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# Study on compatibility of *Trichoderma asperellum* and fungicides for the development of environment friendly and cost-effective disease management strategies

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## Abstract

Chemical fungicides are regularly being used to combat plant pathogens successfully. The escalating concern over human health and environmental safety often put pressure on farmers to lessen the use of chemicals. One of the approaches to minimize the use of fungicides is to integrate it with biological control agents (BCA). *Trichoderma* species are well known for their biological control activity against many plant pathogens. Application of *Trichoderma* is being endorsed for the management of diseases of tropical tuber crops viz., collar rot of elephant foot yam, tuber rot of cassava, stem and root rot of cassava, yam anthracnose and taro leaf blight. For an effective disease management, the activity of bio-control agent should not be stalled by the usage of fungicides. *Trichoderma asperellum* has been studied expansively as a BCA with results reliant on the specificity of the isolate. Present study was conducted at ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram to study the sensitivity of *T. asperellum* isolate tovarious fungicides. Knowledge onthe compatibility of possible bio agents with regularly used and newer agrochemicals is vital for refining/developingan efficient integrated disease management programme. Effect of twelve fungicideson mycelial growth of *Trichoderma* was tested by adopting poison food technique. Fungicides were tested at different concentrations ranging from 3.125 ppm to 3200 ppm. The fungicides, Copper oxychloride 50%, Cymoxanil 8%+ Mancozeb 64%, Mefenoxam 4%+ Mancozeb 64% and Cymoxanil 22.1% +Famoxadone 16.6% were compatible with *T. asperellum*at their recommended concentration by not inhibiting the mycelial growth. WhereasCarbendazim 50%, Carbendazim 25% + Mancozeb 50%, Hexaconazole 5%, Difenoconazole 25% and Metalaxyl–M 3.3%+ Chlorothalonil 33.1% inhibited the mycelial growth of bioagent indicating their incompatibility. A progressive increase in percent inhibition of radial growth in the fungus was observed as the concentrations of fungicides increased. The results obtained from the present study will help in revisiting integrated disease management strategies by combining bioagent, *Trichoderma* and fungicides for managing fungal diseases of tropical tuber crops.

**Keywords:** *Trichoderma asperellum,* Compatibility, Tuber crops, Fungicides

## Introduction

In agriculture, fungicides play a pivotal role around the world and fungicide applications have been the key approach for plant disease management in many crops (Kongcharoen et al., 2020; Seni et al., 2018). However, disadvantages of fungicides have also been reported including residues on plant products and in the environment, deleterious effects on consumers and fungicide users and the environment, and the

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development of resistance to the fungicides (Steinberg and Gurr, 2020). Biocontrol agents (BCA) also take part in the management of plant diseases (Maheswary et al., 2020). Management of plant diseases by employing a potential BCA is always chosen over hazardous chemicals (Pandey et al., 2006). *Trichoderma*, a genus of diverse fungal species classified as anamorphic Hypocreales, have been studied extensively as BCA and have been developed into commercial products that are used worldwide (Kumar et al., 2014). *Trichoderma* has emerged as the most powerful biocontrol agent for management of soil-borne plant diseases (Kumar et al., 2013; Singh et al., 2017; Zaidi et al., 2018 and Kumar et al., 2020a). It demonstrates effective competition in challenging environments due to its natural resistance to antibiotics produced by competing microorganisms. Interaction between *Trichoderma* and plants not only enhances biomass and overall nutrition but also provides protection against various phytopathogens through direct mycoparasitic activity, competition for nutrients, or indirect induction of the plant defense system (Kumar et al., 2014). The use of suitable bio agents in conjunction with fungicides may improve disease control and management of plant diseases. The pathogens can be effectively managed if the fungicide used is compatible with the bioagents without causing any toxic effect (Papavizas and Lumsden, 1980). According to Maheswary et al. (2020), many chemicals inhibit the growth of *Trichoderma* spp. In integrated disease management (IDM), the activity of biological control agents should not be disadvantaged by the application of fungicides.

