 |
| 4. | 1D & 11d | Application of thiram followed by *T.fasciculaum* | 13.67 | 12.60 | 14.855 |
| 5. | 1E & control | No treatment | 18.447 | 18.73 | 18.585 |
| | Mean | — | 14.687 | 13.066 | — |

| Pre-inoculation | Treatment given after inoculation of pathogen | | Post-inoculation | Treatment given before inoculation of pathogen |
|---|---|---|---|---|
| | Method | Treatment | Interaction | |
| Sem | 0.0675 | 0.1068 | 0.1510 | |
| CD (0.05) | 0.1992 | 0.3150 | 0.4455 | |

of thiabendazole (TBZ) reduced *Pencillium digitatum* decay to a level similar to that achieved by the currently recommended concentration of TBZ applied alone[22]. Thus, by adapting an integrated disease management system, we may expect not only to gain effective disease control but we can also maintain very low levels of chemical residues[23]. The biological agent must, however, have low sensitivity to any of the supplemental chemical fungicides. Recent advances in the development of biopesticides offer opportunities for the worldwide exploitation of biocontrol agents as replacement for more hazardous and environmentally unacceptable chemical pesticides and for inclusion in integrated disease management programmes.

Fruit maturity at harvest and at the application of antagonists is another factor affecting post harvest biological control. Late-picked over-mature fruits are some susceptible to decay than are fruits picked at optimal storage maturity[24]. Working with apples and pears, and with different species of the antagonistic yeast *Crytococcus*, Roberts[25,26] found fruit maturity markedly affected biocontrol efficacy: while excellent control was achieved on freshly harvested fruit, treatments of ripened fruit gave much lower levels of control. On the assumption that the infection process can be initiated at harvest, it would be advantageous to treat fruit with biocontrol agents as quickly as possible after harvest and to cool the fruit as rapidly as possible, to retard pathogen development. In fact, studies with *Mucor*-inoculated pears and antagonistic *Cryptococcus* species demonstrated maximal biocontrol effect, when the yeast were applied to the fruit soon after harvest[25]. The principle is to retard pathogen development while allowing the antagonistic microorganisms to colonize wound sites. Thus, during the present investigation *T.fasciculatum*, the compatible potential bioagent would benefit the industry in use of biological product to replace or supplement chemical use would be extremely important. It is therefore clear that standardization of material preparation for fungicidal tolerant bioagents are urgently required. This approach might presumably become good and effective for integrated disease management strategies. The present investigation leads to the exploitation of *T.fasciculatum* (accession no. 6624),

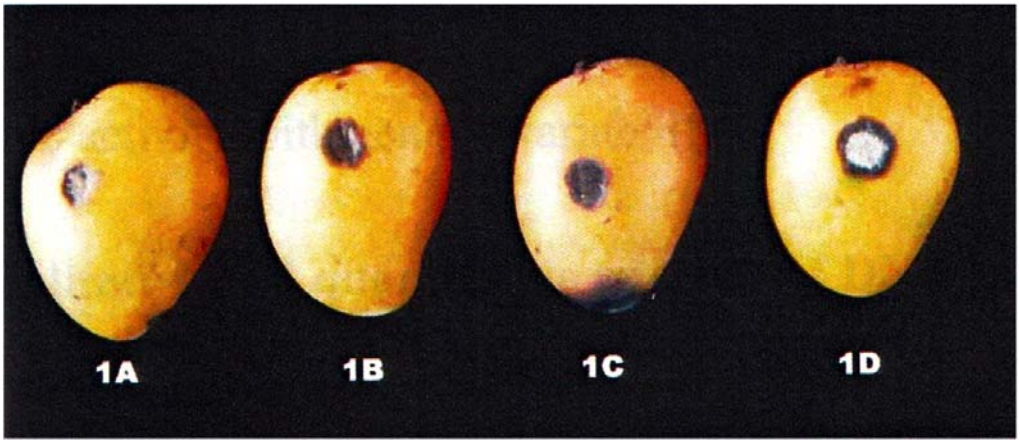


**Fig. 1(a)**

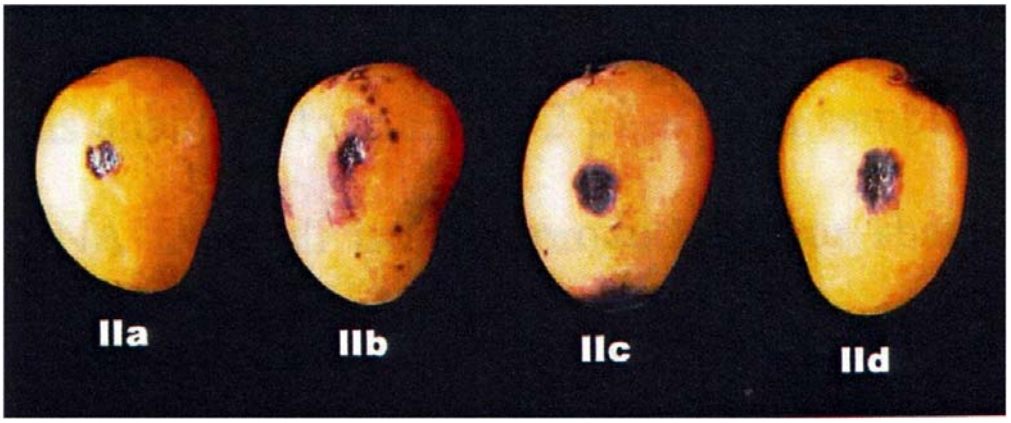


**Fig. 1(b)**

**Control**

**Fig. 1A & 1B.** Integrated management of anthracnose caused by *C.gloeosporioides*; 1A – pre-inoculation; 1B – post-inoculation

a fungicidal compatible antagonist in management of a post harvest anthracnose disease of mango. Moreover, the *T.fasciculatum* has been isolated form fructoplane and as such the viability and survival rate of the antagonist will be high. The integration of non-systemic fungicide thiram along with *T.fasciculatum* in management of post harvest disease of mango anthracnose is preferable compared to systemic fungicides. The non-systemic fungicides have multiple site of action and delays in development of resistance in pathogen population and have less residual effect compared to systemic fungicides. Hence, the present research findings will have significant impact on human health and environment.

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