Key points in disease management are to adopt environment friendly approaches, hinder resistance development in pathogens against chemicals, promote antagonistic populations in the soil, and seek cost-effective disease management methods. Application of *Trichoderma* alone or in combination with chemicals is being practiced for the management of various fungal diseases of tropical tuber crops. *Trichoderma* not only suppress diseases but also offer growth promotion by improved seed germination, increased plant height and weight, root length, increased yield etc. Apart from the presently recommended fungicides, many other chemicals showed excellent activities against fungal/oomycete pathogens causing diseases in tropical tuber crops. In this context, this study was aimed to assess the compatibility of *T. asperellum* with chemical fungicides for developing environment friendly and cost-effective disease management strategies against fungal diseases of tropical tuber crops.

## Materials and Methods

### *Trichoderma* culture

*T. asperellum* (ICAR- CTCRI T2) was isolated from the rhizosphere region of elephant foot yam plant, which was organically grown in the field of ICAR-CTCRI. The identity of the organism was confirmed as *T. asperellum* by ITS and TEF region amplification (gene bank accession number MN176380.1).

### Fungicides

The fungicides recommended for managing the diseases of tropical tuber crops as well as few new fungicides, which inhibited pathogens of tuber crops *(in vitro)* were used for the study (Table 1).

Table1. Details of fungicides used for the study

| Sl. No. | Brand Name | Technical Name/Active ingredient |
|---|---|---|
| 1 | Amistar | Azoxystrobin 23% (SC) |
| 2 | Folio Gold | Metalaxyl – M 3.3% + Chlorothalonil 33.1% (SC) |
| 3 | Equation Pro | Cymoxanil 22.1% + Famoxadone 16.6% (SC) |
| 4 | Score | Difenconazole 25% (EC) |
| 5 | Contaf | Hexaconazole 5% (SC) |
| 6 | Ridomil Gold R | Metalaxyl – M 4%+ 64% Mancozeb (WP) |
| 7 | Indofil M-45 | Mancozeb 75% (WP) |
| 8 | Curzate | Cymoxanil 8% + Mancozeb 64% (WP) |
| 9 | Antracol | Propineb 70% (WP) |
| 10 | Tagstin | Carbendazim 50% (WP) |
| 11 | Blitox | Copper Oxychloride 50% (WP) |
| 12 | Sprint | Carbendazim 25% + Mancozeb 50 % (WS) |

### *In vitro* study on the effect of fungicides on mycelial growth

Compatibility was studied in terms of mycelial growth inhibition and the technique adopted was poisoned food technique (Zentmyer, 1955). Stock solutions of the fungicides were prepared in sterile distilled water. In case of combination fungicides, the content of the desired chemical in the fungicide was taken for calculating the quantity of the fungicide to be added. To begin the experiment, a concentration of 100 ppm was uniformly selected for all the fungicides. Required quantity of the stock solution was incorporated into sterile, molten, and cooled Potato Dextrose Agar medium (HIMEDIA) to get final concentration of 100 ppm. Fungicide amended medium was mixed gently and dispensed into Petri dishes.

*T. asperellum* was cultured on PDA for 48 h and mycelial discs (1cm diameter) were cut from the growing edges of the culture and placed in the centre of plates containing PDA amended with various chemicals at different concentrations. PDA plates inoculated with mycelial disc of *Trichoderma* without fungicide served as control.

Three plates were maintained for each concentration. The plates were incubated at 28±2°C. The growth of the colony was measured after 72 h. The radial growth of mycelium was measured at two points at right angle to each other from each of the three plates maintained for each concentration. The growth of the colony in control sets where no chemical was added was compared with that of various concentrations and the difference was converted into percent inhibition.

Based on the mycelial growth inhibition at 100 ppm, fungicides were grouped into two for further evaluation.The chemicals, which did not inhibit the growth of *Trichoderma* at 100 ppm were tested at higher concentrations, 200, 400, 800, 1600 and 3200 ppm. The chemicals, which inhibited growth at 100ppm were evaluated at lower concentrations of 50, 25, 12.5, 6.25 and 3.125 ppm. The percent inhibition of *Trichoderma* isolates was calculated based on the diameter of growth of the colony by using the formula of Vincent (1947).

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

Where, I is the per cent inhibition, C is the growth of *Trichoderma* isolates in control plates (without fungicide) and T is the growth of *Trichoderma* isolates in test plates (with fungicide).

**Statistical analysis**

The data on mycelial growth of *T. asperellum* at different concentrations of various fungicides was statistically analysed. Mean separation was determined according to Duncan's multiple range test ($p<0.05$)

## Results and Discussion

The growth of *T. asperellum* was recorded at 24h interval until the mycelia growth in control plates completely covered the Petri plates (72 h). The results showed differential inhibitory action of the various fungicides towards mycelia growth of *Trichoderma*. At 100 ppm, seven out of twelve fungicides tested, did not inhibit the growthof *Trichoderma*. The seven fungicides, which did not affect the growth of *Trichoderma* were studied further at higher concentrations *viz*., 200, 400, 800, 1600 and 3200 ppm. At 200 ppm, maximum inhibition was in Azoxystrobin 23% (21.67%), whereas Propineb, Copper oxychloride, Cymoxanil + Mancozeb and Metalaxyl + Mancozebdid not inhibit the growth (Fig. 1a). Copper oxychloride was most compatible with *Trichoderma* and upto 400 ppm, it did not affect the mycelial growth. Other fungicides showed 15.56% to 30.11% inhibition (Fig. 1b). Even at 800ppm, copper oxychloride showed <5% inhibition only (Fig. 1c). However, at 1600 ppm, it showed >75% inhibition (Fig. 1d).

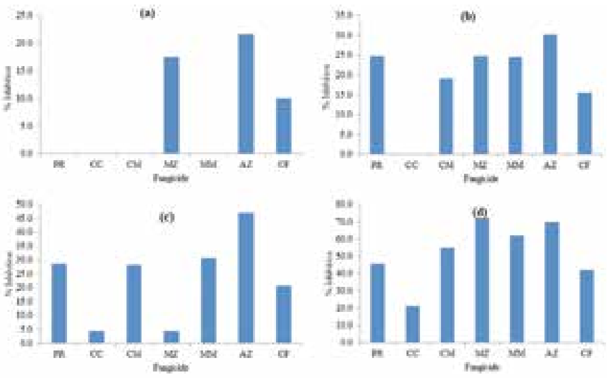


Fig. 1. Percent inhibition of mycelia growth at (a) 200, (b) 400, (c) 800 and (d) 1600 ppm of fungicides

*PR- Propineb 70% (WP); CC- Copper Oxychloride 50% (WP); CM- Cymoxanil 8% + Mancozeb 64% (WP); MZ- Mancozeb 75% (WP); MM- Metalaxyl – M 4%+ 64% Mancozeb (WP); AZ- Azoxystrobin 23% (SC); CF- Cymoxanil 22.1% + Famoxadone 16.6% (SC)

Table 2. Mycelial growth (cm) of *T. asperellum* on seven fungicides amended media (PDA)

| Concentration (ppm) | Type of Medium | | | | | | |
|---|---|---|---|---|---|---|---|
| | Propineb | Copper oxychloride | Cymoxanil 8%+ Mancozeb 64% | Mancozeb | Metalaxyl M 4%+ Mancozeb 64% | Azoxystrobin 23% | Cymoxanil 22.1% +Famoxadone 16.6% |
| 100 | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ |
| 200 | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $7.43^{B}$ | $9.00^{A}$ | $7.05^{B}$ | $8.10^{B}$ |
| 400 | $6.77^{B}$ | $9.00^{A}$ | $7.27^{B}$ | $6.77^{B}$ | $6.80^{B}$ | $6.29^{B}$ | $7.60^{C}$ |
| 800 | $6.43^{C}$ | $8.60^{B}$ | $6.47^{C}$ | $5.53^{C}$ | $6.23^{C}$ | $4.77^{C}$ | $7.13^{D}$ |
| 1600 | $4.90^{C}$ | $7.10^{C}$ | $4.07^{C}$ | $2.50^{D}$ | $3.40^{D}$ | $2.70^{D}$ | $5.20^{E}$ |
| 3200 | $4.57^{C}$ | $0.10^{D}$ | $0.10^{D}$ | $2.00^{D}$ | $2.97^{D}$ | $2.23^{D}$ | $2.67^{F}$ |
| Control | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ | $9.00^{A}$ |
| SE (d) | 0.257 | 0.114 | 0.249 | 0.228 | 0.15 | 0.579 | 0.158 |
| LSD at 1% | 0.7846 | 0.3485 | 0.7619 | 0.6955 | 0.458 | 1.6393 | 0.4624 |

*Mean values with the same alphabet in the superscript did not differ significantly

The concentration of active ingredients in the combination fungicide, Cymoxanil 22.1% + Famoxadone 16.6% at recommended doseis 221 ppm (Cymoxanil) and 166 ppm (Famoxadone). The results of the study showed that the fungicide showed only 10% mycelial inhibition at 200 ppm. Hence, it is suitable for being used with the bioagent, *Trichoderma*. The fungicide Propineb, showed inhibition at 400 ppm (24.78%) and 28.56% inhibition was recorded at 800 ppm, which is above the recommended dose. However, even at the highest concentration tested (3200 ppm), Propineb did not completely arrest the mycelial growth. Propineb recorded least inhibition at 3200 ppm and inhibition was only around 50% (49.22%). This indicates its compatibility with *Trichoderma.*

At 100 ppm, *Trichoderma* showed sensitivity to 5 fungicides among the 12 studied. These five fungicides were separated and the sensitivity at lower concentrations was studied (Table 3). Carbendazim is most inhibitory to *Trichoderma* followed by combination fungicide, Carbendazim 25% + Mancozeb 50%. Even at the lowest concentration (3.125 ppm), these fungicides inhibited mycelial growth of *Trichoderma.* The recommended concentration is 500 ppm of the active ingredient. Hence, combination of these two fungicides with the biocontrol agent, *Trichoderma* is not recommended. Other three fungicides also inhibited the mycelial growth and none of them could support the mycelial growth at their recommended concentrations.

Integrated disease management (IDM) of soil-borne pathogens is the only way of reducing severe impact of chemical pesticides. Thus, studies on compatibility of *Trichoderma* to commonly available commercial pesticides matter very much in developing IDM modules (Kumar et al., 2020c). Based on the results of *in vitro* study, the 12 fungicides can be grouped into three based on their inhibitory effect on mycelial growth of *Trichoderma.* The compatible group involves the fungicides, Copper oxychloride 50%, Cymoxanil 8%+ Mancozeb 64%, Mefenoxam 4%+ Mancozeb 64% and Cymoxanil 22.1% + Famoxadone 16.6%.

The recommended dose of copper oxychloride is 1000 ppm (on active ingredient basis) and at the recommended dose, copper oxychloride and *Trichoderma* was compatible. Hence, copper oxychloride can be considered as a safe fungicide and recommended in IDM module for soil borne diseases in addition to biological control measures. Copper oxychloride (0.2%) was compatible and comparatively safer to *T. harzianum* and *T. viride* (Bagwan, 2010; Manandhar et al., 2020). According to Kumar et al. (2021), minimum inhibitory effect on growth and spore production was recorded with copper oxychloride, and hence can be used with *T. viride* in an integrated disease management practice for managing soil borne pathogens.

Similarly, at recommended dose, Cymoxanil 8% + Mancozeb 64% (WP) and 4% Metalaxyl – M 64% Mancozeb (WP), the contents of Cymoxanil and Metalaxyl were 80 ppm and 40 ppm, respectively. These chemicals at 200 ppm did not inhibit the growth of *Trichoderma* and are also compatible with *Trichoderma* at their recommended concentrations. Least inhibitory effect of Metalaxyl M-4%+ Mancozeb 64% has been reported by many workers (Desai and Kulkarni, 2004; Thoudam and Dutta, 2014; Theerthaet al., 2017; Manjunath et al., 2018 and Manandhar et al., 2020). Compatibility of Mancozeb and *Trichoderma* (Bagwan, 2010; Manjunath et al., 2018; Manandhar et al., 2020) and Cymoxanil 8% + Mancozeb 64% and *Trichoderma* had been reported (Manjunath et al., 2018; Manandhar et al., 2020).

The moderately compatible group includes, Mancozeb, Propineb and Azoxystrobin 23%. Azoxystrobin was highly compatible with *T.harzianum* and *T. viride* with no inhibition (Shashikumar et al., 2019; Manjunath et al., 2018), However, Maheshwary et al., (2020) reported 38.14% inhibition with 100 ppm Azoxystrobin. Propineb was fully compatible with *T. viride* (Madhavi et al., 2011). Theertha et al., (2017) found that Mancozeb at 100 ppm showed 23.0% inhibition and the inhibition rate is positively correlated with the strength of the fungicide.

The incompatible fungicides were Carbendazim 50%, Carbendazim 25% + Mancozeb 50%, Hexaconazole 5%, Difenoconazole 25% and Metalaxyl–M 3.3%+ Chlorothalonil 33.1%. Carbendazim either alone or in mixture proved to be highly toxic to *T. viride*, or should

Table 3. Mycelial growth (cm) of *T. asperellum* on the five fungicides amended media (PDA)

| Concentration (ppm) | Metalaxyl-M 3.3%+ Chlorothalonil 33.1% (SC) | Difenconazole 25% (EC) | Hexaconazole 5% (SC) | Carbendazim 50% (WP) | Carbendazim 25% + Mancozeb 50 % (WS) |
|---|---|---|---|---|---|
| 100 | 2.30 | 2.00 | 0.00 | 0.00 | 0.00 |
| 50 | 3.40 | 2.96 | 0.00 | 0.00 | 0.00 |
| 25 | 5.80 | 4.50 | 0.00 | 0.00 | 0.00 |
| 12.5 | 7.90 | 6,70 | 4.25 | 0.00 | 0.00 |
| 6.25 | 9.00 | 9.00 | 7.30 | 0.00 | 0.00 |
| 3.125 | 9.00 | 9.00 | 9.00 | 0.00 | 1.07 |
| Control | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |

not be combined for seed or soil application along with bio-control agents (Theertha et al., 2017; Dethoup et al., 2022). Growth and sporulation of *T. viride* was totally inhibited by Carbendazim, Hexoconazole, Carbendazim + Mancozeb at all the concentrations (Kumar et al., 2021). Non compatibility of *Trichoderma* spp. with Carbendazim fungicides had been reported by many workers (Sonavane and Venkataravanappa, 2017; Kumar et al., 2018). The high inhibition of benzimidazole compounds like Carbendazim is due to its binding with ß-tubulin of fungal pathogen causing inhibition of microtubule assembly which ultimately hinders cell division and may lead to cell death (Zhou et al., 2016).

Hexaconazole's high inhibition capability is due to the presence of systematic demethylation inhibitors. These inhibitors primarily target the vegetative stage of fungi, disrupting the development of mycelium both internally and externally within the host plant (Khalfallah et al., 1998).

The percent of compatibility decreased with an increase in the concentration of fungicide. Reduced amount of fungicide can weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist. Therefore, rather than applying these chemicals alone, it is imperative to use *Trichoderma* in combination with fungicides at the lower concentration for effective management of fungal pathogens since they do not have a side effect on the environments. Like any other crops, IDM is practiced in tuber crops also for the management of various diseases. In case of taro, cormel treatment with *Trichoderma* and spraying with Metalaxyl+ Mancozeb or Mancozeb are being recommended to combat taro leaf blight caused by *Phytophthora colocasiae*. Result of the study indicated their compatibility and endorses the integration of chemical and biocontrol for the effective management. However, for the management of diseases like collar rot in elephant foot yam and anthracnose in greater yam, corm treatment with *Trichoderma* and Carbendazim or Carbendazim + Mancozeb forms part of IDM. The present study clearly establishes the non-compatibility of *Trichoderma* with the chemicals.

## Conclusion

In sustainable agriculture, improved crop production technologies coupled with plant protection strategies play a vital role in plant disease management thereby enhancing the production and productivity of the crops. Considering the sustainability of the ecosystem and the ever-increasing concern over the presence of toxic chemicals in the food chain, lessening the use of chemicals has become an essential step. The combination of chemicals and bio-agents can take care of these concerns. The compatibility of these strategies needs to be ascertained before making recommendations to the farming community. The present study confirms the compatibility of *T. asperellum* with various agrochemicals is matching with compatibility profile of other species of *Trichoderma* viz., *T. viride* and *T. harzianum*. The class of fungicides, which were classified as compatible or moderately compatible can be recommended or continue to recommend in IDM practices. The present recommendations involving Carbendazimand its combinationshave to be revisited. Being a fungus, *Trichoderma* is affected by many of the fungicides. Hence utmost care may be taken while applying incompatible combination of fungicides and bio-agents or a safe interval must be provided. To draw final conclusions, the effects of agrochemicals on *T. asperellum* must be investigated under field conditions.

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